

GRAÇA MARIA FIGUEIREDO CASAL

**MICROSPORIDIOSES E MIXOSPORIDIOSES DA ICTIOFAUNA
PORTUGUESA E BRASILEIRA: CARACTERIZAÇÃO
ULTRAESTRUTURAL E FILOGENÉTICA**

Dissertação de Candidatura ao grau de
Doutor em Ciências Biomédicas submetida
ao Instituto de Ciências Biomédicas de
Abel Salazar da Universidade do Porto.

Orientador - Doutor Jorge Guimarães da
Costa Eiras

Categoria – Professor Catedrático

Afiliação - Faculdade de Ciências da
Universidade do Porto.

Co-orientadora - Doutora Maria Leonor
Hermenegildo Teles Grilo

Categoria - Professora Associada

Afiliação - Instituto de Ciências Biomédicas
de Abel Salazar da Universidade do Porto.

*Ao Prof. Carlos Azevedo,
Pela amizade e por tudo que me ensinou*

AGRADECIMENTOS

Ao Professor Doutor Carlos Azevedo por ter aceite orientar esta Tese até Março de 2007, apesar do obstante por dispositivos legais em oficialmente dar continuidade, para todos os efeitos fê-lo até à entrega da dissertação para apreciação. Aproveito esta ocasião para manifestar o meu profundo reconhecimento, por me ter dado a oportunidade de estagiar e, posteriormente, ser monitora no Laboratório de Biologia Celular do Instituto de Ciências Biomédicas de Abel Salazar, onde me iniciei na investigação científica, culminando o percurso com a realização desta Tese. Agradeço igualmente os inúmeros ensinamentos de carácter pedagógico e científico, bem como toda a paciência, conselhos e amizade demonstrada durante estes anos.

Ao Professor Doutor Jorge Eiras, por ter aceite em fazer parte da Comissão de acompanhamento e também a responsabilidade de assumir oficialmente a orientação dos trabalhos em substituição do Professor Doutor Carlos Azevedo que entretanto se jubilou. Desejaria aqui expressar o meu sincero agradecimento, bem como reiterar os laços científicos partilhados em diversas reuniões da Sociedade Portuguesa de Parasitologia.

À Professora Doutora Leonor Teles-Grilo o meu agradecimento por ter aceite co-orientar os trabalhos no âmbito da Biologia Molecular, área na qual dei os primeiros passos ao iniciar esta tese. Agradeço igualmente ter-me disponibilizado todo os meios do Laboratório de Genética Molecular, bem como todos os conselhos e apoio dispendido.

Às inúmeras pessoas do Laboratório de Biologia Celular um muito obrigado por me acolherem ainda como aluna do ICBAS e por toda amizade que têm demonstrado. Agradeço ao Professor Doutor Mário Sousa e ao Professor Doutor Alexandre Lobo da Cunha por me terem possibilitado continuar a usufruir das instalações e dos equipamentos do Laboratório de Biologia Celular, após a jubilação do Prof. Carlos Azevedo. Agradeço, igualmente, ao Professor Doutor Alexandre Lobo da Cunha todos os conselhos de índole científica e pessoais, bem como pela amizade e camaradagem demonstrada durante todos estes anos. À Sra. D. Laura Corral pelo ensino das técnicas de microscopia electrónica e preparação de materiais biológicos, ferramenta que serviu de base para o arranque desta Tese. À Sra. D^a. Elsa Oliveira e à Sra. D^a. Ângela Alves agradeço o apoio e conselhos técnicos diários, que sem sombra para dúvida, fazem toda a diferença.

À Doutora Camino Gestal do Instituto de Investigaciones Marinas de Vigo, Espanha pelos inúmeros conselhos diários, bem como pela agradável convivência durante a sua estadia de dois anos no Laboratório de Biologia Celular como bolsreira do Programa Post-Doc

“Fellowships” Marie Curie, supervisionada pelo Prof. Doutor Carlos Azevedo. Da colega Camino, além de uma grande amizade, ficou a saudade de alguém que partilha os mesmos interesses científicos.

Agradeço à Eng.^a Carla Oliveira do Laboratório de Genética Molecular do ICBAS, pela prontidão e amabilidade que sempre demonstrou em me auxiliar nas questões laboratoriais e à Sra. D^a. Matilde Rocha pelo auxílio na esterilização do material. Agradeço também aos alunos de Mestrado, de Estágio para conclusão de licenciatura, Bolseiros e Estagiários a título voluntário que passaram por este laboratório, nomeadamente aos licenciados Joana Tato Costa, Américo Marques, Sérgio Duarte que, pontualmente, me transmitiram um pouco das suas experiências laboratoriais.

Agradeço também ao Técnico Emanuel Monteiro do ICBAS, pelo auxílio na preparação das amostras a serem observadas no Microscópio Electrónico de Varrimento (SEM). Do Centro de Materiais da Universidade do Porto, gostaria também de agradecer à Dr^a Daniela Silva no auxílio da observação das amostras no SEM. Do Departamento de Informática do ICBAS, agradeço, aos Licenciados Rui Claro, João Morais e Nuno Santos a rápida prontidão na resolução dos problemas de informática que foram surgindo. Ao Sr. João Carvalheiro e à Sra D^a. Joana Carvalheiro do Serviço de Iconografia do ICBAS, pela reprodução das fotografias de microscopia electrónica, bem como pelos ensinamentos técnicos sobre fotografia.

À CESPU – Cooperativa de Ensino Superior Politécnico e Universitário pela atribuição de uma bolsa para custear as propinas inerentes à minha inscrição como aluna de Doutoramento no ICBAS. Ao Professor Doutor Victor Seabra, na qualidade de Coordenador do Gabinete de Formação, Investigação e Desenvolvimento, agradeço a disponibilidade e o apoio prestado.

Agradeço ao Professor Doutor Jorge Proença, Director do Instituto Superior de Ciências da Saúde - Norte (ISCS-N), e à Professora Doutora Roxana Moreira, Directora do Departamento de Ciências, as facilidades concedidas na redução da carga horária do serviço docente para o valor mínimo, bem como a compreensão e autorização em repartir a marcação de férias em períodos distintos dos contemplados pela Instituição.

Ao Professor Doutor Hassan Bousbaa, regente das disciplinas do ISCS-N (CESPU) das quais sou Assistente, por todos os ensinamentos teóricos e práticos que tem transmitido, bem como por toda a amizade e confiança depositada durante os últimos anos. A todos colegas de docência, Professora Doutora Carla Batista, Professora Doutora Catarina Lemos, Professor Doutor Frederico Silva, Doutora Manuela Henrique, Mestre Paulo

Barros, Mestre Vanessa Nascimento, Licenciada Georgina Rodrigues e Licenciada Tatiana Resende, o meu obrigado pela inter-ajuda na leccionação das várias disciplinas.

Aproveito esta oportunidade para agradecer a todos os colegas das diferentes instituições, com os quais partilhámos os mesmos anseios, a ajuda nas colheitas efectuadas no Brasil. Ao Professor Doutor Edilson Matos, Director do Laboratório de Pesquisa Carlos Azevedo da Universidade Federal Rural da Amazônia, Belém, Brasil, a quem eu muito agradeço toda a preciosa ajuda, empenho, coordenação e dedicação nas inúmeras colheitas efectuadas, por iniciativa própria e por nós solicitadas, bem como o processamento inicial das mesmas. Agradeço igualmente aos seus colaboradores mais directos, Mestre Patrícia Matos do Laboratório de Animais Aquáticos da Universidade Federal do Pará, Belém e à Mestre Patrícia Garcia do Laboratório de Diagnóstico e Patologia em Aquicultura da Universidade Federal de Santa Catarina. O meu agradecimento também para o Professor Doutor Sérgio Carmona Clemente da Faculdade de Medicina Veterinária da Universidade Federal Fluminense de Niterói pela colaboração num dos trabalhos, bem como à Doutora Débora Marques do Embrapa (Pantanal, Corumbá) e à Professora Ivete Mendonça da Faculdade de Medicina Veterinária da Universidade Federal do Piauí de Teresina, pelo envio de algumas das amostras com material parasitado.

À Professora Doutora Maria de Lurdes Pereira do Departamento de Biologia da Universidade de Aveiro e à Licenciada Fernanda Castilho (Directora do IPIMAR-Matosinhos) agradeço as facilidades concedidas na obtenção de vários espécimes utilizados na nossa investigação.

Ao Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR) e à Fundação Eng.º António de Almeida agradeço os apoios financeiros despendidos durante estes anos, tendo em muito contribuído nos custos inerentes à investigação científica. Gostaria também de agradecer ao Mestre Hugo Santos e ao Sr. Carlos Rosa (CIIMAR- Biotério de Organismos Aquáticos) pelas ocasiões em que necessitei de água salgada para a manutenção de alguns espécimes.

Por último, gostaria de agradecer a algumas pessoas que, apesar de não terem estado envolvidas directamente, foram no entanto importantes pilares emocionais durante os diferentes estados de humor pelos quais passei até concluir esta tese. À Carla Batista amiga e colega de bancada no ICBAS e, simultaneamente, colega na CESPU, pela amizade, camaradagem e pelo espírito de inter-ajuda relativamente ao serviço docente atribuído pelo ISCS-N. À Dolores Resende por toda amizade, apoio e partilha de histórias por um “hobby” comum, que por vezes me deram alento e coragem para continuar. Aos

meus Pais que sempre me apoiaram e providenciaram meios para que nada me faltasse, bem como por toda a paciência que tiveram para aturar as minhas más disposições. Finalmente, a todos os meus amigos mergulhadores, ou não, que sempre me apoiaram nos bons e maus momentos.



DIRECTIVAS LEGAIS

No cumprimento do disposto no Decreto-Lei nº 216/92 de 13 de Outubro, declara-se que a autora desta dissertação participou na concepção e na execução do trabalho experimental que esteve na origem dos resultados apresentados, bem como na sua interpretação e na redacção dos respectivos manuscritos.

Nesta tese incluem-se 10 artigos científicos publicados em revistas internacionais provenientes de uma parte dos resultados obtidos no trabalho experimental, referenciados como:

Casal, G., Matos, E. & Azevedo, C. (2002) Ultrastructural data on the spore of *Myxobolus maculatus* n. sp. (phylum Myxozoa), parasite from the Amazonian fish *Metynnis maculatus* (Teleostei). *Diseases of Aquatic Organisms* **51**: 107-112.

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

Casal, G., Matos, E. & Azevedo, C. (2006) A new myxozoan parasite from the Amazonian fish *Metynnis argenteus* (Teleostei, Characidae): light and electron microscope observations. *Journal of Parasitology* **92**: 817-821.

Casal, G., Costa, G. & Azevedo, C. (2007) Ultrastructural description of *Ceratomyxa tenuispora* (Myxozoa), a parasite of the marine fish *Aphanopus carbo* (Trichiuridae), from the Atlantic coast of Madeira Island (Portugal). *Folia Parasitologica* **54**: 165-171.

Azevedo, C., **Casal, G.**, Matos, P. & Matos, E. (2008) A new species of Myxozoa, *Henneguya rondoni* n. sp. (Myxozoa) from the peripheral nervous system of the Amazonian fish, *Gymnorhamphichthys rondoni* (Teleostei). *The Journal Eukaryotic of Microbiology* **55**: 229-234.

Casal, G., Matos, E., Matos, P. & Azevedo, C. (2008) Ultrastructural description of a new myxosporean parasite *Kudoa aequidens* sp. n. (Myxozoa, Myxosporidia), found in the Sub-opercular musculature of *Aequidens plagiozonatus* (Teleostei) from the Amazon River. *Acta Protozoologica* **47**: 135-141.

Casal, G., Matos, E., Teles-Grilo, M.L. & Azevedo, C. (2008) A new microsporidian parasite, *Potasporea morhaphis* n. gen., n. sp. (Microsporidia) infecting the teleostean fish *Potamorhaphis guianensis* from Amazon River. Morphological, ultrastructural and molecular characterization. *Parasitology* **135**: 1053-1064.

- Casal, G.**, Garcia, P., Matos, P., Monteiro, E., Matos, E. & Azevedo, C. (2009) Fine structure of *Chloromyxum menticirrho* n. sp. (Myxozoa) infecting urinary bladder of the marine teleost *Menticirrhus americanus* (Sciaenidae) in Southern Brazil. *European Journal of Protistology* **45**: 139-146.
- Azevedo, C., **Casal, G.**, Garcia, P., Matos, P., Teles-Grilo, L. & Matos, E. (2009) Ultrastructural and phylogenetic data of *Chloromyxum riorajum* sp. nov. (Myxozoa), a parasite of the stingray *Rioraja agassizii* in Southern Brazil. *Diseases of Aquatic Organisms* **85**: 41-51.
- Casal, G.**, Matos, E., Teles-Grilo, M.L. & Azevedo, C. (2009) Morphological and genetical description of *Loma psittaca* sp. n. isolated from the Amazonian fish *Colomesus psittacus*. *Parasitology Research* (in press)

ÍNDICE

PREÂMBULO	1
RESUMO	3
ABSTRACT	7
RÉSUMÉ	11

PARTE I Introdução Geral

Capítulo 1	17
1.1. Microparasitas da ictiofauna	17
1.2. Microsporidioses	17
1.2.1. Posição taxonómica	18
1.2.2. Esporo	19
Morfologia externa	19
Morfologia interna	20
Aparelho de extrusão	21
Extrusão do filamento polar	22
1.2.3. Ciclo de vida	23
Merogonia e merontes	23
Esporogonia e esporontes	24
Esporogonia e esporoblastos	26
1.2.4. Classificação taxonómica	26
1.2.5. Diagnose dos géneros que parasitam a ictiofauna	27
Listagem das espécies de microsporídios da ictiofauna	30
1.2.6. Patologia: interacção hospedeiro-parasita	36
Desenvolvimento sem formação de xenoma	37
Desenvolvimento com formação de xenoma	37
1.2.7. Estudos moleculares e filogenéticos	38
1.3. Mixosporidioses	43
1.3.1. Posição taxonómica	43
1.3.2. Classificação taxonómica	44
1.3.3. Ciclo de vida	46
1.3.4. Fases de desenvolvimento na ictiofauna	47
Mixosporos	47
Plasmódios	48
Diferenciação celular	49

1.3.5. Diagnose de alguns géneros de mixosporídios	50
1.3.6. Patologia	53
1.3.7. Estudos moleculares e filogenéticos	53
1. 4. Microsporidioses e mixosporidioses da ictiofauna portuguesa e brasileira	57
1. 5. Referências	63
1. 6. Objectivos	87

PARTE II Microsporidioses

Capítulo 2 91

A new microsporidian parasite, *Potasporea morhaphis* n. gen., n. sp. (Microsporidia) infecting the teleostean fish *Potamorhaphis guianensis* from Amazon River. Morphological, ultrastructural and molecular characterization

Capítulo 3 105

Morphological and genetical description of *Loma psittaca* sp. n. isolated from the Amazonian fish species *Colomesus psittacus*

Capítulo 4 119

Ultrastructural and molecular characterization of a new microsporidian parasite from the Amazonian fish, *Gymnorhamphichthys rondoni* (Rhamphichthyidae)

Capítulo 5 139

Fine structure and phylogeny of a new species, *Spraguea gastrophysus* (Phylum Microsporidia), a parasite of the anglerfish *Lophius gastrophysus* (Teleostei, Lophiidae) from Brazil

PARTE III Mixosporidioses

Capítulo 6 159

Ultrastructural data on the spore of *Myxobolus maculatus* n. sp. (Phylum Myxozoa), parasite from the Amazonian fish *Metynnis maculatus* (Teleostei)

Capítulo 7 167

Light and electron microscopic study of the myxosporean, *Henneguya friderici* n. sp. from the Amazonian teleostean fish, *Leporinus friderici*

Capítulo 8 177

A new myxozoan parasite from the Amazonian fish *Metynnis argenteus* (Teleostei, Characidae): light and electron microscope observations

Capítulo 9	185
Ultrastructural description of <i>Ceratomyxa tenuispora</i> (Myxozoa), a parasite of the marine fish <i>Aphanopus carbo</i> (Trichiuridae), from the Atlantic coast of Madeira Island (Portugal)	
Capítulo 10	195
A new species of Myxozoa, <i>Henneguya rondoni</i> n. sp. (Myxozoa) from the peripheral nervous system of the Amazonian fish, <i>Gymnorhamphichthys rondoni</i> (Teleostei)	
Capítulo 11	203
Ultrastructural description of a new myxosporean parasite <i>Kudoa aequidens</i> sp. n. (Myxozoa, myxosporea), found in the sub-opercular musculature of <i>Aequidens plagiozonatus</i> (Teleostei) from the Amazon River	
Capítulo 12	213
Fine structure of <i>Chloromyxum menticirrho</i> n. sp. (Myxozoa) infecting urinary bladder of the marine teleost <i>Menticirrhus americanus</i> (Sciaenidae) in southern Brazil	
Capítulo 13	223
Ultrastructural and phylogenetic data of <i>Chloromyxum riorajum</i> n. sp. (Myxozoa), a parasite of the fish <i>Rioraja agassizii</i> in Southern Brazil	
 PARTE IV Considerações Gerais e Conclusões Finais	
Capítulo 14	239
14.1. Considerações gerais	239
14.2. Conclusões finais	241
14.3. Perspectivas para futuras investigações	244
 ANEXOS	
Anexo 1 - Listagem das microsporidioses diagnosticadas em hospedeiros da ictiofauna portuguesa e brasileira	245
Anexo 2 - Listagem das mixosporidioses diagnosticadas em hospedeiros da ictiofauna portuguesa e brasileira	246
Anexo 3 – Árvore filogenética do gene SSU rRNA de microsporídios de peixes	247
Anexo 4 – Árvore filogenética da região SSU, ITS e LSU do rRNA de microsporídios de peixes	248
Anexo 5 – Árvore filogenética do gene SSU rRNA de espécies de mixosporídios	249

PREÂMBULO

Durante os últimos anos da nossa investigação dedicámos particular atenção ao estudo de microparasitas pertencentes aos filos Microsporidia, Myxozoa, Apicomplexa e Haplosporidia e às suas relações com os hospedeiros, tendo por objectivo o estudo de alguns grupos de animais aquáticos, nomeadamente peixes, crustáceos e moluscos. Para além da pesquisa na fauna portuguesa continental e regiões autónomas, o grupo no qual estou inserida, liderado pelo Professor Doutor Carlos Azevedo, tem também colaborado em trabalhos com colegas espanhóis (Galiza), de Angola e, principalmente, com diversos investigadores a norte e sul do território brasileiro.

As amostras de peixes parasitados correspondentes aos exemplares capturados na fauna brasileira, que constam nesta tese, provêm do baixo Amazonas (Estado do Pará), do Estado de Piauí (Teresina), do Estado do Rio de Janeiro (Niterói), do Estado do Paraná (Curitiba), do Estado do Mato Grosso do Sul (Corumbá) e do Estado de Santa Catarina (Florianópolis), em resultado de várias colaborações efectuadas pelo Professor Doutor Carlos Azevedo ao longo dos últimos anos. Inicialmente, nesta tese não estava previsto caracterizar parasitoses provenientes das regiões autónomas portuguesas. Contudo, pareceu-nos pertinente incluir uma importante parasitose que ocorre, frequentemente, no peixe-espada preto capturado na costa marítima da ilha da Madeira, tendo sido caracterizada ultrastruturalmente.

Relativamente ao material proveniente do Brasil, os colaboradores de cada laboratório de apoio das Universidades correspondentes a cada local de colheita, enviaram as amostras fixadas para o Laboratório de Pesquisa Carlos Azevedo da Universidade Federal Rural da Amazônia (Belém, Pará), dirigido pelo Professor Doutor Edilson Matos, onde prosseguiu o processamento das amostras até à formação do bloco (Epon), para posteriormente serem observadas no TEM, do Laboratório de Biologia Celular do ICBAS. As amostras destinadas ao SEM foram somente fixadas em glutaraldeído, enquanto que as destinadas aos estudos de biologia molecular foram preservadas em etanol a 80% e, posteriormente, enviadas para o nosso laboratório onde foram processadas consoante os estudos previstos.

RESUMO

A identificação das possíveis parasitoses da ictiofauna tem sido considerada de grande interesse em piscicultura. A nível mundial tem-se assistido, nas últimas décadas, à sua expansão, prevendo-se que, cada vez mais, espécies de peixes, crustáceos e moluscos possam vir a ser introduzidas em aquacultura. Sabe-se também que os peixes cultivados, em comparação com os nativos, são particularmente susceptíveis de adquirir várias infecções parasitárias, devido ao facto de se encontrarem em elevadas densidades populacionais. Assim, a caracterização e a identificação dos organismos patogénicos são fundamentais, tendo em vista o desenvolvimento de métodos de rápida detecção dos agentes parasitários, bem como a pesquisa de drogas e de vacinas susceptíveis de combater essas infecções. Dada a grande variedade de agentes patogénicos que ocorrem na ictiofauna, na presente tese foram eleitos dois grupos importantes de parasitas, os microsporídios (filo Microsporidia Balbiani, 1882) e os mixosporídios (filo Myxozoa Grassé, 1970), com o objectivo de os caracterizar a nível morfológico, ultrastrutural e filogenético.

Os microsporídios são microrganismos de reduzidas dimensões, unicelulares, com um ciclo de vida obrigatoriamente intracelular. Este grupo de parasitas possui características celulares e moleculares invulgares e tem como hospedeiros variados grupos de animais invertebrados e vertebrados de diferentes habitats de diversas áreas geográficas. Considerando os microsporídios como agentes patogénicos que além de provocarem grande mortalidade em várias espécies, podem entrar na cadeia alimentar animal, inclusive na humana, o seu estudo torna-se fundamental em várias vertentes.

Por seu lado, os mixosporídios são agentes patogénicos multicelulares que têm sido descritos, principalmente, em peixes de vários habitats de diferentes áreas geográficas. As parasitoses por mixosporídios são, geralmente, um grave problema, principalmente quando se encontram associadas ao tecido muscular esquelético, uma vez que podem induzir uma generalizada liquefacção do músculo infectado, acarretando perdas avultadas no seu valor comercial, chegando mesmo a inviabilizar a sua comercialização.

Os estudos destes dois grupos de parasitas de animais aquáticos provenientes da fauna portuguesa e brasileira são escassos, comparativamente com os de outras regiões geográficas. Neste sentido, a pesquisa de material biológico parasitado por microsporídios e por mixosporídios foi direccionada para algumas espécies de peixes marinhos e de água doce, com valor comercial, da fauna portuguesa e brasileira. Da costa atlântica portuguesa, a região norte foi a zona seleccionada para a amostragem de peixes. Por outro lado, os exemplares provenientes da fauna brasileira abrangeram vários

Estados (Pará, Piauí, Rio de Janeiro, Paraná, Mato Grosso do Sul e Santa Catarina) do norte e sul do país.

As amostras de tecido parasitado foram processadas para microscopia de luz (LM), microscopia electrónica de transmissão (TEM) e microscopia electrónica de varrimento (SEM). Adicionalmente, parte do material parasitado foi processado com vista à obtenção do DNA genómico, seguida da sua amplificação, clonagem, sequenciação de genes ou porções de genes, tais como SSU rDNA e LSU rDNA, incluindo a região ITS.

Assim, este estudo incidiu essencialmente em duas vertentes, tendo como objectivo a classificação taxonómica das espécies de parasitas diagnosticadas: a caracterização morfológica e ultrastrutural dos diferentes estádios do ciclo de vida dos parasitas (microsporídios e mixosporídios) e, paralelamente para algumas espécies, a caracterização molecular de genes conservados com o objectivo de estabelecer relações filogenéticas com espécies afins. Nos estudos filogenéticos, a análise foi efectuada consoante os casos, pelos métodos máximo parcimónio, máxima verossimilhança e inferência Bayesiana. Foram tidos em conta, igualmente, os aspectos relacionados com a histopatologia associada às respectivas parasitoses.

No decurso desta tese, foram pesquisados e diagnosticados vários microsporídios e mixosporídios em peixes provenientes de ambas as origens descritas. Relativamente aos microsporídios caracterizados (**Parte II**), foi criado um novo género e descritas 4 novas espécies com base na ultraestrutura da esporogénese e na filogenia do gene SSU rRNA. Três dos parasitas provêm do Estado do Pará, sendo elas *Potasporea morhaphis* n. gen., n. sp., que desenvolve xenomas encontrados na parede da cavidade celómica abdominal, localizada na região posterior, do peixe de água doce *Potamorhaphis guianensis* (Belontiidae) (**Capítulo 2**); *Loma spittaca* n. sp., espécie que também diferencia xenomas, na mucosa intestinal de *Colomesus psittacus* (Tetraodontidae) (**Capítulo 3**); e uma terceira espécie localizada no tecido muscular esquelético de *Gymnorhamphichthys rondoni* (Rhamphichthyidae), sem a formação de xenomas. Esta espécie foi incluída, provisoriamente, no grupo colectivo dos microsporídios, tendo sido classificada como *Microsporidium rondoni* n. sp., dado que os resultados ultrastruturais e moleculares não foram conclusivos (**Capítulo 4**). Por último, foi descrito um microsporídio identificado como pertencendo ao género *Spraguea*, localizado nos nervos da medula espinal do tamboril *Lophius gastrophysus* (Lophiidae), peixe de grande importância económica, capturado perto da cidade de Niterói (Estado do Rio de Janeiro) (**Capítulo 5**).

Relativamente às mixosporidioses estudadas (**Parte III**), foram identificadas 7 novas espécies com base em resultados obtidos através de microscopia óptica (DIC), TEM e,

em alguns casos, recorreu-se também a observações efectuadas no SEM. Cinco das mixosporidioses ocorreram em peixes capturados no Estado do Pará: 2 espécies pertencentes ao género *Myxobolus*, 2 ao género *Henneguya* e uma outra do género *Kudoa* (ordem Multivalvulida). A espécie *M. maculatus* parasita o rim do peixe de água doce *Metynnis maculatus* (Characidae), enquanto que a espécie *M. metynnis* ocorre nos tecidos conjuntivos subcutâneos da região orbicular do peixe *Metynnis argenteus* (Characidae), descritas nos **Capítulos 6 e 8**, respectivamente. A parasitose por *H. friderici* foi observada em vários órgãos, tais como filamentos branquiais, intestino, rim e fígado de *Leporinus friderici* (Anostomidae) (**Capítulo 7**). Já a espécie *H. rondoni* ocorre no sistema nervoso periférico do peixe de água doce, conhecido por peixe-faca, *Gymnorhamphichthys rondoni* (Rhamphichthyidae) (**Capítulo 10**). Foi ainda descrita como nova espécie, *Kudoa aequidens*, encontrada na musculatura subopercular do peixe de água doce *Aequidens plagiozonatus* (Cichlidae) (**Capítulo 11**). Nos peixes oriundos do Estado de Santa Catarina foram descritas mais duas novas espécies de mixosporídios pertencentes ao género *Chloromyxum*. A espécie *C. menticirrhii* foi encontrada na vesícula urinária do peixe teleósteo marinho *Menticirrhus americanus* (Sciaenidae) (**Capítulo 12**), enquanto que a espécie *C. riorajum* foi diagnosticada na vesícula biliar do peixe cartilagíneo marinho *Rioraja agassizii* (Rajidae) (**Capítulo 13**). Em peixes capturados na costa portuguesa da ilha da Madeira, foi feita a caracterização dos estádios de desenvolvimento do ciclo de vida inerentes à esporogénese da espécie *Ceratomyxa tenuispora* (**Capítulo 9**). Este mixosporídio parasita a vesícula biliar do peixe-espada, *Aphanopus carbo* (Trichiuridae), espécie de grande interesse comercial. Apenas para o mixosporídio *C. riorajum*, foram realizadas análises moleculares e filogenéticas com base na sequenciação do gene SSU rDNA.

Pela análise destes resultados, constata-se que a classificação de qualquer grupo de organismos não deveria ser baseada numa única característica, mas tendo em conta uma combinação de vários factores, tais como: habitat, especificidade do hospedeiro, local de infecção, interacção com as células hospedeiras e as características morfológicas ultraestruturais do ciclo de vida do parasita. Adicionalmente, a análise de sequências moleculares e, consequentemente, as inferências filogenéticas estabelecidas entre espécies afins são de grande relevância para uma classificação mais precisa. Assim, o conjunto destes resultados é um contributo significativo para o conhecimento deste grupo de parasitas, servindo de ponto de partida para estudos de investigação abrangendo outras áreas, bem como uma aplicação mais directa, como por exemplo, no desenvolvimento de tratamentos específicos contra estas espécies.

ABSTRACT

The identification of the possible parasitosis in the ichthyofauna has been considered of a great interest in fisheries. Globally, in the last decades, their enlargement has been observed suspecting that more fish, crustaceans and clams species could be introduced in aquaculture. It is also known that fishes from captivity, when compared to the natives, are particularly susceptible to be infected by some parasites, due to high population densities. Thus, the characterization and the identification of the pathogenic organisms are fundamental, taking into account the development of fast detection methods of the parasitic agent, as well as the research of drugs and susceptible vaccines against these infections. Recognized the wide pathogens variety that occur in fish species, this thesis was focused on two important groups of parasites, the microsporidian (phylum Microsporidia Balbiani, 1882) and the myxosporidian (phylum Myxozoa Grassé, 1970), which occurred frequently in the ichthyofauna, aiming their morphological, ultrastructural and phylogenetic characterization.

Microsporidian are microorganisms of reduced dimensions, unicellular, with an obligatorily intracellular life cycle. This group of parasites possesses unusual cellular and molecular characteristics. They are hosted by several groups of invertebrate and vertebrate organisms from different habitats and distinct geographic areas. Because microsporidian can be considered as pathogenic agents that cause great mortality in some species and they are able to be introduced in the animal food chain, including in humans, their study becomes fundamental in several aspects.

On the other hand, myxosporidian are multicellular pathogenic agents, which have been described, mainly, in fishes from several habitats and different geographic areas. In general, the parasitosis by myxosporidian are a serious problem, mainly when associated with muscular tissues, because they can induce a generalized liquefaction of the infected muscle, causing high losses of its commercial value, leading to impracticable commercialization.

Studies in these parasite groups of aquatic animals proceeding from the Portuguese and Brazilian fauna are limited, when compared to other from different geographic regions. Thus, this work focused on biological samples parasitized by microsporidian and myxosporidian from some marine and freshwater fish species with commercial value from Portuguese and Brazilian coasts. From the Portuguese Atlantic coast, the north region was the elected zone for the fish sampling. On the other hand, the specimens from the Brazilian fauna were caught in different States, from north and south of the country (Pará, Piauí, Rio de Janeiro, Paraná, Mato Grosso do Sul, Santa Catarina).

Samples of parasitized tissues were processed for light microscopy (LM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Additionally, part of the samples were processed in order to obtain genomic DNA, as well as the amplification, cloning and sequentiation of genes or conserved gene portions, such as SSU rDNA and LSU rDNA, including ITS region.

Thus, the aim of this study consisted in the taxonomic classification of the parasite species diagnosticated (microsporidian and myxosporidian), in particular taking into account the morphological and ultrastructural characterization of different life cycle stages and, simultaneously in some species, the molecular characterization of conserved genes with the objective to establish phylogenetic relations with similar species. In the phylogenetic studies, the analysis for maximum parsimony, maximum likelihood methods and Bayesian inference were performed. In addition, histopathological aspects associated with the parasitosis were considered.

Hence, in this thesis, some microsporidian and myxosporidian of fishes originated from the referred habitat were studied and diagnosticated. In relation to the microsporidian (**Part II**), a new genus and 4 new species were named and described, based on the ultrastructure of the sporogenesis and on SSU rRNA gene phylogeny. Three parasites were from the State of Pará, namely *Potasporea morhaphis* n. gen., n. sp., which develops xenomas in the wall of the posterior region of the abdominal celomic cavity in the freshwater fish *Potamorhaphis guianensis* (Belonidae) (**Chapter 2**); *Loma spittaca* n. sp., a species that also forms xenomas in the intestinal mucosa of *Colomesus psittacus* (Tetraodontidae) (**Chapter 3**); and a third species located in the skeletal muscular tissue of *Gymnorhamphichthys rondoni* (Rhamphichthyidae), without the xenoma formation. This last parasite species was included in the collective group of microsporidian, and was classified as *Microsporidium species rondoni* n. sp., because the ultrastructural and molecular results were not conclusive (**Chapter 4**). Finally, a microsporidian was described and identified as belonging to the genus *Spraguea*. It was found in the spinal marrow nerves of the anglerfish *Lophius gastrophysus* (Lophiidae), a fish with great economic importance, captured close to the city of Niterói (State of Rio de Janeiro) (**Chapter 5**).

In relation to the studied myxosporidiosis (**Part III**), 7 new species were identified based on results obtained by optical microscopy (DIC), TEM and, in some cases, on SEM observations. Five of the myxosporidiosis occurred in fish caught in the State of Pará: 2 species belonging to the genus *Myxobolus*, 2 to the genus *Henneguya* and another one from genus *Kudoa* (Order Multivalvulida). *Myxobolus maculatus* n. sp. parasites the kidney of freshwater fish *Metynnis maculatus* (Characidae), while *Myxobolus metynnis*

species occurs in the subcutaneous conjunctive tissue from the orbicular region of the fish *Metynnis argenteus* (Characidae), described in **chapters 6** and **8** respectively. The *Henneguya friderici* n. sp. parasitosis was observed in some organs, such as gill filaments, intestine, kidney and liver of *Leporinus friderici* (Anostomidae) (**Chapter 7**). On the other hand the species *Henneguya rondoni* occurs in the peripheral nervous system of a freshwater fish, known as sand knifefish, *Gymnorhamphichthys rondoni* (Rhamphichthyidae) (**Chapter 10**). *Kudoa aequidens* was also described as a new species, which was found in the sub-opercular musculature of the freshwater fish *Aequidens plagiozonatus* (Cichlidae) (**Chapter 11**). In fishes from the State of Santa Catarina two new myxosporidian species belonging to genus *Chloromyxum* were described. The *C. menticirrho* was found in the urinary bladder of the marine teleostean fish, *Menticirrhus americanus* (Sciaenidae) (**Chapter 12**), while the *C. riorajum* was diagnosed in the gall bladder of the cartilaginous marine fish *Rioraja agassizii* (Rajidae) (**Chapter 13**). From the fishes captured on the Madeira island coast, the characterization of the *Ceratomyxa tenuispora* life cycle stages inherent to the sporogenesis stage was carried out (**Chapter 9**). This myxosporidian infects the gall bladder of the black-scabbard fish, *Aphanopus carbo* (Trichiuridae), being a species of great commercial interest. Only for the myxosporidian *C. riorajum*, molecular analyses and phylogenetic relationships were carried out based on SSU rDNA gene sequencing.

From the examination of these results, it seems that the classification of any group of organisms should not be based on a single characteristic. It would consider a combination of several factors, such as the habitat, host specificity, local of infection, interaction with host cells and the morphological and ultrastructural details of the parasite life cycle. In addition, molecular sequences analysis and, consequently, the phylogenetic inferences established between related species are of great importance for an accurate classification. Thus, all these results contribute significantly to the knowledge of this parasite group, being a baseline for research in other areas, as well as for a practical application, e. g., in the development of specific treatments against these species.

RÉSUMÉ

L'identification des possibles parasitoses de l'ichthyofaune a été considérée de grand intérêt en pisciculture. À niveau mondial il s'est assisté, les dernières décennies, à son expansion, en se prévoyant que, de plus en plus, des espèces de poissons, crustacés et mollusques puissent venir à être introduits dans l'aquaculture. On sait aussi que les poissons cultivés, par rapport aux indigènes, sont particulièrement susceptibles de contracter plusieurs infections parasitaires, dû au fait d'ils se trouvers dans de hautes densités populationnels. Ainsi, la caractérisation et l'identification des organismes pathogènes sont fondamentales, en vue du développement des méthodes de détection rapide des agents parasitaires bien aussi dans la recherche des drogues et des vaccins susceptibles de combattre les infections. En vue de la grande variété d'agents pathogènes qui se produisent dans l'ichthyofaune, dans la présente thèse ont été élus deux groupes importants de parasites, les microsporidies (phylum Microsporidia Balbiani, 1882) et les myxosporidies (phylum Myxozoa Grassé, 1970), avec l'objectif de les caractériser à travers la morphologie, de l'ultrastructure et de la phylogénie.

Les microsporidies sont des microorganismes de dimensions réduites, unicellulaires, avec un cycle de vie obligatoirement intracellulaire. Ce groupe de parasites possède des caractéristiques cellulaires et moléculaires rares et ont, comme hôte, différents groupes d'animaux invertébrés et vertébrés de différents habitats de divers régions géographiques. Considérant que les microsporidies sont agents pathogènes qui causent grande mortalité dans plusieurs espèces, en pouvant entrer dans la chaîne alimentaire animale et humaine, il se rend fondamental son étude dans plusieurs aspects.

D'autre côté, les myxosporidies sont agents pathogènes multicellulaires qui ont été décrits, principalement, dans des poissons d'eau douce et marins dans de différentes régions géographiques. Les parasitoses par des myxosporidies sont un grave problème quand ils se trouvent associés, principalement, au tissue musculaire squelettique, une fois que peuvent induire une liquéfaction généralisée du muscle qui cause des pertes importantes de leur valeur commerciale, en arrivant même à rendre impraticables leur commercialisation.

Des études de ces deux groupes de parasites des animaux aquatiques provenant de la faune portugaise et brésilienne sont insuffisantes, par rapport aux autres régions géographiques. Dans ce sens, la recherche du matériel biologique parasité par des microsporidies et par des myxosporidies a été dirigée pour quelques espèces de poissons marins et d'eau douce, avec valeur commerciale, de la faune portugaise et brésilienne. De la côte atlantique portugaise, la région nord a été la zone sélectionnée pour

l'échantillonnage de poissons. D'autre part, les exemplaires provenant de la faune brésilienne ont inclus plusieurs états (Pará, Piauí, Rio de Janeiro, Paraná, Mato Grosso do Sul et Santa Catarina) du nord et du sud du pays.

Les échantillons de tissu parasités ont été préparés pour la microscopie de lumière (LM), microscopie électronique de transmission (TEM) et microscopie électronique à balayage (SEM). D'autre part, une portion du matériel parasité a été traitée pour obtenir du DNA génomique, suivi par son amplification, clonage et séquençage des gènes ou de portions des gènes, tels que SSU rDNA et LSU rDNA, y compris la région ITS.

Ainsi, l'étude il est arrivé, essentiellement, dans deux aspects, en ayant comme objectif la classification taxonomique des espèces: la caractérisation morphologique et ultrastructure des différents stades du cycle de vie des parasites (microsporidies et myxosporidies) et, parallèlement pour quelques espèces, la caractérisation moléculaire des gènes conservés avec l'objectif d'établir des relations phylogénétiques avec les espèces semblables. Dans des études phylogénétiques, l'analyse a été effectuée selon les cas, par les méthodes maximum parcimonie, maximum de vraisemblance et inférence Bayésienne. Ils ont été tenus compte, également, des aspects rapportés avec l'histopathologie associé aux respectives parasitoses.

Au cours de cette thèse, ils ont été cherchés et diagnostiqués plusieurs microsporidies et myxosporidies dans des poissons provenant des deux faunes. En relation aux microsporidies caractérisées (**Partie II**), il été créé un nouveau genre et décrites 4 nouvelles espèces basées sur l'ultrastructure de l'esporenèse et sur la phylogénie du gène SSU rRNA. Trois des parasites viennent de l'État du Pará, sont elles *Potasporea morhaphis* n. gen. et n. sp., qui développe des xenomes trouvées dans la paroi de la cavité coelomique abdominale, localisée dans la région postérieure, du poisson d'eau douce *Potamorhaphis guianensis* (Belontiidae) (**Chapitre 2**) ; *Loma spittaca* n. sp. espèce qui aussi forme des xenomes dans la muqueuse intestinale de *Colomesus psittacus* (Tetraodontidae) (**Chapitre 3**) et la troisième espèce localisée dans le tissu musculaire squelettique de *Gymnorhamphichthys rondoni* (Rhamphichthyidae), sans la formation de xenomes. Cette espèce a été introduit, provisoirement, dans le groupe collectif des microsporidies, en ayant été classifié comme *Microsporidium rondoni* n. sp., étant donné que les résultats ultrastructuraux et moléculaires n'ont pas été concluants (**Chapitre 4**). Dernièrement, une microsporidie identifiée comme appartenant au genre *Spraguea*, a été décrit dans les nerfs de la moelle épinière de baudroie pêcheuse *Lophius gastrophysus* (Lophiidae), poisson de grande importance économique, capturée près de la ville de Niterói (l'État du Rio de Janeiro) (**Chapitre 5**).

En relation aux myxosporidioses étudiées (**Partie III**), ont été identifiées 7 nouvelles espèces basées sur des résultats obtenus par microscopie optique (DIC), TEM et dans quelques cas il s'est fait appel, aussi, à des observations effectuées dans ce SEM. Cinq de myxosporidioses s'ont produits dans des poissons capturés dans l'État du Pará: 2 espèces appartenant au genre *Myxobolus*, 2 au genre *Henneguya* et une autre au genre *Kudoa* (ordre Multivalvulida). L'espèce *M. maculatus* parasite le rein du poisson d'eau douce *Metynnis maculatus* (Characidae), tandis que l'espèce *M. metynnis* se retrouve dans les tissus conjonctifs sous-cutanées de la région orbiculaire du poisson *Metynnis argenteus* (Characidae), ont été décrites dans les **Chapitres 6** et **8**, respectivement. La parasitose par *H. friderici* a été observée dans plusieurs organes, tels que des filaments branchiaux, intestin, rein et foie de *Leporinus friderici* (Anostomidae) (**Chapitre 7**). Déjà l'espèce *H. rondoni* se produit dans le système nerveux périphérique du poisson d'eau douce, connu par poisson électrique, *Gymnorhamphichthys rondoni* (Rhamphichthyidae) (**Chapitre 10**). Le parasite décrit comme nouvelle espèce, *Kudoa aequidens*, a été trouvé dans la musculature sub-operculaire du poisson d'eau douce *Aequidens plagiozonatus* (Cichlidae) (**Chapitre 11**). Dans les poissons originaires de l'État de Santa Catarina ont été décrits plus deux nouvelles espèces de myxosporidies appartenant au genre *Chloromyxum*. L'espèce *C. menticirrho* a été trouvée dans la vésicule urinaire du poisson téléostéen marin, *Menticirrhus americanus* (Sciaenidae) (**Chapitre 12**), tandis que l'espèce *C. riorajum* a été diagnostiquée dans la vésicule biliaire du poisson cartilagineux marin *Rioraja agassizii* (Rajidae) (**Chapitre 13**). Dans des poissons capturés dans la côte portugaise d'Île de Madère, a été faite la caractérisation des stades de développement du cycle de vie inhérents à l'esporegénèse de l'espèce *Ceratomyxa tenuispora* (**Chapitre 9**). Cette myxosporidie parasite la vésicule biliaire du sabre noir, *Aphanopus carbo* (Trichiuridae), espèce de grand intérêt commercial. Seulement pour la myxosporidie *C. riorajum*, ont été réalisées des analyses moléculaires et phylogénétiques basées sur la séquenciation du gène SSU rDNA.

Par l'analyse de ces résultats, se constate que le classement de tout groupe d'organismes ne doit pas être basé sur une seule caractéristique, mais vu une combinaison de plusieurs facteurs, comme l'habitat, la spécificité de l'hôte, lieu d'infection, interaction avec les cellules hôtes, les caractéristiques morphologiques et les détails ultrastructurales du cycle de vie du parasite. Supplémentairement, l'analyse des séquences moléculaires et, en conséquence, les inférences phylogénétiques établies entre des espèces semblables sont de grande importance pour un classement plus précis. Ainsi, l'ensemble de ces résultats sont une contribution significative pour la connaissance de ce groupe de parasites, en servant de point de départ pour recherche dans d'autres contextes, ainsi

qu'une application plus directe, comme par exemple, dans le développement de traitements spécifiques contre ces espèces.

PARTE I

INTRODUÇÃO GERAL

Capítulo 1

1.1. Microparasitoses da ictiofauna

A fauna aquática dos diferentes meios ambientes e das variadas áreas geográficas estão sujeitos à acção nefasta de diferentes tipos de microparasitoses. Esta situação tem grande relevância quando se trata de animais de interesse económico, como peixes, moluscos e crustáceos, os quais concorrem para uma baixa de produção.

De entre os agentes patogénicos que ocorrem geralmente na fauna aquática, como vírus, bactérias, rickettsias, apicomplexos, haplosporídios, ciliados, entre outros, destacamos dois grupos de agentes que ocorrem, frequentemente, na fauna ictiológica, induzindo microsporidioses e mixosporidioses.

1.2. Microsporidioses

As microsporidioses são doenças provocadas pela acção parasitária dos microsporídios (filo Microsporidia Balbiani, 1882), organismos unicelulares eucariotas de reduzidas dimensões, que têm um ciclo de vida obrigatoriamente intracelular. Estes parasitas podem causar enormes malefícios e, em muitos casos, são a causa da morte dos seus hospedeiros. Este grupo de parasitas patogénicos ocorre em alguns organismos unicelulares (ciliados e gregarinas) e em quase todos os filos dos metazoários, tais como mixosporídios, celenterados, platelmintas, nemátodes, rotíferos, anelados, moluscos, briozoários, artrópodes e em todas as classes de vertebrados, incluindo os humanos. Neste caso, as parasitoses estão, muitas vezes, associadas a infecções provocadas pelo vírus da imunodeficiência humana (HIV) (Desportes *et al.* 1985). Alguns géneros destes microrganismos são também referidos como sendo a causa primária de diarreias crónicas em pacientes com a síndrome da imunodeficiência adquirida (SIDA) (Wasson & Peper 2000, Didier & Weiss 2006).

Actualmente são reconhecidas mais de 1300 espécies pertencendo a 144 géneros (Larsson 1999), números com tendência a aumentar com a descoberta de novos géneros e espécies, que têm como hospedeiro, em larga maioria, espécies de artrópodes e de peixes (Sprague 1977, Canning & Lom 1986, Larsson 1986, Lom & Dyková 1992a, Sprague *et al.* 1992, Lom 2002, Lom & Nilsen 2003).

O estudo dos microsporídios tem suscitado um grande interesse por parte dos investigadores e das entidades sanitárias. Tem sido de fundamental importância o estudo destes parasitas nas vertentes morfológica, fisiológica, citoquímica, imunológica,

molecular e filogenética. Também tem havido a preocupação de tentar encontrar métodos mais eficazes de diagnóstico das microsporidioses, assim como de tentar a otimização de recursos à profilaxia mais apropriada, de forma a combater as infecções oportunistas causadas por este tipo de microrganismos.

1.2.1. Posição taxonómica

A primeira referência a microsporídios data do século XIX, com a identificação de um parasita em França, encontrado em insectos produtores de seda (*Bombyx mori*) e associado à doença conhecida por “Pebrina”, da qual resultaram graves prejuízos económicos (Becnel & Andreadis 1999). Este parasita foi, inicialmente, classificado como pertencendo ao grupo comum das leveduras e bactérias (Schizomycetes), ao qual se deu o nome de *Nosema bombycis* Naegli, 1857 (Sprague & Becnel 1998).

Durante muitos anos, os microsporídios foram incluídos nos protozoários (Protozoa). Só na última década do século XX, a taxonomia sofreu grandes transformações, tendo sido reconhecidos como dos mais primitivos seres da árvore filogenética dos eucariotas, divergindo antes de ocorrer a endossimbiose mitocondrial (Vossbrinck *et al.* 1987). Em 1993, Cavalier-Smith agrupou-os no reino designado por Archezoa, juntamente com os Archamoebae, Metamonada e Parabasalia. Entre as características citológicas e moleculares invulgares que possuem, salientam-se uma aparente ausência de mitocôndrias, estruturas comparáveis aos cinetossomas, peroxissomas, lisossomas e flagelos (Marquardt & Demeree 1985, Larsson 1986, 1999 Cavalier-Smith 1987, Perkins 1991). Por outro lado, os núcleos são constituídos por um invólucro nuclear, constituído por duas membranas, mas com uma divisão nuclear considerada primitiva, embora mostrem evidentes características dos eucariotas (Vossbrinck *et al.* 1987). Os ribossomas e os RNAs ribossomais têm afinidades, simultaneamente, com os seres procariotas e eucariotas (Vossbrinck & Woese 1986, Vossbrinck *et al.* 1987). Várias teorias foram propostas com o intuito de explicar o seu primitivismo. Segundo Cavalier-Smith (1993), os microsporídios tiveram origem a partir de formas pré-mitocondriais, ou então, como outra hipótese, estes organismos perderam as suas mitocôndrias, em consequência do tipo de vida parasitária.

Nos finais do século XX, a sequenciação do gene HSP70 (codifica proteínas de choque de 70 kDa, do tipo chaperone, normalmente funcionais nas mitocôndrias dos eucariotas) do microsporídio *Vairimorpha necatrix* sugere que, em períodos ancestrais, este grupo de parasitas possuiu mitocôndrias, acabando por as perder (Germot *et al.* 1997, Hirt *et al.* 1997). Presentemente, com o conhecimento na íntegra do genoma do microsporídio

Encephalitozoon cuniculi (Katinka *et al.* 2001), foram descobertas apenas 22 proteínas envolvidas em processos mitocondriais, tais como a formação dos complexos Fe-S da mitocôndria não intervindo nenhuma delas, no entanto, em funções mitocondriais canónicas, tais como a respiração aeróbica (Goldberg *et al.* 2008). A elaboração de árvores filogenéticas com base na sequenciação dos genes que codificam para as proteínas tubulina α e β (Keeling & Doolittle 1996, Edlind *et al.* 1996), nas sequências genéticas que codificam os factores de alongação da tradução EF-1 α e EF-2 (Hashimoto *et al.* 1997), proteínas de ligação à “TATA box” (Fast *et al.* 1999), valil-tRNA sintetase (Weiss *et al.* 1999), a grande subunidade da RNA polimerase II (RPB1) (Hirt *et al.* 1999) apontam para uma grande proximidade dos microsporídios com o reino Fungi (Gill & Fast 2006). Estas evidências genéticas e moleculares, bem como a presença de quitina e trehalose nos microsporídios, componente igualmente presente nos fungos (Keeling & McFadden 1998), vêm reforçar a 2ª hipótese proposta por Cavalier-Smith (1993). Como resultado do acumular de inúmeras evidências filogenéticas, aparentemente, os microsporídios encontram-se incluídos no reino Fungi (Cavalier-Smith 1998) persistindo a dúvida se eles partilharam o mesmo ancestral com os fungos ou, se então, derivaram a partir destes (Gill & Fast 2006). Recentes análises filogenéticas, efectuadas por Lee e colaboradores (2008), demonstram que os microsporídios são fungos verdadeiros, especificamente relacionados com os zigomicetes, que possuem componentes reguladores genéticos que poderiam funcionar na determinação do sexo e na reprodução sexual.

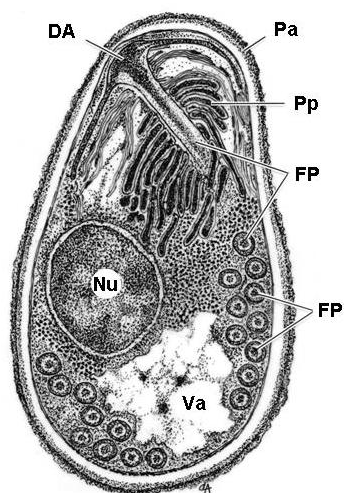
1.2.2. Esporo

Morfologia externa

Ultrastruturalmente caracterizam-se por possuir esporos unicelulares com parede rígida e espessa sem qualquer tipo de perfuração. Os esporos encontrados na ictiofauna são, na maior parte dos casos, de características morfológicas similares, de forma oval ou elipsoidal (Larsson 1986, Lom & Dyková 1992a). As suas dimensões oscilam entre os limites de 2 μm de comprimento na espécie *Nucleospora salmonis* (Hedrick *et al.* 1991) até 20 μm na espécie *Jirovecia piscicola*, descrita no peixe *Gadus merlangus* (Lom & Dyková 1992a) (Esquema 1).

Externamente, a superfície é geralmente lisa, no entanto em algumas espécies, podem existir sulcos de diferente forma e organização, que lhe confere uma certa especificidade (Lom & Weiser 1972). A parede é espessa, excepto no local de extrusão do filamento polar, e constituída por duas camadas finas. A exterior, exosporo, é electrodensa,

proteica e de pequena espessura (15-100 nm), recobre uma camada mais interna, endosporo, que é mais espessa (150-200 nm), electrolucente e de natureza quitinosa e proteica (Erickson & Blanquet 1969, Vávra 1976). A parede do esporo é uma estrutura que funciona como uma barreira protectora ambiental e, simultaneamente, as proteínas da parede intervêm no processo de aderência aos glicosaminoglicanos sulfatados da superfície das células hospedeiras (Hayman *et al.* 2005). A identificação das proteínas da parede do esporo pode ser útil no diagnóstico e na elaboração de drogas apropriadas no combate a organismos patogénicos. Até ao momento foram identificadas em *Encephalitozoon* spp. 2 proteínas exosporais SWP1 e SWP2 (Bohne *et al.* 2000, Hayman



et al. 2001) e 3 proteínas endosporais EnP2 ou SWP3, EnP1 (Peuvel-Fanget *et al.* 2006, Xu *et al.* 2006) e EcCDA (Brosson *et al.* 2005). Em *Nosema bombycis* estão descritas 3 proteínas, SW30, SW32 (Wu *et al.* 2008) e SW26 (Li *et al.* 2009).

Esquema 1 – Desenho esquemático do esporo de um microsporídeo, em corte longitudinal mostrando a parede (Pa), disco de ancoragem (DA), polaroplasto (Pp), núcleo (Nu), vacúolo (Va) e o filamento polar (FP).

Morfologia interna

Internamente, envolvida pela parede, encontra-se a célula germinal chamada esporoplasma. Esta é delimitada por uma membrana simples e diferencia as estruturas típicas deste grupo de parasitas, sendo elas um núcleo individualizado idêntico aos eucariotas (Larsson 1986), podendo este ser 1 simples ou 2 associados em que as superfícies adjacentes achatadas estabelecem contacto, formando um diplocário (Sprague & Vernick 1974) e um aparelho de extrusão de origem golgiana que serve para injectar o esporoplasma dentro da célula hospedeira (Canning & Lom 1986) (Esquema 1). Nesta célula também estão presentes ribossomas, por vezes organizados numa disposição linear ou em espiral semelhante aos polirribossomas. Os ribossomas tipo-procariotas têm um coeficiente de sedimentação 70S e dissociam-se nas subunidades 50S e 30S (Ishihara & Hayashi 1968); cisternas de RE liso e rugoso; microtúbulos também estão presentes, no entanto, foram somente observados associados à divisão nuclear (Vávra 1976). Aparentemente, durante todo o ciclo de vida, não existem mitocôndrias, substâncias de reserva, bem como estruturas comparáveis aos cinetossomas, peroxissomas e lisossomas (Larsson 1986, Perkins 1991). Em 2002, Williams e colaboradores, ao imunolocalizar a proteína mitocondrial HSP70 em

Trachipleistophora hominis, provaram a existência de um pequeno organelo sem cristas delimitado por dupla membrana, designado de mitossoma (Tovar *et al.* 1999). Com base nas observações ultraestruturais efectuadas por Vávra (2005), este organelo existe em várias espécies de microsporídios, sendo referido como mitocôndria rudimentar.

Aparelho de extrusão

O aparelho de extrusão é constituído por quatro estruturas, que determinam a polaridade do esporo, conhecidas pelo nome de disco de ancoragem (DA), polaroplasto (Pp), filamento polar (FP) e vacúolo posterior (VP) (Esquema 1).

O DA é uma estrutura laminar achatada, revestida por membrana, em forma de cogumelo, localizado no pólo anterior do esporo (neste local o endosporo é menos espesso) e que reage positivamente à reacção do PAS para os polissacarídios (Perkins 1991). No DA insere-se a primeira porção do FP, designada de manúbrio, numa zona proximal e central, projectando-se rectilínea e obliquamente em relação ao eixo do esporo, enrolando-se de seguida em várias voltas, ficando estas dispostas numa ou mais fiadas. O número de voltas tem sido considerado como um dos critérios na identificação de espécies pertencentes ao mesmo género (Perkins 1991). Existem espécies, como *Neonosemoides tilapiae*, com um número diminuto de enrolamentos (Faye *et al.* 1996) e, contrariamente, existem outras, como *Ichthyosporidium giganteum*, com mais de 40 enrolamentos em volta do vacúolo (Casal & Azevedo 1995).

Em secção transversal, o FP apresenta-se constituído por 3 a 20 camadas concêntricas, alternadamente electrodensas e electrolucentes (Franzen 2004). Os polissacarídios fazem parte da composição do FP (Takizawa *et al.* 1975). Contudo, o principal componente são proteínas (Weidner 1976), tendo sido identificadas 4, respectivamente com 23, 27, 34 e 43 kDa (Keohane *et al.* 1996). As proteínas do FP (PTPs) foram descritas em alguns microsporídios, inclusive em 2 espécies parasitas de peixes, *Spraguea americana* e *Glugea atherinae* (Keohane & Weiss 1999). Presentemente, conhecem-se 3 tipos de PTPs (PTP1, PTP2 e PTP3), havendo evidências que possam exercer uma função de controlo na extrusão do filamento polar (Delbac *et al.* 2001, Peuvel *et al.* 2002). Em muitas espécies, o FP é isofilar, isto é, do mesmo diâmetro em toda a sua extensão, enquanto que, noutras espécies, o manúbrio tem maior diâmetro do que a porção posterior designando-se de anisofilar. O manúbrio pode, em alguns casos, ser a única porção constituinte do FP (Faye *et al.* 1991).

A membrana do DA está em continuidade com a membrana que reveste o FP (Petri & Schiødt 1966). Esta, em volta do manúbrio, diferencia-se no principal organelo do

aparelho de extrusão, o polaroplasto, que consiste num empilhamento de membranas, resultante de projecções consecutivas da mesma (Larsson 1986). Este organelo, de origem golgiana, apresenta uma organização lamelar, excepto na extremidade posterior que, geralmente, é de menor periodicidade e mais desorganizada (Weidner 1972, Azevedo & Matos 2002a, 2003a), podendo ocupar grande parte do volume do esporo (Perkins 1991). Por último, um VP delimitado por uma membrana, geralmente de grandes proporções nas espécies que parasitam peixes, contém no seu interior, frequentemente, corpos densos designados de posterossomas (Matthews & Matthews 1980, Lom *et al.* 1999, 2001, McGourty *et al.* 2007, Casal *et al.* 2008b). Relativamente ao VP têm surgido algumas opiniões contraditórias, nomeadamente uma suposta ligação com a extremidade posterior do FP (Larsson 1986, Perkins 1991). A inexistência de observações microscópicas da extremidade do FP faz com que a grande maioria dos autores pense na descontinuidade destas estruturas (Vávra 1976, Vinckier *et al.* 1993). Recentemente, foram detectadas dentro do VP, moléculas marcadoras dos peroxissomas, tais como catalase, oxidase actil-CoA e ácido gordo nervónico, que muito provavelmente estão envolvidas no processo de extrusão do FP (Weidner & Findley 2002, Findley *et al.* 2005).

Extrusão do filamento polar

Os microsporídios podem ser transmitidos a um novo hospedeiro por diferentes vias. A entrada mais comum parece ser por via do tracto digestivo. Uma vez dentro do hospedeiro, mais precisamente no intestino, sob acção de apropriados estímulos, nomeadamente o aumento de pH (Weidner *et al.* 1984) e o aumento da pressão osmótica, gera-se um aumento da pressão dentro do esporo, que desencadeia a extrusão do filamento polar (Undeen & Frixione 1990). Por outro lado, a presença nos esporos de grandes concentrações do dissacarídeo trehalose (Wood *et al.* 1970), bem como da enzima trehalase (Vandermeer & Gochnauer 1971), degradando-o em moléculas mais pequenas de glucose ou outros açúcares (Undeen 1990), também contribui para o aumento da pressão osmótica (Undeen & Frixione 1990, Undeen & Vander Meer 1994).

O aumento da pressão osmótica, associado à dilatação do Pp e do VP, desencadeia a extrusão do FP, com início na sua porção posterior (processo semelhante à inversão dos dedos de uma luva). A acção combinada do Pp e do VP conduz o conteúdo do esporo para dentro do filamento oco, que possui rigidez suficiente para permitir a penetração no citoplasma ou no nucleoplasma da célula hospedeira e, conseqüentemente, a libertação do esporoplasma (Weidner 1972, Lom & Dyková 1992a). A ruptura da membrana da

célula hospedeira pelo FP ocorre sem haver perda do citoplasma (Perkins 1991). Através da microscopia de fluorescência, Weidner e colaboradores (1984) verificaram que, após a extrusão do filamento, a membrana celular do esporoplasma é proveniente da membrana do polaroplasto. Por vezes, a pressão exercida pelo FP extrudido é tal, que permite que o tubo polar atravesse grandes porções de citoplasma e as membranas dos núcleos das células hospedeiras (Lom & Pekkarinen 1999, Matos *et al.* 2003).

1.2.3. Ciclo de vida

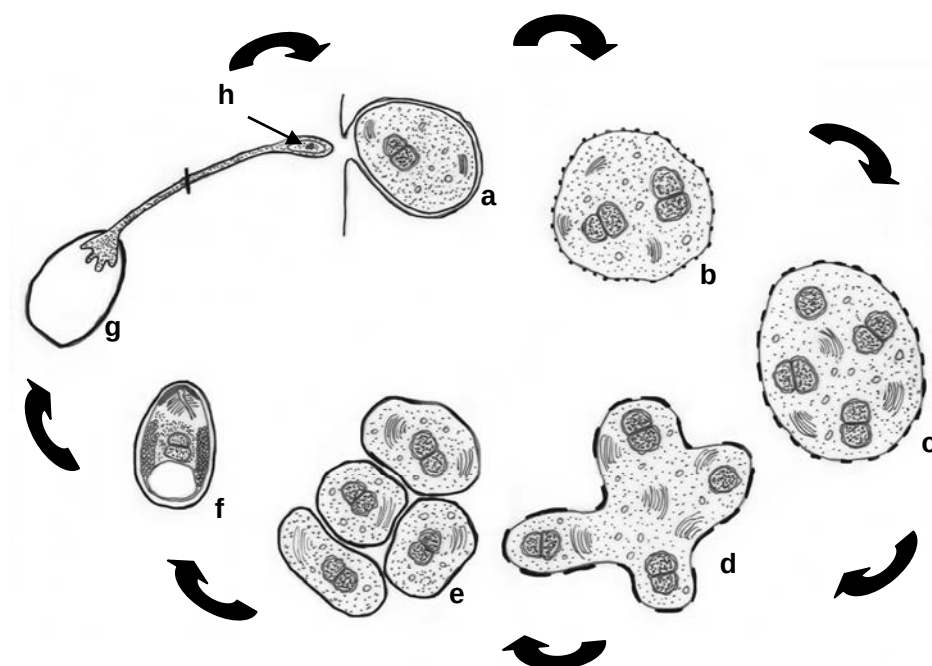
Os esporos maduros podem ser libertados dos seus hospedeiros (ou pelas fezes, ruptura da pele e brânquias ou após a sua morte), resistindo às condições externas até determinado ponto de secura do meio ambiente (Lom 2008). Após a ingestão dos esporos, o esporoplasma é libertado do esporo, no intestino, infectando as células epiteliais. O desenvolvimento pode dar-se no local de contacto do esporo, ou como também sucede, em tecidos situados a longa distância do local de infecção. Neste caso, presume-se que sejam células transportadoras, nomeadamente células mesenquimatosas indiferenciadas, macrófagos e fluídos corporais que possibilitam, por vezes, uma generalizada distribuição (Canning & Lom 1986, Lom & Dyková 1992a).

O ciclo de vida (Esquema 2) compreende sequências proliferativas: merogonia (também conhecida de esquizogonia), que produz um grande número de células, as quais, numa segunda fase, a esporogonia, originam os esporoblastos. Estas células, mediante profundas alterações ultraestruturais, diferenciam-se em esporos altamente especializados, com capacidade de transmissão (Canning & Lom 1986, Lom & Dyková 1992a). Nos microsporídios da ictiofauna não existe nenhuma referência de propagação dependente de hospedeiros intermediários (Lom & Nilsen 2003).

Merogonia e merontes

Quando o esporoplasma penetra uma célula hospedeira, num curto espaço de tempo perde a compartimentação citoplasmática característica (Perkins 1991). Posteriormente, esta célula, possuindo em regra um núcleo isolado ou dois em diplocário, como sucede nos géneros *Ichthyosporidium* (Casal *et al.* 1995) e *Neonosemoides* (Faye *et al.* 1996), aumenta de tamanho e adquire uma forma irregular arredondada ou alongada. No citoplasma observam-se poucos organelos, entre eles um retículo endoplasmático (RE) e um complexo de Golgi (Youssef & Hammond 1971, Canning & Lom 1986). Estas células, designadas de merontes, podem dividir-se por fissão binária ou múltipla. Em alguns casos, pode formar-se um plasmódio merogonial, que, posteriormente, se divide por

plasmotomia (Lom & Dyková 1992a). Regra geral, os merontes encontram-se em contacto directo com o citoplasma da célula hospedeira, excepto para o género *Nucleospora*, que se desenvolve no nucleoplasma das células hospedeiras (Hedrick *et al.* 1991, Lom & Dyková 2002). Em alguns géneros podem diferenciar-se estruturas invulgares, tais como: uma cutícula electrodensa intimamente associada a cisternas de RE liso, no género *Pleistophora* (Canning & Nicholas 1980), que pode acabar por desaparecer durante a esporogénese no género *Glugea* (Canning *et al.* 1982); os merontes localizados dentro de um vacúolo, originado pelo hospedeiro, são observados em *Tetramicra brevifilum* (Matthews & Matthews 1980); o envolvimento dos merontes por uma cisterna de RE, como sucede nas espécies *Microgemma* (Ralphs & Matthews 1986).



Esquema 2 - Desenho esquemático do ciclo de vida simplificado do microsporídio *Ichthyosporidium giganteum*. **a** – meronte com núcleo em diplocário; **b, c, d** – esporontes (fases sequenciais da esporogonia tetrasporoblástica); **e** – quatro esporoblastos; **f** - esporo maduro; **g** - esporo sem conteúdo celular mostrando a extrusão do filamento polar; **h** - núcleo na extremidade do filamento polar extrudido.

Esporogonia e esporontes

A esporogonia caracteriza-se pela diferenciação de merontes em esporontes e pela divisão destes últimos em células designadas de esporoblastos (Perkins 1991). Durante este processo, na superfície externa do plasmalema dos esporontes, ou em ambas as faces, ocorre gradualmente uma deposição de material electrodense que, posteriormente, tornar-se-á no exosporo da parede celular (Lom & Dyková 1992a). Perto de cada invaginação da membrana citoplasmática dos esporontes pode ocorrer um organelo

designado de corpo paramural. Este corpo consiste num aglomerado de túbulos limitados por membrana, semelhantes aos mesossomas das bactérias (Vávra 1976).

As sequências de divisão são variáveis e características para cada género (Canning & Lom 1986). Esporontes binucleados, originando dois esporoblastos, foram observados na espécie *Microsporidium chloroscombri* (Toguebaye *et al.* 1989). Contudo, é mais frequente a esporogénese da qual resulta a formação de vários esporoblastos. Neste caso, ocorrem várias nucleocineses formando esporontes multinucleados, designados de plasmódios esporogoniais, que se podem dividir directamente por fissão múltipla. A esporogénese tetrasporoblástica é uma característica dos géneros *Microfilum* (Faye *et al.* 1991), *Potaspora* (Casal *et al.* 2008b) e *Tetramicra* (Matthews & Matthews 1980). Nos géneros *Loma*, *Nucleospora* e *Pleistophora*, os esporoblastos formam-se por plasmotomia sucessiva, a partir de plasmódios mais pequenos. No género *Glugea*, os plasmódios esporogoniais, através de fissão múltipla, originam muitos estádios intermédios, designados células-mãe dos esporoblastos, que se dividem, posteriormente, por fissão binária originando dois esporoblastos (Canning & Lom 1986, Perkins 1991).

Somente em alguns géneros de microsporídios, todos os estádios de desenvolvimento esporogoniais ocorrem em directo contacto com o citoplasma da célula hospedeira: *Amazonspora*, *Ichthyosporidium*, *Kabatana*, *Microgemma*, *Microfilum*, *Neonosemoides*, *Nucleospora*, *Potaspora* e *Tetramicra*. Contudo, nos géneros *Glugea*, *Heterosporis*, *Loma*, *Myosporidium* e *Pleistophora*, diferencia-se um espaço entre os esporontes e o citoplasma da célula hospedeira em resultado da formação de uma membrana à volta do parasita. Consoante a sua origem, é designada de vesícula esporófora (VE), quando se forma a partir do parasita, e de vacúolo parasitóforo (VPa) se for originada pelo hospedeiro. Em *Glugea* spp., o VPa não é mais do que uma frágil membrana (Canning *et al.* 1982). Pelo contrário, no género *Pleistophora*, o VPa desenvolve-se a partir de uma camada amorfa da superfície do esporonte, numa parede persistente espessa (com mais de 0,5 µm) constituída por 3 camadas distintas (Canning & Nicholas 1980). No espaço episporal, espaço confinado pelo VPa ou VE, podem diferenciar-se estruturas tubulares de função desconhecida.

A divisão mitótica nos microsporídios tem sido, frequentemente, observada em esporontes em fase de divisão. O invólucro nuclear não se fragmenta durante a divisão e o fuso mitótico forma-se internamente no núcleo, sem a presença de centríolos (Vávra 1976, Canning & Lom 1986). O aparelho mitótico consiste em duas placas centriolares, associadas e localizadas em depressões do invólucro nuclear, para as quais convergem os microtúbulos (Youssef & Hammond 1971, Sprague & Vernick 1974, Canning & Hazard 1982, Ralphs & Matthews 1986, Lom & Pekkarinen 1999). Estes têm 15 nm de diâmetro e

encontram-se ligados numa das extremidades aos cinetocoros, enquanto que a outra extremidade se liga ao centro organizador microtubular (placa centriolar) (Larsson 1986). Por vezes, podem existir no citoplasma discos electrodensos, em associação com as placas centriolares (Morrison & Sprague 1981b).

Esporogonia e esporoblastos

Estas células são geralmente de forma ovóide, na qual se diferenciam os organelos típicos dos esporos. Constata-se, ao nível do esporoplasma, um aumento considerável de RE liso e rugoso e de inúmeras vesículas golgianas, que irão ser responsáveis pela formação do FP e do DA. Contrariamente ao que ocorre com mais frequência, no género *Nucleospora* inicia-se a diferenciação do aparelho de extrusão ainda na fase de meronte (Hedrick *et al.* 1991, Lom & Dyková 2002). Na parede celular, o endosporo forma-se gradualmente sob a camada externa já sintetizada, exosporo (Larsson 1986). Após a conclusão do processo de maturação, as vesículas do complexo de Golgi excedentes confluem e formam o VPa (Canning & Lom 1986).

Em regra, verifica-se uma uniformidade, quer em tamanho quer em forma, nos esporos maduros, no entanto, existem algumas excepções, como no caso dos géneros *Pleistophora* (Canning & Nicholas 1980) e *Heterosporis* (Michel *et al.* 1989), em que se observa uma heterogeneidade de tamanhos, com a formação de macrosporos e microsporos, em resultado do desigual número de divisões celulares precedentes à formação de esporoblastos. O dimorfismo, que envolve todo o ciclo de vida, é muito vulgar em géneros de microsporídios que têm insectos como hospedeiros. *Spraguea* é o único género parasita de vertebrados, em que, simultaneamente no mesmo hospedeiro e no mesmo xenoma, ocorrem dois ciclos distintos. Um deles origina esporos uninucleados sem a formação de VPa, enquanto que o outro ciclo permite a formação de esporos em diplocário em contacto directo com a célula hospedeira (Loubès *et al.* 1979).

1.2.4. Classificação taxonómica

Em 1909, Stempel elaborou, pela primeira vez, uma classificação dos microsporídios em que estes eram distinguidos dos mixosporídios, grupo com o qual, até então, eram frequentemente confundidos. Posteriormente, esta classificação foi alterada por Léger e Hesse (1922) e Kudo (1924), vigorando até meados da década 70 (consultar a publicação, Sprague 1977). No final deste período, já existiam inúmeras publicações com dados obtidos de microscopia electrónica, consequentemente tornava-se primordial haver uma reestruturação da classificação até então utilizada. Os primeiros modelos modernos

foram propostos por Weiser (1976) e Sprague (1977). Este último autor propôs a designação de filo Microspora, bem como reclassificou as espécies-tipo em géneros, segundo aspectos inerentes ao desenvolvimento do ciclo de vida do parasita. Nos anos seguintes, Sprague (1982) propôs um segundo modelo, que serviria, posteriormente, de suporte às classificações de Larsson (1986, 1988) e de Canning (1990), contudo não existiam critérios em relação aos taxa superiores à família. Em 1992, numa revisão elaborada por Sprague e colaboradores foram introduzidos na taxonomia, como critério principal, os aspectos referentes à divisão nuclear, sem que isso implicasse profundas alterações em relação à definição dos taxa família e género. Assim, o modelo proposto tornou-se complexo e de difícil utilização, constatando-se um uso preferencial do modelo proposto por Canning (1990).

Com o acumular de dados moleculares e filogenéticos obtidos durante a última década do século XX, constatou-se que os microsporídios são aparentados com os fungos. Cavalier-Smith (1998) transferiu o filo Microsporidia Balbiani, 1882 para o sub-Reino Eomycota Cavalier-Smith, 1998 e subdividiu o filo em duas classes: **Minisporea** Cavalier-Smith, 1993 e **Microsporea** Levine & Corliss, 1963. Esta última classe é composta por duas sub-classes: Pleistophorea Cavalier-Smith, 1993 (microsporídios com divisão em plasmotomia e diferenciação de um único tipo de esporo) e Disporea Cavalier-Smith, 1993 (fissão binária e diferenciação de 2 tipos de esporos). Por outro lado, as análises moleculares filogenéticas revelaram-se inconsistentes, isto é, os agrupamentos dos taxa diferiram substancialmente da classificação proposta por Cavalier-Smith (Baker *et al.* 1998, Nilsen 2000, Lom & Nilsen 2003) chegando mesmo a ser proposta a sua subdivisão em 3 classes, **Aquasporidia**, **Marinosporidia** e **Terresporidia** reflectindo o habitat de cada grupo (Vossbrinck & Debrunner-Vossbrinck 2005). Relativamente aos microsporídios que ocorrem na ictiofauna, os cladogramas, tendo por base os genes ribossomais, sugerem 5 agrupamentos, alguns deles correspondendo ao *taxon* família (Lom & Nilsen 2003, McGourty *et al.* 2007, Casal *et al.* 2008b).

1.2.5. Diagnose dos géneros que parasitam a ictiofauna

Os peixes teleósteos de água doce, estuarina e salgada são o segundo maior grupo parasitado por microsporídios, existindo referências em praticamente todas as famílias. O género *Nosema* Naegeli, 1857, foi o primeiro a ter sido identificado, contudo, presentemente, não tem representatividade na ictiofauna. Actualmente estão identificados 17 géneros (Tabela 1) e 91 espécies. Existe, aproximadamente, igual número de espécies nomeadas provisoriamente num dos géneros ou no grupo colectivo *Microsporidium* (Tabela 2, páginas 28 a 33).

Tabela 1 - Diagnose dos géneros de microsporídios da ictiofauna

Género Família	Local da infecção	Núcleo	Merogonia: Merontes (Me)	Esporogonia: esporontes (Sp), esporoblastos (Sb), esporos (Sp)	Interface	Xenoma	Habitat	Tecido/órgão
<i>Amazonspora</i> Azevedo & Matos, 2003 (Fam. Glugeidae)	Citop	Mono	Merontes uninucleados	Tetrasporoblástica	Contacto directo	(+) estruturas ~ a microvilosidades anastomosadas; na parede 22 camadas de fibras de colagénio justapostas com alternância de orientação	D	Brânquias
<i>Glugea</i> Thélohan, 1891 (Fam. Glugeidae)	Citop	Mono	Merontes multinucleados Cisterna de RER	2 fases: 1ª divisão por fissão múltipla; 2ª divisão por fissão binária	VPa – membrana espessa	(+) de grandes dimensões; externamente encapsulado por uma parede refráctil formada por várias camadas justapostas de material opaco	M / D	Vários órgãos
<i>Heterosporis</i> Schubert, 1969 (Fam. Glugeidae)	Citop	Mono	Merontes encapsulados numa parede sintetizada pelo parasita (esporoforcisto) durante a merogonia e esporogonia	Macrosporos e microsporos	VPa	(-)	D	Maioritariamente, o músculo esquelético
<i>Ichthyosporidium</i> Caullery & Mesnil, 1905 (Fam. Ichthyosporidiidae)	Citop	Diplo	Fissão binária dentro de cápsulas císticas globulares compartimentadas, originadas por coalescência e hipertrofia de fibroblastos infectados	Tetrasporoblástica; esporos com um filamento polar enrolado cerca de 40 voltas	Contacto directo	(+) xenomas lobulados de grandes dimensões (4 mm) induzindo extensas alterações aos tecidos envolventes	M	Tecido conjuntivo, fígado, brânquia
<i>Kabatana</i> Lom, Dyková & Tonguthai, 2000	Citop	Mono	Multinucleada com divisão por plasmotomia ou fissão binária	Plasmódio esporogonial que por segmentação forma células-mãe esporoblásticas; origina dois esporoblastos	Contacto directo	(-)	M / D	Músculo esquelético
<i>Loma</i> Morrison & Sprague, 1981 (Fam. Glugeidae)	Citop	Mono	Plasmódios multinucleados revestidos por uma cisterna de RER	Esporogonia polisporoblástica que se divide por plasmotomia originando 8 esporos	VPa; diferenciação de estruturas no espaço episporal	(+) com 1 a 1,5 mm de diâmetro; externamente, parede espessa e amorfa	M / D	Filamentos branquiais, aparelho digestivo
<i>Microfilum</i> Faye, Toguebaye & Bouix, 1991 (Fam. Microfilidae)	Citop	Mono	Divisão binária	Tetrasporoblástica; exosporo muito espesso; (DA) com grandes alterações; manúbrio que termina num filamento polar muito curto, sem enrolamento, e em forma de gancho	Contacto directo	(+) diferenciação de microvilosidades na superfície	M	Filamentos branquiais
<i>Microgemma</i> Ralphs & Matthews, 1986 (Fam. Tetramicridae)	Citop	Mono	Multinucleados. Divisão por plasmotomia	Divisão gemulação exógena simples e múltipla, ou então por fragmentação do plasmódio	Contacto directo	(+) com 0,5 mm de diâmetro; microvilosidades na membrana citoplasmática	M	Fígado
<i>Myosporidium</i> Baquero, Rubio, Moura, Pieniazek & Jordana, 2005	Citop	Mono	Não foram observados	Polisporoblástica; origina 30 a 50 esporos; filamento polar anisofilar	VE	(+) filamentosos de coloração negra, revestidos por várias camadas de fibroblastos	M	Músculo esquelético

Género	Local da infecção	Núcleo	Merogonia: Merontes (Me)	Esporogonia: esporontes (Sp), esporoblastos (Sb), esporos (Sp)	Interface	Xenoma	Habitat	Tecido/órgão
<i>Neonosemoides</i> Faye, Toguebaye & Bouix, 1996 (Fam. Neonosemoidiidae)	Citop	Mono / Diplo	2 fases: diplocariótica com divisão por fissão binária; plasmódios moniliformes monocariontes dividem-se por plasmotomia	Fissão múltipla com um número indeterminado de esporos; filamento polar anisofilar	Contacto directo	(+) Região periférica extremamente vacuolizada.	Estuarino	Brânquia e intestino
<i>Nucleospora</i> Hedrick, Graff & Baxa, 1991 (Fam. Enterocytozoonidae)	Nu	Mono	Multinucleada; início da diferenciação do aparelho de extrusão	Multinucleada	Contacto directo	(-)	M / D	Células hematopoiéticas e enterócitos
<i>Ovipleistophora</i> Pekkarinen, Lom & Nilsen, 2002 (Fam. Pleistophoridae)	Citop	Mono	(Me) uni ou multinucleados revestidos por uma cutícula que acompanha a divisão. 2º revestimento espesso que não se divide, constituído por vesículas e material granular	Polisporoblástica; número variado de esporos; Macrosporos e microsporos	VE	(-)	D	Ovócitos e testículo
<i>Pleistophora</i> Gurley, 1893 (Fam. Pleistophoridae)	Citop	Mono	Merontes multinucleados com parede amorfa espessa; Divisão por plasmotomia	Polisporoblástica, 4 a 200 (Sb) por VP; Diferenciação de uma 2ª camada na superfície dos (Sp); macro e microsporos similares	VPa - parede espessa	(-)	M / D	Maioritariamente, o músculo esquelético
<i>Potaspora</i> Casal, Matos, Teles-Grilo & Azevedo, 2008 (Fam. Tetramicridae)	Citop	Mono	Divisão por fissão binária	Tetrasporoblástica; esporoblastos diferenciam um corpo de forma irregular electrodense	Contacto directo	(+) Diferenciação de estruturas filamentosas e anastomosadas, ~ a microvilosidades ao nível do plasmalema	D	Cavidade celómica perto da região anal
<i>Pseudoloma</i> Matthews, Brown, Larison, Bishop-Stewart & Kent, 2001	Citop	Mono	Não foram observados	Diferenciação de 8 a 16 esporos uninucleados	VE	(+)	D	Sistema nervosa central
<i>Spraguea</i> Weissenberg, 1976 (Fam. Spraguidae)	Citop	Mono / Diplo	Merontes multinucleados	Dimórfica (2 tipos de esporos): monocariontes e polisporoblásticos por divisão radial; diferenciação de diplocariontes disporoblásticos somente na espécie tipo.	Contacto directo	(+) de grandes dimensões sem parede espessa; o volume da célula hospedeira não é transformado numa estrutura xenómica	M	Células ganglionares do sistema nervoso central
<i>Tetramicra</i> Matthews & Matthews, 1980 (Fam. Tetramicridae)	Citop	Mono	Merontes binucleados	Tetrasporoblástica; esporoblastos permanecem interligados pelas porções posteriores, em forma semelhante a um trevo	Todo ciclo de vida em vacúolos originados pelo hospedeiro	(+) numerosas projecções na superfície, semelhantes a microvilosidades	M	Tecido conjuntivo da musculatura esquelética

Diagnose dos géneros de microsporídios de peixes: citoplasma (Citop), núcleo (Nu) monocário (Mono), diplocário (Diplo), disco de ancoragem (DA), vacúolo parasitóforo (VPa), vesícula esporófora (VE), sem formação de xenoma (-), com formação de xenoma (+), marinho (M), água doce (D).

Tabela 2 - Listagem das espécies de microsporídios da ictiofauna

ESPÉCIES	HOSPEDEIROS	LOCAIS DE INFECÇÃO	HABITAT	REGIÃO / PAÍS	REFERÊNCIAS BIBLIOGRÁFICAS
<i>Amazonspora hassar</i>	<i>Hassar orestis</i>	Brânquias	Água doce	Pará, Brasil	Azevedo & Matos 2003a
<i>Glugea anomala</i>	<i>Gasterosteus aculeatus</i> , <i>Pungitius pungitius</i>	Tecidos conjuntivos de vários órgãos	Água doce, eurihalino	Europa, Ásia, América do Norte	Canning <i>et al.</i> 1982
<i>Glugea acuta</i>	<i>Synganthus acus</i> , <i>Nerophis aequorus</i>	Tec. conj. do músculo da barbatana dorsal	Marinho	França - costa Atlântica	Thélohan 1895 (*)
<i>Glugea atherinae</i>	<i>Atherina boyeri</i>	Tecidos conjuntivos de vários órgãos	Eurihalino, salobro	França – costa Mediterrânica	Berrebi & Bouix 1978 (*)
<i>Glugea berglax</i>	<i>Macrourus berglax</i>	Vesícula biliar	Marinho	Terranova, Canadá	Lom & Laird 1976 (*)
<i>Glugea bychowskyi</i>	<i>Alosa kessleri volgensis</i>	Intestino, testículo	Água doce	Mar Cáspio	Gasimagomedov & Issi 1970 (*)
<i>Glugea capverdensis</i>	<i>Myctophum punctatum</i>	Intestino	Marinho	Cabo Verde	Lom, Gaevskaya & Dyková 1980 (*)
<i>Glugea cepedianae</i>	<i>Dorosoma cepedianum</i>	Cavidade visceral	Água doce	USA	Canning & Lom 1986
<i>Glugea cordis</i>	<i>Sardina pilchardus sardine</i>	Tec. conjuntivo e musculatura cardíaca	Marinho	França – costa Mediterrânica	Thélohan 1895 (*)
<i>Glugea depressa</i>	<i>Coris julis</i>	Fígado	Marinho	França – costa Mediterrânica	Thélohan 1895 (*)
<i>Glugea destruens</i>	<i>Callionymus lyra</i>	Músculos	Marinho	França - costa Atlântica	Gaevskaya & Kovaleva 1975 (*)
<i>Glugea fennica</i>	<i>Lota lota</i>	Tecidos subcutâneos e barbatanas	Água doce	Finlândia, Polónia e Russia	Lom & Laird 1976 (*)
<i>Glugea heraldi</i>	<i>Hippocampus erectus</i>	Tecidos subcutâneos	Marinho	Florida	Blasiola 1979 (*)
<i>Glugea hertwigi</i>	<i>Osmerus eperlanus</i> , outras espécies	Intestino e outros órgãos	Eurihalino	Holoártico	Fantham, Porter & Richardson 1941 (*)
<i>Glugea intestinalis</i>	<i>Mylopharyngodon piceus</i>	Intestino	Água doce	China	Chen 1956 (*)
<i>Glugea luciopercae</i>	<i>Stizostedion lucioperca</i>	Intestino, ovário e brânquias	Água doce, salobro	Rússia e Bulgária	Dogiel & Bykhowsky 1939 (*)
<i>Glugea machari</i>	<i>Dentex dentex</i>	Fígado	Marinho	Croácia	Sprague 1977
<i>Glugea nemipteri</i>	<i>Nemipterus japonicus</i>	Músculo liso, gónadas	Marinho	Índia	Weiser, Kalavati & Sandeep 1981 (*)
<i>Glugea pimephales</i>	<i>Pimephales promelas</i>	Mesentério	Água doce	USA	Fantham, Porter & Richardson 1941 (*)
<i>Glugea plecoglossi</i>	<i>Plecoglossus altivelis</i>	Vários órgãos	Água doce	Japão	Takahashi & Egusa 1977 (*)
<i>Glugea punctifera</i>	<i>Pollachius virens</i> ; <i>Theragra chalcogramma</i>	Tec. conjuntivo do músculo ocular	Marinho	França - costa Atlântica; Japão	Thélohan 1895, Akhmerov 1951 (*)
<i>Glugea rodei</i>	<i>Rhodeus sericeus amarus</i>	Intestino	Água doce	Azerbaijão	Kazieva & Voronin 1981 (*)
<i>Glugea shiplei</i>	<i>Trisopterus luscus</i>	Músculo esquelético, estômago e intestino	Marinho	Inglaterra	Drew 1910 (*)
<i>Glugea schulmani</i>	<i>Neogobius caspius</i> , outras espécies	Intestino	Marinho	Mar Cáspio	Gasimagomedov & Issi 1970 (*)
<i>Glugea stephani</i>	<i>Pleuronectes flesus</i> , outras espécies	Tracto intestinal	Marinho	Holoártico	Lom & Dyková 1992a
<i>Glugea tisae</i>	<i>Silurus glanis</i>	Intestino	Água doce	Hungria	Lom & Laird 1976 (*)
<i>Glugea truttae</i>	<i>Salmo trutta fario</i>	Saco vitelino	Água doce	Suíça	Berrebi 1979
<i>Glugea vincentiae</i>	<i>Vincentia conspersa</i>	Tec. subcutâneo do corpo e nas barbatanas	Marinho	Australia	Vagelli <i>et al.</i> 2005
<i>Glugea sp.</i>	<i>Abramis ballerus</i>	Parede intestinal	Água doce	Rio Volga	Bogdanova 1961 (*)
<i>Glugea sp.</i>	<i>Fundulus heteroclitus</i>	Mucosa estomacal, ducros biliares	Marinho	USA	Bond 1938 (*)
<i>Glugea sp.</i>	<i>Pseudopleuronectes americanus</i>	Submucosa intestinal	Marinho	USA	Canning & Lom 1986

(*) Consultar a publicação de Lom (2002)

ESPÉCIES	HOSPEDEIROS	LOCAIS DE INFECÇÃO	HABITAT	REGIÃO / PAÍS	REFERÊNCIAS BIBLIOGRÁFICAS
<i>Glugea</i> sp.	<i>Sphaeroides maculatus</i>	Mucosa intestinal	Marinho	Costa Atlântica dos USA	Canning & Lom 1986
<i>Glugea</i> sp.	<i>Gambusia affinis</i>	Fígado, ovários, tec. conjuntivo subcutâneo	Água doce	Califórnia, USA	Crandall & Bowser 1981 (*)
<i>Glugea</i> sp.	<i>Sparus auratus</i>	Tec. conj. (vários órgãos)	Marinho	Aquacultura em França	Mathieu <i>et al.</i> 1992 (*)
<i>Glugea</i> sp.	<i>Cyprinodon variegatus</i>	Órgãos abdominais	Estuarino	Inglaterra	Majeed, Douglas & Jolly 1985 (*)
<i>Glugea</i> sp.	<i>Gobius niger</i> , <i>G. paganellus</i> , <i>G. cobitis</i> , <i>G. ophiocephalus</i> , <i>G. ratan</i> , <i>G. platystris</i> , <i>Neogobius fluviatus</i> , <i>N. melanostomus</i> , <i>N. cephalarges</i> , <i>Mesogobius batrachocephalus</i> , <i>Proterorhinus marmoratus</i>	Submucosa intestinal, raramente o fígado	Estuarino, Marinho	Mar Negro e Mar de Azov	Naidenova 1974 (*)
<i>Glugea</i> sp.	<i>Phoxinus phoxinus</i>	-	Água doce	Alemanha	Pfeiffer 1895 (*)
<i>Glugea</i> sp.	<i>Abudefduf saxatilis</i>	Intestino	Marinho	Florida, USA	Reimchuessel <i>et al.</i> 1987 (*)
<i>Glugea</i> sp.	<i>Perca fluviatilis</i>	-	Água doce	Rio Danúbio, Roménia	Roman 1955 (*)
<i>Glugea</i> sp.	<i>Lota lota</i>	Pele	Água doce	Lago Vrevo, Russia	Voronin 1974
<i>Heterosporis finiki</i>	<i>Pterophyllum scalare</i>	T. muscular e conjuntivo do esófago	Água doce	Alemanha, França (aquário)	Schubert 1969
<i>Heterosporis anguillarum</i>	<i>Anguilla japonica</i>	Tecido muscular	Eurialina	Japão	Lom <i>et al.</i> 2000b
<i>Heterosporis cichlidarum</i>	<i>Hemichromis bimaculatus</i>	Brânquias	Água doce	França	Coste & Bouix 1998
<i>Heterosporis schuberti</i>	<i>Pseudocrenilabrus multicolor</i> , <i>Ancistrus cirrhosus</i>	Tecido muscular	Água doce	Alemnaha (Aquário)	Lom <i>et al.</i> 1989a
<i>Heterosporis</i> sp.	<i>Betta splendens</i>	Tecido muscular	Água doce	Tailândia	Lom <i>et al.</i> 1993
<i>Heterosporis</i> sp.	<i>Perca flavescens</i>	Tecido muscular	Água doce	Winconsin e Minnesota, USA	Sutherland <i>et al.</i> 2000
<i>Ichthyosporidium gigateum</i>	<i>Crenilabrus melops</i> , <i>C. ocellatus</i> , <i>Leiostomus xanthurus</i> , <i>Ctenolabrus rupestris</i>	T. conjuntivo subcutâneo, tecido adiposo, fígado	Marinho	França (costa Atlântica) Holanda, Portugal; Mar Negro, Ucrânia	Swarczewsky 1914 (*); Schwartz 1963; Casal & Azevedo 1995
<i>Ichthyosporidium herwigi</i>	<i>Crenilabrus tinca</i>	Brânquias	Marinho	Mar Negro, Ucrânia	Swarczewsky 1914 (*)
<i>Kabatana arthuri</i>	<i>Pangasius sutchi</i>	Tecido muscular esquelético	Água doce	Tailândia	Lom <i>et al.</i> 1990, 1999, 2000a
<i>Kabatana seriola</i>	<i>Seriola quinqueradiata</i> , <i>Pagrus major</i>	Tecido muscular	Marinho	Japão	Egusa 1982, Lom <i>et al.</i> 1999
<i>Kabatana takedai</i>	<i>Oncorhynchus mykiss</i>	Tecido muscular cardíaco, esquelético	Água doce	Japão, Rússia	Lom <i>et al.</i> 2001
<i>Kabatana newberryi</i>	<i>Eucyclogobius newberryi</i> ; <i>Gobiusculus flavescens</i>	Tecido muscular esquelético	Estuarino, Marinho	Pacífico, USA; Oceano Atlântico	McGourty <i>et al.</i> 2007; Barber <i>et al.</i> 2009
<i>Loma branchialis</i>	<i>Melanogrammus aeglefinus</i>	Filamentos brânquiais	Marinho	Boreo-ártico	Morrison & Sprague 1981a
<i>Loma acerinae</i>	<i>Gymnocapalus cernuus</i>	Parede intestinal	Água doce	República Checa	Lom & Pekkarinen 1999
<i>Loma boopsi</i>	<i>Boops boops</i>	Tracto intestinal e fígado	Marinho	Senegal	Faye <i>et al.</i> 1995
<i>Loma camerounensis</i>	<i>Oerochromis niloticus</i>	Intestino e esófago	Água doce	Camarões	Fomena <i>et al.</i> 1992

(*) Consultar a publicação de Lom (2002)

ESPÉCIES	HOSPEDEIROS	LOCAIS DE INFECÇÃO	HABITAT	REGIÃO / PAÍS	REFERÊNCIAS BIBLIOGRÁFICAS
<i>Loma dimorpha</i>	<i>Gobius niger</i> , <i>Zosterisessor ophiocephalus</i> , <i>Lipophrys pholis</i>	Tecido conjuntivo do intestino	Marinho	França, costa Atlântica Espanhola	Loubès <i>et al.</i> 1984; Arias <i>et al.</i> 1999 ; Leiro <i>et al.</i> 1994
<i>Loma diplodae</i>	<i>Diplodus sargus</i>	Filamentos brânquiais	Marinho	França	Bekhti & Bouix 1985
<i>Loma embiotocia</i>	<i>Cymatogaster aggregate</i>	Filamentos brânquiais	Marinho	Canadá	Shaw <i>et al.</i> 1997
<i>Loma fontinalis</i>	<i>Salvelinus fontinalis</i>	Filamentos brânquiais	Água doce	Canadá	Morrison & Sprague 1983
<i>Loma myrophis</i>	<i>Myrophis platyrhynchus</i>	Tecido epitelial do intestino	Água doce	Brasil	Azevedo & Matos 2002a
<i>Loma salmonae</i>	<i>Oncorhynchus mykiss</i>	Filamentos brânquiais	Água doce	América do Norte, Japão, França	Putz <i>et al.</i> 1965
<i>Loma trichiuri</i>	<i>Trichurus savala</i>	Filamentos brânquiais	Marinho	Índia	Sandeep & Kalavati 1985
<i>Loma psittaca</i>	<i>Colomesus psittacus</i>	Parede intestinal	Água doce	Brasil	Casal <i>et al.</i> 2009b
<i>Loma</i> spp.	<i>Tilapia zillii</i>	Músculo aductor dos filamentos branquiais	Água doce	Benin, Africa	Lom 2002
<i>Loma</i> spp.	<i>Anoploma fimbria</i> , <i>Cymatogaster aggregata</i> , <i>Gadus macrocephalus</i> , <i>Microgadus proximus</i> , <i>Ophiodon elongatus</i> , <i>Theragra chalcogramma</i>	Sem dados	Marinho	Canadá	Kent, Shaw, Dawe, Higgins, Brown, & Adamsonb1998 (*)
<i>Microfilum lutjani</i>	<i>Lutjanus fulgens</i>	Filamentos brânquias	Marinho	Senegal	Faye <i>et al.</i> 1991
<i>Microgemma hepaticus</i>	<i>Chelon labrosus</i>	Fígado	Marinho	Reino Unido	Ralphs & Matthews 1986
<i>Microgemma caulleryi</i>	<i>Hyperoplus lanceolatus</i>	Fígado	Marinho	Costa Atlântica da França, Espanha	Leiro <i>et al.</i> 1999
<i>Microgemma ovoidea</i>	<i>Motella tricirrata</i> , <i>Cepola rubescens</i> , <i>C. macrophthalma</i> , <i>Merluccius hubbsi</i> , <i>M. gayi</i>	Fígado	Marinho	Mar Mediterrâneo, costa Atlântica da França, Peru e Patagônia	Canning & Lom 1986, Amigó <i>et al.</i> 1996
<i>Microgemma tincae</i>	<i>Symphodus tinca</i>		Marinho	Tunísia	Mansour <i>et al.</i> 2005
<i>Microgemma vivaresi</i>	<i>Taurulus bubalis</i>	Fígado, tecido muscular	Marinho		Canning <i>et al.</i> 2005
<i>Myosporidium merluccius</i>	<i>Merluccius</i> sp.	Tecido muscular esquelético	Marinho	Namíbia	Baquero <i>et al.</i> 2005
<i>Neonosemoides tilapiae</i>	<i>Tilapia zillii</i> , <i>T. guineensis</i> , <i>Sarotherodon melanotheron</i>	Intestino e brânquias	Salobro	Benin	Faye <i>et al.</i> 1996
<i>Nucleospora salmonis</i>	<i>Oncorhynchus tshawytscha</i> , <i>O. mykiss</i>	Núcleos das células hematopoiéticas	Marinho	Costa Pacífica da América do norte	Hedrick <i>et al.</i> 1991
<i>Nucleospora secunda</i>	<i>Nothobranchius rubripinis</i>	Núcleos de enterócitos	Água doce	Aquário na República Checa	Lom & Dyková 2002
<i>Nucleospora</i> sp.	<i>Cyclopterus lumpus</i>	Núcleos das células hematopoiéticas	Marinho	Canadá	Mullins <i>et al.</i> 1994
<i>Nucleospora</i> sp.	<i>Hippoglossus hippoglossus</i>	Núcleos das células hematopoiéticas	Marinho	Noruega	Nilsen <i>et al.</i> 1995
<i>Ovipleistophora mirandellae</i>	<i>Alburnus alburnus</i> , <i>Barbus barbus</i> , <i>Rutilus rutilus</i> , <i>Leuciscus cephalus</i> , <i>L. leuciscus</i> , <i>Abramis brama</i> , <i>Gobio gobio</i> , <i>Gymnocephalus cernuus</i> , raramente <i>Exocoetis lucius</i> , <i>Hucho hucho</i>	Ovócitos e testículo	Água doce	Alemanha, Filândia	Pekkarinen <i>et al.</i> 2002
<i>Ovipleistophora ovariae</i>	<i>Notemigonus crysoleucas</i>	Ovócitos	Água doce	USA	Summerfelt 1964, Pekkarinen <i>et al.</i> 2002

(*) Consultar a publicação de Lom (2002)

ESPÉCIES	HOSPEDEIROS	LOCAIS DE INFECÇÃO	HABITAT	REGIÃO / PAÍS	REFERÊNCIAS BIBLIOGRÁFICAS
<i>Pleistophora typicalis</i>	<i>Myoxocephalus scorpius</i> , <i>M. quadricornis</i> <i>labradoricus</i> e outras espécies	Tec. muscular esquelético	Marinho	Costa Atlântica da França, Escócia; Mar Báltico e Branco	Canning & Nicholas 1980
<i>Pleistophora acerinae</i>	<i>Gymnocephalus cernuus</i> , <i>Perca schrenki</i>	Mesentério, Intestino	Água doce	França, Ucrânia, Rússia	Agapova 1966 (*)
<i>Pleistophora aegytiacus</i>	<i>Tilapia zilli</i>	Tecido muscular do estômago	Água doce	Nilo, Egito	Negm-Eldin 1992 (*)
<i>Pleistophora carangoidi</i>	<i>Carangoides malabaricus</i>	Tec. muscular esquelético	Marinho	Oceano Índico	Narasimhamurti & Sonabai 1977 (*)
<i>Pleistophora dallii</i>	<i>Dallia pectoralis</i>	Tec. conj. subcutâneo perto das barbatanas	Água doce	Rússia	Zhukov 1964 (*)
<i>Pleistophora destruens</i>	<i>Mugil auratus</i>	Tecido muscular	Marinho	Island Tatiou, Cherbourg, França	Delphy 1916 (*)
<i>Pleistophora duodecimae</i>	<i>Coryphaenoides nasutus</i>	Tec. muscular esquelético	Marinho	Northern Oceano Atlântico	Gaevskaya & Dyková 1980 (*)
<i>Pleistophora ehrenbaumi</i>	<i>Anarhichas lúpus</i> , <i>A. Minor</i>	Tec. muscular esquelético	Marinho	Mar do Norte	Reichenow 1929 (*)
<i>Pleistophora finisterrensis</i>	<i>Micromesistius poutassou</i>	Tecido muscular	Marinho	Galiza, Espanha	Leiro <i>et al.</i> 1996
<i>Pleistophora gadi</i>	<i>Gadus morhua morhua</i>	Tec. muscular esquelético	Marinho	Mar de Barents	Polyansky 1955 (*)
<i>Pleistophora hippoglossoides</i>	<i>Drepanopsetta hippoglossoides</i> , <i>Hippoglossoides</i> <i>platessoides</i> , <i>Solea solea</i>	Parede da cavidade abdominal, músculo das barbatanas	Marinho	Nordeste do Mar do Norte	Bosanquet 1910 (*)
<i>Pleistophora hyphessobryconis</i>	<i>Paracheirodon inessi</i> , várias espécies	Tec. muscular esquelético e outros órgãos	—	—	Schäperclaus 1941 (*); Canning & Lom 1986
<i>Pleistophora ladogensis</i>	<i>Lota lota</i> , <i>Osmerus eperlanus eperlanus</i>	Tec. muscular esquelético	Água doce, eurialino	Lagos, S. Peterburgo, Rússia	Voronin 1978 (*)
<i>Pleistophora littoralis</i>	<i>Blennius pholis</i>	Tec. muscular esquelético	Marinho	Reino Unido	Canning <i>et al.</i> 1979
<i>Pleistophora macrospora</i>	<i>Noemacheilus barbatulus</i>	Tecido muscular da região abdominal	Água doce	França, Mar Negro	Issi & Voronin 1984 (*)
<i>Pleistophora macrozoarcidis</i>	<i>Macrozoarces americanus</i>	Tec. muscular esquelético	Marinho	Atlântico Norte na região oeste	Nigrelli 1946 (*)
<i>Pleistophora oolyticus</i>	<i>Saurida tumbil</i>	Ovários	Marinho	Mar Vermelho, Egito	Negm-Eldin 1992 (*)
<i>Pleistophora priacanthicola</i>	<i>Priacanthus tayenus</i> , <i>P. macrocanthus</i>	Ceg. pilóricos, pericárdio, intestino, gónadas	Marinho	Mar Sul da China	He 1982 (*)
<i>Pleistophora sauridae</i>	<i>Saurida tumbil</i>	Tecido muscular liso	Marinho	Índia	Narasimhamurti & Kalavati 1972 (*)
<i>Pleistophora senegalensis</i>	<i>Sparus auratus</i>	Parede intestinal	Marinho	Senegal	Faye <i>et al.</i> 1990
<i>Pleistophora siluri</i>	<i>Silurus glanis</i>	Parede intestinal	Água doce	Mar Cáspio	Gasimagomedov & Issi 1970 (*)
<i>Pleistophora tahoensis</i>	<i>Cottus beldingi</i>	Músculo esquelético abdominal	Água doce	Califórnia, USA	Summerfelt & Ebert 1969 (*)
<i>Pleistophora tuberifera</i>	<i>Neogobius kessleri gorlap</i> , <i>N. caspius</i> , <i>N.</i> <i>melanostomus affinis</i>	Músculos subcutâneos	Água doce	Mar Cáspio	Gasimagomedov & Issi 1970 (*)
<i>Pleistophora vermiformis</i>	<i>Cottus gobio</i>	Músculo esquelético	Água doce	Rio Danúbio, França e Áustria	Léger 1905 (*)
<i>Pleistophora</i> spp.	<i>Theragra chalcogramma</i> , <i>Gobiodon okinawae</i> , <i>Fundulus heteroclitus</i> , <i>Noemacheilus malapterus</i> <i>longicauda</i> , <i>Salmo salar</i> , <i>Dorosoma petenense</i>	—	—	—	Lom 2002

(*) Consultar a publicação de Lom (2002)

ESPÉCIES	HOSPEDEIROS	LOCAIS DE INFECÇÃO	HABITAT	REGIÃO / PAÍS	REFERÊNCIAS BIBLIOGRÁFICAS
<i>Potasporea morhaphis</i>	<i>Potamorhaphis guianensis</i>	Cavidade celômica perto da região anal	Água doce	Pará, Brasil	Casal <i>et al.</i> 2008b
<i>Pseudoloma neutrophila</i>	<i>Danio rerio</i>	Sistema nervosa central	Água doce	Universidade de Oregon, USA	Matthews <i>et al.</i> 2001
<i>Spraguea lophii</i>	<i>Lophius piscatorius</i> , <i>L. budegassa</i> ,	Células ganglionares do sistema nervoso central	Marinho	Costa europeia Atlântica e mediterrânica (Reino Unido, Noruega e Islândia);	Weissenberg 1976, Loubès <i>et al.</i> 1979
<i>Spraguea americana</i>	<i>Lophius americanus</i> , <i>L. litulon</i>	Células ganglionares do sistema nervoso central	Marinho	Costa Atlântica dos USA, Japão	Takvorian & Cali 1986, Freeman <i>et al.</i> 2004
<i>Spraguea sp.</i>	<i>Lophius gastrophysus</i>	Musculatura abdominal interna perto do gânglio dorsal	Marinho	Rio de Janeiro, Brasil	Jakowska 1964
<i>Tetramicra brevifilum</i>	<i>Scophthalmus maximus</i> , <i>Lophius budegassa</i>	Tecido conectivo da musculatura esquelética	Marinho	Reino Unido, costa Atlântica espanhola; costa mediterrânica espanhola	Matthews & Matthews 1980, Estevez <i>et al.</i> 1992 Maillo <i>et al.</i> 1998
Microsporidium (grupo colectivo)					
<i>Microsporidium bengalis</i>	<i>Nemipterus japonicus</i>	Brânquias	Marinho	Golfo de Bengal, Índia	Weiser, Kalavati & Sandeep 1981 (*)
<i>Microsporidium brevirostris</i>	<i>Brachyhypopomus brevirostris</i>	Músculo da cavidade abdominal	Estuarino	Pará, Brasil	Matos & Azevedo 2004
<i>Microsporidium cerebri</i>	<i>Salmo salar</i>	Cerebro	Marinho	Aquacultura, Canadá	Brocklebank, Speare & Kent 1995 (*)
<i>Microsporidium chloroscombr</i>	<i>Chloroscombrus chrysurus</i>	Fígado	Marinho	Senegal	Toguebaye, Marchand & Faye 1989 (*)
<i>Microsporidium cypselurus</i>	<i>Cypselurus pinnatibarbus</i>	Tecido muscular esquelético	Marinho	Japão	Yokoyama <i>et al.</i> 2002
<i>Microsporidium dicologlossae</i>	<i>Dicologlossa cuneata</i>	Fígado	Marinho	Senegal	Faye <i>et al.</i> 2004
<i>Microsporidium girardini</i>	<i>Girardinus caudimaculatus</i>	Pele, músculo, intestino	Água doce	São Paulo, Brasil	Sprague 1977
<i>Microsporidium peponoides</i>	<i>Percottus plehni</i>	Tecido subcutâneo conectivo	Água doce	Rússia	Sprague 1977
<i>Microsporidium pseudotumefaciens</i>	<i>Xiphophorus maculatus</i> , <i>Molienesia sphenops</i> , <i>Colisa lalia</i>	Vários órgãos	Água doce	Aquário na Alemanha	Canning & Lom 1986
<i>Microsporidium prosopium</i>	<i>Prosopium williamsoni</i>	Tecido muscular esquelético	Água doce	Canadá	Kent <i>et al.</i> 1999
<i>Microsporidium rhabdophila</i>	<i>Oncorhynchus tshawytscha</i> , <i>O. mykiss</i>	Núcleo de células rodlet da pele, brânquias, intestino	Água doce	Califórnia, USA	Modin 1981
<i>Microsporidium sauridae</i>	<i>Saurida tumbil</i>	Musculatura visceral	Marinho	Índia	Narasinhamurti & Kalavati 1972 (*)
<i>Microsporidium sciaenae</i>	<i>Sciaena australis</i>	Tec. conjuntivo à volta do ovário	Água doce	Australia	Canning & Lom 1986
<i>Microsporidium sulci</i>	<i>Acipenser ruthenus</i> , <i>A. guldenstadti</i>	Ovócitos	Água doce	Rio Danúbio, Volga, Kura	Sprague 1977
<i>Microsporidium synapturae</i>	<i>Synaptura cadenati</i>	Fígado	Marinho	Senegal	Faye <i>et al.</i> 2004
<i>Microsporidium valamugili</i>	<i>Valamugil sp.</i>	Parede intestinal	Estuarino	Índia	Canning & Lom 1986
<i>Microsporidium vantraeleniae</i>	<i>Vantraelenia chirophthalmus</i>	Fígado	Marinho	Senegal	Faye <i>et al.</i> 2004

<i>Microsporidium zhanjiangensis</i>	<i>Priacanthus tayenus</i> , <i>P. macracanthus</i>	Parede intestinal e baço	Marinho	China	Hua & Zhang 1988 (*)
<i>Microsporidium</i> spp.	<i>Pagrus major</i> e <i>Sparus aurata</i>		Marinho	Japão; Malta	Bell <i>et al.</i> 2001
(30 espécies)	<i>Branchiostegus semifaciatus</i> , <i>Caranx crysos</i> , <i>C. senegallus</i> , <i>Selene dorsalis</i> , <i>Trachurus</i> <i>trachurus</i> , <i>Erythrocles monodi</i> , <i>Eucinostomus</i> <i>melanopterus</i> , <i>Galeoides decadactylus</i> , <i>Umbrina canariensis</i> , <i>Dicologlossa cuneata</i> , <i>Synaptura cadenati</i> , <i>S. lusitanica</i> , <i>Dentex</i> <i>canarensis</i> , <i>D. maroccanus</i> , <i>Sparus</i> <i>caeruleosticus</i> , <i>S. pagrus pagrus</i>	Fígado	Marinho	Senegal	Lom 2002 (Doutoral Thesis Faye, 1992)
	<i>Chilomycterus reticulatus</i>	Intestino	Marinho		Doutoral Thesis Faye, 1992 (*)
	<i>Trichiurus lepturus</i>	Ovário	Marinho		Doutoral Thesis Faye, 1992 (*)
	<i>Vimba vimba</i>	Intestino e rim	Eurihalino	Mar Cáspio	Lom 2002
	<i>Ictalurus punctatus</i> , <i>Lycodopsis pacifica</i>	Músculo cardíaco e intestino	Água doce	USA	Lom 2002
	<i>Trachurus declivis</i>	Cavidade pericardial e nervos	Marinho	Nova Zelândia	Lom 2002
	<i>Mallotus villosus</i>	Epitélio peritoneal, ovários	Marinho	Newfoundland	Lom 2002
	<i>Salmo trutta</i>	Ovário	Eurihalino	Noruega	Lom 2002
	<i>Pleuronectes flesus</i>	Pele	Marinho	Polónia	Lom 2002

(*) Consultar a publicação de Lom (2002)

Grupo colectivo *Microsporidium* Balbiani, 1884

As espécies que não foram detalhadamente identificadas, devido à escassez de informação disponível relativamente às fases da merogonia e esporogonia são, temporariamente, incluídas no género *Microsporidium*. Existem, pelo menos, 18 espécies descritas em peixes, algumas de significativo interesse comercial (Tabela 2).

Microsporídios de classificação taxonómica duvidosa

Algumas referências de microsporídios em peixes necessitam de ser devidamente caracterizadas, nomeadamente através da análise molecular, dado terem sido classificadas em géneros típicos de platelmintos, crustáceos e insectos, entre os quais espécies do género *Jirovecia* (Sprague 1977), *Nosemoides* (Faye et al. 1994) e *Thelohania* (Voronin 1974), respectivamente.

Casos de **hiperparasitismo** foram descritos em mixosporídios: *Nosema notabilis* foi observada em plasmódios de *Ortholinea polymorpha*, a parasitar a bexiga urinária de um peixe-sapo da costa Atlântica dos EUA (Lom & Dyková 1992a); *N. ceratomyxae* ocorre em plasmódios de *Ceratomyxa* sp. na bexiga natatória de um peixe-coelho do Mar Vermelho (Diamant & Paperna 1985, 1989); microsporídios por identificar foram igualmente observados nos trofozóitos dos mixosporídios *Leptotheca fugu* e *Myxidium fugu*, por sua vez localizados no epitélio intestinal de *Takifugu rubripes*, peixe marinho das costas japonesas (Tun et al. 2000).

1.2.6. Patologia: interacção hospedeiro-parasita

Os microsporídios desenvolvem-se directamente no citoplasma, eventualmente no nucleoplasma, das células hospedeiras, induzindo-lhes uma generalizada destruição celular ou uma hipertrofia celular, culminando com a formação de xenomas. A proliferação dos estádios merogoniais e esporogoniais ocasiona progressivas degradações do citoplasma e dos organelos da célula hospedeira, acabando por a destruir praticamente na sua totalidade, sendo o espaço preenchido por esporos maduros. As mitocôndrias são, provavelmente, a única excepção, uma vez que permanecem inalteradas e em grandes concentrações à volta dos parasitas. Dada a ausência de mitocôndrias em qualquer estágio de desenvolvimento do parasita, sabe-se que, energeticamente, este depende totalmente das mitocôndrias circundantes (Dyková & Lom 1980, 2000, Lom & Dyková 2005, Lom & Nilsen 2003).

Desenvolvimento sem formação de xenoma

Em alguns géneros (*Heterosporis*, *Kabatana*, *Nucleospora*, *Ovipleistophora* e *Pleistophora*), os estádios de desenvolvimento desenrolam-se no citoplasma, eventualmente no núcleo da célula hospedeira, sem que haja a diferenciação de uma estrutura protectora para ambos, isto é parasita-hospedeiro, designada de xenoma. O género *Kabatana* parasita preferencialmente o tecido muscular esquelético, no qual os estádios proliferativos formam gradualmente focos de infecção dentro das fibras musculares, induzindo uma generalizada desorganização do sarcoplasma (Dyková & Lom 2000). Em *Pleistophora typicalis*, os estádios de desenvolvimento estão separados das miofibrilas intactas, apenas por uma camada amorfa com cerca de 0,2 a 0,6 µm de espessura. Também se pode observar à volta de *Pleistophora hyphessobryconis* uma auréola que, ultrastruturalmente, traduz uma acção lítica local, resultante da desorganização do sarcoplasma, pelo que essa zona se apresenta desprovida de miofibrilas (Dyková & Lom 1980, 2000). A diferenciação de invólucros densos (esporoforocistos) de origem parasítica dentro do sarcoplasma, contendo exclusivamente estádios do parasita sem citoplasma e núcleo da célula hospedeira, verifica-se em espécies de *Heterosporis* (Schubert 1969, Lom *et al.* 1989a, Michel *et al.* 1989).

Desenvolvimento com formação de xenoma

A célula hospedeira e o parasita encontram-se fisiologica e morfologicamente integrados, formando uma entidade distinta do hospedeiro, com um desenvolvimento próprio e capacidade de crescimento. Ambos parecem beneficiar da formação de xenomas: ao parasita oferece um meio susceptível para poder proliferar e, simultaneamente, protege-o contra os ataques do hospedeiro, uma vez que se encontra camuflado por estruturas celulares da célula hospedeira: em contrapartida, esta última também fica beneficiada na medida que restringe a propagação do parasita às células vizinhas. Externamente, pode diferenciar-se uma parede refráctil e, perifericamente a esta, pode haver a deposição de camadas de tecido conjuntivo, resultantes da resposta do hospedeiro. Internamente, a célula hospedeira possui um núcleo hipertrófico, muitas vezes ramificado ou fragmentado, por um processo amitótico, em numerosos pequenos núcleos. O citoplasma torna-se hipertrófico à medida que o número de esporos aumenta, podendo a célula hospedeira atingir dimensões superiores a 1 mm. O plasmalema pode apresentar modificações que se traduzem por um aumento da área de absorção (Weissenberg 1968, Lom & Dyková 1992a, Lom 2008).

Segundo Lom e Dyková (2005) os xenomas podem ser classificados em:

- a) Xenomas delimitados por uma única membrana, em que o volume total da célula hospedeira não é transformado num xenoma. No género *Spraguea* formam-se xenomas de grandes dimensões, com semelhanças a “cachos de uvas”, que infectam principalmente grandes gânglios extracraniais do cérebro, nervos espinais, bem como qualquer outra célula ganglionar (Freeman *et al.* 2004).
- b) Xenomas delimitados por uma única membrana, em que o volume total da célula hospedeira é transformado num xenoma. Nos géneros *Ichthyosporidium* (Sprague & Vernick 1974 Casal & Azevedo 1995), *Microgemma* (Ralphs & Matthews 1986, Amigó *et al.* 1996, Leiro *et al.* 1999), *Microfilum* (Faye *et al.* 1991), *Potasporea* (Casal *et al.* 2008b) e *Tetramicra* (Matthews & Matthews 1980), o plasmalema expande-se, formando estruturas semelhantes a microvilosidades e, externamente, não se diferencia qualquer revestimento estratificado ou hialinizado.
- c) Xenomas em que o plasmalema é recoberto por fibras de colagénio. No género *Amazonspora* dispõem-se 22 camadas de colagénio justapostas e orientadas longitudinal e transversalmente (Azevedo & Matos 2003a). Plasmalema recoberto por fina camada de glicocálice, seguida de fibras de colagénio, foi descrito no género *Neonosemoides* (Faye *et al.* 1996).
- d) Xenomas de parede espessa. Formação típica nos géneros *Glugea*, *Loma* e *Pseudoloma*. Parede estratificada formada por camadas laminares, podendo diferenciar 60 camadas em alguns casos, e aderente ao revestimento do plasmalema, observa-se em espécies do género *Glugea*. Internamente, o núcleo ocupa uma posição central, é hipertrófico e ramificado (Canning *et al.* 1982, Vagelli *et al.* 2005). No género *Loma* a parede é estreita, formada por uma substância fibrosa ou granular. Externamente, é notória a presença de várias camadas de fibroblastos (Morrison & Sprague 1981a, 1983, Azevedo & Matos 2002a). Presentemente não existem dados referentes à caracterização do xenoma no género *Pseudoloma*.

1.2.7. Estudos moleculares e filogenéticos

Análises moleculares revelaram que os microsporídios são organismos eucariotas com um genoma muito reduzido com a amplitude de 2,3 Mb para a espécie *Encephalitozoon intestinalis* e 19,5 Mb em *Glugea atherinae*, chegando a ser inferior ao da bactéria *E. coli* (4,6 Mb) (Keeling & Slamovits 2004). A redução genómica terá implicado o desenvolvimento de estratégias de compactação da informação genética ou, então, os microsporídios, no decurso da evolução, perderam informação genética correspondente a vias metabólicas

dependo assim dos recursos dos seus hospedeiros (Keeling *et al.* 2005). Sabe-se também que o genoma está organizado sob a forma de múltiplos cromossomas lineares (8 a 18) com as extremidades organizadas em telómeros (Weiss & Vossbrinck 1999, Keeling & Fast 2002). Em 2001, Katinka e colaboradores, com a sequenciação na íntegra do genoma de *E. cuniculi*, observaram a existência de somente 1997 genes codificantes para proteínas. Entre elas, as envolvidas nos processos de replicação de DNA, formação dos ribossomas e via da glicólise, bem como proteínas mitocondriais. Por outro lado, verificaram a inexistência de genes correspondentes à biossíntese de vários componentes, tais como nucleótidos, ácidos gordos e alguns aminoácidos.

De todas as moléculas, o rDNA é a molécula “alvo” em muitos dos estudos filogenéticos devido à presença de regiões hiper-conservadas na sequência, tornando susceptível o seu uso para efeito de comparação entre organismos distantes (Weiss & Vossbrinck 1999). Além disso, a estrutura secundária do rRNA torna possível a realização de alinhamentos baseados na sua estrutura e, assim, assegurar a comparação de caracteres homólogos nas análises filogenéticas (De Rijk & Wachter 1997, Lom & Nilsen 2003). Há muito que se sabe que ribossomas dos microsporídios assemelham-se aos dos procariotas (Ishihara & Hayashi 1968), no entanto, apresenta também algumas particularidades dos eucariotas (Vossbrinck *et al.* 1987). A SSU possui uma molécula de rRNA de 16S, enquanto que a LSU uma molécula de rRNA de 23S. A sequência do rRNA de 5,8S individualizada, tal como se encontra nos eucariotas, não existe nos microsporídios, mas existem algumas sequências homólogas da região 5,8S contidas no início da subunidade 23S, em resultado da ausência, entre ambas, da região espaço transcricional interno 2 (ITS2) (Vossbrinck & Woese 1986, Vossbrinck *et al.* 1987, Cavalier-Smith 1993). Esta invulgar característica, igualmente reportada em bactérias, nunca foi descrita noutros grupos de eucariotas.

Apesar da sequenciação do gene para o SSU rRNA ser largamente utilizada como marcador molecular para muitas espécies, recentemente tem sido sugerida a sequenciação preferencial do espaço ITS e do gene para o LSU rRNA para efeitos de comparações filogenéticas entre espécies muito afins (Tsai *et al.* 2005), nomeadamente entre as espécies que ocorrem na ictiofauna (Cheney *et al.* 2000). Presentemente, é conhecida a região ITS e o LSU rDNA (parcialmente) para algumas espécies. Dos microsporídios presentes na ictiofauna, somente para a espécie *Heterosporis anguillarum*, o gene LSU rDNA foi sequenciado na sua totalidade (Tsai *et al.* 2002). A estrutura da unidade do rDNA na íntegra é conhecida unicamente para a espécie *Encephalitozoon cuniculi*, parasita que ocorre em várias espécies de mamíferos, incluindo os humanos (Peyretailade *et al.* 1998).

Infelizmente, os caracteres que são, geralmente, usados na classificação dos microsporídios para diferenciar os níveis superiores, isto é, o número de núcleos/célula, a presença de uma membrana a rodear o parasita (vesícula esporófora ou vacúolo parasitóforo) e o tipo de divisão nuclear, são incoerentes ao nível dos taxa género e espécie (Vossbrinck & Debrunner-Vossbrinck 2005). Sabe-se que a sequenciação unicamente do gene para o SSU rRNA contribuiu para a posição basal dos microsporídios nos cladogramas dos eucariotas, posicionamento erróneo até finais do século XX, devido ao artefacto gerado pela atracção dos ramos longos (Van der Peer *et al.* 2000, Fast *et al.* 2003). Contudo, a sequenciação do mesmo tem provado ser bastante útil no estabelecimento das relações filogenéticas dentro do grupo dos microsporídios, nomeadamente, para os taxa superiores ao género (Lom & Nilsen 2003). Idealmente, quando se pretende inferir em termos filogenéticos, devem ser usadas várias diferentes moléculas. Contudo, a análise dos resultados obtidos por sequenciação dos genes que codificam as proteínas tem-se revelado ser mais difícil devido à presença de codões degenerados para muitos aminoácidos, bem como devido da existência de várias formas de genes codificantes de proteínas estarem presentes no genoma (parálogos) fazendo com que seja mais difícil a identificação de homólogos (Lom & Nilsen 2003).

Pela análise filogenética efectuada por Vossbrinck e Debrunner-Vossbrinck (2005) num total de 125 sequências do gene para o SSU rRNA, disponíveis em várias bases de dados (p. e. GenBank), verificou-se que os microsporídios agrupam em 5 clados principais correspondentes a 3 classes: classe **Aquasporidia** é um grupo parafilético constituído por 3 clados, maioritariamente formado por microsporídios, que têm como hospedeiro animais de água doce; classe **Marinosporidia** correspondente aos microsporídios que ocorrem em animais aquáticos marinhos, salvo algumas excepções, tais como parasitas que têm como hospedeiro peixes de água doce, bem como microsporídios pertencentes ao género *Dictyocoela*, que ocorrem em anfípodes de água doce. Adaptação do hospedeiro a um novo habitat, aparentemente, é a explicação mais provável. O mesmo poderá ter acontecido a 2 microsporídios do género *Vavraia* e à espécie *Trachipleistophora hominis*, respectivamente parasitas de insectos e de humanos. Por sua vez, a classe **Terresporidia** engloba microsporídios que têm como hospedeiro, maioritariamente, insectos, répteis, aves e mamíferos, inclusivamente, humanos. O microsporídio *Vittaforma corneae* é uma das excepções, levando a crer tratar-se de um falso microsporídio de humanos, uma vez que parasita pacientes imunodeficientes. Segundo Lom e Nilsen (2003), as análises filogenéticas para os microsporídios que ocorrem na ictiofauna, sugere a formação de 5 grupos. Excepto o grupo 5, os restantes encontram-se englobados na classe **Marinosporidia**, recentemente

designada por Vossbrinck e Debrunner-Vossbrinck (2005).

Classificação segundo Lom & Nilsen 2003

Grupo 1

(Grupo correspondente a nenhum taxon): *Loma*, *Ichthyosporidium* e *Pseudoloma*.

Grupo 2

(Família **Glugeidae** Thélohan, 1892): *Glugea*.

Grupo 3

(Fam. **Pleistophoridae** Doflein, 1901): *Pleistophora*, *Heterosporis* e *Ovipleistophora*.

Grupo 4

(Família **Spragueidae** Weissenberg, 1976): *Spraguea*; (Família **Tetramicridae** Matthews & Matthews, 1980) *Microgemma*, *Tetramicra* e *Potasporea*. Inclui também o género *Kabatana*.

Grupo 5

(Família **Enterocytozoonidae** Cali & Owen, 1990): *Nucleospora*.

A análise filogenética do gene SSU rRNA do microsporídio *Myosporidium* não permitiu agrupá-lo em nenhum dos grupos (Baquero *et al.* 2005). Para os géneros com uma única espécie, *Amazonspora* (Azevedo & Matos 2003a), *Microfilum* (Faye *et al.* 1991) e *Neonosemoides* (Faye *et al.* 1996), não existe informação molecular disponível.

Grupo 1

Existe informação molecular referente ao gene SSU rRNA para 4 das 12 espécies pertencentes ao género *Loma*, tratando-se de um grupo parafilético em que a espécie tipo, *Loma branchialis*, não está sequenciada. Para além de *Loma* spp., este grupo engloba sequências referentes a parasitas dos géneros *Pseudoloma* (Matthews *et al.* 2001) e *Ichthyosporidium* (Sprague & Vernick 1974). Os caracteres morfológicos do ciclo de vida do género *Ichthyosporidium* diferem em muito dos restantes do grupo, entre eles inclui-se núcleo em diplocário e todo o desenvolvimento em directo contacto com citoplasma da célula hospedeira. Possivelmente, a sequência (GenBank L39110) referente ao *Ichthyosporidium* sp., corresponderá a um parasita erroneamente classificado.

Grupo 2

Grupo monofilético com um alto “bootstrap”, que reúne todas as espécies do género *Glugea*. Para além das espécies que ocorrem em peixes, o microsporídio marinho

Tuzetia weidneri (= *Pleistophora*), descrito num crustáceo, agrupa com a espécie tipo *G. anomala*. Em todos os cladogramas elaborados, a espécie descrita como *Pleistophora finisterrensis*, parasita do verdinho *Micromesistius poutassou*, não agrupa com as restantes do género, mas sim com *G. anomala*, situação que, indiscutivelmente, carece de uma reclassificação.

Grupo 3

Este grupo apresenta uma heterogeneidade, na medida em que engloba representantes que parasitam diferentes hospedeiros. O bootstrap para as sequências de *Heterosporis* spp. e *Ovipleistophora* spp. é elevado, constituindo um grupo formado, exclusivamente, por microsporídios em que o hospedeiro é de água doce. Apesar do género *Pleistophora* ocorrer, simultaneamente, em espécies marinhas e de água doce, não é de estranhar bootstrap superiores a 98% em vários cladogramas, uma vez que todas as sequências disponíveis dizem respeito a microsporídios marinhos. Este grupo engloba também espécies dos géneros *Dictyocoela* e *Vavraia*, bem como a espécie *Trachipleistophora hominis*.

Grupo 4

Grupo composto, exclusivamente, por microsporídios que parasitam peixes, que alberga 5 géneros estritamente relacionados, tendo em conta os caracteres morfológicos. Entre eles incluem-se a ausência da diferenciação de uma vesícula esporófora/ vacúolo parasitóforo, bem como núcleo unicário (excepto uma das fases do microsporídio dimórfico *Spraguea* spp.) durante todo o ciclo de vida. Os géneros *Tetramicra* e *Microgemma*, aparentemente, constituem um grupo monofilético em todos os cladogramas. O mesmo não sucede ao género *Kabatana*, que se caracteriza por parasitar, exclusivamente, o tecido muscular esquelético de peixes de água doce e de água salgada, motivo pelo qual, muito possivelmente, explica a parafilia do género.

Grupo 5

Nos cladogramas elaborados por Vossbrinck e Debrunner-Vossbrinck (2005), este grupo encontra-se excluído da classe Marinosporidia, onde se encontram agrupados todos os microsporídios que parasitam a ictiofauna, para se posicionar na classe Terresporidia, formando um clado com o microsporídio *Enterocytozoon bieneusi*, parasita de mamíferos, incluindo humanos. Não é de estranhar, que em todas as análises filogenéticas, o género *Nucleospora* se posiciona agrupado com as sequências seleccionadas como “outgroup” (Docker *et al.* 1997, Lom & Nilsen 2003), visto que possui a invulgar característica de parasitar o nucleoplasma, em vez do citoplasma, como sucede com os outros microsporídios (Lom & Dyková 2002).

1.3. Mixosporidioses

As mixosporidioses são doenças causadas pela acção de parasitas do grupo dos mixosporídios (= mixozoários, como também são designados). Estes parasitas são metazoários, pertencentes taxonomicamente ao filo Myxozoa Grassé, 1970, e representam um grupo de grande importância económica, devido aos seus efeitos nefastos na aquacultura e na natureza. São parasitas, quase exclusivamente, de vertebrados poiquilotérmicos, principalmente peixes de água doce e salgada, bem como de alguns invertebrados. Presentemente, conhecem-se mais de 2200 espécies, agrupadas em 16 famílias e 65 géneros, a parasitar vertebrados (Lom & Dyková 2006, Køie *et al.* 2007b, Prunescu *et al.* 2007). Apesar de se saber que têm um ciclo de vida indirecto, envolvendo um invertebrado como hospedeiro definitivo e um peixe como hospedeiro intermediário, estes parasitas são conhecidos, principalmente, por provocarem infecções em peixes, visto que somente 180 tipos (17 grupos) de actinosporos foram descritos em invertebrados (Lom & Dyková 2006). Algumas ocorrências em platelmintos, anfíbios, répteis, aves e mamíferos foram também documentadas (Friedrich *et al.* 2000, Kent *et al.* 2001, Duncan *et al.* 2004, Eiras 2005, Garner *et al.* 2005, Jirků *et al.* 2006, Prunescu *et al.* 2007, Bartholomew *et al.* 2008). Em humanos verificaram-se esporádicas ocorrências, que indicam tratar-se de presenças acidentais. Em alguns trabalhos foram descritos esporos encontrados nas fezes de pacientes que, muito provavelmente, serão provenientes da ingestão de peixes infectados (McClelland *et al.* 1997, Boreham *et al.* 1998, Moncada *et al.* 2001). O estudo dos mixosporídios tem sido desenvolvido nas mais variadas vertentes de investigação, nomeadamente nos aspectos morfológicos do seu ciclo de vida, processo de transmissão, taxonomia e identificação filogenética.

1.3.1. Posição taxonómica

Os esporos de mixosporídios foram, pela primeira vez, identificados por Jurine (1825), tendo sido classificados, mais tarde, por Otto Bütschli (1882) e incluídos na subclasse Myxosporida, pertencente à classe designada de Sporozoa (consultar a publicação, Lom & Dyková 2006). Desde então, os mixosporídios foram considerados protistas, em parte devido ao tamanho e à forma dos esporos, apesar de na época lhes ter sido reconhecido o parentesco com os organismos do filo Cnidaria. Após alguma hesitação inicial, os mixosporídios acabaram por ser reconhecidos como organismos metazoários. Presentemente, constituem uma superclasse do infrafilos Metazoa dentro do filo Opisthoconta (Cavalier-Smith 1998, Hausmann *et al.* 2003), em que a morfologia e

ultraestrutura está bem documentada através das inúmeras publicações (Azevedo *et al.* 1989, 2005, Current *et al.* 1979, Desser *et al.* 1983, Lom & Puytorac 1965a, 1965b, Lom 1969, Lom & Dyková 1992b, Sitjà-Bobadilla & Álvarez-Pellitero 1992, 1993a, 1993b, 1995, 2001), Por outro lado, vários estudos de biologia molecular sugerem semelhanças filogenéticas com os metazoários (Siddall *et al.* 1995), nomeadamente com os que apresentam simetria bilateral (Smothers *et al.* 1994, Anderson *et al.* 1998, Okamura *et al.* 2002, Siddall & Whiting 1999, Zrzavý & Hypša 2003).

1.3.2. Classificação Taxonómica

Phylum Myxozoa Grassé, 1970

Tipicamente, são organismos formados por células eucarióticas sem centríolos e sem flagelos, no entanto, observam-se, frequentemente, muitos microtúbulos intimamente envolvidos na divisão nuclear e na diferenciação celular, mitocôndrias com cristas de forma tubular, discóide ou então achadas, bem como junções celulares de aderência semelhantes a desmossomas e junções comunicantes. Parasitam alternadamente seres invertebrados e vertebrados, caracterizando-se por diferenciarem esporos de forma e estrutura variada. Os esporos são constituídos por uma ou mais valvas, podendo eventualmente possuir projecções simples ou elaboradas. Internamente, localizam-se uma ou várias células amibóides germinais infectivas (esporoplasmas) e uma ou várias cápsulas polares (semelhantes aos nematocistos dos cnidários), cada uma apetrechada com um filamento polar extrusível. Este filo divide-se em 2 classes: **Malacosporea** (esporos de valvas sem rigidez que infectam os briozoários e os peixes) e **Myxosporea** (esporos de valvas rígidas que ocorrem em anelados e peixes). Esta última classe é composta por duas ordens: Bivalvulida (esporos com 2 valvas e geralmente com 2 cápsulas polares) e Multivalvulida (esporos formados por mais de 2 valvas e por mais de 2 cápsulas polares (Kent *et al.* 2001, Lom & Dyková 1992b, 2006).

Classe Malacosporea Canning, Curry, Feist, Longshaw & Okamura, 2000

Os malacosporos são parasitas de briozoários de água doce (filo Bryozoa, classe Phylactolaemata). Os estádios vegetativos desenvolvem-se dentro da cavidade do corpo dos briozoários em forma de sacos multicelulares fechados ou, então, em organismos em forma de verme (Canning *et al.* 1996, 2000, 2002, Okamura *et al.* 2002, Morris & Adams 2008).

Filo Myxozoa

Classe Myxosporea

Ordem **Bivalvulida**

Subordem Sphaeromyxina

Família: **Sphaeromyxidae** - *Sphaeromyxa*

Subordem Variisporina

Família: **Myxidiidae** - *Myxidium*, *Enteromyxum*, *Zschokkella*, *Coccomyxa*, *Soricimyxum*

Família: **Ortholineidae** - *Ortholinea*, *Neomyxobolus*, *Cardimyxobolus*, *Triangula*, *Triangulamyxa*, *Kentmoseria*

Família: **Sinuolineidae** - *Sinuolinea*, *Myxodavisia*, *Myxoproteus*, *Bipteria*, *Paramyxoproteus*, *Neobipteria*, *Schulmania*, *Noblea*

Família: **Fabesporidae** - *Fabespora*

Família: **Ceratomyxidae** - *Ceratomyxa*, *Leptotheca*, *Meglitschia*, *Ellipsomyxa*

Família: **Sphaerosporidae** - *Sphaerospora*, *Polysporoplasma*, *Hoferellus*, *Wardia*, *Palliatum*, *Myxobilatus*

Família: **Chloromyxidae** - *Chloromyxum*, *Caudomyxum*, *Agarella*

Família: **Auerbachiiidae** - *Auerbachia*, *Globospora*

Família: **Alatosporidae** - *Alatospora*, *Pseudoalatospora*, *Renispora*

Família: **Parvicapsulidae** - *Parvicapsula*, *Neoparvicapsula*, *Gadimyxa*

Subordem Platysporina

Família: **Myxobolidae** – *Myxobolus*, *Spirosuturia*, *Unicauda*, *Dicauda*, *Phlogospora*, *Laterocaudata*, *Henneguya*, *Hennegoides*, *Tetrauronema*, *Thelohanellus*, *Neothelohanellus*, *Neohenneguya*, *Trigonosporus*

Ordem **Multivalvulida**

Família: **Trilosporidae** – *Trilospora*, *Unicapsula*

Família: **Kudoidae** - *Kudoa*

Família: **Spinavaculidae** - *Octospina*

Trilosporoides (*incertae sedis*)

Classe Malacosporea

Ordem Malacovalvulida

Família: **Saccosporidae** – *Buddenbrockia* (sinónimo: *Tetracapsula*), *Tetracapsuloides*

Classificação taxonómica do filo Myxozoa:

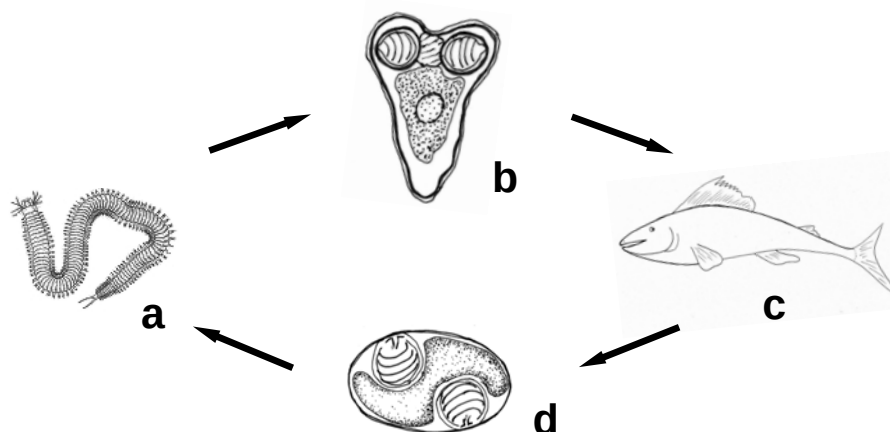
Adaptado da última revisão efectuada por Lom e Dyková (2006) com a reestruturação e introdução de novos géneros. *Kudoa* (Whipps et al. 2004), *Gadimyxa* n. gen. (Køie et al. 2007b), *Soricimyxum* n. gen. (Prunescu et al. 2007) e *Myxodavisia* (= *Davisia*) (Zhao et al. 2008b).

Classe Myxosporea Bütschli, 1881

O ciclo de vida é indirecto e compreende duas fases: num peixe (hospedeiro intermediário), proliferam e diferenciam-se esporos multicelulares resistentes ao ambiente externo (**mixosporos**) que, por sua vez, infectam um anelado, raramente sipunculídeos, (hospedeiro definitivo) onde se reproduzem, sexuadamente, originando o agente infectivo dos peixes, denominado de **actinosporo** (Lom & Dyková 2006). Dado que no âmbito deste trabalho os peixes são a fauna alvo, a descrição morfológica e ultrastrutural restringir-se-á aos mixosporos, tendo por base os trabalhos de revisão efectuados por Lom e Dyková (1992, 2006), Kent e colaboradores (2001) e Yokoyama (2003).

1.3.3. Ciclo de vida

Com o crescente interesse nos actinosporos como agentes infectivos dos peixes, vários estudos têm sido conduzidos em oligoquetas e em poliquetas em habitat natural (Xiao & Desser 1998a, 1998b, Negredo & Mulcahy 2001, Székely *et al.* 2000, 2003, 2005), bem como a partir da fauna dos tanques, onde existem mixosporídios como agentes patogénicos de espécies de peixes cultivadas (Burtle *et al.* 1991, Yokoyama *et al.* 1993, Özer *et al.* 2002, Oumouna *et al.* 2003) (esquema 3).



Esquema 3 – Desenho esquemático do ciclo de vida de um mixosporídio: a) hospedeiro definitivo (género *Nereis*); b) actinosporo; c) hospedeiro intermediário; d) mixosporo.

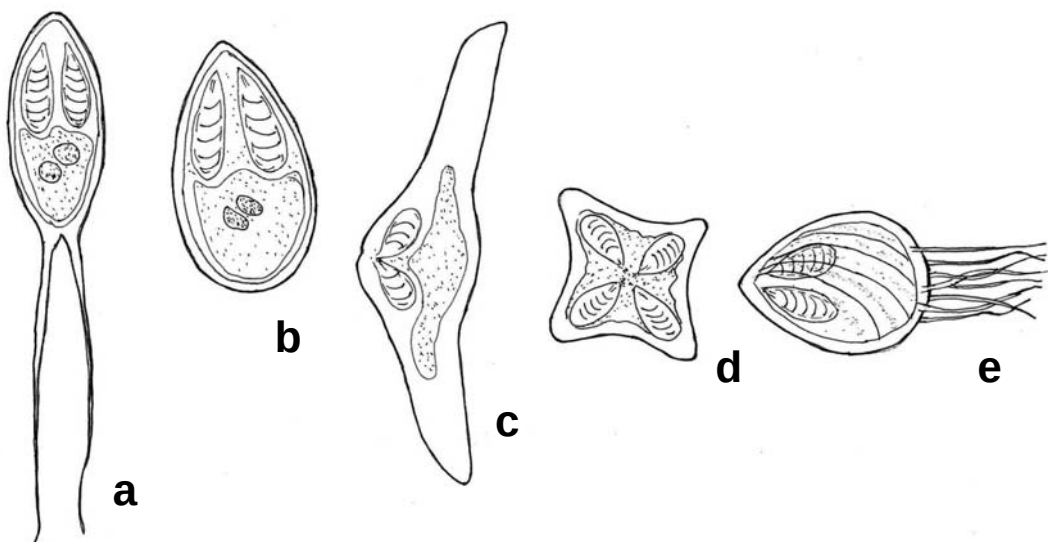
Desde a descoberta de estádios de actinosporos, do tipo triactinomyxon, no ciclo de vida do mixosporídio *Myxobolus cerebralis* (Wolf & Markiw 1984), foram caracterizados até à data, pelo menos 40 ciclos de vida de diferentes mixosporídios (Kent *et al.* 2001, Køie *et al.* 2004, Atkinson & Bartholomew 2009). Os estádios alternativos de actinosporos e mixosporos do ciclo de vida de espécies do filo Myxozoa podem ser identificados através de estudos do controlo das infecções de ambos os hospedeiros ou, então, através da

análise das sequências de DNA obtidas de ambos as fases. Presentemente, já se conhecem as sequências para alguns actinosporídios (El-Mansy *et al.* 1998, Hallett *et al.* 1999, Negredo *et al.* 2003, Holzer *et al.* 2004). A utilização do gene de SSU rRNA tem permitido, com sucesso, a confirmação de diferentes estádios do ciclo de vida dos mixosporídios, pertencentes a diferentes géneros, tais como *Myxobolus cerebralis* (Andree *et al.* 1997), *Ceratomyxa shasta* (Bartholomew *et al.* 1997), *Tetracapsuloides bryosalmonae* (Anderson *et al.* 1999), *Henneguya ictaluri* (Lin *et al.* 1999), *Thelohanellus hovorkai* (Anderson *et al.* 2000), *Ellipsomyxa gobii* (Køie *et al.* 2004), *Chloromyxum auratum* (Atkinson *et al.* 2007), *Gadimyxa atlantica* (Køie *et al.* 2007b), *Ceratomyxa auerbachii* (Køie *et al.* 2008) e *Myxobilatus gasterostei* (Atkinson & Bartholomew 2009).

1.3.4. Fases de desenvolvimento na ictiofauna

Mixosporos

Os mixosporos, muitas vezes, são diagnosticados através da detecção macroscópica ou microscópica de pequenos plasmódios alojados nos tecidos. Em alguns casos, encontram-se localizados livremente dentro da cavidade de órgãos. O corpo dos mixosporos maduros pode apresentar variadas formas, tais como: ovóide, elipsóide piriforme, fusiforme, encurvada, arredondada, quadrangular, triangular atingindo, habitualmente, entre 10 a 20 µm de comprimento ou de espessura variável de acordo com o género (esquema 4).



Esquema 4 - Desenhos esquemáticos de cinco mixosporos correspondentes a diferentes géneros: a) *Henneguya*; b) *Myxobolus*; c) *Ceratomyxa*; d) *Kudoa*; e) *Chloromyxum*.

Algumas espécies do género *Ceratomyxa* podem alcançar algumas centenas de µm de comprimento total (Eiras *et al.* 2006, Casal *et al.* 2007). Os esporos podem possuir 2 a 12 valvas, 1 a 13 cápsulas polares e 1 esporoplasma infectivo, formado por uma célula binucleada ou por 2 células uninucleadas como sucede no género *Kudoa* (Lom & Dyková 2006). Excepcionalmente, o género *Polysporoplasma* pode conter até 12 células esporoplasmáticas uninucleadas (Sitjà-Bobadilla & Álvarez-Pellitero 1995).

Plasmódios

Nos hospedeiros intermediários, o ciclo inicia-se, quando os actinosporos ou os malacosporos, uma vez livres na coluna de água, extrudem os seus filamentos polares, se fixam nos tecidos epiteliais (intestino, brânquia, pele, rim) dos peixes e, conseqüentemente, a abertura das valvas permitem a libertação da ou das células esporoplasmáticas no tecido hospedeiro (Kallert *et al.* 2007). Simultaneamente, dá-se a fusão dos núcleos haplóides, transformando-se num trofozóito (fase pré-esporogónica). Os trofozóitos migram do local de infecção e continuam a desenvolver-se num **plasmódio** ou num **pseudoplasmodio**. Dentro do plasmódio existem estádios pré-esporogónicos e esporogónicos do parasita, que, eventualmente, acabam por se diferenciar em esporos, adquirindo as características morfológicas típicas através das quais são frequentemente classificados. Os plasmódios desenvolvem-se por dois processos: intercelular ou intracelularmente nos tecidos (**histozóicos**), assemelhando-se a cistos ou, então, na cavidade dos órgãos nomeadamente no tracto urinário e na vesícula biliar, onde estabelecem ligações com o epitélio do órgão e/ou flutuam livremente no fluído dentro da cavidade (**parasitas coelozóicos**) (El-Matbouli *et al.* 1992, Lom & Dyková 2006). Os plasmódios histozóicos são delimitados por uma membrana pinocítica, por vezes com invaginações através da qual os nutrientes alcançam o interior do plasmódio (Lom & Puytorac 1965a, Casal *et al.* 1997, 2002, Rocha *et al.* 1992). Nos plasmódios coelozóicos, a superfície de absorção é incrementada devido à diferenciação de inúmeras extensões citoplasmáticas em torno da membrana (Sitjà-Bobadilla & Álvarez-Pellitero 1993b, Canning *et al.* 1999, Casal *et al.* 2007). Geralmente, os plasmódios polispóricos são macroscópicos, podendo mesmo alcançar cerca de 2 cm de diâmetro (*Sphaeromyxa maiyai*). Em contrapartida, alguns géneros formam pseudoplasmodios (plasmódios monospóricos ou dispóricos), que contêm um núcleo e as células generativas necessárias para se formar um ou dois esporos (Lom & Dyková 1992b, 2006).

No interior dos plasmódios encontram-se núcleos vegetativos e células generativas, que iniciam o processo da esporogonia através do envolvimento completo de uma célula

generativa por uma outra célula generativa, que irá diferenciar-se no pericito. A célula envolvida, esporogónica, divide-se várias vezes consoante o género de mixosporídio que irá originar, formando agregados de células envolvidas pelo pericito designado de pansporoblasto. As células diferenciam-se gradualmente em células valvogénicas, capsulogénicas e no esporoplasma binucleado, culminando com a formação dos esporos (Lom & Puytorac 1965a, Feist 2008). Os esporos de algumas espécies do género *Kudoa* diferenciam-se, sem que haja a formação de pansporoblastos (Moran *et al.* 1999).

Diferenciação celular

As **células valvogénicas** envolvem as células capsulogénicas e a/as células esporoplasmáticas. Estas, gradualmente, tornam-se electronodensas, acabando por ficar ligadas entre si ao nível da linha de sutura, através de junções semelhantes a desmossomas. Frequentemente, observam-se feixes de microtúbulos dispostos ao longo das futuras valvas (Lom 1969, Desser *et al.* 1983, Azevedo *et al.* 1989, Casal *et al.* 2002). A linha de sutura é linear, raramente sinuosa como sucede nos géneros pertencentes à família Sinuolineidae: *Sinuolinea*, *Davisia*, *Myxoproteus*, *Bipteria*, *Paramyxoproteus*, *Neobipteria*, *Schulmania*, *Noblea* (Lom & Dyková 1992b). A superfície externa das valvas é geralmente lisa. Contudo, em algumas espécies dos géneros *Chloromyxum* (Azevedo *et al.* 2009a, Lom & Dyková 1993), *Myxidium* (Azevedo *et al.* 1989, Canning *et al.* 1999), *Hoferellus*, *Myxobilatus*, *Neomyxobolus*, *Ortholinea*, *Sphaeromyxa*, *Zschokkella* (Lom & Dyková 1992b) e *Triangulamyxa* (Azevedo *et al.* 2005) caracteriza-se por ser enrugada. Diferenciação de projecções caudais em continuidade com as valvas é típica, entre outros, dos géneros *Henneguya* (Azevedo & Matos 1995, 1996a, 2002b, 2003b, Casal *et al.* 1996, 2003) e *Tetrauronema* (Azevedo & Matos 1996b). Por outro lado, prolongamentos de diferente natureza associados às valvas são característicos dos géneros *Unicauda* e *Dicauda* (Lom & Dyková 1992b).

Nas **células capsulogénicas** diferencia-se um primórdio capsular arredondado em continuidade com um tubo externo rodeado de microtúbulos. O tubo externo invagina-se no primórdio, gradualmente, enrolando-se obliquamente várias vezes no seu interior, acabando por se transformar no filamento polar (Lom & Puytorac 1965b, Lom 1969, Current *et al.* 1979, Desser *et al.* 1983). A matriz capsular densifica-se, podendo, eventualmente, diferenciarem-se também estruturas electronodensas concêntricas (Lom *et al.* 1989b), estruturas semelhantes a microfilamentos (Casal *et al.* 2002) ou inclusões cristalóides (Casal *et al.* 2007). Feixes de tubulina na matriz capsular foram descritos nos géneros *Sphaeromyxa* (Lom 1969), *Henneguya* (Rocha *et al.* 1992) e *Myxobolus* (Casal

et al. 2002).

A **célula germinal**, denominada **esporoplasma**, é geralmente binucleada, rica em RE, mitocôndrias e em vesículas designadas de esporoplasmosomas. Estas vesículas podem possuir 1 ou 2 membranas e ter grande heterogeneidade quanto à forma e electrodensidade (Lom et al. 1989b), permitindo diferenciar espécies pertencentes ao mesmo género, tais como *Henneguya* (Rocha et al. 1992, Azevedo & Matos 2002b, Casal et al. 2003, Vita et al. 2003, Azevedo et al. 2008) e *Myxobolus* (Azevedo et al. 2002, Casal et al. 1996, 2006). Em alguns géneros diferencia-se, eventualmente, um vacúolo iodínforo. No género *Kudoa*, formam-se 2 células esporoplasmáticas uninucleadas, uma designada de primária que envolve completamente uma outra, designada de secundária (Moran et al. 1999, Casal et al. 2008a, Dyková et al. 2009).

1.3.5. Diagnose de alguns géneros de mixosporídios

No âmbito desta tese foram caracterizadas algumas parasitoses correspondentes a 5 géneros do filo Myxozoa: 4 géneros pertencentes à ordem Bivalvulida (*Ceratomyxa*, *Chloromyxum*, *Henneguya*, *Myxobolus*) e 1 género pertencente à ordem Multivalvulida (*Kudoa*) (ver esquema 4).

***Ceratomyxa* Thélohan, 1892**

Bivalvulida; 2 cápsulas polares próximas uma da outra; simetria bilateral; cápsulas polares (CPs) em plano perpendicular à linha de sutura; CPs próximas da região apical; esporos sem projecções ou estruturas membranosas; esporos em forma de meia-lua, extremamente alongados na direcção perpendicular à linha de sutura. Diferencia trofozóitos mono a polispóricos, preferencialmente dispóricos. O esporoplasma binucleado não preenche completamente a cavidade. São parasitas coelozóicos de peixes marinhos, excepcionalmente parasitam peixes de água doce e, raramente, são histozóicos. Existem, pelo menos, 5 referências em peixes de água doce e entre elas inclui-se a espécie, *Ceratomyxa shasta*, parasita histozóico do intestino de salmonídeos, sendo uma das poucas espécies em que se conhece o seu ciclo de vida. A fase dos actinosporos do tipo tetractinomyxon desenvolve-se na poliqueta de água doce *Manyunkia speciosa* (Bartholomew et al. 1997, Lom & Dyková 1992b, 2006). Segundo a revisão elaborada por Eiras (2006), foram descritas pelo menos 147 espécies em diferentes áreas geográficas. Recentemente, foram descritas mais 32 espécies: 1 num tunídeo do Mar Mediterrânico (Mladineo & Bocina 2006), 3 na África do Sul (Reed et al. 2007), 1 no tamboril do Atlântico Norte (Afonso-Dias et al. 2007), 1 no tamboril do Japão (Freeman et al. 2008), 4 no Mar Vermelho (Abdel-Ghaffar et al. 2008a). Na vesícula biliar

da fauna australiana foram descritas 11 espécies pertencentes à família Pomacentridae (Gunter *et al.* 2008), 4 em labrídeos (Heiniger *et al.* 2008) e 7 em serranídeos (Gunder & Adlard 2009).

***Chloromyum* Mingazzini, 1890**

Bivalvulida; 4 cápsulas polares posicionadas na região apical do esporo, por vezes de tamanho desigual; 1 par de CP paralelo à linha de sutura e um segundo par perpendicular à linha de sutura; esporos esféricos em que valvas podem ser lisas ou apresentar sulcos; algumas espécies possuem projecções filamentosas caudais; esporoplasma binucleado; em regra são parasitas coelozóicos de peixes de água doce e salgada, raramente são histozóicos. No hospedeiro definitivo foram descritos actinosporos do tipo neoactinomyxon, antonactinomyxon e aurantiactinomyxon em espécies de água doce e salgada. Existem descritas pelo menos 118 espécies em peixes (Lom & Dyková 2006, Abdel-Baki 2007, Azevedo *et al.* 2009a, Casal *et al.* 2009a) e 3 referências em anfíbios (Lom & Dyková 2006).

***Henneguya* Thélohan, 1892**

Bivalvulida, 2 CP próximas uma da outra; simetria bilateral; CPs alongadas geralmente de igual tamanho posicionadas paralelamente à linha de sutura; CPs próximas da região apical; esporos de forma ovóide, fusiforme ou arredondada em perspectiva valvular; achatados paralelamente à linha de sutura; valvas lisas que se prolongam na região posterior em 2 projecções caudais independentes, muitas vezes revestidas por material hialino (Azevedo & Matos 1995, 1996a) ou floculento (Azevedo & Matos 2003b, Casal *et al.* 1997). Diferencia trofozóitos grandes, polispóricos, com formação pansporoblástica. O esporoplasma binucleado, por vezes com uma inclusão polissacarídica esférica. São parasitas histozóicos, maioritariamente de peixes de água doce, existindo escassas referências em peixes marinhos. O ciclo de vida é conhecido somente para 3 espécies: *H. exilis* e *H. ictaluri* diferenciam aurantiactinomyxon em oligoquetas (Kent *et al.* 2001) e actinosporos tipo triactinomyxon formam-se na espécie *H. nuesslii* (Kallert *et al.* 2005). Estão descritas pelo menos 204 espécies (Eiras 2002, Lom & Dyková 2006). Recentemente foram descritas mais 14 espécies (Adriano *et al.* 2005a, 2005b, Brickle *et al.* 2006, Martins & Onaka 2006, Molnár *et al.* 2006b, 2006c, Reed *et al.* 2007, Azevedo *et al.* 2008, Eiras *et al.* 2008, Feijó *et al.* 2008, Work *et al.* 2008, Azevedo *et al.* 2009c, Eiras *et al.* 2009, Kageyama *et al.* 2009, Székely *et al.* 2009a).

***Myxobolus* Bütschli, 1882**

Bivalvulida, 2 CP próximas uma da outra; simetria bilateral; CPs posicionadas paralelamente à linha de sutura e próximas da região apical; esporos de forma elipsoidal,

ovóide, arredondada em perspectiva valvular, achatados paralelamente à linha de sutura que se apresenta direita; o bordo sutural pode, eventualmente, estender-se na porção posterior; ausência de projecções ou estruturas membranosas, Diferencia trofozóitos grandes, polispóricos, com formação de pansporoblastos. CPs, geralmente, piriformes e de igual tamanho. O esporoplasma binucleado, por vezes com um vacúolo iodínóforo. São parasitas histozóicos maioritariamente de peixes de água doce. Nas 14 espécies em que o ciclo de vida foi descrito, na fase dos actinosporos diferenciam-se triactinomyxon, hexactinomyxon ou raabeia de acordo com a espécie. Das cerca de 792 espécies, 30 ocorrem em peixes marinhos, sendo em maioria espécies coelozóicas (Eiras *et al.* 2005, Lom & Dyková 2006), existindo ainda 7 referenciadas em anfíbios e répteis (Eiras 2005). Nos últimos 3 anos foram descritas mais 30 espécies (Adriano *et al.* 2006, Casal *et al.* 2006, Martins & Onaka 2006, Molnár *et al.* 2006a, 2006b, Ali *et al.* 2007, Diamanka *et al.* 2007, Eiras *et al.* 2007, Molnár *et al.* 2007, Yokoyama *et al.* 2007, Abdel-Ghaffar *et al.* 2008b, Ferguson *et al.* 2008, Hogge *et al.* 2008, Molnár *et al.* 2008, Zhao *et al.* 2008a, Adriano *et al.* 2009, Azevedo *et al.* 2009b, Hemananda *et al.* 2009, Molnár *et al.* 2009, Székely *et al.* 2009a, 2009b).

***Kudoa* Meglitsch, 1947**

Multivalvulida; esporos com 4 ou mais valvas de simetria radial em forma de estrela, quadrada ou redondo quadrangular; por vezes são assimétricos, sendo uma das valvas maior; a face posterior é achatada ou semiesférica; 1 cápsula polar piriforme por valva. Dois esporoplasmas uninucleados em que uma célula é completamente envolvida pela outra. Trofozóitos pequenos, originando 1 a 7 esporos, ou grandes e polispóricos. Ausência de formação de pansporoblastos; parasitas histozóicos de peixes marinhos, maioritariamente, ocorrem no tecido muscular; excepcionalmente podem ser coelozóicos, bem como parasitar outros órgãos. Salvo raras excepções, conforme se verificou na espécie *K. permulticapsula*, constituída por 13 valvas e por 13 CPs (Whipps *et al.* 2003b), em regra, as espécies de *Kudoa* possuem 4 valvas e 4 CPs. Recentemente, evidências filogenéticas implicaram uma reestruturação da família Kudoidae e, conseqüentemente, 6 espécies (espécies com mais de 4 valvas e 4 CPs) foram transferidas para o género *Kudoa* em resultado da renomeação dos géneros *Pentacapsula*, *Hexacapsula* e *Septemcapsula* (Whipps *et al.* 2004). Em nenhuma das 69 espécies descritas foram identificados estádios da fase dos actinosporos (Moran *et al.* 1999, Swearer & Robertson 1999, Lom & Dyková 2006). Recentemente, foram descritas mais 12 espécies (Wang *et al.* 2005, Adlard *et al.* 2005, Gunter *et al.* 2006, Holzer *et al.* 2006a, Burger *et al.* 2007, Yurakhno *et al.* 2007, Casal *et al.* 2008a, Quraishy *et al.* 2008, Dyková *et al.* 2009).

1.3.6. Patologia

A maioria das infecções por mixosporídios são relativamente benignas, existindo algumas espécies patogénicas que podem causar mortalidade ou então infringir severos danos aos seus hospedeiros. Dos mixosporídios conhecidos, o que provoca a doença “whirling” é de longe o mais estudado, tendo como agente a espécie *Myxobolus cerebralis* que parasita as cartilagens da cabeça e da coluna vertebral de salmonídeos, induzindo deformações consideráveis aos seus hospedeiros (Kent *et al.* 2001). Para além deste mixosporídio, foram descritos outros agentes igualmente nefastos para os seus hospedeiros: O malacosporo, *Tetracapsuloides brysalmonae* também conhecido por PKX, provoca a doença renal proliferativa (PKD) no salmão do Pacífico (Kent *et al.* 2001), principalmente em espécimes em aquacultura. Este hospedeiro é, igualmente, susceptível a infecções pelo agente *Ceratomyxa shasta* (Bartholomew *et al.* 1997) e por espécies pertencentes ao género *Parvicapsula* (Feist 2008).

Como exemplos de outras patologias, podem referir-se enterites devido à presença de *Enteromyxum leei* no intestino (Diamant 1992), castração parasítica por *Sphaerospora testicularis* (Sitjà-Bobadilla & Alvarez-Pellitero 1990) no robalo do Mediterrâneo e mixosporidioses por *Henneguya exilis* (Current & Janovy 1977) e *H. ictaluri* (Pote *et al.* 2000) em peixe-gato de aquacultura, originando a doença da brânquia proliferativa.

Sabe-se que várias espécies de interesse comercial, tais como o arenque do Atlântico, o espadarte, a pescada do Pacífico e a cavala, estão parasitadas ao nível do tecido muscular esquelético por espécies pertencentes ao género *Kudoa* (Moran *et al.* (1999). A presença deste grupo de parasitas está associada, frequentemente, à liquefacção pós-morte das fibras musculares causando um aspecto leitoso dos músculos, e consequentemente inviabilizando a sua comercialização (Feist 2008, Lom & Dyková 1992b). Existem também casos de infecções das gónadas dos peixes por *Kudoa* spp. A espécie *K. ovivora* ocorre ao nível dos ovócitos implicando neste caso uma redução do crescimento e da fecundidade dos animais (Swearer & Robertson 1999).

1.3.7. Estudos moleculares e filogenéticos

Nos estudos iniciais, as sequências de DNA eram usadas em análises filogenéticas com o objectivo de investigar as relações do filo Myxozoa com os outros filos aparentemente mais afins (Smothers 1994, Siddall *et al.* 1995). A utilização de sequências de DNA em estudos comparativos das diferentes espécies de mixosporídios tem demonstrado ser fundamental na classificação de novas espécies, nomeadamente em géneros detentores de centenas de espécies descritas com morfologia muito semelhante (Andree *et al.* 1999,

Hervio *et al.* 1997, Kent *et al.* 2001, Fiala 2006). Para o efeito, recorre-se à informação genética contida no SSU rDNA. Existem também dados referentes à sequenciação do ITS, bem como do gene LSU rDNA, situação análoga ao que acontece com outros organismos, devido ao facto de serem genes altamente conservados, susceptíveis de serem alinhados com sequências afins (Hillis & Dixon 1991). Outro ponto favorável para a escolha destes genes prende-se com o facto de cada célula conter múltiplas cópias dos genes que transcrevem para os rRNAs.

Presentemente existem, pelo menos, 141 sequências referentes ao SSU rDNA do grupo dos mixosporídios, pertencentes a 19 géneros (Fiala 2006). Ao nível dos taxa superior ao género, as análises moleculares têm-se revelado claramente consistentes com a taxonomia tradicional baseada na morfologia ultrastrutural. Contrariamente ao que verifica entre os géneros e dentro de um mesmo género, a análise de sequências de SSU rDNA de tetracapsuloides (Classe Malacosporea) revela bem as diferenças morfológicas, visto que o grupo diverge bastante dos restantes mixosporídios, encontrando-se posicionado na raiz de todos os cladogramas (Canning *et al.* 1996, 2000, 2002, Okamura *et al.* 2002).

Há muito que as análises filogenéticas prevêm uma nítida separação dos mixosporídios em 2 grupos, em função do habitat, isto é, os de água doce e salgada. Para a grande maioria das espécies, este é o principal critério taxonómico, no entanto existem várias excepções (Kent *et al.* 2001) nas quais estão incluídos dois mixosporídios parasitas de peixes cartilagíneos marinhos, *Chloromyxum leydigi* (Fiala & Dyková 2004) e *C. riorajum* (Azevedo *et al.* 2009a). A posição basal destas duas espécies de água doce é explicada pelo tamanho da sequência do gene SSU rDNA. Para a maioria das espécies marinhas, o comprimento do gene SSU rDNA revelou ser mais curto em relação às espécies de água doce, devido ao facto de lhes faltar as sequências nucleotídicas correspondentes à região V7 do gene. Curiosamente, o comprimento intermédio do gene SSU rDNA destas duas espécies de *Chloromyxum* reflecte bem a posição filogenética ocupada dentro do grupo dos mixosporídios de água doce (Fiala & Dyková 2004, Fiala 2006, Holzer *et al.* 2006b, Azevedo *et al.* 2009a). Segundo Fiala (2006), além das 2 principais linhagens de mixosporídios (água doce e salgada), existe um terceiro grupo formado pelas espécies *Sphaerospora truttae*, *S. elegans* e *Leptotheca ramae* correspondendo a organismos com sequências do gene SSU rDNA muito longas, na ordem dos 2500 nucleótidos.

À medida que novas sequências nucleotídicas vão sendo incorporadas aos cladogramas já existentes, deparamo-nos com um crescente número de excepções, isto é, de espécies marinhas agrupadas filogeneticamente com as de água doce e vice-versa, situação que, frequentemente, se verifica nos géneros mais representativos da fauna dos

mixosporídios (Kent *et al.* 2001, Fiala 2006). Bahri e colaboradores (2003) descreveram 6 *Myxobolus* spp. marinhas, de mugilídeos, filogeneticamente posicionados no grupo correspondente ao habitat de água doce, sucedendo o mesmo a 2 *Sphaerospora* spp. marinhas parasitas da bexiga biliar. Entre as várias explicações possíveis para este fenómeno, inclui-se o facto de alguns hospedeiros poderem co-habitar, simultaneamente, em ambientes marinhos e de água doce (Fiala 2006). O contrário também se verifica e, como exemplo disso, *Ceratomyxa shasta*, parasita de salmonídeos de água doce, é uma das poucas espécies para qual se conhece o ciclo de vida (Kent *et al.* 2001). Uma possível explicação prende-se com o facto de ocorrerem em poliquetas marinhas na fase correspondente à formação dos actinosporos, agrupando-se assim, com os mixosporídios marinhos, no entanto, constituindo uma linhagem independente (Bartholomew *et al.* 1997, Køie *et al.* 2004).

Através da análise do gene SSU rDNA pode-se, igualmente, constatar que o local de infecção é um factor importante que influi na evolução dos mixosporídios. Andree e colaboradores (1999) constataram haver afinidades entre as 10 espécies de *Myxobolus* analisadas em função do local da infecção. A análise molecular de 5 espécies, de géneros diferentes, todas de água doce e a parasitar a bexiga urinária, demonstrou estarem filogeneticamente muito próximas (Holzer *et al.* 2004). Whipps e colaboradores (2004), provaram que o local de infecção é um critério muito importante no estabelecimento das relações dentro do grupo dos Multivalvulida, nomeadamente entre as espécies de *Kudoa*. Sabe-se, também, que os mixosporídios, que ocorrem na vesícula biliar de peixes marinhos e de água doce, estão filogeneticamente relacionados em ambos os cladogramas, onde estão incluídos representantes, de forma muito similar, pertencentes aos géneros *Myxidium* e *Zschokkella* (Fiala 2006). São igualmente notórias as relações monofiléticas dos mixosporídios entéricos do género *Enteromyxum* (Palenzuela *et al.* 2002, Yanagida *et al.* 2004), bem como entre as espécies que ocorrem no sistema urinário pertencentes aos géneros *Parvicapsula* (Køie *et al.* 2007a) e *Gadimyxa* (Køie *et al.* 2007b). Entre os géneros de mixosporídios que necessitam de serem revistos, dadas as discrepâncias classificativas morfológicas e moleculares, incluem-se alguns géneros coelozóicos: *Myxidium*, *Zschokkella* e *Ceratomyxa* (Fiala, 2006).

Em regra, as espécies coelozóicas parasitam a vesícula biliar e ocupam em ambos os cladogramas uma posição basal relativamente às histozóicas, levando a pressupor que estas últimas evoluíram a partir das primeiras (Kent *et al.* 2001, Fiala 2006). Entre os géneros ditos quase exclusivamente histozóicos, incluem-se 3 dos mais representativos dos Myxozoa: *Myxobolus*, *Henneguya* e *Kudoa*. As espécies de *Myxobolus* parasitam,

preferencialmente, as brânquias, mas também outros órgãos. Segundo Fiala (2006), das cerca de 50 espécies sequenciadas (aproximadamente um 1/10 do total), somente algumas formam um grupo monofilético, tendo como local preferencial de infecção as brânquias. Recentemente, evidências filogenéticas do grupo Multivalvulida colocam em causa a monofilia do género *Kudoa*, caracterizado por ter 4 valvas e 4 CPs e por ocorrer predominantemente no tecido muscular de peixes marinhos. A análise do gene SSU rDNA de parasitas com uma forma tão distinta, isto é, com 5 valvas e cápsulas (*Pentacapsula* spp.), 6 (*Hexacapsula* spp.) e 7 (*Septemcapsula* spp.), revelou uma grande proximidade evolutiva com o género *Kudoa*. Whipps e colaboradores (2004) propuseram uma nova descrição do género *Kudoa*, de forma a albergar estas espécies, enquanto que Lom e Dyková (2006), apesar de considerarem ser pertinente esta fusão, optaram por conservar os referidos géneros como distintos.

À partida, a forma dos esporos, de acordo com o modelo cladístico, deveria predizer que as espécies filogeneticamente relacionadas pertencem ao mesmo género. Tal pressuposto não se verifica nos géneros *Mixidium*, *Sphaerospora* e *Zschokkella*, visto que parasitam peixes de água doce e salgada, formando, por sua vez, vários subgrupos com outros parasitas. Uma das questões taxonómicas para a qual ainda não existe uma resposta (Kunz 2002), prende-se com a dificuldade em se definir quais as diferenças qualitativas e / ou quantitativas entre duas sequências nucleotídicas, seja dos genes para os rRNAs ou de outros, que confirmem diversidade intragenómica significativa para que possam ser reconhecidas como pertencentes a indivíduos distintos (Buckler *et al.* 1997). Designar espécies com base unicamente nos agrupamentos filogenéticos e nas distâncias genéticas é problemático. Em populações de *Mixidium lieberkuehni*, verificou-se que distavam entre si na ordem dos 2,6% (Schlegel *et al.* 1996), o mesmo sucedendo a *Zschokkella nova* proveniente de diferentes áreas geográficas (Fiala 2006). Em contrapartida, existem espécies de morfologia muito distinta, *Kudoa thyrsites* e *K. minithyrsites*, que distam entre si 1,5% (Whipps *et al.* 2003a), ou somente 0,1%, como sucede com as espécies *Myxobolus pellicides* e *M. pendula* (Kent *et al.* 2001).

É bem evidente que vários géneros são parafiléticos ou polifiléticos, tendo em conta que mixosporídios com a mesma morfologia se agrupam em linhagens bem distintas (Kent *et al.* 2001, Fiala 2006). No entanto, é indiscutível que existe uma relação directa entre o comprimento do gene SSU rDNA, o habitat, área geográfica e o local de desenvolvimento dos mixosporos, colocando em causa, muitas vezes, os relacionamentos filogenéticos baseados unicamente na morfologia do espora.

1. 4. Microsporidioses e mixosporidioses da ictiofauna portuguesa e brasileira

No âmbito desta tese foi elaborada uma listagem de todas as microsporidioses da ictiofauna (**Tabela 2**) descritas até à data. Das 109 espécies descritas em peixes de água doce e salgada, apenas 12 ocorrem em hospedeiros da fauna Ibérica e brasileira (**Tabela 3**). Entre as espécies descritas incluem-se os géneros *Amazonspora*, *Ichthyosporidium*, *Loma*, *Glugea*, *Microgemma*, *Pleistophora*, *Potaspota* e *Tetramicra*. Algumas espécies foram incluídas no género colectivo (*Microsporidium*). Dada a proximidade geográfica, incluíram-se todas as ocorrências na Península Ibérica quer para as microsporidioses como para as mixosporidioses.

Na **Tabela 4** encontram-se listadas todas as mixosporidioses diagnosticadas na ictiofauna portuguesa, bem como as provenientes da fauna espanhola tendo em conta a proximidade geográfica. A maioria dos trabalhos referem-se a mixosporídios que parasitam espécies nativas marinhas, capturadas em águas mediterrânicas, bem como espécies introduzidas em piscicultura intensiva na região de Valência. Na Península Ibérica existem 3 referências de actinosporídios: *Aurantiactinomyxon* em specimens de oligoquetas *Brachiura sowerbyi* capturadas em águas espanholas (Székely *et al.* 2000) e *Synactinomyxon* em oligoquetas *Tubifex tubifex* capturadas no rio Sousa em Portugal (Székely *et al.* 2005). Infecções experimentais da oligoqueta *Tubifex tubifex*, com esporos das espécies *Myxobolus bramae* e *M. pseudodispar*, foram efectuadas com êxito, permitindo assim a caracterização dos estádios correspondentes dos triactinosporos (Álvarez-Pellitero *et al.* 2002).

Relativamente à fauna brasileira, foram, até ao momento, descritos 12 géneros de mixosporídios num total de 83 espécies, predominando em larga escala espécies de habitat de água doce (**Tabelas 5**). Das cerca 744 *Myxobolus* spp. descritas (Eiras *et al.* 2005), apenas 26 espécies têm como hospedeiro espécies brasileiras. Sabe-se que este número está muito aquém do que seria esperado, atendendo que os peixes brasileiros representam cerca de 24% de todas as espécies existentes (Cellere *et al.* 2002).

Na fauna brasileira foram também identificados 3 mixosporídios em anfíbios: *Leptotheca chagasi*, *Myxidium immersum* e *Myxidium* sp. (Gioia & Cordeiro 1996). Békési e colaboradores (2002) efectuaram o primeiro estudo de actinosporos na fauna brasileira, tendo sido identificado, positivamente, o actinosporo do tipo – Raabeia, em oligoquetas pertencentes à família Ocnerodrilidae.

Tabela 3 - Microsporídios diagnosticados na ictiofauna da Península Ibérica e do Brasil

ESPÉCIES	HOSPEDEIROS	LOCAIS DE INFECÇÃO	HABITAT	REGIÕES	REFERÊNCIAS BIBLIOGRÁFICAS
<i>Amazonspora hassar</i>	<i>Hassar orentis</i>	Brânquia	Água doce	Pará, Brasil	Azevedo & Matos 2003b
<i>Ichthyosporidium giganteum</i>	<i>Ctenolabrus rupestris</i>	T. conjuntivo subcutâneo, tecido adiposo,	Marinho	Apúlia, Portugal	Casal & Azevedo 1995
<i>Loma dimorpha</i>	<i>Gobius niger</i> , <i>Lipophrys pholis</i>	Tecido conjuntivo do intestino	Marinho	Galiza, Espanha	Leiro <i>et al.</i> 1994, Arias <i>et al.</i> 1999
<i>Loma myrophis</i>	<i>Myrophis platyrhynchus</i>	Tecido sub-epitelial do intestino	Água doce	Pará, Brasil	Azevedo & Matos 2002b
<i>Loma psittaca</i>	<i>Colomesus psittacus</i>	Mucosa intestinal	Água doce	Pará, Brasil	Casal <i>et al.</i> 2009b
<i>Microgemma caulleryi</i>	<i>Hyperoplus lanceolatus</i>	Fígado	Marinho	Galiza, Espanha	Leiro <i>et al.</i> 1999
<i>Microgemma ovoidea</i>	<i>Cepola macrophthalma</i>	Fígado	Marinho	Catalunha, Espanha	Amigó <i>et al.</i> 1996
<i>Pleistophora finisterrensis</i>	<i>Micromesistius poutassou</i>	Tecido muscular	Marinho	Galiza, Espanha	Leiro <i>et al.</i> 1996
<i>Potaspora morhaphis</i>	<i>Potamorhaphis guianensis</i>	Cavidade celômica perto da região anal	Água doce	Pará, Brasil	Casal <i>et al.</i> 2008b
<i>Spraguea lophii</i>	<i>Lophius piscatorius</i> , <i>L. budegassa</i>	Células ganglionares do sistema nervoso central	Marinho	Catalunha, Espanha,	Amigó <i>et al.</i> 1995
<i>Tetramicra brevifilum</i>	<i>Scophthalmus maximus</i>	Tecido connectivo da musculatura esquelética	Marinho	Galiza, Espanha	Estevez <i>et al.</i> 1992
	<i>Lophius budegassa</i>			Catalunha, Espanha	Maíllo <i>et al.</i> 1998
<i>Microsporidium brevirostris</i>	<i>Brachyhypopomus brevirostris</i>	Tecido muscular da cavidade abdominal	Água doce	Pará, Brasil	Matos & Azevedo 2004
<i>Microsporidium</i> sp.	<i>Lophius gastrophissus</i>	Musculatura abdominal interna perto do gânglio dorsal	Marinho	Rio de Janeiro, Brasil	Jakowska 1964

Tabela 4 - Mixosporídios diagnosticados na ictiofauna portuguesa e espanhola

ESPÉCIES	HOSPEDEIROS	LOCAIS DE INFECÇÃO	HABITAT	REGIÕES	REFERÊNCIAS BIBLIOGRÁFICAS
<i>Alataspora budegassai</i>	<i>Lophius budegassa</i>	Vesícula biliar	Marinho	Costa Algarvia	Afonso-Dias <i>et al.</i> 2007
<i>Ceratomyxa appendiculata</i>	<i>Lophius budegassa</i>	Vesícula biliar	Marinho	Mditerrâneo	Maillo-Bellon & Gracia-Royo 2007
<i>Ceratomyxa diplodae</i>	<i>Dicentrarchus labrax</i>	Vesícula biliar	Marinho	Mediterrâneo	Sitjà-Bobadilla & Álvarez-Pellitero 1993a
<i>Ceratomyxa labracis</i>	<i>Dicentrarchus labrax</i>	Vesícula biliar	Marinho	Mediterrâneo	Sitjà-Bobadilla & Álvarez-Pellitero 1993a
<i>Ceratomyxa sparusaurati</i>	<i>Sparus aurata</i>	Vesícula biliar	Marinho	Mediterrâneo	Sitjà-Bobadilla <i>et al.</i> 1995; Costa <i>et al.</i> 1998
<i>Ceratomyxa tenuispora</i>	<i>Aphanopus carbo</i>	Vesícula biliar	Marinho	Ilha da Madeira	Casal <i>et al.</i> 2007
<i>Ceratomyxa</i> sp.	<i>Dentex dentex</i>	Vesícula biliar	Marinho	Mediterrâneo	Company <i>et al.</i> 1999
<i>Enteromyxum scopthalmi</i>	<i>Scophthalmus maximus</i>	Intestino	Marinho	Mediterrâneo	Palenzuela <i>et al.</i> 2002
<i>Enteromyxum leei</i>	<i>Coris julis</i> , <i>Symphodus tinca</i> , <i>S. ocellatus</i> , <i>S. mediterraneus</i> , <i>S. rostratus</i> , <i>S. roissali</i> , <i>S. cinereus</i> , <i>S. melops</i> , <i>Thalassoma pavo</i> , <i>Labrus viridis</i> , <i>L. merula</i> , <i>L. bergylta</i> , <i>Xyrichtys novacula</i> , <i>Spicara maena</i> , <i>Sparus aurata</i> , <i>Diplodus sargus</i> , <i>D. vulgaris</i> , <i>Mola mola</i> , <i>Mullus surmuletus</i> , <i>Halobatrachus didactylus</i> , <i>Chromis chromis</i> , <i>Lipophrys pavo</i> , <i>Gobius niger</i> , <i>Scorpaena porcus</i>	Intestino	Marinho	Mediterrâneo	Padrós <i>et al.</i> 2001
<i>Kudoa trifolia</i>	<i>Liza aurata</i> , <i>L. ramada</i>	Baço, v. biliar, intest., brânquias	Marinho	Mediterrâneo	Holzer <i>et al.</i> 2006a
<i>Kudoa unicapsula</i>	<i>Liza aurata</i> , <i>L. ramada</i>	Intestino, cecos pilóricos	Marinho	Mediterrâneo	Yurakhno <i>et al.</i> 2007
<i>Kudoa</i> sp.	<i>Trachurus trachurus</i>	Músculo	Marinho	Norte, Portugal	Cruz <i>et al.</i> 2003
<i>Leptotheca sparidarum</i>	<i>Dentex dentex</i> , <i>Sparus aurata</i>	Rim	Marinho	Mediterrâneo	Sitjà-Bobadilla & Álvarez-Pellitero 2001
<i>Myxidium giardi</i>	<i>Anguilla anguilla</i>	Brânquias	Estuarino	Norte de Portugal; Galiza, Espanha	Azevedo <i>et al.</i> 1989; Aguilar <i>et al.</i> 2005
<i>Myxidium rhodei</i>	<i>Leuciscus cephalus cabeda</i> , <i>Chondrostoma polylepis</i>	Rim	Água doce	Noroeste, Espanha e no Norte, Portugal	Álvarez-Pellitero 1989; Saraiva <i>et al.</i> 2000
<i>Myxobolus</i> sp.	<i>Seriola dumerili</i>	Cérebro	Marinho	Baleares, Espanha	Grau <i>et al.</i> 1999
<i>Myxobolus pseudodispar</i>	<i>Chondrostoma polylepis</i> , <i>Leuciscus cephalus</i>	Músculo, rim, ductos urinários, fígado baço, brânquias		Portugal	Cruz <i>et al.</i> 2000
<i>Myxobolus portucalensis</i>	<i>Anguilla anguilla</i>	Barbatanas	Água doce	Norte, Portugal	Saraiva & Molnár 1990
<i>Pentacapsula cutanea</i>	<i>Serranus atricauda</i>	Subcutâneo	Marinho	Canárias	Cuyas <i>et al.</i> 2004
<i>Polysporoplasma sparis</i>	<i>Sparus aurata</i>	Rim	Marinho	Mediterrâneo	Sitjà-Bobadilla & Álvarez-Pellitero 1995
<i>Polysporoplasma mugilis</i>	<i>Liza aurata</i>	Rim	Marinho	Mediterrâneo	Sitjà-Bobadilla & Álvarez-Pellitero 1995
<i>Sphaeromyxa balbiani</i>	<i>Cepola macrophthalma</i>	Vesícula biliar	Marinho	Catalunha, Espanha	Gracia <i>et al.</i> 1997
<i>Sphaerospora dicentrarchi</i>	<i>Dicentrarchus labrax</i>		Marinho	Mediterrâneo	Sitjà-Bobadilla & Álvarez-Pellitero 1992
<i>Sphaerospora testicularis</i>	<i>Dicentrarchus labrax</i>	Testículo	Marinho	Mediterrâneo	Sitjà-Bobadilla & Álvarez-Pellitero 1990
<i>Unicapsula pflugfelderii</i>	<i>Lithognathus mormyrus</i> , <i>Spicara smar</i>	Tecido muscular	Marinho	Mediterrâneo	Alama-Bermejo <i>et al.</i> 2009
<i>Zschokkella mugilis</i>	<i>Mugil capito</i> , <i>Mugil cephalus</i> , <i>Liza saliens</i> ,	Vesícula biliar	Marinho	Mediterrâneo	Sitjà-Bobadilla & Álvarez-Pellitero 1993b
PKX - Myxozoa desconhecido	Salmonídeos - doença proliferativa do rim	Rim	Água doce	Aragão, Espanha	Peribanez <i>et al.</i> 1997

Tabela 5a - Mixosporídios diagnosticados na ictiofauna brasileira

ESPÉCIES	HOSPEDEIROS	LOCAIS DE INFECÇÃO	ESTADOS	HABITAT	REFERÊNCIAS BIBLIOGRÁFICAS
<i>Agarella gracilis</i>	<i>Lepidosiren paradoxa</i>	Testículo e rim	Pará	Água doce	Pinto 1928; Vita <i>et al.</i> 2004
<i>Ceratomyxa sphaerulosa</i>	<i>Sphyrna tudes</i>	Vesícula biliar	Rio de Janeiro	Marinho	Pinto 1928
<i>Ceratomyxa truncata</i>	?	Vesícula biliar	Brasil	-	Pinto 1928
<i>Ceratomyxa curvata</i>	<i>Odontaspis americanus</i>	Vesícula biliar	Rio de Janeiro	Marinho	Pinto 1928
<i>Ceratomyxa hippocampi</i>	<i>Hippocampus punctulatus</i>	Vesícula biliar	Brasil	Marinho	Pinto 1928
<i>Chloromyxum leydigi</i>	<i>Scoliodon terra-novae</i>	Vesícula biliar	Rio de Janeiro	Marinho	Pinto 1928
<i>Chloromyxum menticirrho</i>	<i>Menticirrhus americanus</i>	Vesícula urinária	Florianópolis	Marinho	Casal <i>et al.</i> 2009a
<i>Chloromyxum riorajum</i>	<i>Rioraja agassizii</i>	Vesícula biliar	Florianópolis	Marinho	Azevedo <i>et al.</i> 2009a
<i>Chloromyxum sphyrnae</i>	<i>Sphyrna tibura</i>	Vesícula biliar	Rio de Janeiro	Marinho	Cunha & Fonseca, 1918
<i>Coccomyxa claviforme</i>	<i>Chilomycterus spinosus</i>	Vesícula biliar	Brasil		Pinto 1928
<i>Kudoa aequidens</i>	<i>Aequidens plagiozonatus</i>	Musculatura sub-opercular	Pará	Água doce	Casal <i>et al.</i> 2008a
<i>Henneguya</i> spp. (Consultar a tabela 5b)					
<i>Myxidium striatum</i>	<i>Menticirrhus americanus</i>	Vesícula biliar	Rio de Janeiro	Marinho	Jayasri & Hoffman 1982
<i>Myxidium fonsecai</i>	<i>Carapus fasciatus</i>	Vesícula biliar	Mato Grosso	Água doce	Jayasri & Hoffman 1982
<i>Myxidium cruzi</i>	<i>Chalcinus nematurus</i>	Vesícula biliar	Mato Grosso	Água doce	Jayasri & Hoffman 1982
<i>Myxidium gurgeli</i>	<i>Acestrorhamphus</i> sp.	Vesícula biliar	São Paulo	Água doce	Jayasri & Hoffman 1982
<i>Myxidium cholecysticum</i>	<i>Astyanax scabripinnis</i>	Vesícula biliar	São Paulo	Água doce	Cordeiro & Gioia 1990
<i>Myxobolus</i> spp. (Consultar a tabela 5c)					
<i>Sphaeromyxa balbiani</i>	<i>Scorpena plumieri</i>	Vesícula biliar	Brasil	Marinho	Pinto 1928
<i>Tetrauronema desaequalis</i>	<i>Hoplias malabaricus</i>	Base da barbatana ventral	Pará	Água doce	Azevedo & Matos 1996b
<i>Triangulamyxa amazonica</i>	<i>Sphoeroides testudineus</i>	Intestino	Pará	Água doce	Azevedo <i>et al.</i> 2005

Tabela 5b - Comparação morfológica dos esporos de diferentes espécies de *Henneguya* diagnosticadas em peixes da fauna brasileira.

Espécies	CT	CC	LC	CCa	CCP	LCP	NFP	VI	R	Hospedeiros	Habitat	Locais de infecção	Estados	Referências Bibliográficas
<i>H. occulta</i>	36–46	16	8	20		8	–	–	–	<i>Loricaila</i> sp.	D	Brânquias	Rio de Janeiro	Nemeczek 1926
<i>H. leporini</i>	28–33	13–15	5	15–18		5–8	–	–	–	<i>Leporinus mormyrops</i>	D	Ductos urinários	Minas Gerais	Nemeczek 1926
<i>H. wenyoni</i>	21	11–12	5,2	10,8	3,7		1,5	–	–	<i>Tetragonopterus</i> sp.	D	Brânquias	São Paulo	Pinto 1928
<i>H. travassosi</i>	27,3	10,6	4,3	16,7		3,6	–	–	–	<i>Astyanax fasciatus</i>	D	D	São Paulo	Guimarães & Bergamin 1933
<i>H. santae</i>	21,0	9,6	5,3	11,2		2,9	–	Sim	–	<i>Tetragonopterus santae</i>	D	Brânquias	São Paulo	Guimarães & Bergamin 1934
<i>H. visceralis</i>	22–24	11–12	5–6,5	11–12	6,5–8		2	–	–	D	D	Rim, fígado, coração	Brasil	Jakowska & Nigrelli 1953
<i>H. electrica</i>	35–39	11–13	6–8	24–27	5–7		2	–	–	<i>Electrophorus electricus</i>	D	Órgãos eléctricos	Brasil	Jakowska & Nigrelli 1953
<i>H. pisciforme</i>	20,4	–	6,1	10,7	4,3		1,7	–	Sim	<i>Hyphessobrycon anisitsi</i>	D	Brânquias	São Paulo	Cordeiro <i>et al.</i> 1984
<i>H. theca</i>	48,0	24,8	3,5	23,2	11,1		1,4	–	Sim	<i>Eigemannia virescens</i>	D	Cérebro	Brasil	Kent & Hoffman 1984
<i>H. intracornea</i>	42,4	–	6,7	24,3	8,6		2,4	–	Sim	<i>Astyanax scabripinnis</i>	D	Córnea	São Paulo	Gioia <i>et al.</i> 1986
<i>H. hoimba</i>	24,7	–	7,5	–	4,4		1,9	–	Sim	<i>Astyanax fasciatus</i>	D	Brânquias	São Paulo	Cordeiro & Gioia 1987
<i>H. artigasi</i>	16,4	–	4,4	–	3,3		1,5	–	–	<i>Astyanax scabripinnis</i>	D	Brânquias	São Paulo	Gioia & Cordeiro 1987
<i>H. amazonica</i>	59,3	13,9	5,7	45,4	3,3		1,5	6	–	<i>Crenicichla lepidota</i>	D	Brânquias	Pará	Rocha <i>et al.</i> 1992
<i>H. adherens</i>	32,3	12,4	5,8	20,5	3,1		1,2	3–4	–	<i>Acestrorhynchus falcatus</i>	D	Brânquias	Pará	Azevedo & Matos 1995
<i>H. malabarica</i>	28,3	12,6	4,8	17,1	3,7		1,8	6–7	–	<i>Hoplias malabaricus</i>	D	Brânquias	Pará	Azevedo & Matos 1996a
<i>H. piaractus</i>	52,5	12,7	3,6	41,2	6,7		1,2	8–9	Sim	<i>Piaractus mesopotamicus</i>	D	Brânquias	São Paulo	Martins & Souza 1997
<i>H. testicularis</i>	27,5	14,0	6,5	13,5	9,0		2,0	12–13	–	<i>Moenkhausia oligolepis</i>	D	Testículo	Pará	Azevedo <i>et al.</i> 1997
<i>H. striolata</i>	42,2	15,8	5,3	25,9	6,8		1,2	13–14	Não	<i>Serrasalmus striolatus</i>	D	Brânquias	Pará	Casal <i>et al.</i> 1997
<i>H. leporinicola</i>	–	5,5–8,7	3,6–4,9	12,9–32,2	2,0–3,6		1,2–2,0	–	–	<i>Leporinus macrocephalus</i>	D	F. branquiais secund.	São Paulo	Martins <i>et al.</i> 1999
<i>H. curimata</i>	35,4	16,6	6,2	19,1	6,5		1,2	10–11	–	<i>Curimata inornata</i>	D	Rim	Pará	Azevedo & Matos 2002b
<i>H. astyanax</i>	47,8	15,2	5,7	32,6	5,0		1,5	8–9	–	<i>Astyanax keithi</i>	D	Brânquias	Pará	Vita <i>et al.</i> 2003
<i>H. chydadea</i>	17,6–20	8,8–11,2	3,2–5,6	8–9,6	3,2–4,4		1,2–1,6	9–10	Não	<i>Astyanax altiparanae</i>	D	Brânquias	São Paulo	Barassa <i>et al.</i> 2003b
<i>H. curvata</i>	41,7	16,4	4,7	25,3	7,8		1,4	10–11	–	<i>Serrasalmus spilopleura</i>	D	Brânquias	São Paulo	Barassa <i>et al.</i> 2003a
<i>H. friderici</i>	28,7–39,3	9,6–11,8	4,8–6,6	19,1–28,7	4,2–5,9		1,5–2,6	7–8	Sim	<i>Leporinus friderici</i>	D	Vários órgãos	Pará	Casal <i>et al.</i> 2003
<i>H. pilosa</i>	52,3–56,0	20,0–23,1	5,5–6,3	30,5–34,9	7,1–7,6		1–1,3	11–12	–	<i>Serrasalmus altuvei</i>	D	Brânquias	Pará	Azevedo & Matos 2003b
<i>H. schizodon</i>	27–30	12–14	3–4	15–17	5–6		1–1,5	8–10	–	<i>Schizodon fasciatum</i>	D	Rim	Amazonas	Eiras <i>et al.</i> 2004a
<i>H. paranaensis</i>	56–63	14–17	6–7	41–46	8–9 e 6–7		2	10–12	–	<i>Prochilodus lineatus</i>	D	F. branquiais secund.	Paraná	Eiras <i>et al.</i> 2004b
<i>H. caudalongula</i>	71	16,6	4,6	52,6	6,1		1,6	10–11	–	<i>Prochilodus lineatus</i>	D	Brânquias	São Paulo	Adriano <i>et al.</i> 2005a
<i>H. pellucida</i>	33,3	11,4	4,1	24,1	4,0		1,6	6–7	–	<i>Piaractus mesopotamicus</i>	D	Cavidade visceral		Adriano <i>et al.</i> 2005b
<i>H. rhamdia</i>	48,2–51,8	12,0–14,2	4,7–5,7	35,3–38,5	4,3–5,1		0,9–1,3	10–11	–	<i>Rhamdia quelen</i>	D	Lamelas branquiais	Pará	Matos <i>et al.</i> 2005
<i>H. garavelli</i>	41,2–51,5	12,0–14,4	3,9–4,1	31,4–35,6	4,8–6,0		1,0–1,5	8–9	Sim	<i>Cyphocharax nagelli</i>	D	Brânquias	São Paulo	Martins & Onaka 2006
<i>H. cyphocharax</i>	29,6–44,4	7,7–13,4	2,9–6,3	20,8–31,5	4,2–6,3 / 3,4–5,2		1,5–2,3 / 1,3–2,2	7–9	–	<i>Cyphocharax gilbert</i>	D	Brânquias	Rio de Janeiro	Abdallah <i>et al.</i> 2007
<i>H. guanduensis</i>	27,3–38,1	11,4–16,7	4,9–7,6	15,6–22,5	3,3–5,6 / 3,3–5,3		1,6–2,3 / 1,5–2,8	3–6	–	<i>Hoplosternum littorale</i>	D	Brânquias	Rio de Janeiro	Abdallah <i>et al.</i> 2007
<i>H. caudicula</i>	14–16	11–12	5–6	3,4	3–4		1,5	3	Sim	<i>Leporinus lacustris</i>	D	Brânquias	Paraná	Eiras <i>et al.</i> 2008
<i>H. arapaima</i>	48,4–53,1	13,5–15,2	5,1–6,1	38,0–41,2	6,3–6,8 / 6,2–6,6		1,4–1,6	5	–	<i>Arapaima gigas</i>	D	Vesícula biliar	Goias	Feijó <i>et al.</i> 2008
<i>H. rondoni</i>	16,9–18,1	6,8–7,3	3,0–3,9	10,3–11	2,2–2,7		0,8–0,9	6–7	Não	<i>Gymnorhamphichthys rondoni</i>	D	Sist. nervoso periférico	Pará	Azevedo <i>et al.</i> 2008
<i>H. corruscans</i>	25,0–29,0	13–15	5,0	12–15	6,8		2,0	5–6	Não	<i>Pseudoplatystoma corruscans</i>	D	Brânquias	Paraná	Eiras <i>et al.</i> 2009
<i>H. hemiodopsis</i>	18,8–20,6	10,3–11,3	2,9–3,7	8,1–9,3	3,2–3,8		0,8–1,2	5–6	Não	<i>Hemiodopsis microlepes</i>	D	Brânquias	Piauí	Azevedo <i>et al.</i> 2009c

Abreviaturas: CT: comprimento total; CC: comprimento do corpo; LC: largura do corpo; CCa: Comprimento das caudas; CCP: comprimento da cápsula polar; LCP: largura da cápsula polar; NFP: número de voltas do filamento polar; VI: vacúolo iodínoro; R: + revestimento em torno das caudas; Habitat: água doce (D), marinho (M); -: sem dados. (medidas em µm)

Tabela 5c - Comparação morfológica dos esporos de diferentes espécies de *Myxobolus* diagnosticadas em peixes da fauna brasileira.

Espécies	TL	LC	CCP	LCP	NFP	VI	PI	R	Hospedeiros	Habitat	Locais de infecção	Estados	Referências Bibliográficas
<i>M. inaequalis</i>	5,2	3,3	Desiguais	–	–	–	–	–	<i>Pimelodus clarias</i>	D	Pele da cabeça	América do Sul	Pinto 1928
<i>M. lutzi</i>	10	7	–	–	–	–	–	–	<i>Girardinus januaris</i>	D	Testículo	Rio de Janeiro	Pinto 1928
<i>M. chondrophilus</i>	6	4,5	3	–	–	–	–	–	<i>Sardinella anchovia</i>	M	Brânquia	Rio de Janeiro	Nemeczek 1926
<i>M. associates</i>	15	10	7	–	–	–	–	–	<i>Leporinus mormyrops</i>	D	Rim	Minas Gerais	Nemeczek 1926
<i>M. pygocentris</i>	15-16	9-11	9-11	3-4	–	–	–	–	<i>Pygocentrus piraya</i>	D	Conteúdo intestinal	Mato Grosso	Penido 1927
<i>M. cunhai</i>	9-11	4-6	Desiguais	–	–	–	–	–	<i>Pygocentrus piraya</i>	D	Conteúdo intestinal	Mato Grosso	Penido 1927
<i>M. noguchii</i>	13,6	8,5	6,8	2,2	–	–	–	–	<i>Serrasalmus spilopleura</i>	D	Brânquias?	São Paulo	Pinto 1928
<i>M. stokesi</i>	8,5	5,3	3,4	1,7	–	–	–	–	<i>Pimelodella</i> sp.	D	Tec. subcutâneos	São Paulo	Pinto 1928
<i>M. kudoii</i>	8,5-8,9	6,5-7,3	3,5-4,2	1,3-2	–	–	–	–	<i>Nematognatha</i> sp.	D	Pele	São Paulo	Guimarães 1938
<i>M. serrasalmi</i>	12,5-18	7-10	6-9	2,5-4	–	–	–	–	<i>Serrasalmus rhombeus</i>	D	Baço, rim, fígado	Amazonas	Walliker 1969
	7-9,5	3,5-5	5-7,5	1-2									
<i>M. inaequus</i>	19,8	8,6	11,8	–	–	Sim	–	–	<i>Eigemannia virescens</i>	D	Cérebro	Brasil	Kent & Hoffman 1984
			4,8										
<i>M. colossomatis</i>	11,8	6,9	6,0	2,1	7-8	–	Sim	–	<i>Colossoma macropomum</i> , <i>Hybrid tambacu</i>	D	Tec. subcutâneos	Ceará São Paulo	Molnár & Békési 1993
<i>M. braziliensis</i>	10,2	5,3	5,3	1,4	9-11	Não	Não	–	<i>Bunocephalus coracoideus</i>	D	Brânquias	Pará	Casal et al. 1996
<i>M. macropasmodialis</i>	11,0	8,5	4,5	2,8	6		Sim	–	<i>Salminus maxillosus</i>	D	Cavidade abdominal	São Paulo	Molnár et al. 1998
<i>M. porofilus</i>	5,7	4,8	1,6	1,1	3			–	<i>Prochilodus lineatus</i>	D	Cavidade visceral	São Paulo	Adriano et al. 2002
<i>M. desaequalis</i>	18,3	11,2	11,2	4,9	11-12	Não	Não	–	<i>Apteronotus albifrons</i>	D	Brânquias	Pará	Azevedo et al. 2002
			4,6	2,8	4-5								
<i>M. maculatus</i>	21,0	8,9	12,7	3,2	14-15	Sim	Não	–	<i>Metynnis maculatus</i>	D	Rim	Pará	Casal et al. 2002
<i>M. absonus</i>	15,7	10,2	6,4	3,6	5		Sim	–	<i>Pimelodus maculatus</i>	D	Cavidade opercular	São Paulo	Cellere et al. 2002
			4,2	2,5	3								
<i>M. insignis</i>	14,5	11,3	7,6	4,2	6	Não	Sim		<i>Semaprochilodus insignis</i>	D	Brânquias	Manaus	Eiras et al. 2005
<i>M. testicularis</i>	8,6	7,2	3,5	1,7	5-6	Não	Não	Sim	<i>Hemiodopsis microlepis</i>	D	Testículo	Pará	Tajdari et al. 2005
<i>M. cuneus</i>	10,0	5,1	5,7	1,7	8-9	–	–	–	<i>Piaractus mesopotamicus</i>	D	Tecidos conectivos		Adriano et al. 2006
<i>M. metynnis</i>	13,1	7,8	5,2	2,3	8-9	Sim	Não	Sim	<i>Metynnis argenteus</i>	D	Tecidos subcutâneos	Pará	Casal et al. 2006
<i>M. peculiaris</i>	25,2	15,4	10,7	4,4	4-5				<i>Cyphocharax nagelli</i>	D	Brânquias	São Paulo	Martins & Onaka 2006
<i>M. platanus</i>	10,7	10,8	7,7	3,8	5-6	Não	Sim	Não	<i>Mugil platanus</i>	D	Baço	Rio Grande do Sul	Eiras et al. 2007
<i>M. cordeiroi</i>	10,9-11,3	7,1-7,5	5,3-5,4	1,4-1,5	5-6	–	–	–	<i>Zungaro jahu</i>	D	Vários órgãos	Pantanal	Adriano et al. 2009
<i>M. heckelii</i>	12,2-13,2	6,3-6,9	2,7-3,1	1,4-2,0	4-5	Sim	–	Sim	<i>Centromochlus heckelii</i>	D	Brânquias	Pará	Azevedo et al. 2009b

Abreviaturas: TL: comprimento total; LC: largura do corpo; CCP: comprimento da cápsula polar; LCP: largura da cápsula polar; NFP: número de voltas do filamento polar; VI: vacúolo iodíniforo; PI: processo intercapsular; R: revestimento; Habitat: água doce (D), marinho (M); –: sem dados. (medidas em µm)

1. 5. Referências Bibliográficas

- Abdallah, V.D., Azevedo, R.K., Luque, J.L. & Bomfim, T.C.B. (2007) Two new species of *Henneguya* Thélohan, 1892 (Myxozoa, Myxobolidae), parasitic on the gills of *Hoplosternum littorale* (Callichthyidae) and *Cyphocharax gilbert* (Curimatidae) from Guandu River, State of Rio de Janeiro, Brazil. *Parasitol. Latinoamer.* 62: 35-41.
- Abdel-Baki, A.A.S. (2007) *Chloromyxum alii* sp. n. (Myxozoa: Myxosporea) infecting the gallbladder of African butter catfish *Schilbe mystus* (Linnaeus, 1758) from the River Nile, Egypt: Light and scanning electron microscopy. *Acta Protozool.* 46: 263-267.
- Abdel-Ghaffar, F., Ali, M.A., Al Quraishy, S., Al Rasheid, K.A.S., Al Farraj, S., Abdel-Baki, A.S. & Bashtar, A.R. (2008a) Four new species of *Ceratomyxa* Thélohan 1892 (Myxozoa: Myxosporea: Ceratomyxidae) infecting the gallbladder of some Red Sea fishes. *Parasitol. Res.* 103: 559-565.
- Abdel-Ghaffar, F., El-Toukhy, A., Al-Quraishy, S., Al-Rasheid, K.A.S., Abdel-Baki, A.S., Hegazy, A. & Bashtar, A.R. (2008b) Five new myxosporean species (Myxozoa: Myxosporea) infecting the Nile Tilapia *Oreochromis niloticus* in Bahr Shebin, Nile Tributary, Nile Delta, Egypt. *Parasitol. Res.* 103: 1197-1205.
- Adlard, R.D., Bryant, M.S., Whipps, C.M. & Kent, M.L. (2005) Multivalvulid myxozoans from eastern Australia: Three new species of *kudoa* from scombrid and labrid fishes of the Great Barrier Reef, Queensland, Australia. *J. Parasitol.* 91: 1138-1142.
- Adriano, E.A., Arana, S., Ceccarelli, P.S. & Cordeiro, N.S. (2002) Light and scanning microscopy of *Myxobolus porofilus* sp. n. (Myxosporea: Myxobolidae) infecting the visceral cavity of *Prochilodus lineatus* (Pisces: Characiformes: Prochilodontidae) cultivated in Brazil. *Folia Parasitol.* 49: 259-262.
- Adriano, E.A., Arana, S. & Cordeiro, N.S. (2005a) Histopathology and ultrastructure of *Henneguya caudalongula* sp. n. infecting *Prochilodus lineatus* (Pisces: Prochilodontidae) cultivated in the state of São Paulo, Brazil. *Mem. Inst. Oswaldo Cruz* 100: 177-181.
- Adriano, E.A., Arana, S. & Cordeiro, N.S. (2005b) An ultrastructural and histopathological study of *Henneguya pellucida* n. sp. (Myxosporea: Myxobolidae) infecting *Piaractus mesopotamicus* (Characidae) cultivated in Brazil. *Parasite* 12: 221-227.
- Adriano, E.A., Arana, S. & Cordeiro, N.S. (2006) *Myxobolus cuneus* n. sp (Myxosporea) infecting the connective tissue of *Piaractus mesopotamicus* (Pisces: Characidae) in Brazil: Histopathology and ultrastructure. *Parasite* 13: 137-142.
- Adriano, E.A., Arana, S., Alves, A.L., Silva, M.R.M. & Ceccarelli, P.S. (2009) *Myxobolus cordeiroi* n. sp, a parasite of *Zungaro jahu* (Siluriformes: Pimelodiade) from Brazilian Pantanal: morphology, phylogeny and histopathology. *Vet. Parasitol.* 162: 221-229.
- Afonso-Dias, I., Kalavati, C., Mackenzie, K. & Mackenzie, K.S. (2007) Three new species of Myxosporea (Bivalvulida: Ceratomyxidae: Alatasporidae) from the gall bladders of anglerfishes *Lophius* spp. (Teleostei: Lophiidae) in the Northeast Atlantic Ocean. *Zootaxa* 1466: 35-46.
- Aguilar, A., Alvarez, M.F., Leiro, J.M. & Sanmartin, M.L. (2005) Parasite populations of the European eel (*Anguilla anguilla* L.) in the Rivers Ulla and Tea (Galicia, northwest Spain). *Aquaculture* 249: 85-94.
- Alama-Bermejo, G., Cuadrado, M., Raga, J.A. & Holzer, A.S. (2009) Morphological and molecular redescription of the myxozoan *Unicapsula pflugfelderi* Schubert, Sprague & Reinboth, 1975 from two

- teleost hosts in the Mediterranean. A review of the genus *Unicapsula* Davis, 1924. *J. Fish Dis.* 32: 335-350.
- Ali, M.A., Abdel-Baki, A.S., Sakran, T., Entzeroth, R. & Abdel-Ghaffar, F. (2007) *Myxobolus lubati* n. sp. (Myxosporea: Myxobolidae), a new parasite of haffara seabream *Rhabdosargus haffara* (Forsskal, 1775), Red Sea, Egypt: a light and transmission electron microscopy. *Parasitol. Res.* 100: 819-827.
- Al-Quraishy, S., Koura, E., Abdel-Baki, A.S., Bashtar, A.R., El Deed, N., Al-Rasheid, K.A.S. & Abdel-Ghaffar, F. (2008) Light and electron microscopic studies on *Kudoa pagrusi* sp. n. (Myxosporea: Multivalvulida) infecting the heart of sea bream *Pagrus pagrus* (L.) from the Red Sea. *Parasitol. Res.* 102: 205-209.
- Álvarez-Pellitero, P. (1989) *Myxidium rhodei* (Protozoa, Myxozoa, Myxosporea) in cyprinid fish from NW Spain. *Dis. Aquat. Org.* 7: 13-16.
- Álvarez-Pellitero, P., Molnár, K., Sitjà-Bobadilla, A. & Székely, C. (2002) Comparative ultrastructure of the actinosporean stages of *Myxoholus bramae* and *M. pseadodispar* (Myxozoa). *Parasitol. Res.* 88:198-207.
- Amigó, J. M. (1995) Estudi de microsporidiosis en peixos d'interès comercial. *Ph. D. Thesis*, Universitat de Barcelona, Espanya.
- Amigó, J.M., Salvadó, H., Gracia, M.P. & Vivarès, C.P. (1996) Ultrastructure and development of *Microsporidium ovoideum* (Thélohan, 1895) Sprague, 1977, a microsporidian parasite of the red band fish (*Cepola macrophthalmia* L.) – redescription of the organism and reassignment to the genus *Microgemma*, Ralphs & Matthees 1986. *Eur. J. Protistol.* 32: 532-538.
- Anderson, C.L., Canning, E.U. & Okamura, B. (1998) A triploblast origin for Myxozoa? *Nature* 392: 346.
- Anderson, C.L., Canning, E.U. & Okamura, B. (1999) Molecular data implicate bryozoans as hosts for PKX (phylum Myxozoa) and identify a clade of bryozoan parasites within the Myxozoa. *Parasitology* 119: 555–561.
- Anderson, C.L., Canning, E.U., Schaefer, S.M., Yoyama, H. & Okamura, B. (2000) Molecular confirmation of the life cycle of *Thelohanellus hovorkai* Achmerov, 1960 (Myxozoa: Myxosporea). *Bull. Eur. Ass. Fish Pathol.* 20: 111–115.
- Andree, K.B., Gresoviac, S.J. & Hedrick, R.P. (1997) Small subunit ribosomal RNA sequences unite alternate actinosporean and myxosporean stages of *Myxobolus cerebralis*, the causative agent of whirling disease in salmonid fish. *J. Eukaryot. Microbiol.* 44: 208–215.
- Andree, K.B., Székely, C., Molnár, K., Gresoviac, S.J. & Hedrick, R.P. (1999) Relationships among members of the genus *Myxobolus* (Myxozoa: Bivalvulidae) based on small subunit ribosomal DNA sequences. *J. Parasitol.* 85: 68–74.
- Arias, C., Estévez, J., Lores, B., Sanmartín, M.L. & Leiro, J. (1999) Discovery of *Loma dimorpha* Loubès, Maurant, Gasc, De Buron & Barral 1984, in a new host, the shanny, *Lipophrys pholis* (L.) from the north-west coast of Spain. *J. Fish Dis.* 22: 155–159.
- Atkinson, S.D. & Bartholomew, J.L. (2009) Alternate spore stages of *Myxobilatus gasterostei*, a myxosporean parasite of three-spined sticklebacks (*Gasterosteus aculeatus*) and oligochaetes (*Nais communis*). *Parasitol. Res.* 104: 1173–1181.
- Atkinson, S.D., Hallett, S.L. & Bartholomew, J.L. (2007) The life cycle of *Chloromyxum auratum* (Myxozoa) from goldfish, *Carassius auratus* (L.), involves an antonactinomyxon actinospore. *J. Fish Dis.* 30: 149–156.

- Azevedo, C., Casal, G., Garcia, P., Matos, P., Teles-Grilo, L. & Matos, E. (2009a) Ultrastructural and phylogenetic data of *Chloromyxum riorajum* sp. nov. (Myxozoa), a parasite of the stingray *Rioraja agassizii* in Southern Brazil. *Dis. Aquat. Org.* 85: 41-51.
- Azevedo, C., Casal, G., Matos, P., Ferreira, I. & Matos, E. (2009b) Ultrastructural data on the spore of *Myxobolus heckelii* n. sp. (Myxozoa), parasite from the Brazilian fish *Centromochlus heckelii* (Teleostei, Auchenipteridae). *J. Eukaryot. Microbiol.* (in press)
- Azevedo, C., Casal, G., Matos, P. & Matos, E. (2008) A new species of Myxozoa, *Henneguya rondoni* n. sp. (Myxozoa), from the peripheral nervous system of the Amazonian fish, *Gymnorhamphichthys rondoni* (Teleostei). *J. Eukaryot. Microbiol.* 55: 229-234.
- Azevedo, C., Casal, G., Mendonça, I. & Matos, E. (2009c) Fine structure of *Henneguya hemiodopsis* n. sp. (Myxozoa), a parasite of the gills of Brazilian teleostean fish, *Hemiodopsis microlepes* (Hemiodontidae). *Mem. Inst. Oswaldo Cruz* (in press)
- Azevedo, C., Corral, L. & Matos, E. (1997) Light and ultrastructural data on *Henneguya testicularis* n. sp. (Myxozoa, Myxobolidae), a parasite from the testis of the Amazonian fish *Moenkhausia oligolepis*. *Syst. Parasitol.* 37: 111-114.
- Azevedo, C., Corral, L. & Matos, E. (2002) *Myxobolus desaequalis* n. sp. (Myxozoa, Myxosporea), parasite of the Amazonian freshwater fish, *Apteronotus albifrons* (Teleostei, Apteronotidae). *J. Eukaryot. Microbiol.* 49: 485-488.
- Azevedo, C., Corral, L. & Matos, E. (2005) Ultrastructure of *Triangulamyxa amazonica* n. gen. and n. sp. (Myxozoa, Myxosporea), a parasite of the Amazonian freshwater fish, *Sphoeroides testudineus* (Teleostei, Tetraodontidae). *Eur. J. Protistol.* 41: 57-63.
- Azevedo, C., Lom, J. & Corral, L. (1989) Fine ultrastructural aspects of *Myxidium giardi* (Myxozoa, Myxosporea) from Portuguese eels (*Anguilla anguilla*). *Dis. Aquat. Org.* 6: 55-61.
- Azevedo, C. & Matos, E. (1995) *Henneguya adherens* n. sp. (Myxozoa, Myxosporea), parasite of the Amazonian fish, *Acestrorhynchus falcatus*. *J. Eukaryot. Microbiol.* 42: 515-518.
- Azevedo, C. & Matos, E. (1996a) *Henneguya malabarica* sp. nov. (Myxozoa, Myxobolidae) in the Amazonian fish *Hoplias malabaricus*. *Parasitol. Res.* 82: 222-224.
- Azevedo, C. & Matos, E. (1996b) Light and electron microscopic study of a myxosporean, *Tetrauronema desaequalis* n. sp. (Fam. Tetrauronematidae) from an Amazonian fish. *J. Parasitol.* 82: 288-291.
- Azevedo, C. & Matos, E. (2002a) Fine structure of a new species, *Loma myrophis* (Phylum Microsporidia), parasite of the Amazonian fish *Myrophis platyrhynchus* (Teleostei, Ophichthidae). *Eur. J. Protistol.* 37: 445-452.
- Azevedo, C. & Matos, E. (2002b) Fine structure of the myxosporean, *Henneguya curimata* n. sp., parasite of the Amazonian fish, *Curimata inornata* (Teleostei, Curimatidae). *J. Eukaryot. Microbiol.* 49: 197-200.
- Azevedo, C. & Matos, E. (2003a) *Amazonspora hassar* n. gen. and n. sp. (phylum Microsporidia, fam. Glugeidae), a parasite of the Amazonian teleost *Hassar orestis* (fam. Doradidae). *J. Parasitol.* 89: 336-341.
- Azevedo, C. & Matos, E. (2003b) Fine structure of *Henneguya pilosa* sp. n. (Myxozoa: Myxosporea), parasite of *Serrasalmus altuvei* (Characidae), in Brazil. *Folia Parasitol.* 50: 37-42.

- Bahri, S., Andree, K.B. & Hedrick, R.P. (2003) Morphological and phylogenetic studies of marine *Myxobolus*. *J. Eukaryot. Microbiol.* 50: 463–470.
- Baker, M.D., Vossbrinck, C.R., Becnel, J.J. & Andreadis, T.G. (1998) Phylogeny of *Amblyospora* (Microsporida: Amblyosporidae) and related genera based on small subunit ribosomal DNA data: A possible example of host parasite cospeciation. *J. Invertebr. Pathol.* 71: 199–206.
- Baquero, E., Rubio, M., Moura, I.N.S., Pieniazek, J. & Jordana, R. (2005) *Myosporidium merluccius* n. g., n. sp. infecting muscle of commercial hake (*Merluccius* sp.) from fisheries near Namibia. *J. Eukaryot. Microbiol.* 52: 476–483.
- Barassa, B., Adriano, E. A., Arana, S. & Cordeiro, N.C. (2003a) *Henneguya curvata* sp. n. (Myxosporea: Myxobolidae) parasitizing the gills of *Serrasalmus spilopleura* (Characidae: Serrasalminae), a South American freshwater fish. *Folia Parasitol.* 50: 151–153.
- Barassa, B., Cordeiro, N.S. & Arana, S. (2003b) A new species of *Henneguya*, a gill parasite of *Astyanax altiparanae* (Pisces: Characidae) from Brazil, with comments on histopathology and seasonality. *Mem. Inst. Oswaldo Cruz* 98: 761–765.
- Barber, I., Davies, A.J., Ironside, J.E., Forsgren, E., & Amundsen, T. (2009) First record of a *Kabatana* sp. microsporidium infecting fish in the Atlantic Ocean. *Dis. Aquat. Org.* 83: 145–152.
- Bartholomew, J.L., Atkinson, S.D., Hallett, S.L., Lowenstine, L.J., Garner, M.M., Gardiner, C.H., Rideout, B.A., Keel, M.K. & Brown, J.D. (2008) Myxozoan parasitism in waterfowl. *Int. J. Parasitol.* 38: 1199–1207.
- Bartholomew, J.L., Whipple, M.J., Stevens, D.G. & Fryer, J.L. (1997) The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *J. Parasitol.* 83: 859–868.
- Becnel, J.J. & Andreadis, T.G. (1999) Microsporidia of Insects. In: The Microsporidia and Microsporidiosis. (Wittner, M. & Weiss, L. M. Eds.), ASM Press, Washington D. C. pp. 447–501.
- Bekhti, M. & Bouix, G. (1985) *Loma salmonae* (Putz, Hoffmann et Dunbar, 1965) et *Loma diplodae* n. sp., Microsporidies parasites de branchies de Poissons Téléostéens: implantation et données ultrastructurales. *Protistologica* 21: 47–59.
- Békési, L., Székely, C. & Molnár, K. (2002) Atuais conhecimentos sobre Myxosporea (Myxozoa), parasitas de peixes. Um estágio alternativo dos parasitas no Brasil. *Braz. J. Vet. Res. Anim. Sci.* 39: 271–276.
- Bell, A.S., Aoki, T. & Yokoyama, H. (2001) Phylogenetic relationships among microsporidia based on rDNA sequences data, with particular reference to fish-infecting *Microsporidium* Balbiani, 1884 species. *J. Eukaryot. Microbiol.* 48: 258–265.
- Berrebi, P. (1979) Étude ultrastructurale de *Glugea atherinae* n. sp., microsporidie parasite de l'athérine *Atherina boyeri* Risso 1810 (poisson téléostéen) dans les Lagunes du Languedoc et de Provence. *Z. Parasitenkd.* 60: 105–122.
- Bohne, W., Ferguson, D.J., Kohler, K. & Gross, U. (2000) Developmental expression of a tandemly repeated, glycine- and serine-rich spore wall protein in the microsporidian pathogen *Encephalitozoon cuniculi*. *Infect. Immun.* 68: 2268–2275.
- Boreham, R.E., Hendrick, S., O'Donoghue, P.J. & Stenzel, D.J. (1998) Incidental finding of *Myxobolus* spores (Protozoa: Myxozoa) in stool samples from patients with gastrointestinal symptoms. *J. Clin. Microbiol.* 36: 3728–3730.

- Brickle, P., Kalavati, C. & MacKenzie, K. (2006) *Henneguya shackletoni* sp. nov. (Myxosporea, Bivalvulida, Myxobolidae) from the Falklands mullet, *Eleginops maclovinus* (Cuvier) (Teleostei, Eleginopidae) in the Falkland Islands. *Acta Parasitol.* 51: 36-39.
- Brosson, D., Kuhn, L., Prensier, G., Vivarès, C.P. & Texier, C. (2005) The putative chitin deacetylase of *Encephalitozoon cuniculi*: a surface protein implicated in microsporidian spore-wall formation. *FEMS Microbiol. Lett.* 247: 81–90.
- Buckler, E.S., Ippolito, A. & Holtsford, T.P. (1997) The evolution of ribosomal DNA: divergent paralogues and phylogenetic implications. *Genetics* 145: 821–832.
- Burger, M.A.A., Cribb, T.H. & Adlard, R.D. (2007) Patterns of relatedness in the Kudoidae with descriptions of *Kudoa chaetodonti* n. sp. and *K. lethrini* n. sp. (Myxosporea: Multivalvulida). *Parasitology* 134: 669-681.
- Burtle, G.J., Harrison, L.R. & Styer, E.L. (1991) Detection of a triactinomyxid myxozoan in an oligochaete from ponds with proliferative gill disease in channel catfish. *J. Aquat. Anim. Health* 3: 281–287.
- Canning, E.U. (1990) Phylum Microspora. In: The Handbook of Protoctists (Margulis, L., Chapman, D.J., Melkonian, M. & Corliss, J.O. Eds.), Jones & Bartlett, Boston, pp. 53-72.
- Canning, E.U., Feist, S.W., Longshaw, M., Okamura, B., Anderson, C.L., Tse, M.T. & Curry, A. (2005) *Microgemma vivaresi* n. sp. (Microsporidia, Tetramicridae), infecting liver and skeletal muscle of sea scorpions, *Taurulus bubalis* (Euphrasen 1786) (Osteichthyes, Cottidae), an inshore, littoral fish. *J. Eukaryot. Microbiol.* 52: 123–131.
- Canning, E.U., Curry, A., Anderson, C.L. & Okamura, B. (1999) Ultrastructure of *Myxidium trachinorum* sp. nov. from the gallbladder of the lesser weever fish *Echiichthys vipera*. *Parasitol. Res.* 85: 910-919.
- Canning, E.U., Curry, A., Feist, S.W., Longshaw, M. & Okamura, B. (2000) A new class and order of myxozoans to accommodate parasites of bryozoans with ultrastructural observations on *Tetracapsula bryosalmonae* (PKX organism). *J. Eukaryot. Microbiol.* 47: 456-468.
- Canning, E.U. & Hazard, E.I. (1982) Genus *Pleistophora* Gurley, 1893: An assemblage of at least three genera. *J. Protozool.* 29: 38-49.
- Canning, E.U., Hazard, E.I. & Nicholas, J.P. (1979) Light and electron microscopy of *Pleistophora* sp. from skeletal muscle of *Blennius pholis*. *Protistologica*, 15: 317-332.
- Canning, E.U. & Lom, J. (1986) The Microsporidia of Vertebrates, Academic Press, London.
- Canning, E.U., Lom, J. & Nicholas, J.P. (1982) Genus *Glugea* Thélohan, 1891 (Phylum Microspora): redescription of the type species *Glugea anomala* (Moniez, 1887) and recognition of its sporogonic development within sporophorous vesicles (pansporoblastic membranes). *Protistologica* 18: 193-210.
- Canning, E.U. & Nicholas, J.P. (1980) Genus *Pleistophora* (Phylum Microspora): redescription of the type species, *Pleistophora typicalis* Gurley, 1893 and ultrastructural characterization of the genus. *J. Fish Dis.* 3: 317-338.
- Canning, E.U., Okamura, B. & Curry, A. (1996) *Tetracapsula bryozoides* gen. n. et sp. n. in the body cavity of *Cristatella mucedo* (Bryozoa, Phylactolaemata). *Folia Parasitol.* 43: 249-261.
- Canning, E.U., Tops, S., Curry, A., Wood, T.S. & Okamura, B. (2002) Ecology, development and pathogenicity of *Buddenbrockia plumatellae* Schröder, 1910 (Myxozoa, Malacosporea) (syn. *Tetracapsula bryozoides*) and establishment of *Tetracapsuloides* n. gen. for *Tetracapsula bryosalmonae*. *J. Eukaryot. Microbiol.* 49:

280-295.

- Casal, G. & Azevedo, C. (1995) New ultrastructural data on the microsporidian *Ichthyosporidium giganteum* infecting the marine teleostean fish *Ctenolabrus rupestris*. *J. Fish Dis.* 18: 191-194.
- Casal, G., Costa, G. & Azevedo, C. (2007) Ultrastructural description of *Ceratomyxa tenuispora* (Myxozoa), a parasite of the marine fish *Aphanopus carbo* (Trichiuridae), from the Atlantic coast of Madeira Island (Portugal). *Folia Parasitol.* 54: 165-171.
- Casal, G., Garcia, P., Matos, P., Monteiro, E., Matos, E. & Azevedo, C. (2009a) Fine structure of *Chloromyxum menticirrhii* n. sp. (Myxozoa) infecting urinary bladder of the marine teleost *Menticirrhus americanus* (Sciaenidae) in Southern Brazil. *Eur. J. Protistol.* 45: 139-146.
- Casal, G., Matos, E. & Azevedo, C. (1996) Ultrastructural data on the life stages of *Myxobolus braziliensis* n. sp., parasite of an Amazonian fish. *Eur. J. Protistol.* 32: 123-127.
- Casal, G., Matos, E. & Azevedo, C. (1997) Some ultrastructural aspects of *Henneguya striolata* sp. nov. (Myxozoa, Myxosporea), a parasite of the Amazonian fish *Serrasalmus striolatus*. *Parasitol. Res.* 83: 93-95.
- Casal, G., Matos, E. & Azevedo, C. (2002) Ultrastructural data on the spore of *Myxobolus maculatus* n. sp. (phylum Myxozoa), parasite from the Amazonian fish *Metynnis maculatus* (Teleostei). *Dis. Aquat. Org.* 51: 107-112.
- Casal, G., Matos, E. & Azevedo, C. (2003) Light and electron microscopic study of the myxosporean, *Henneguya friderici* n. sp. from the Amazonian teleostean fish, *Leporinus friderici*. *Parasitology* 126: 313-319.
- Casal, G., Matos, E. & Azevedo, C. (2006) A new myxozoan parasite from the Amazonian fish *Metynnis argenteus* (Teleostei, Characidae): Light and electron microscope observations. *J. Parasitol.* 92: 817-821.
- Casal, G., Matos, E., Matos, P. & Azevedo, C. (2008a) Ultrastructural description of a new myxosporean parasite *Kudoa aequidens* sp. n. (Myxozoa, Myxosporea), found in the sub-opercular musculature of *Aequidens plagiazonatus* (Teleostei) from the Amazon River. *Acta Protozool.* 47: 135-141.
- Casal, G., Matos, E., Teles-Grilo, M.L. & Azevedo, C. (2008b) A new microsporidian parasite, *Potasporea morhaphis* n. gen., n. sp. (Microsporidia) infecting the teleostean fish *Potamorhaphis guianensis* from the River Amazon. Morphological, ultrastructural and molecular characterization. *Parasitology* 135: 1053-1064.
- Casal, G., Matos, E., Teles-Grilo, M.L. & Azevedo, C. (2009b) Morphological and genetical description of *Loma psittaca* sp. n. isolated from the Amazonian fish *Colomesus psittacus*. *Parasitol. Res. (in press)*
- Cavalier-Smith, T. (1987) Eukaryotes with no mitochondria. *Nature* (London) 326: 332-333.
- Cavalier-Smith, T. (1993) Kingdom Protozoa and its 18 Phyla. *Microbiol. Rev.* 57: 953-994.
- Cavalier-Smith, T. (1998) A revised six-kingdom system of life. *Biol. Rev.* 73: 203-266.
- Cellere, E.F., Cordeiro, N.S. & Adriano, E.A. (2002) *Myxobolus absonus* sp. n. (Myxozoa: Myxosporea) parasitizing *Pimelodus maculatus* (Siluriformes: Pimelodidae), a South American freshwater fish. *Mem. Inst. Oswaldo Cruz* 97: 79-80.
- Cheney, S.A., Lafranchi-Tristem, N.J. & Canning, E.U. (2000) Phylogenetic relationships of *Pleistophora*-like microsporidia based on small subunit ribosomal DNA sequences and implications for the source of

- Trachipleistophora hominis* infections. *J. Eukaryot. Microbiol.* 47: 280-287.
- Company, R., Sitjà-Bobadilla, A., Pujalte, M.J., Garay, E., Álvarez-Pellitero, P. & Perez-Sanchez, J. (1999) Bacterial and parasitic pathogens in cultured common dentex, *Dentex dentex* L. *J. Fish Dis.* 22: 299-309.
- Cordeiro, N.S., Artigas, P.T., Gioia, I. & Lima, R.S. (1984) *Henneguya pisciforme* n. sp., mixosporídeo parasito de brânquias do lambari *Hyphessobrycon anisitsi* (Pisces, Characidae). *Mem. Inst. Butantan* 47/48: 61–69.
- Cordeiro, N.S. & Gioia, I. (1987) Mixosporídios da ictiofauna brasileira. II. *Henneguya hoimba* n. sp. (Myxosporea: Myxobolidae). *X Cong. Soc. Bras. Parasitol., Salvador, Brasil*, 176.
- Cordeiro, N.S. & Gioia, I. (1990) On a new myxosporean parasite (Myxozoa), *Myxidium cholecysticum* sp. n. from the freshwater fish *Astyanax scabripinnis* (Jenyns, 1842). *Acta Protozool.* 29: 157-161.
- Costa, G., Lom, J., Andrade, C. & Barradas, R. (1998) First report of *Ceratomyxa sparusaurati* (Protozoa: Myxosporea) and the occurrence of epitheliocystis in cultured sea bream, *Sparus aurata* L. from Madeira. *Bull. Eur. Ass. Fish Pathol.* 18: 165-167.
- Coste, F. & Bouix, G. (1998) *Heterosporis cichlidarum* n. sp. (Microspora), a parasite of the ornamental cichlid fish *Hemichromis bimaculatus* Gill, 1862. *Bull. Eur. Ass. Fish Pathol.* 18: 157–161.
- Cruz, C., Saraiva, A. & Ferreira, S. (2000) Preliminary observations on *Myxobolus* sp. from cyprinid fish in Portugal. *Bull. Eur. Assoc. Fish Pathol.* 20: 65-69.
- Cruz, C., Vaz, A. & Saraiva, A. (2003) Occurrence of *Kudoa* sp. (Myxozoa) in *Trachurus trachurus* L. (Osteichthyes) in Portugal. *Parasite*, 10: 165-167.
- Cunha, A.M. & Fonseca, O. (1918) Sobre os myxosporídeos dos peixes brasileiros. *Bras. Med.* 32: 691-695.
- Current, W.L. & Janovy, J. (1977) Sporogenesis in *Henneguya exilis* infecting the channel catfish: an ultrastructural study. *Protistologica* 13: 157–167.
- Current, W.L., Janovy, J.Jr. & Knight, S.A. (1979) *Myxosoma funduli* Kudo (Myxosporida) in *Fundulus kansae*: ultrastructure of the plasmodium wall and of sporogenesis. *J. Protozool.* 26: 574–583.
- Cuyas, C., Castro, J.J., Santana-Ortega, A.T. & Carbonell, E. (2004) Insular stock identification of *Serranus atricauda* (Pisces: Serranidae) through the presence of *Ceratothoa steindachneri* (Isopoda: Cymothoidae) and *Pentacapsula cutanea* (Myxozoa: Pentacapsulidae) in the Canary Islands. *Sci. Mar.* 68: 159-163.
- Delbac, F., Peuve, I.I., Méténier, G., Peyretailade, E. & Vivarès C.P. (2001) Microsporidian invasion apparatus: identification of a novel polar tube protein and evidence for clustering of ptp1 and ptp2 genes in three *Encephalitozoon* species. *Infect. Immun.* 69: 1016-1024.
- De Rijk, P. & De Wachter, R. (1997) RnaViz, a program for the visualization of RNA secondary structure. *Nucleic Acids Res.* 25: 4679-4684.
- Desportes, I., Le Charpentier, Y., Galian, A., Bernard, F., Cochand-Priollet, B., Lavergne, A., Ravisse, P. & Modigliani, R. (1985) Occurrence of a new microsporidian *Enterocytozoon bieneusi* n. g., n. sp., in the enterocytes of a human patient with AIDS. *J. Protozool.* 32: 250-254.
- Desser, S.S., Molnár, K. & Weller, I. (1983) Ultrastructure of sporogenesis of *Thelohanellus nikolskii* Akhmerov, 1955 (Myxozoa: Myxosporea) from the common carp, *Cyprinus carpio*. *J. Parasitol.* 69: 504–518.

- Diamanka, A., Faye, N., Fall, M. & Toguebaye, B.S. (2007) Myxosporidian parasites of the genus *Myxobolus* Bütschli, 1882 found for the first time in cichlid fishes from Senegal River (West Africa). *Acta Protozool.* 46: 257-262.
- Diamant, A. (1992) A new pathogenic histozoic *Myxidium* (Myxosporea) in cultured gilt-head sea bream *Sparus aurata* L. *Bull. Eur. Assoc. Fish Pathol.* 12: 64-66.
- Diamant, A. & Paperna, I. (1985) The development and ultrastructure of *Nosema ceratomyxae* sp. nov., a microsporidian hyperparasite of the myxosporean *Ceratomyxa* sp. from red sea rabbitfish (Siganidae). *Protistologica*, 21: 249-258.
- Diamant, A. & Paperna, I. (1989) Cytopathology of *Ceratomyxa* sp. (Myxosporea) hyperparasitized with the microsporidian *Nosema ceratomyxae*. *Dis. Aquat. Org.* 6: 75-79.
- Didier, E.S. & Weiss, L.M. (2006) Microsporidiosis: current status. *Curr. Opin. Infect. Dis.* 19: 485-492.
- Docker, M.F., Kent, M.L., Hervio, D.M.L., Khattra, J.S., Weiss, L.M., Cali, A. & Devlin, R.H. (1997) Ribosomal DNA sequence of *Nucleospora salmonis* Hedrick, Groff and Baxa, 1991 (Microsporea: Enterocytozoonidae): Implications for phylogeny and nomenclature. *J. Eukaryot. Microbiol.* 44: 55-60.
- Duncan, A.E., Garner, M.M., Bartholomew, J.L., Reichard, T.A. & Nordhausen, R.W. (2004) Renal myxosporidiasis in Asian horned frogs (*Megophrys nasuta*). *J. Zoo Wild. Dis.* 35: 381-386.
- Dyková, I. & Lom, J. (1980) Tissue reactions to microsporidian infections in fish. *J. Fish Dis.* 3: 265-283.
- Dyková, I. & Lom, J. (2000) Histopathology of *Kabatana arthuri* (Microspora) infection in sutchi catfish, *Pangasius sutchi*. *Folia Parasitol.* 47: 161-166.
- Dyková, I., Buron, I., Fiala I. & Roumillat, W. A. (2009) *Kudoa inornata* sp. n. (Myxosporea: Multivalvulida) from the skeletal muscles of *Cynoscion nebulosus* (Teleostei: Sciaenidae). *Folia Parasitol.* 56: 91-98.
- Edlind, T.D., Li, J., Visvesvara, G.S., Vodkin, M.H., McLaughlin, G.L. & Katiyar, S.K. (1996) Phylogenetic analysis of beta-tubulin sequences from amitochondrial protozoa. *Mol. Phylogen. Evol.* 5: 359-367.
- Egusa, S. (1982) A microsporidian species from yellowtail juveniles, *Seriola quinqueradiata*, with 'Beko' disease. *Fish Pathol.* 16: 187-192.
- Eiras, J.C. (2002) Synopsis of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa: Myxosporea: Myxobolidae). *Syst. Parasitol.* 52: 43-54.
- Eiras, J.C. (2005) An overview on the myxosporean parasites in amphibians and reptiles. *Acta Parasitol.* 50: 267-275.
- Eiras, J.C. (2006) Synopsis of the species of *Ceratomyxa* Thélohan, 1892 (Myxozoa: Myxosporea: Ceratomyxidae). *Syst. Parasitol.* 65: 49-71.
- Eiras, J.C., Molnár, K. & Lu, Y.S. (2005) Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). *Syst. Parasitol.* 61: 1-46.
- Eiras, J.C., Abreu, P.C., Robaldo, R. & Pereira, J. (2007) *Myxobolus platanus* n. sp (Myxosporea, Myxobolidae), a parasite of *Mugil platanus* Gunther, 1880 (Osteichthyes, Mugilidae) from Lagoa dos Patos, RS, Brazil. *Arq. Bras. Med. Veter. Zootecn.* 59: 895-898.
- Eiras, J.C., Malta, J.C., Varela, A. & Pavanelli, G.C. (2004a) *Henneguya schizodon* n. sp. (Myxozoa, Myxobolidae), a parasite of the Amazonian teleost fish *Schizodon fasciatus* (Characiformes,

- Anostomidae). *Parasite* 11: 169–173.
- Eiras, J.C., Pavanelli, G.C. & Takemoto, R.M. (2004b) *Henneguya paranaensis* sp. n. (Myxozoa, Myxobolidae), a parasite of the teleost fish *Prochilodus lineatus* (Characiformes, Prochilodontidae) from the Paraná River, Brazil. *Bull. Eur. Assoc. Fish Pathol.* 34: 308–311.
- Eiras, J.C., Takemoto, R.M. & Pavanelli, G.C. (2008) *Henneguya caudicula* n. sp. (Myxozoa, Myxobolidae) a parasite of *Leporinus lacustris* (Osteichthyes, Anostomidae) from the high Paraná River, Brazil, with a revision of *Henneguya* spp. infecting south American fish. *Acta Protozool.* 47: 149–154.
- Eiras, J.C., Takemoto, R.M. & Pavanelli, G.C. (2009) *Henneguya corruscans* n. sp. (Myxozoa, Myxosporea, Myxobolidae), a parasite of *Pseudoplatystoma corruscans* (Osteichthyes, Pimelodidae) from the Paraná River, Brazil: A morphological and morphometric study. *Vet. Parasitol.* 159: 154–158.
- El-Mansy, A., Székely, C. & Molnár, K. (1998) Studies on the occurrence of actinosporean stages of fish myxosporeans in a fish farm of Hungary, with the description of triactinomyxon, raabeia, aurantiactinomyxon and neoactinomyxon types. *Acta Vet. Hung.* 46: 259–284.
- El-Matbouli, M., Fischer-Scherl, T. & Hoffmann, R.W. (1992) Present knowledge on the life cycle, taxonomy, pathology, and therapy of some *Myxosporea* spp. important for freshwater fish. *Ann. Rev. Fish Dis.* 3: 367–402.
- Erickson, B.W. & Blanquet, R.S. (1969) The occurrence of chitin in the spore wall of *Glugea weissenbergi*. *J. Invertebr. Pathol.* 14: 358–364.
- Estévez, J., Iglesias, R., Leiro, J., Ubeira, F.M. & Sanmartín, M.L. (1992) An unusual site of infection by a microsporean in the turbot *Scophthalmus maximus*. *Dis. Aquat. Org.* 13: 139–142.
- Fast, N.M., Law, J.S., Williams, B.A.P. & Keeling, P.J. (2003) Bacterial catalase in the microsporidian *Nosema locustae*: implications for microsporidian metabolism and genome evolution. *Eukaryot. Cell* 2: 1069–1075.
- Fast, N.M., Logsdon, Jr., J.M. & Doolittle, W.F. (1999) Phylogenetic analysis of the TATA box binding protein (TBP) gene from *Nosema locustae*: evidence for a microsporidia-fungi relationship and spliceosomal intron loss. *Mol. Biol. Evol.* 16: 1415–1419.
- Faye, A., Toguebaye, B.S. & Bouix, G. (1990) Ultrastructure and development of *Pleistophora senegalensis* sp. nov. from the gilthead sea bream, *Sparus aurata* L. (Teleost, Sparidae) from the coast of Senegal. *J. Fish Dis.* 3: 179–192.
- Faye, N., Toguebaye, B.S. & Bouix, G. (1991) *Microfilum lutjani* n. g. n. sp. (Protozoa, Microsporida), a gill parasite of the golden African snapper *Lutjanus fulgens* (Valenciennes, 1830) (Teleostei, Lutjanidae): Developmental cycle and ultrastructure. *J. Protozool.* 38: 30–40.
- Faye, N., Toguebaye, B.S. & Bouix, G. (1994) *Nosemoides syacii* n. sp., a microsporidian parasite of the west African turbot, *Syacium micrurum* Ranzani, 1840. *Syst. Parasitol.* 28: 403–50.
- Faye, N., Toguebaye, B.S. & Bouix, G. (1995) On the cytology and development of *Loma boopsi* n. sp. (Microspora, Glugeidae), parasite of *Boops boops* (Pisces, Teleostei, Sparidae) from the coasts of Senegal. *Arch. Protistenkd.* 146: 85–93.
- Faye, N., Toguebaye, B.S. & Bouix, G. (1996) Ultrastructure and development of *Neonosemoides tilapiae* (Sakiti & Bouix, 1987) n. g., n. comb. (Protozoa, Microspora) from African cichlid fish. *Eur. J. Protistol.* 32: 320–326.

- Faye, N., Toguebaye, B.S. & Bouix, G. (2004) First report of microsporidian infections in solefishes from Senegal coast (West Africa). *Bull. Eur. Ass. Fish Pathol.* 24: 115-118.
- Feijó, M.M., Arana, S., Ceccarelli, P.S. & Adriano, E.A. (2008) Light and scanning electron microscopy of *Henneguya arapaima* n. sp. (Myxozoa: Myxobolidae) and histology of infected sites in pirarucu (*Arapaima gigas*: Pisces: Arapaimidae) from the Araguaia River, Brazil. *Vet. Parasitol.* 157: 59-64.
- Feist, S.W. (2008) Myxozoan Disases. In: Fish Diseases (Eiras, J., Segner, H., Wahli, T.H. & Kapoor, B.G., Eds.), vol 2. Science Publishers Phymouth, pp. 613-682.
- Ferguson, J.A., Atkinson, S.D., Whipps, C.M. & Kent, M.L. (2008) Molecular and morphological analysis of *Myxobolus* spp. of salmonid fishes with the description of a new *Myxobolus* species. *J. Parasitol.* 94: 1322-1334.
- Fiala, I. (2006) The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. *Int. J. Parasitol.* 36:1521-1534.
- Fiala, I. & Dyková, I. (2004) The phylogeny of marine and freshwater species of the genus *Chloromyxum* Mingazzini, 1890 (Myxosporea: Bivalvulida) based on small subunit ribosomal RNA gene sequences. *Folia Parasitol.* 51: 211-214.
- Findley, A.M., Weidner, E.H., Carman, K.R., Xu, Z. & Godbar, J.S. (2005) Role of the posterior vacuole in *Spraguea lophii* (Microsporidia) spore hatching. *Folia Parasitol.* 52: 111-117.
- Fomena, A., Coste, F. & Bouix, G. (1992) *Loma camerounensis* sp. nov. (Protozoa: Microsporida) a parasite of *Oreochromis niloticus* Linnaeus 1757 (Teleost: Cichlidae) in fish-rearing ponds in Melen, Yaoundé, Cameroon. *Parasitol. Res.* 78: 201-208.
- Franzen, C. (2004) Microsporidia: how can they invade other cells? *Trends Parasitol.* 20: 275-279.
- Freeman, M.A., Yokoyama, H. & Ogawa, K. (2004) A microsporidian parasite of the genus *Spraguea* in the nervous tissues of the Japanese anglerfish *Lophius litulon*. *Folia Parasitol.* 51: 167-176.
- Freeman, M.A., Yokoyama, H. & Ogawa, K. (2008) Description and phylogeny of *Ceratomyxa anko* sp. n. and *Zschokkella lophii* sp. n. from the Japanese anglerfish, *Lophius litulon* (Jordan). *J. Fish Dis.* 31: 921-930.
- Friedrich, C., Ingolic, E., Freitag, B., Kastberger, G., Hohmann, V., Skofitsch, G., Neumeister, U. & Kepka, O. (2000) A myxozoan-like parasite causing xenomas in the brain of the mole, *Talpa europaea* L., 1758 (Vertebrata, Mammalia). *Parasitology* 121: 483-492.
- Garner, M.M., Bartholomew, J.L., Whipps, C.M., Nordhausen, R.W. & Raiti, P. (2005) Renal myxozoanosis in Crowned River Turtles *Hardella thurjii*: description of the putative agent *Myxidium hardella* n. sp. by histopathology, electron microscopy, and DNA sequencing. *Vet. Pathol.* 42: 589-595.
- Gill, E.E. & Fast, N.M. (2006) Assessing the microsporidia-fungi relationship: Combined phylogenetic analysis of eight genes. *Gene* 375: 103-109.
- Gioia, I. & Cordeiro, N.S. (1987) Mixosporídeos da ictiofauna brasileira: *Henneguya artigasi* n. sp. (Myxosporea: Myxobolidae). *XIV Cong. Bras. Zool., Juiz de Fora, Brasil*, 186.
- Gioia, I. & Cordeiro, N.S. (1996) Brazilian myxosporidians' checklist (Myxozoa). *Acta Protozool.* 35: 137-149.
- Gioia, I., Cordeiro, N.S. & Artigas, P.T. (1986) *Henneguya intracornea* n. sp. (Myxozoa: Myxosporea) parasita do olho do lambari, *Astyanax scabripinnis* (Jenyns, 1842) (Osteichthyes, Characidae). *Mem. Inst. Oswaldo Cruz* 81: 401-407.

- Germot, A., Philippe, H. & Le Guyader, H. (1997) Evidence for loss of mitochondria in microsporidia from a mitochondrial-type HSP70 in *Nosema locustae*. *Mol. Biochem. Parasitol.* 87: 159-168.
- Goldberg, A.V., Molik, S., Tsoulos, A.D., Neumann, K., Kuhnke, G., Delbac, F., Vivarès, C.P., Hirt, R.P., Lill, R. & Embley T.M. (2008) Localization and functionality of microsporidian iron-sulphur cluster assembly proteins. *Nature* 452: 624–628.
- Gracia, M.P., Maillo, P.A., Amigó, J.M. & Salvado, H. (1997) Ultrastructural study of *Sphaeromyxa balbianii* Thélohan, 1892 (Myxozoa, Myxosporea: Bivalvulida), a parasite of *Cepola macrophthalmia* Linnaeus, 1758. *Acta Protozool.* 36: 171-179.
- Grau, A., Riera, F. & Carbonell, E. (1999) Some protozoan and metazoan parasites of the amberjack from the Balearic sea (Western Mediterranean). *Aquacult. Int.* 7: 307-317.
- Guimarães, J.R.A. & Bergamin, F. (1933) Considerações sobre as ictiopizootias produzidas pelos Mixosporídeos do género *Henneguya* Thélohan, 1892. *Rev. Indust. Anim., São Paulo* 10: 1151–1156.
- Guimarães, J.R.A. & Bergamin, F. (1934) *Henneguya santae* sp. n. – um novo mixosporídeo parasito de *Tetragonopterus* sp. *Rev. Indust. Anim.* 12: 110–113.
- Guimarães, J.R.A. & Bergamin, F. (1938) Sobre um novo myxosporídeo parasito de peixe de água doce, *Myxobolus kudo* sp. n. *Rev. Indust. Anim.* 1: 65-67.
- Gunter, N.L. & Adlard, R.D. (2008) Bivalvulidan (Myxozoa: Myxosporea) parasites of damselfishes with description of twelve novel species from Australia's Great Barrier Reef. *Parasitology* 135: 1165-1178.
- Gunter, N.L., Cribb, T.H., Whipps, C.M. & Adlard, R.D. (2006) Characterization of *Kudoa monodactyli* n. sp. (Myxosporea: Multivalvulida) from the muscle of *Monodactylus argenteus* (Teleostei: Monodactylidae) from Moreton Bay, Queensland, Australia. *J. Eukaryot. Microbiol.* 53: 374-378.
- Gunder, N.L. & Adlard, R. (2009) Seven new species of *Ceratomyxa* Thélohan, 1892 (Myxozoa) from the gall-bladders of serranid fishes from the great barrier reef, Australia. *Syst. Parasitol.* 73: 1-11.
- Hallett, S.L., Erseus, C. & Lester, R.J.G. (1999) Actinosporeans (Myxozoa) from marine oligochaetes of the Great Barrier Reef. *Syst. Parasitol.* 44: 49–57.
- Hausmann, K., Hülsmann, N. & Radek, R. (2003) *Protistology*. E. Schweizerbart'sche Verlagsbuchhandlung. Berlin-Stuttgart, 379 pp.
- Hayman, J.R., Hayes, S.F., Amon, J. & Nash, T.E. (2001) Developmental expression of two spore wall proteins during maturation of the microsporidian *Encephalitozoon intestinalis*. *Infect. Immun.* 69: 7057–7066.
- Hashimoto, T., Nakamura, Y., Kamaishi, T. & Hasegawa, M. (1997) Early evolution of eukaryotes inferred from protein phylogenies of translation elongation factors 1 α and 2. *Arch. Protistenkd.* 148: 287-295.
- Hayman, J.R., Southern, T.R. & Nash, T.E. (2005) Role of sulfated glycans in adherence of the microsporidian *Encephalitozoon intestinalis* to host cells in vitro. *Infect. Immun.* 73: 841–848.
- Hedrick, R.P., Groff, J.M. & Baxa, D.A. (1991) Experimental infections with *Enterocytozoon salmonis* Chilmonczyk, Cox, Hedrick (Microsporea): an intranuclear microsporidium from chinook salmon *Oncorhynchus tshawytscha*. *Dis. Aquat. Org.* 10: 103-108.
- Heiniger, H., Gunter, N.L. & Adlard, R.D. (2008) Relationships between four novel ceratomyxid parasites from the gall bladders of labrid fishes from Heron Island, Queensland, Australia. *Parasitol. Int.* 57: 158-165.

- Hemananda, T., Mohilal, N., Bandyopadhyay, P.K. & Mitra, A.K. (2009) Two new Myxosporidia (Myxozoa: Myxosporea) of the genus *Myxobolus* Bütschli, 1882 from cornea of *Clarias batrachus* (Linnaeus, 1758) caught from a fish farm in India. *North-West J. Zool.* 5: 165-169.
- Hervio, D.M.L., Kent, M.L., Khattra, J., Sakanari, J., Yokoyama, H. & Devlin, R.H. (1997) Taxonomy of *Kudoa* species (Myxosporea), using a small-subunit ribosomal DNA sequence. *Can. J. Zool.* 75: 2112-2119.
- Hillis, D.M. & Dixon, M.T. (1991) Ribosomal DNA: Molecular evolution and phylogenetic inference. *Qrtl. Rev. Biol.* 66: 411-453.
- Hirt, R.P., Healy, B., Vossbrinck, C.R., Canning, E.U. & Embley, T.M. (1997) A mitochondrial Hsp70 orthologue in *Vairimorpha necatrix*: molecular evidence that microsporidia once contained mitochondria. *Curr. Biol.* 7: 995-998.
- Hirt, R.P., Longsdon, J.M., Healy, B., Dorey, M.W., Doolittle, W.F. & Embley, T.M. (1999) Microsporidia are related to Fungi: evidence from the largest subunit of RNA polymerase II and other proteins. *Proc. Natl. Acad. Sci.* 96: 580-585.
- Hogge, C.I., Campbell, M.R. & Johnson, K.A. (2008) A new species of myxozoan (Myxosporea) from the brain and spinal cord of rainbow trout (*Oncorhynchus mykiss*) from Idaho. *J. Parasitol.* 94: 218-222.
- Holzer, A.S., Blasco-Costa, I., Sarabeev, V.L., Ovcharenko, M.O. & Balbuena, J.A. (2006a) *Kudoa trifolia* sp n. - molecular phylogeny suggests a new spore morphology and unusual tissue location for a well-known genus. *J. Fish Dis.* 29: 743-755.
- Holzer, A.S., Sommerville, C. & Wootten, R. (2004) Molecular relationships and phylogeny in a community of myxosporeans and actinosporeans based on their 18S rDNA sequences. *Int. J. Parasitol.* 34: 1099-1111.
- Holzer, A.S., Sommerville, C. & Wooden, R. (2006b) Molecular identity, phylogeny and life cycle of *Chloromyxum schurovi* Shul'man and Ieshko, 2003. *Parasitol. Res.* 99: 90-96.
- Ishihara, R. & Hayashi, Y. (1968) Some properties of ribosomes from the sporoplasm of *Nosema bombycis*. *J. Invertebr. Pathol.* 11: 377-385.
- Jakowska, S. (1964) Infecção microsporídea das células nervosas numa população de peixes marinhos, *Lophius americanus*. Annual 2nd Congress Latin- American Zoology (S. Paulo, Brazil, 1962). 1: 265-273 (In Portuguese).
- Jakowska, S. & Nigrelli, R.F. (1953) The pathology of myxosporidiosis in the electric eel, *Electrophorus electricus* (Linnaeus), caused by *Henneguya visceralis* and *Henneguya electrica* spp. nov. *Zoologica* 38: 183-191.
- Jayasri, M. & Hoffman, G.L. (1982) Review of *Myxidium* (Protozoa: Myxozoa: Myxosporea). *Protozool. Abst.* 6: 61-91.
- Jirků, M., Bolek, M.G., Whipps, C.M., Janovy Jr.J., Kent, M.L. & Modrý, D. (2006) A new species of *Myxidium* (Myxosporea: Myxidiidae), from the western chorus frog, *Pseudacris triseriata triseriata*, and Blanchard's cricket frog, *Acris crepitans blanchardi* (Hylidae), from Eastern Nebraska: morphology, phylogeny, and critical comment. *J. Parasitol.* 92: 611-619.
- Kageyama, T., Yanagida, T., Ohara, K. & Yokoyama, H. (2009) *Henneguya pseudorhinogobii* n. sp (Myxozoa: Myxosporea) parasitizing the gills of the freshwater goby *Rhinogobius* sp. OR from the Nagara River and redescription of *Henneguya rhinogobii*. *Fish. Sci.* 75: 657-663.

- Kallert, D.M., Ponader, S., Eszterbauer, E., El-Matbouli, M. & Haas, W. (2007) Myxozoan transmission via actinospores: new insights into mechanisms and adaptations for host invasion. *Parasitology* 134: 1741-1750.
- Kallert, D.M., Eszterbauer, E., Ereséus, C., El-Matbouli, M. & Haas, W. (2005) Life cycle studies of *Myxobolus parviformis* sp. n. (Myxozoa: Myxobolidae) from bream. *Dis. Aquat. Org.* 66: 233-243.
- Katinka, M.D., Duprat, S., Cornillot, E., Metenier, G., Thomarat, F., Prensier, G., Barbe, V., Peyretailade, E., Brottier, P., Wincker, P., Delbac, F., El Alaoui, H., Peyret, P., Saurin, W., Gouy, M., Weissenbach, J. & Vivarès, C.P. (2001) Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. *Nature* 414: 450-453.
- Keeling, P.J. & Doolittle, W.F. (1996) Alpha-tubulin from early-diverging eukaryotic lineages and the evolution of the tubulin family. *Mol. Biol. Evol.* 13: 1297-1305.
- Keeling, P.J. & Fast, N.M. (2002) Microsporidia: biology and evolution of highly reduced intracellular parasites. *Ann. Rev. Microbiol.*, 56: 93-116.
- Keeling, P.J. & Fast, N.M., Joyce, S.L., Williams, B.A.P. & Slamovits C.H. (2005) Comparative genomics of microsporidia. *Folia Parasitol.* 52: 8-14.
- Keeling, P.J. & McFadden, G.I. (1998) Origins of microsporidia. *Trends Microbiol.* 6: 19-23.
- Keeling, P.J. & Slamovits, C.H. (2004) Simplicity and complexity of microsporidian genomes. *Eukaryot. Cell* 3: 1363-1369.
- Kent, M.L., Andree, K.B., Bartholomew, J.L., El-Matbouli, M., Desser, S.S., Devlin, R.H., Feist, S.W., Hedrick, R.P., Hoffmann, R.W., Khattra, J., Hallett, S.L., Lester, R.J.G., Longshaw, M., Palenzeula, O., Siddall, M.E. & Xiao, C. (2001) Recent advances in our understanding of the Myxozoa. *J. Eukaryot. Microbiol.* 48: 395-413.
- Kent, M.L., Docker, M., Khattra, J., Vossbrinck, C.R., Speare, D.J. & Devlin, R.H. (1999) A new *Microsporidium* sp. (Microsporidia) from the musculature of the mountain white fish *Prosopium williamsoni* from British Columbia: morphology and phylogeny. *J. Parasitol.* 85: 1114-1119.
- Kent, M.L. & Hoffman, G.L. (1984) Two new species of Myxozoa, *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the brain of a South American knife fish, *Eigemannia virescens* (V.). *J. Protozool.* 31: 91-94.
- Keohane, E.M., Takvorian, P.M., Cali, A., Tanowitz, H.B., Wittner, M. & Weiss, L.M. (1996) Identification of a microsporidian polar tube protein reactive monoclonal antibody. *J. Eukaryot. Microbiol.* 43: 26-31.
- Keohane, E.M. & Weiss, L.M. (1999) The structure, function, and composition of the microsporidian polar tube. In: *The Microsporidia and Microsporidiosis* (Wittner, M. & Weiss, L., Eds.), *Am. Soc. Microbiol.*, Washington, DC, pp. 196-224.
- Køie, M., Karlsbakk, E. & Nylund, A. (2007a) *Parvicapsula bicornis* n. sp. and *P. limandae* n. sp. (Myxozoa, Parvicapsulidae) in Pleuronectidae (Teleostei, Heterosomata) from Denmark. *Dis. Aquat. Org.* 63: 123-129.
- Køie, M., Karlsbakk, E. & Nylund, A. (2007b) A new genus *Gadimyxa* with three species (Myxozoa, Parvicapsulidae) parasitic in marine fish (Gadidae) and the two-host life cycle of *Gadimyxa atlantica* n. sp. *J. Parasitol.* 93: 1459-1467.

- Køie, M., Karlsbakk, E. & Nylund, A. (2008) The marine herring myxozoan *Ceratomyxa auerbachii* (Myxozoa: Ceratomyxidae) uses *Chone infundibuliformis* (Annelida: Polychaeta: Sabellidae) as invertebrate host. *Folia Parasitol.* 55: 100–104.
- Køie, M., Whipps, C.M. & Kent, M.L. (2004) *Ellipsomyxa gobbii* (Myxozoa: Ceratomyxidae) in the common goby *Pomatoschistus microps* (Teleostei: Gobiidae) uses *Nereis* spp. (Annelida: Polychaeta) as invertebrate hosts. *Folia Parasitol.* 51: 14–18.
- Kunz, W. (2002) When is a parasite species a species? *Trends Parasitol.* 18: 121–124.
- Larsson, J.I.R. (1986) Ultrastructure, function, and classification of Microsporidia. In: Progress in Protistology (Corliss J.O. & Patterson, D.J., Eds.), vol. 1. Biopress, Bristol, pp. 325–390.
- Larsson, J.I.R. (1988) Identification of microsporidian genera (Protozoa, Microspora): a guide with comments on the taxonomy. *Arch. Protistenkd.*, 136: 1–37.
- Larsson, J.I.R. (1999) Identification of Microsporidia. *Acta Protozool.* 38: 161–197.
- Lee, S.C., Corradi, N., Byrnes, E.J., Torres-Martinez, S., Dietrich, F.S., Keeling, P.J. & Heitman, J. (2008) Microsporidia evolved from ancestral sexual Fungi. *Curr. Biol.* 18: 1675–1679.
- Leiro, J., Ortega, M., Iglesias, R., Estevez, J. & Sanmartin, M.L. (1996) *Pleistophora finisterrensis* n. sp., a microsporidian parasite of blue whiting *Micromesistius poutassou*. *Syst. Parasitol.* 34: 163–170.
- Leiro, J., Paramá, A., Ortega, M., Santamarina, M.T. & Sanmartin, M.L. (1999) Redescription of *Glugea caulleryi*, a microsporidian parasite of the greater sand-eel, *Hyperoplus lanceolatus* (Le sauvage), (Teleostei: Ammodytidae), as *Microgemma caulleryi* comb. nov. *J. Fish Dis.* 22: 101–110.
- Leiro, J., Bos, J., Peris, D., Estevez, J., Santamarina, M.T. & Sanmartin, M.L. (1994) Infection of black goby (*Gobius niger* L.) from the northwest coast of Spain by *Loma dimorpha* (Protozoa: Microspora). *Res. Rev. Parasitol.* 54: 217–223.
- Li, Y., Wu, Z., Pan, G., He, W., Zhang, R., Hu, J. & Zhou, Z. (2009) Identification of a novel spore wall protein (SWP26) from microsporidia *Nosema bombycis*. *Int. J. Parasitol.* 39: 391–398.
- Lin, D., Hanson, L.A. & Pote, L.M. (1999) Small subunit ribosomal RNA sequence of *Henneguya exilis* (Class Myxosporea) identifies the actinosporean stage from an oligochaete host. *J. Eukaryot. Microbiol.* 46: 66–68.
- Lom, J. (1969) Notes on the ultrastructure and sporoblast development in fish parasitizing myxosporidian of the genus *Sphaeromyxa*. *Z. Zellforsch.* 97: 416–437.
- Lom, J. (2002) A catalogue of described genera and species of microsporidians parasitic in fish. *Syst. Parasitol.* 53:81–99.
- Lom, J. (2008) Microsporidia. In: Fish Diseases (Eiras, J. & Segner, H. Wahli, T.H. & Kapoor, B.G., Eds.), vol 1. Science Publishers Phymouth, pp. 351–395.
- Lom, J. & Dyková, I. (1992a) Microsporidia (Phylum Microspora Sprague, 1977). In: Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science (Lom, J. & Dyková, I., Eds), vol 26, Elsevier, Amsterdam, pp. 125–157.
- Lom, J. & Dyková, I. (1992b) Myxosporidia (Phylum Myxozoa) In: Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science (Lom, J. & Dyková, I., Eds), vol 26, Elsevier, Amsterdam, pp. 159–235.

- Lom, J. & Dyková, I. (1993) Scanning electron microscopic revision of common species of the genus *Chloromyxum* (Myxozoa: Myxosporaea) infecting European freshwater fishes. *Folia Parasitol.* 40: 161-174.
- Lom, J. & Dyková, I. (2002) Ultrastructure of *Nucleospora secunda* n. sp. (Microsporidia), parasite of enterocytes of *Nothobranchius rubripinnis*. *Eur. J. Protistol.* 38: 19–27.
- Lom, J. & Dyková, I. (2005) Microsporidian xenomas in fish seen in wider perspective. *Folia Parasitol.* 52: 69-81.
- Lom, J. & Dyková, I. (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol.* 43: 1-36.
- Lom, J., Dyková, I., Körting, W. & Klinger, H. (1989a) *Heterosporis schuberti* n. sp., a new microsporidian parasite of aquarium fish. *Eur. J. Protistol.* 25: 129-135.
- Lom, J., Feist, S.W., Dyková, I. & Kepr, T. (1989b) Brain myxoboliasis of bullhead, *Cottus gobio* L., due to *Myxobolus jiroveci* sp. nov.: light and electron microscope observations. *J. Fish Dis.* 12: 15–27.
- Lom, J., Dyková, I. & Shaharom, F. (1990) *Microsporidium arthuri* n. sp., parasite of *Pangasius sutchi* (Pangasiidae, Siluroidea) in South-East Asia. *Dis. Aquat. Org.* 8: 65-67.
- Lom, J., Dyková, I. & Tonguthai, K. (1999) *Kabataia* gen. n., a new genus proposed for *Microsporidium* spp. infecting trunk muscles of fishes. *Dis. Aquat. Org.* 38: 39-46.
- Lom, J., Dyková, I. & Tonguthai, K. (2000a) *Kabatana* gen. n., new name for the microsporidian genus *Kabataia* Lom, Dyková et Tonguthai, 1999. *Folia Parasitol.* 47: 78.
- Lom, J., Dyková, I., Tonguthai, K. & Chinabut, S. (1993) Muscle infection due to *Heterosporis* sp. in the Siamese fighting fish, *Betta splendens* Regan. *J. Fish Dis.* 16: 513–516.
- Lom, J., Dyková, I., Wang, C.H., Lo, C.F. & Kou, G.H. (2000b) Ultrastructural justification for the transfer of *Pleistophora anguillarum* Hoshina, 1959 to the genus *Heterosporis* Schubert, 1969. *Dis. Aquat. Org.* 43: 225-231.
- Lom, J. & Nilsen, F. (2003) Fish microsporidia: fine structural diversity and phylogeny. *Int. J. Parasitol.* 33: 107-127.
- Lom, J., Nilsen, F. & Urawa, S. (2001) Redescription of *Microsporidium takedai* (Awakura, 1974) as *Kabatana takedai* (Awakura, 1974) comb. n. *Dis. Aquat. Org.* 44: 223-230.
- Lom, J. & Pekkarinen, M. (1999) Ultrastructural observations on *Loma acerinae* (Jirovec, 1930) comb. Nov. (Phylum Microsporidia). *Acta Protozool.* 38: 61-74.
- Lom, J. & Puytorac, P. (1965a) Observations sur l'ultrastructure des trophozoites des myxosporidies. *C. R. Acad. Sci. Paris* 260: 2588-2590.
- Lom, J. & Puytorac, P. (1965b) Studies on the myxosporidian ultrastructure and polar capsule development. *Protistologica* 1: 53-65.
- Lom, J. & Weiser, J. (1972) Surface pattern of some microsporidian spores as seen in the scanning electron microscope. *Folia Parasitol.* 19: 359-363.
- Loubès, C., Maurand, J., Gasc, C., De Buron, I. & Barral, J. (1984) Étude ultrastructurale de *Loma dimorpha* n. sp., Microsporidie parasite de poissons Gobiidae lauguedociens. *Protistologica* 22: 579-589.
- Loubès, C., Maurand, J. & Ormières, R. (1979) Étude ultrastructurale de *Spraguea lophii* (Doflein, 1898),

- microsporidie parasite de la Baudroie: essai d'interpretation du dimorphisme sporal. *Protistologica* 15: 43-54.
- Maíllo, P.A., Amigó, J.M., Baena, R., Salvadó, H. & Gracia, M.P. (1998) *Tetramicra brevifilum* (Matthews & Matthews, 1980) (Microsporita: Tetramicriidae) in a new fish host, *Lophius budegassa* (Spinola, 1807) in Spain. *Parasitol. Res.* 84: 208-212.
- Maillo-Bellon, P.A. & Gracia-Royo, M.P. (2007) Vegetative stages, sporogenesis and spore morphology of *Ceratomyxa appendiculata*, Thélohan, 1892 (Myxozoa: Bibalvulida), from the gall bladder of *Lophius budegassa* Spinola, 1807 (Teleostei: Lophiidae). *Acta Protozool.* 46: 247-256.
- Mansour, L., Prensier, G., Jemaa, S.B., Hassine, O.K.B., Metenier, G., Vivarès, C.P. & Cornillot, E. (2005) Description of a xenoma-inducing microsporidian, *Microgemma tincae* n. sp., a parasite of the teleost fish *Symphodus tinca* from Tunisian coasts. *Dis. Aquat. Org.* 65: 217-226.
- Marquardt, W.C. & Demeree, R.S. (1985) Microspora. In: Parasitology, Macmillan, New York, pp. 197-204.
- Martins, M.L. & Onaka, E.M. (2006) *Henneguya garavelli* n. sp. and *Myxobolus peculiaris* n. sp. (Myxozoa: Myxobolidae) in the gills of *Cyphocarax nagelli* (Osteichthyes: Curimatidae) from Rio do Peixe Reservoir, São José do Rio Pardo, São Paulo, Brazil. *Vet. Parasitol.* 137: 253-261.
- Martins, M.L. & Souza, V.N. (1997) *Henneguya piaractus* n. sp. (Myxozoa: Myxobolidae), a gill parasite of *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae), in Brazil. *Rev. Brasil. Biol.* 57: 239-245.
- Martins, M.L., Souza, V.N., Moraes, J.R.E. & Moraes F.R. (1999) Gill infection of *Leporinus macrocephalus* Garavello & Britski, 1988 (Osteichthyes: Anostomidae) by *Henneguya leporinicola* n. sp. (Myxozoa: Myxobolidae). Description, histopathology and treatment. *Rev. Brasil. Biol.* 59: 527-534.
- Matos, E. & Azevedo, C. (2004) Ultrastructural description of *Microsporidium brevirostris* sp. n., parasite of the teleostean *Brachyhypopomus brevirostris* (Hypopomidae) from the Amazon River. *Acta Protozool.* 43: 261-267.
- Matos, E., Corral, L. & Azevedo, C. (2003) Ultrastructural details of the xenoma of *Loma myrophis* (phylum Microsporidia) and extrusion of the polar tube during autoinfection. *Dis. Aquat. Org.* 54: 203-207.
- Matos, E., Tajdari, J. & Azevedo, C. (2005) Ultrastructural studies of *Henneguya rhamdia* n. sp. (Myxozoa) a parasite from the Amazon teleost fish *Rhamdia quelen* (Pimelodidae). *J. Eukaryot. Microbiol.* 52: 532-537.
- Matthews, J.L., Brown, A.M.V., Larison, K., Bishop-Stewart, J.K. & Kent, M.L. (2001) *Pseudoloma neurophilia*, n. g., n. sp., a new microsporidium from the central nervous system of the zebrafish. *J. Eukaryot. Microbiol.* 48: 227-233.
- Matthews, R.A. & Matthews, B.F. (1980) Cell and tissue reactions of turbot *Scophthalmus maximus* (L.) to *Tetramicra brevifilum* gen. n., sp. n. (Microspora). *J. Fish Dis.* 3: 495-515.
- McGourty, K.R., Kinziger, A.P., Hendrickson, G.L., Goldsmith, G.L., Casal, G. & Azevedo, C. (2007) A new microsporidian infecting the musculature of the endangered tidewater goby (Gobiidae). *J. Parasitol.* 93: 655-660.
- McClelland, R.S., Murphy, D.M. & Cone, D.K. (1997) Report of spores of *Henneguya salminicola* (Myxozoa) in human stool specimens: possible source of confusion with human spermatozoa. *J. Clin. Microbiol.* 35: 2815-2818.

- Michel, C., Maurand, J., Loubes, C., Chilmoneczyk, S. & de Kinkelin, P. (1989) *Heterosporis finki*, a microsporidian parasite of the angel fish *Pterophyllum scalare*: pathology and ultrastructure. *Dis. Aquat. Org.* 7: 103-109.
- Mladineo, I. & Bocina, I. (2006) *Ceratomyxa thunni* sp. n. (Myxozoa: Ceratomyxidae) in Atlantic northern bluefin tuna (*Thunnus thynnus*) caught in the Adriatic Sea, Island of Jabuka. *Zootaxa* 1224: 59-68.
- Modin, J.C. (1981) *Microsporidium rhabdophilia* n. sp. from rodlet cells of salmonid fishes. *J. Fish Dis.* 4: 203-211.
- Molnár, K. & Békési, L. (1993) Description of a new *Myxobolus* species, *M. colossomatis* n. sp. from the teleost *Colossoma macropomum* of the Amazon River basin. *J. Appl. Ichthyol.* 9: 57-63.
- Molnár, K., Cech, G. & Székely, C. (2008) *Myxobolus* species infecting the cartilaginous rays of the gill filaments in cyprinid fishes. *Acta Parasitol.* 53: 330-338.
- Molnár, K., Eszterbauer, E., Marton, S., Cech, G. & Székely, C. (2009) *Myxobolus erythrophthalmi* sp. n. and *Myxobolus shaharomae* sp. n. (Myxozoa: Myxobolidae) from the internal organs of rudd, *Scardinius erythrophthalmus* (L.), and bleak, *Alburnus alburnus* (L.). *J. Fish Dis.* 32: 219-231.
- Molnár, K., Marton, S., Eszterbauer, E. & Székely, C. (2006a) Comparative morphological and molecular studies on *Myxobolus* spp. infecting chub from the River Danube, Hungary, and description of *M. muellericus* sp. n. *Dis. Aquat. Org.* 73: 49-61.
- Molnár, K., Marton, S., Eszterbauer, E. & Székely, C. (2007) Description of *Myxobolus gayerae* sp. n. and re-description of *M. leuciscini* infecting European chub from the Hungarian stretch of the River Danube. *Dis. Aquat. Org.* 78: 147-153.
- Molnár, K., Ranzani-Paiva, M.J., Eiras, J.C. & Rodrigues, E.L. (1998) *Myxobolus macroplasmoidalis* sp. n. (Myxozoa: Myxosporidia), a parasite of the abdominal cavity of the characid teleost, *Salminus maxillosus*, in Brazil. *Acta Protozool.* 37: 241-245.
- Molnár, K., Székely, C., Mohamed, K. & Shaharom-Harrison, F. (2006b) Myxozoan pathogens in cultured Malaysian fishes. I. Myxozoan infections of the sutchi catfish *Pangasius hyphothermum* in freshwater cage cultures. *Dis. Aquat. Org.* 68: 209-218.
- Molnár, K., Székely, C., Mohamed, K. & Shaharom-Harrison, F. (2006c) Myxozoan pathogens in cultured Malaysian fishes. II. Myxozoan infections of redtail catfish *Hemibagrus nemurus* in freshwater cage cultures. *Dis. Aquat. Org.* 68: 219-226.
- Moncada, L.I., López, M.C., Murcia, M.I., Nicholls, S., León, F., Guío, O.L. & Corredor, A. (2001) *Myxobolus* sp., another opportunistic parasite in immunosuppressed patients. *J. Clin. Microbiol.* 39: 1938-1940.
- Moran, J.D.W., Whitaker, D.J. & Kent, M.L. (1999) A review of the myxosporean genus *Kudoa* Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture* 172: 163-196.
- Moris, D.J. & Adams, A. (2008) Sporogony of *Tetracapsuloides bryosalmonae* in the brown trout *Salmo trutta* and the role of the tertiary cell during the vertebrate phase of myxozoan life cycles. *Parasitology* 135: 1075-1092.
- Morrison, C.M. & Sprague, V. (1981a) Electron microscopical study of a new genus and new species of microsporidia in the gills of Atlantic cod *Gadus morhua* L. *J. Fish Dis.* 4: 15-32.

- Morrison, C.M. & Sprague, V. (1981b) Microsporidian parasites in the gills of salmonid fishes. *J. Fish Dis.* 4: 371-386.
- Morrison, C.M. & Sprague, V. (1983) *Loma salmonae* (Putz, Hoffman & Dunbar, 1965) in the rainbow trout, *Salmo gairdneri* Richardson, and *L. fontinalis* sp. nov. (Microsporida) in the brook trout, *Salvelinus fontinalis* (Mitchell). *J. Fish Dis.* 6: 345-353.
- Mullins, J.E., Powell, M., Speare, D.J. & Cawthorn, R. (1994) An intranuclear microsporidian in lumpfish *Cyclopterus lumpus*. *Dis. Aquat. Org.* 20: 7-13.
- Negredo, C. & Mulcahy, M.F. (2001) Actinosporean infections in oligochaetes in a river system in southwest Ireland with descriptions of three new forms. *Dis. Aquat. Org.* 46: 67-77.
- Negredo, C., Dillane, E. & Mulcahy, M.F. (2003) Small subunit ribosomal DNA characterisation of an unidentified aurantiactinomoxon form and its oligochaete host *Tubifex ignotus*. *Dis. Aquat. Org.* 54: 229-241.
- Nemeczek, A. (1926) Beiträge zur Kenntnis der Myxosporidienfauna Brasiliens. *Arch. Protistenk.* 54: 137-149.
- Nilsen, F. (2000) Small subunit ribosomal DNA phylogeny of Microsporidia with particular reference to genera that infect fish. *J. Parasitol.* 86: 128-133.
- Nilsen, F., Ness, A. & Nylund, A. (1995) Observations on an intracellular microsporidian in lymphoblasts from farmed Atlantic halibut larvae (*Hippoglossus hippoglossus* L.). *J. Eukaryot. Microbiol.* 42: 131-135.
- Okamura, B., Curry, A., Wood, T.S. & Canning, E.U. (2002) Ultrastructure of *Buddenbrockia* identifies it as a myxozoan and verifies the bilaterian origin of the Myxozoa. *Parasitology* 124: 215-223.
- Oumouna, M., Hallett, S.L., Hoffmann, R.W. & El-Matbouli, M. (2003) Seasonal occurrence of actinosporeans (Myxozoa) and oligochaetes (Annelida) at a trout hatchery in Bavaria, Germany. *Parasitol. Res.* 89: 170-184.
- Özer, A., Wootten, R. & Shinn, A.P. (2002) Survey of actinosporean types (Myxozoa) belonging to seven collective groups found in a freshwater salmon farm in Northern Scotland. *Folia Parasitol.* 49: 189-210.
- Padros, F., Palenzuela, O., Hispano, C., Tosas, O., Zarza, C., Crespo, S. & Álvarez-Pellitero, P. (2001) *Myxidium leei* (Myxozoa) infections in aquarium-reared Mediterranean fish species. *Dis. Aquat. Org.* 47: 57-62.
- Palenzuela, O., Redondo, M.J. & Álvarez-Pellitero, P. (2002) Description of *Enteromyxum scopthalmi* gen. nov., sp. nov. (Myxozoa), an intestinal parasite of turbot (*Scophthalmus maximus* L.) using morphological and ribosomal RNA sequence data. *Parasitology* 124: 369-379.
- Pekkarinen, M., Lom, J. & Nilsen, F. (2002) *Ovipleistophora* gen. n., a new genus for *Pleistophora mirandellae*-like microsporidia. *Dis. Aquat. Org.* 48: 133-142.
- Penido, J.C.N. (1927) Quelques nouvelles myxosporidies parasites des poissons d'eau douce du Brésil. *C. R. Séances Soc. Biol.* 97: 850-852.
- Peribanez, M.A., Luco, D.F., Garcia, L. & Castillo, J.A. (1997) The prevalence of proliferative kidney disease from the kidney and muscle of rainbow and brown trout in Aragon (Spain). *Prev. Vet. Med.* 32: 287-297.
- Perkins, F.O. (1991) 'Sporozoa': Apicomplexa, Microsporidia, Haplosporidia, Paramyxia, Myxosporidia and Actinosporidia. In: *Microscopic Anatomy of Invertebrates (Protozoa)* (Harrison, F. W. & Corliss, J. O., Eds.), vol. 1, Wiley-Liss Inc., New York, NY, pp. 261-331.

- Petri, M. & Schiødt, T. (1966) On the ultrastructural study of microsporidian development. *Z. Zellforsch.* 105: 33-54.
- Peuvel, I., Peyret, P., Méténier, G., Vivarès, C. P. & Delbac, F. (2002) The microsporidian polar tube : evidence for a third polar tube protein (PTP3) in *Encephalitozoon cuniculi*. *Mol. Biochem. Parasitol.* 122: 69–80.
- Peuvel-Fanget, I., Polonais, V., Brosseau, D., Texier, C., Kuhn, L., Peyret, P., Vivarès, C.P. & Delbac, F. (2006) EnP1 and EnP2, two proteins associated with the *Encephalitozoon cuniculi* endospore, the chitin-rich inner layer of the microsporidian spore wall. *Int. J. Parasitol.* 36: 309–318.
- Peyretailade, E., Biderre, C., Peyret, P., Duffieux, F., Metenier, G., Gouy, M., Michot, B. & Vivarès, C.P. (1998) Microsporidian *Encephalitozoon cuniculi*, a unicellular eukaryote with an unusual chromosomal dispersion of ribosomal genes and a LSU rRNA reduced to the universal core. *Nucleic Acids Res.* 26: 3513–3520.
- Pinto, C. (1928) Myxosporídeos e outros protozoários intestinaes de peixes observados na América do Sul. *Arch. Inst. Biol.* 1: 102–136.
- Pote, L.M., Hanson, L.A. & Shivaji. R. (2000) Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to *Henneguya* n. sp. (Myxozoa: Myxosporea). *J. Aquat. Anim. Health* 12: 230-240.
- Prunescu, C.C., Prunescu, P., Pucek, Z. & Lom, J. (2007) The first finding of myxosporean development from plasmodia to spores in terrestrial mammals: *Soricimyxum fegati* gen. et sp. n. (Myxozoa) from *Sorex araneus* (Soricomorpha). *Folia Parasitol.* 54: 159-164.
- Putz, R.E., Hoffman, G.L. & Dunbar, C.E. (1965) Two new species of *Pleistophora* (Microsporidea) from North America fish with a synopsis of Microsporidea of freshwater and euryhaline fishes. *J. Protozool.* 12: 228-236.
- Ralphs, J.R. & Matthews, R.A. (1986) Hepatic microsporidiosis of juvenile grey mullet, *Chelon labrosus* (Risso), due to *Microgemma hepaticus* gen. nov. sp. nov. *J. Fish Dis.* 9: 225-242.
- Reed, C.C., Basson, L., Van As, L.L. & Dyková, I. (2007) Four new myxozoans (Myxosporea: Bivalvulida) from intertidal fishes along the south coast of Africa. *Folia Parasitol.* 54: 283-292.
- Rocha, E., Matos, E. & Azevedo, C. (1992) *Henneguya amazonica* n. sp. (Myxozoa, Myxobolidae), parasitizing the gills of *Crenicichla lepidota* Heckel, 1840 (Teleostei, Cichlidae) from Amazon River. *Eur. J. Protistol.* 28: 273–277.
- Sandeep, B.V. & Kalavati, C. (1985) A new microsporidian, *Loma trichiuri* n. sp., from the gill of a marine fish, *Trichiurus salva* Cuv. (Trichiuridae). *Indian J. Parasitol.* 9: 257-259.
- Saraiva, A., Cruz, C. & Ferreira, S. (2000) Studies of *Myxidium rhodei* Léger, 1905 (Myxozoa: Myxosporea) on *Chondrostoma polylepis* from River Ave, North Portugal. *Bull. Eur. Assoc. Fish Pathol.* 20: 106-110.
- Saraiva, A. & Molnár, K. (1990) *Myxobolus portucalensis* n. sp. in the fins of European eel *Anguilla anguilla* (L.) in Portugal. *Rev. Ibér. Parasitol.* 50: 31-35.
- Schubert, G. (1969) Ultrascytologische untersuchungen na der spore des mikrosporidienart, *Heterosporis finki* gen. n., sp. n. *Z. Parasitenkd.* 32: 59-79.
- Schlegel, M., Lom, J., Stechmann, A., Bernhard, D., Leipe, D., Dyková, I. & Sogin, M.L. (1996) Phylogenetic

- analysis of complete small subunit ribosomal RNA coding region of *Myxidium lieberkuehni*: evidence that Myxozoa are Metazoa and related to the Bilateria. *Arch. Protist.* 147: 1–9.
- Schwartz, F.J. (1963) A new *Ichthyosporidium* parasite of the spot (*Leiostomus xanthurus*): a possible answer to recent oyster mortalities. *Progr. Fish Cult.* 25: 181-186.
- Shaw, R.W., Kent, M.L., Docker, M.F., Brown, A.M.V., Devlin, R.H. & Adamson, M.L. (1997) A new species of *Loma* (Microsporea) in shiner perch (*Cymatogaster aggregata*). *J. Parasitol.* 83: 296-301.
- Siddall, M.E., Martin, D.S., Bridge, D., Dessler, S.S. & Cone, D.K. (1995) The demise of a phylum of protists: phylogeny of the Myxozoa and other parasitic cnidaria. *J. Parasitol.* 81: 961-967.
- Siddall, M.E. & Whiting, M.F. (1999) Long-branch abstractions. *Cladistics* 15: 9-24.
- Sitjà-Bobadilla, A. & Álvarez-Pellitero, P. (1990) *Sphaerospora testiculares* sp. nov. (Myxosporea: Sphaerosporidae) in wild and cultured sea bass, *Dicentrarchus labrax* (L.), from the Spanish Mediterranean area. *J. Fish Dis.* 13: 193-203.
- Sitjà-Bobadilla, A. & Álvarez-Pellitero, P. (1992) Light and electron microscopic description of *Sphaerospora dicentrarchi* n. sp. (Myxosporea: Sphaerosporidae) from wild and cultured sea bass, *Dicentrarchus labrax* L. *J. Protozool.* 39: 273-281.
- Sitjà-Bobadilla, A. & Álvarez-Pellitero, P. (1993a) Light and electron microscopic description of *Ceratomyxa labracis* n. sp. and a redescription of *C. diplodae* (Myxosporea, Bivalvulida) from wild and cultured Mediterranean-sea bass *Dicentrarchus labrax* (L.) (Teleostei, Serranidae). *Syst. Parasitol.* 26: 215-223.
- Sitjà-Bobadilla, A. & Álvarez-Pellitero, P. (1993b) *Zschokkella mugilis* n. sp. (Myxosporea, Bivalvulida) from mullets (Teleostei, Mugilidae) of Mediterranean waters - light and electron-microscopic description. *J. Eukaryot. Microbiol.* 40: 755-764.
- Sitjà-Bobadilla, A. & Álvarez-Pellitero, P. (1995) Light and electron microscopic description of *Polysporoplasma* n. g. (Myxosporea, Bivalvulida), *Polysporoplasma sparis* n. sp. from *Sparus aurata* (L) and *Polysporoplasma mugilis* n. sp. from *Liza aurata* L. *Eur. J. Protistol.* 31: 77-89.
- Sitjà-Bobadilla, A. & Álvarez-Pellitero, P. (2001) *Leptotheca sparidarum* n. sp. (Myxosporea: Bivalvulida), a parasite from cultured common dentex (*Dentex dentex* L.) and Gilthead sea bream (*Sparus aurata* L.) (Teleostei: Sparidae). *J. Eukaryot. Microbiol.* 48: 627-639.
- Sitjà-Bobadilla, A., Palenzuela, O. & Álvarez-Pellitero, P. (1995) *Ceratomyxa sparusaurati* n. sp. (Myxosporea, Bivalvulida), a new parasite from cultured gilthead seabream (*Sparus aurata* L.) (Teleostei, Sparidae). Light and electron-microscopic description *J. Eukaryot. Microbiol.* 42: 529-539.
- Smothers, J.F., von Dohlen, C.D., Smithe, L.H.Jr. & Spall, R.D. (1994) Molecular evidence that the myxozoan protists are metazoans. *Science* 265: 1719-1721.
- Sprague, V. (1977) Systematics of the microsporidia. In: Comparative Pathobiology, (Bulla L.A. & Cheng T.C., Eds.), vol 2, Plenum Press, New York, pp. 1-510.
- Sprague, V. (1982) Microspora. In: Synopsis and classification of living organisms (Parker S.B. Ed.), Vol 1, McGraw-Hill, London, pp. 589-594.
- Sprague, V. & Becnel, J.J. (1998) Note on the name-author-date combination for the taxon Microsporidies Balbiani, 1882, when ranked as a Phylum. *J. Invertebr. Pathol.* 71: 91-94.
- Sprague, V., Becnel, J.J. & Hazard, E.I. (1992) Taxonomy of phylum Microspora. *Crit. Rev. Microbiol.* 18: 285-

395.

- Sprague, V. & Vernick, S.H. (1974) Fine structure of the cyst and some sporulation stages of *Ichthyosporidium* (Microsporida). *J. Protozool.* 21: 667-677.
- Summerfelt, R.C. (1964) A new microsporidian parasite from the golden shiner, *Notemigonus crysoleucas*. *Trans. Am. Fish. Soc.* 93: 6-10.
- Sutherland, D., Marcquenski, S., Marcino, J., Lom, J., Dyková, I., Hsu, H.M., Jahns, W., & Nilsen, F. (2000) *Heterosporis* sp. (Microspora: Glugeidae): a new parasite from *Perca flavescens* in Wisconsin and Minnesota. *Proc. of the 62nd Midwest Fish & Wildlife Conference, Minneapolis, MN, December 3-6, 2000*.
- Swearer, S.E. & Roberston, D.R. (1999) Life history, pathology, and description of *Kudoa ovivora* n. sp. (Myxozoa, Myxosporea): An ovarian parasite of Caribbean labroid fishes. *J. Parasitol.* 85: 337-353.
- Székely, C., Eiras, J.C. & Eszterbauer, E. (2005) Description of a new synactinomyxon type from the River Sousa, Portugal. *Dis. Aquat. Org.* 66: 9-14.
- Székely, C., Shaharom-Harrison, F., Cech, G., Mohamed, K. & Molnár, K. (2009a) Myxozoan pathogens of Malaysian fishes cultured in ponds and net-cages. *Dis. Aquat. Org.* 83: 49-57.
- Székely, C., Shaharom-Harrison, F., Cech, G., Ostoros, G. & Molnár, K. (2009b) Myxozoan infections in fishes of the Tasik Kenyir Water Reservoir, Terengganu, Malaysia. *Dis. Aquat. Org.* 83: 37-48.
- Székely, C., Sitjà-Bobadilla, A. & Álvarez-Pellitero, P. (2000) First report on the occurrence of an actinosporean stage (Myxozoa) in oligochaetes from Spanish freshwaters. *Acta Vet. Hung.* 48: 433-441.
- Székely, C., Yokoyama, H., Urawa, S., Timm, T. & Ogawa, K. (2003) Description of two new actinosporean types from a brook of Fuji Mountain, Honshu, and from Chitose River, Hokkaido, Japan. *Dis. Aquat. Org.* 53: 127-132.
- Tajdari, J., Matos, E., Mendonça, I. & Azevedo, C. (2005) Ultrastructural morphology of *Myxobolus testicularis* sp. n., parasite of the testis of *Hemiodopsis microlepis* (Teleostei: Hemiodontidae) from the NE of Brazil. *Acta Protozool.* 44: 377-384.
- Takizawa, H., Vivier, E. & Petriprez, A. (1975) Recherches cytochimiques sur la Microsporidie *Nosema bombycis* au cours de son développement chez le ver à soie (*Bombyx mori*). *J. Protozool.* 22: 359-368.
- Takvorian, P.M. & Cali, A. (1986) The ultrastructure of spores (Protozoa: Microsporida) from *Lophius americanus*, the angler fish. *J. Protozool.* 33: 570-575.
- Toguebaye, B.S., Marchand, B. & Faye, A. (1989) Preliminary observations on a microsporidian parasite of *Chloroscombrus chrysurus* Linnaeus, 1776 (Teleost fish). *Ann. Parasitol. Hum. Comp.* 64: 157-160.
- Tovar, J., Fischer, A. & Clark, C.G. (1999) The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. *Mol. Microbiol.* 32: 1013-1021.
- Tsai, S.H., Huang, W.F. & Wang, C.H. (2005) Complete sequence and gene organization of the *Nosema spodopterae*. *J. Eukaryot. Microbiol.* 52: 52-54.
- Tsai, S.J., Kou, G.H., Lo, C.F. & Wang, C.H. (2002) Complete sequence and structure of ribosomal RNA gene of *Heterosporis anguillarum*. *Dis. Aquat. Org.* 49: 199-206.
- Tun, T., Yokoyama, H., Ogawa, K. & Wakabayashi, H. (2000) Myxosporeans and their hyperparasitic microsporeans in the intestine of emaciated tiger puffer. *Fish Pathol.* 35: 145-156.

- Undeen, A.H. (1990) A proposed mechanism for the germination of microsporidian (Protozoa: Microspora) spores. *J. Theor. Biol.* 142: 223-235.
- Undeen, A.H. & Frixione, E. (1990) The role of osmotic pressure in the germination of *Nosema algerae* spores. *J. Protozool.* 37: 561-567.
- Undeen, A.H. & Vander Meer, R.K. (1994) Conversion of intrasporal trehalose into reducing sugars during germination of *Nosema algerae* (Protista: Microspora) spores: a quantitative study. *J. Eukaryot. Microbiol.* 41: 129-132.
- Vagelli, A., Parama, A., Sanmartin, M.L. & Leiro, J. (2005) *Glugea vincentiae* n. sp. (Microsporidia: Glugeidae) infecting the Australian marine fish *Vincentia conspersa* (Teleostei: Apogonidae). *J. Parasitol.* 91:152-157.
- Van de Peer, Y., Ali, A.B. & Meyer, A. (2000) Microsporidia: accumulating molecular evidence that a group of amitochondriate and suspectedly primitive eukaryotes are just curious fungi. *Gene* 246: 1-8.
- Vandermeer, J.W. & Gochnaeur, T.A. (1971) Trehalase activity associated with spores of *Nosema apis*. *J. Invertebr. Pathol.* 17: 38-41.
- Vávra, J. (1976) Structure of the Microsporidia. In: Comparative Pathobiology, Biology of the Microsporidia (A. Bulla, A., Jr. & T.C. Cheng, T.C., Eds.), vol. 1, Plenum Press, New York, pp. 1-86.
- Vávra, J. (2005) "Polar vesicles" of microsporidia are mitochondrial remnants ("mitosomes")? *Folia Parasitol.* 52: 193-195.
- Vinckier, D., Porchet, E., Vivier, E., Vávra, J. & Torpier, G. (1993) A freeze-fracture study of microsporidia (Protozoa: Microspora). II. The extrusion apparatus: polar filament, polaroplast, posterior vacuole. *Eur. J. Protistol.* 29: 370-380.
- Vita, P., Corral, L., Matos, E. & Azevedo, C. (2003) Ultrastructural aspects of the myxosporean *Henneguya astyanax* n. sp. (Myxozoa: Myxobolidae), a parasite of the Amazonian teleost *Astyanax keithi* (Characidae). *Dis. Aquat. Org.* 53: 55-60.
- Vita, P., Corral, L., Matos, E. & Azevedo, C. (2004) Ultrastructural description of *Agarella gracilis* Dunkerly, 1915 (Myxozoa, Chloromyxidae) parasite of the dipnoan *Lepidosiren paradoxa* from the River Amazon. *Eur. J. Protistol.* 40: 213-218.
- Voronin, V.N. (1974) Some microsporidians (Microsporidia, Nosematidae) from sticklebacks *Pungitius pungitius* and *Gasterosteus aculeatus* of the Finnish Bay. *Acta Protozool.* 13: 211-220.
- Vossbrinck, C.R. & Debrunner-Vossbrinck, B.A. (2005) Molecular phylogeny of the Microsporidia: ecological, ultrastructural and taxonomic considerations. *Folia Parasitol.* 52: 131-142.
- Vossbrinck, C.R., Maddox, J.V., Friedman, S., Debrunner-Vossbrinck, B.A. & Woese, C.R. (1987) Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature*, 6111: 411-414.
- Vossbrinck, C.R. & Woese, C.R. (1986) Eucaryotic ribosomes that lack a 5.8S RNA. *Nature*, 320: 287-288.
- Walliker, D. (1969) Myxosporidia of some Brazilian freshwater fishes. *J. Parasitol.* 55: 942-948.
- Wang, P.C., Huang, J.P., Tsai, M.A., Cheng, S.Y., Tsai, S.S., Chen, S.D., Chen, S.P., Chiu, S.H., Liaw, L.L., Chang, L.T. & Chen, S.C. (2005) Systemic infection of *Kudoa lutjanus* n. sp. (Myxozoa: Myxosporidia) in red snapper *Lutjanus erythropterus* from Taiwan. *Dis. Aquat. Org.* 67: 115-124.
- Wasson, K. & Peper, R.L. (2000) Mammalian microsporidiosis. *Vet. Pathol.* 37: 113-128.

- Weidner, E. (1972) Ultrastructural study of microsporidian invasion into cells. *Z. Parasitenkd.* 40: 227-242.
- Weidner, E. (1976) The microsporidian spore invasion tube. The ultrastructure, isolation and characterization of the protein comprising the tube. *J. Cell Biol.* 71: 23-34.
- Weidner, E., Byrd, W., Scarborough, A., Pleshinger, J. & Sibley, D. (1984) Microsporidian spore discharge and the transfer of polaroplast organelle membrane into plasma membrane. *J. Protozool.* 31: 195-198.
- Weidner, E. & Findley, A. (2002) Peroxisomal catalase in extrusion apparatus posterior vacuole of microsporidian spores. *Biol. Bull.* 203: 212.
- Weiser, J. (1976) Contribution to the classification of microsporidia. *Vest. Cesk. Spol. Zool.* 41: 308-320.
- Weiss, L.M., Edlind, T.D., Vossbrinck, C.R. & Hashimoto, T. (1999) Microsporidian molecular phylogeny: the fungal connection. *J. Eukaryot. Microbiol.* 46: 17S-18S.
- Weiss, L. & Vossbrinck, C. (1999) Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the microsporidia. In: The Microsporidia and Microsporidiosis (Wittner, M. & Weiss, L., Eds.), *Am. Soc. Microbiol.*, Washington, DC, pp. 129-171.
- Weissenberg, R. (1976) Microsporidium interactions with the host cells. In: Comparative Pathobiology, Biology of the Microsporidia (A. Bulla, A., Jr. & T. C. Cheng, T. C., Eds.), vol. 1, Plenum Press, New York, pp. 203-238.
- Weissenberg, R., (1968) Intracellular development of the microsporidian *Glugea anomala* Moniez in hypertrophying migratory cells of the fish *Gasterosteus aculeatus* L., an example of the formation of "xenoma tumors". *J. Protozool.* 15: 44-57.
- Whipps, C.M., Adlard, R.D., Bryant, M.S., Lester, R.J.G., Findlay, V. & Kent, M.L. (2003a) First report of three *Kudoa* species from Eastern Australia: *Kudoa thyrsites* from Mahi mahi (*Coryphaena hippurus*), *Kudoa amamiensis* and *Kudoa minithyrsites* n. sp. from sweeper (*Pempheris ypsilychnus*). *J. Eukaryot. Microbiol.* 50: 215-219.
- Whipps, C.M., Adlard, R.D., Bryant, M.S. & Kent, M.L. (2003b) Two unusual myxozoans, *Kudoa quadricornis* n. sp. (Multivalvulida) from the muscle of goldspotted trevally (*Carangoides fulvoguttatus*) and *Kudoa permulticapsula* n. sp. (Multivalvulida) from the muscle of Spanish mackerel (*Scomberomorus commerson*) from the Great Barrier Reef, Australia. *J. Parasitol.* 89: 168-173.
- Whipps, C.M., Grossel, G., Adlard, R.D., Yokoyama, H., Bryant, M.S., Munday, B.L. & Kent, M.L. (2004) Phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based on comparative ribosomal DNA sequence analysis. *J. Parasitol.* 90: 618-622.
- Williams, B.A., Hirt, R.P., Lucocq, J.M. & Embley, T.M. (2002) A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. *Nature* 418: 865-869.
- Wolf, K. & Markiw, M.E. (1984) Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. *Science* 255: 1449-1452.
- Wood, P.J., Siddiqui, I.R., Vandermeer, J.W. & Gochnauer, T.A. (1970) Carbohydrates of *Nosema apis* spores. *Carbohydr. Res.* 15: 154-158.
- Work, T.M., Takata, G., Whipps, C.M. & Kent, M.L. (2008) A new species of *Henneguya* (Myxozoa) in the big-eyed scad (*Selar crumenophthalmus*) from Hawaii. *J. Parasitol.* 94: 524-529.
- Wu, Z., Li, Y., Pan, G., Tan, X., Hu, J., Zhou, Z. & Xiang, Z. (2008) Proteomic analysis of spore wall proteins

- and identification of two spore wall proteins from *Nosema bombycis* (Microsporidia). *Proteomics* 8: 2447–2461.
- Xiao, C.X. & Desser, S.S. (1998a) Actinosporean stages of myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario: new forms of triactinomyxon and raabeia. *J. Parasitol.* 84: 998–1009.
- Xiao, C.X. & Desser, S.S. (1998b) Actinosporean stages of myxozoan parasites of oligochaetes from Lake Sasajewun, Algonquin Park, Ontario: new forms of Echinactinomyxon, Neoactinomyxon, Aurantiactinomyxon, Guyenotia, Synactinomyxon and Antonactinomyxon. *J. Parasitol.* 84: 1010–1019.
- Xu, Y., Takvorian, P., Cali, A., Wang, F., Zhang, H., Orr, G. & Weiss, L.M. (2006) Identification of a new spore wall protein from *Encephalitozoon cuniculi*. *Infect. Immun.* 74: 239–247.
- Yanagida, T., Nomura, Y., Kimura, T., Fukuda, Y., Yokoyama, H. & Ogawa, K. (2004) Molecular and morphological redescrptions of enteric myxozoans, *Enteromyxum leei* (formerly *Myxidium* sp. TFI) and *Enteromyxum fugu* comb. n. (syn. *Myxidium fugu*) from cultured tiger puffer. *Fish Pathol.* 39: 137–143.
- Yokoyama, H. (2003) A review: gaps in our knowledge on myxozoan parasites of fishes. *Fish Pathol.* 38: 125–136.
- Yokoyama, H., Kageyama, T., Ohara, K. & Yanagida, T. (2007) *Myxobolus nagaraensis* n. sp. (Myxozoa: Myxosporea) causes abdominal distension of freshwater goby *Rhinogobius* sp. OR type from the Nagara River. *Fish. Sci.* 73: 633–639.
- Yokoyama, H., Lee, S.J. & Bell, A. (2002) Occurrence of a new microsporidian in the skeletal muscle of the flying fish *Cypselurus pinnibarbus japonicus* (Exocoetidae) from Yakushima, Japan. *Folia Parasitol.* 49: 9–15.
- Yokoyama, H., Ogawa, K. & Wakabayashi, H. (1993) Involvement of *Branchiura sowerbyi* (Oligochaeta, Annelida) in the transmission of *Hoferellus carassii* (Myxosporea, Myxozoa), the causative agent of kidney enlargement disease (KED) of goldfish *Carassius auratus*. *Fish Pathol.* 28: 135–139.
- Youssef, N.N. & Hammond, D.M. (1971) The fine structure of the developmental stages of the microsporidian *Nosema apis* Zander. *Tis. Cell* 3: 283–294.
- Yurakhno, V.M., Ovcharenko, M.O., Holzer, A.S., Sarabeev, V.L. & Balbuena, J.A. (2007) *Kudoa unicipsula* n. sp. (Myxosporea: Kudoidae) a parasite of the Mediterranean mullets *Liza ramada* and *L. aurata* (Teleostei: Mugilidae). *Parasitol. Res.* 101: 1671–1680.
- Zhao, Y.J., Sun, C.Y., Kent, M.L., Deng, J.L. & Whipps, C.M. (2008a) Description of a new species of *Myxobolus* (Myxozoa: Myxobolidae) based on morphological and molecular data. *J. Parasitol.* 94: 737–742.
- Zhao, Y.J., Zhou, Y., Kent, M.L. & Whipps, C.M. (2008b) Replacement of the preoccupied name *Davisia* Laird, 1953 and description of a new myxozoan species (Myxosporea: Sinuolineidae) from *Sebastiscus marmoratus* (Cuvier, 1829) in the East China Sea. *J. Parasitol.* 94: 269–279.
- Zrzavý, J. & Hypša, V. (2003) Myxozoa, *Polypodium*, and the origin of the Bilateria: The phylogenetic position of “Endocnidozoa” in light of the rediscovery of *Buddenbrockia*. *Cladistics* 19: 164–169.

1. 6. Objectivos

Os peixes são hospedeiros susceptíveis de serem infectados, entre outros, por vários organismos microscópicos, tais como vírus, bactérias, fungos, amibas, apicomplexos, flagelos, ciliados, microsporídios e mixosporídios. Estes dois últimos grupos foram descritos, pela primeira vez, nos finais do século XIX, a parasitar peixes, induzindo em alguns casos grandes mortalidades. A presença dos microsporídios e/ou mixosporídios é facilmente detectada, devido ao facto de algumas espécies desenvolverem, em vários órgãos, estruturas macroscópicas semelhantes a cistos/xenomas.

A identificação e caracterização do agente infectivo em peixes é muito importante em termos sanitários. Esta situação reveste-se de carácter primordial, principalmente em aquacultura intensiva, quer para efeitos de consumo alimentar ou de ornamentação. Estudos experimentais têm indicado que a identificação dos microsporídios e mixosporídios ao nível da espécie, bem como a sua detecção em estádios iniciais, são aspectos importantes, na medida em que interfere na escolha das drogas a utilizar para fins terapêuticos.

Após uma pesquisa bibliográfica aos grupos dos microsporídios e mixosporídios, constatou-se que são escassos e, em alguns casos, superficiais, os trabalhos efectuados na ictiofauna proveniente do território português e brasileiro, comparativamente a outras regiões geográficas. Assim, nesta tese foram delineados alguns objectivos, com a finalidade de contribuir para o estado da arte destes grupos de parasitas:

1. Diagnosticar parasitoses por microsporídios e mixosporídios em peixes teleósteos e cartilagíneos de diferentes habitats (água doce, salobra e salgada) provenientes da fauna portuguesa e brasileira. A escolha das espécies a estudar teve, por base, a ausência de registo de parasitoses, muito possivelmente devido à inexistência de estudos. Na medida do possível, procurou-se estudar parasitoses em espécies com possível interesse comercial em aquacultura.
2. Caracterizar os microsporídios e mixosporídios com base em estudos morfológicos e ultrastruturais, nomeadamente das diferentes fases do ciclo de vida, com a finalidade de os classificar em termos taxonómicos.
3. Caracterizar, através da biologia molecular, os genes ribossomais, nomeadamente o SSU rDNA, dos microsporídios e mixosporídios previamente diagnosticados em estudos anteriores, visando o estabelecimento de relações filogenéticas com as espécies afins.
4. Avaliar epidemiologicamente a infecção das microsporidioses e mixosporidioses

diagnosticadas e analisar, igualmente, o grau de patogenicidade (interacções parasita-hospedeiro) através de estudos microscópicos.

5. Identificação de eventuais novas taxa (géneros e espécies) de microsporídios e mixosporídios, após análise dos dados obtidos através dos estudos morfológicos e filogenéticos.

PARTE II

MICROSPORIDIOSES

Capítulo 2

**A NEW MICROSPORIDIAN PARASITE, *POTASPORA MORHAPHIS* N. GEN., N. SP.
(MICROSPORIDIA) INFECTING THE TELEOSTEAN FISH
POTAMORHAPHIS GUIANENSIS FROM AMAZON RIVER. MORPHOLOGICAL,
ULTRASTRUCTURAL AND MOLECULAR CHARACTERIZATION**

Parasitology (2008) 135: 1053-1064

Graça Casal, Edilson Matos, M. Leonor Teles-Grilo & Carlos Azevedo

A new microsporidian parasite, *Potasporea morhaphis* n. gen., n. sp. (Microsporidia) infecting the Teleostean fish, *Potamorhaphis guianensis* from the River Amazon. Morphological, ultrastructural and molecular characterization

G. CASAL^{1,2,3}, E. MATOS⁴, M. L. TELES-GRILLO⁵ and C. AZEVEDO^{1,3*}

¹Department of Cell Biology, Institute of Biomedical Sciences, University of Porto (ICBAS/UP), Lg. A. Salazar no. 2, P-4099-003 Porto, Portugal

²Department of Sciences, High Institute of Health Sciences, P-4585-116 Gandra, Portugal

³Laboratory of Pathology, Centre for Marine Environmental Research (CIIMAR/UP), 4050-123 Porto, Portugal

⁴Carlos Azevedo Research Laboratory, Federal Rural University of Amazonia, 66.077-530 Belém (Pará), Brazil

⁵Genetics Molecular Laboratory, Institute of Biomedical Sciences, University of Porto (ICBAS/UP), Lg. A. Salazar no. 2, P-4099-003 Porto, Portugal

(Received 3 March 2008; revised 9 May 2008; accepted 10 May 2008)

SUMMARY

A fish-infecting Microsporidia *Potasporea morhaphis* n. gen., n. sp. found adherent to the wall of the coelomic cavity of the freshwater fish, *Potamorhaphis guianensis*, from lower Amazon River is described, based on light microscope and ultrastructural characteristics. This microsporidian forms whitish xenomas distinguished by the numerous filiform and anastomosed microvilli. The xenoma was completely filled by several developmental stages. In all of these stages, the nuclei are monokaryotic and develop in direct contact with host cell cytoplasm. The merogonial plasmodium divides by binary fission and the disporoblastic pyriform spores of sporont origin measure $2.8 \pm 0.3 \times 1.5 \pm 0.2 \mu\text{m}$. In mature spores the polar filament was arranged into 9–10 coils in 2 layers. The polaroplast had 2 distinct regions around the manubrium and an electron-dense globule was observed. The small subunit, intergenic space and partial large subunit rRNA gene were sequenced and maximum parsimony analysis placed the microsporidian described here in the clade that includes the genera *Kabatana*, *Microgemma*, *Spraguea* and *Tetramicra*. The ultrastructural morphology of the xenoma, and the developmental stages including the spores of this microsporidian parasite, as well as the phylogenetic analysis, suggest the erection of a new genus and species.

Key words: Amazonian fish, parasite, Microsporidia, ultrastructure, developmental stages, phylogeny, *Potasporea morhaphis* n. gen., n. sp.

INTRODUCTION

The phylum Microsporidia Balbiani, 1882 is represented by at least 144 available genera. It is characterized by unicellular eukaryotic microorganisms living as obligate intracellular parasites, commonly infecting fishes, insects, crustaceans, and other invertebrate and vertebrate groups from different geographical areas (Lom and Dyková, 1992; Sprague *et al.* 1992; Larsson, 1999; Lom, 2002). In a recent paper, Lom and Nilsen (2003) described the following 15 microsporidian genera as infecting fish: *Glugea* Thélohan, 1891; *Pleistophora* Gurley,

1893; *Ichthyosporidium* Caullery and Mesnil, 1905; *Heterosporis* Schubert, 1969; *Nosemoides* Vinckier, 1975; *Spraguea* Weissenberg, 1976; *Loma* Morrison and Sprague, 1981; *Tetramicra* Matthews and Matthews, 1980; *Microgemma* Ralphs and Matthews, 1986; *Microfilum* Faye, Toguebaye and Bouix, 1991; *Nucleospora* Hedrick, Graff and Baxa, 1991; *Neonosemoides* Faye, Toguebaye and Bouix, 1996; *Kabatana* Lom, Dyková and Tonguthai, 1999; *Pseudoloma* Matthews, Brown, Larison, Bishop-Stewart, Rogers and Kent, 2001; *Ovipleistophora* Pekkarinen, Lom and Nilsen, 2002. Recently 2 new genera were identified as infecting fish: *Amazonspora* in the gills of an Amazonian fish (Azevedo and Matos, 2003) and *Myosporidium* in muscle of commercial hake (*Merluccius* sp.) from fisheries near Namibia (Baquero *et al.* 2005).

There is very little knowledge about microsporidiosis in the ichthyological fauna of South

* Corresponding author: Department of Cell Biology, Institute of Biomedical Sciences, University of Porto, Lg. A. Salazar no. 2, P-4099-003 Porto, Portugal. Tel: +351 22 206 22 00. Fax: +351 22 206 22 32/33. E-mail: azevedoc@icbas.up.pt, gcasal@icbas.up.pt

America. Two Microsporidia were found in Amazonian fishes *Loma myrophis* (Azevedo and Matos, 2002; Matos *et al.* 2003) and *Microsporidium brevirostris* (Matos and Azevedo, 2004) in *Myrophis platyrhynchus* and *Brachyhyppopomus brevirostris* host species respectively.

In this paper, we described a new genus and new species of a microsporidian through morphological and ultrastructural observations, with special reference to the ultrastructural aspects of the xenoma wall and the spore differentiation. Phylogenetic relationships comparing the *Potasporea morhaphis* SSU rRNA gene with that of other fish infecting microsporidian species was also done. The morphological characteristics and taxonomic position are discussed.

MATERIALS AND METHODS

Fish, location of infection and prevalence

Thirty specimens of freshwater teleost fish *Potamorhaphis guianensis* Schomburgk, 1843 (Teleostei, Belontiidae) (Brazilian common name 'Peixe-Agulha'), were collected from the estuarine region of the Amazon River (01°11'S/47°18'W) near the city of Belém (Pará State), Brazil. The specimens were anaesthetized by MS 222 (Merck) and later measured (19–25 cm in length). Infection was determined by the presence of several xenomas located in the coelomatic cavity near the anal region. The prevalence of infection was 40% (12 fishes in 30 examined), in both sexes.

Light (LM) and transmission electron microscopy (TEM)

For LM smears of xenoma and free spores were observed directly without any fixation or stain by a light microscope equipped with Nomarski interference-contrast (DIC) optics.

For ultrastructural studies, the xenomas were excised and fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4 °C for 24 h. After washing overnight in the same buffer at 4 °C and post-fixation in 2% osmium tetroxide in the same buffer and temperature for 3 h, the fragments were dehydrated through a graded ethanol ascending series, followed by propylene oxide (3 changes of 2 h each) and embedded in Epon (12 h in each change). Semi-thin sections were stained with methylene blue-Azur II and observed by DIC optics. Ultrathin sections were contrasted with aqueous uranyl acetate and lead citrate and observed with a JEOL 100CXII TEM, operated at 60 kV.

DNA isolation and PCR amplification

Several cysts were dissected from fishes, following homogenization to isolate the spores, and were then

stored in 80% ethanol at 4 °C. The genomic DNA of about 5×10^6 spores was extracted using a GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma) following the manufacturer's instructions for animal tissue, except for the incubation time. The DNA was stored in 50 µl of TE buffer at –20 °C until used. The DNA concentration was estimated with the Qubit™ Fluorometer (Invitrogen). The majority of the region coding for the small subunit (SSU) rRNA gene was amplified by PCR using the primers V1f (5'CACCAGG-TTGATTCCTGCC3') and 1492r (5'GGTTACCTTGTTACGACTT3') (Vossbrinck *et al.* 1993; Nilsen, 2000). To amplify the 3'-end of the SSU, internal transcribed spacer (ITS) and 5'-end of the large subunit (LSU) rRNA gene, HG4F (5'GCGGCTTAATTTGACTCAAC) and HG4R (5'TCTCCTTGGTCCGTGTTTCAA) primers were used (Gatehouse and Malone, 1998). To obtain the 5'-end of the SSU gene region a primer was designed (454r – 5'AATTAAGCCGCACACTCCAC). PCR was carried out in 50 µl reactions using 10 pmol of each primer, 10 nmol of each dNTP, 2 mM of MgCl₂, 5 µl of 10X Taq polymerase buffer, 1.25 units Taq DNA polymerase (Invitrogen products), and 3 µl of the genomic DNA. The reactions were run on Hybaid PxE Thermocycler (Thermo Electron Corporation, Milford, MA). The amplification program consisted of 94 °C denaturation for 5 min, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min. A final elongation step was performed at 72 °C for 10 min. Five µl aliquots of PCR products were visualized with ethidium bromide staining after running on a 1% agarose gel.

DNA sequencing

PCR products for the SSU gene and ITS region have approximate sizes of 1400 bp and 1100 bp respectively. They were cleaned using the MinElute PCR purification kit (QIAGEN) and then 3 purified PCR products were sequenced in both directions. Sequencing was done using BigDye Terminator v1.1 of Applied Biosystems Kit and the sequence reactions were run on an ABI3700 DNA analyser Perkin-Elmer, Applied Biosystems, Stabvida, Co., Oeiras, Portugal).

Distance and phylogenetic analysis

To evaluate the relationship of *Potasporea morhaphis* to other Microsporidia, we have used the 42 rDNA sequences, listed with their hosts in Table 1, obtained from GenBank data. The corresponding sequences and GenBank/NCBI Accession number of *Endoreticulatus schubergi* (L39109), *Enterocytozoon bieneusi* (L07123), *Vairimorpha necatrix* (Y00266) and *Vittaforma corneae* (L39112) were used as the

Table 1. Hosts and GenBank Accession numbers for the SSU rRNA sequences of 42 microsporidian that parasite fishes species used in the phylogenetic analyses

Microsporidian	Host	Accession number
<i>Glugea anomala</i>	<i>Gasterosteus aculeatus</i>	AF044391
<i>Glugea atherinae</i>	<i>Atherina prebyster</i>	U15987
<i>Glugea plecoglossi</i>	<i>Plecoglossus altivelis</i>	AJ295326
<i>Glugea stephani</i>	<i>Platichthys flesus</i>	AF056015
<i>Glugea</i> sp. GS1	<i>Gasterosteus aculeatus</i>	AJ295325
<i>Glugea</i> sp.	<i>Epinephelus awoara</i>	AY090038
<i>Heterosporis anguillarum</i>	<i>Anguilla japonica</i>	AF387331
<i>Heterosporis</i> sp. PF	<i>Perca flavescens</i>	AF356225
<i>Ichthyosporidium</i> sp.	<i>Leiostomus xanthurus</i>	L39110
<i>Kabatana takedai</i>	<i>Oncorhynchus masu</i>	AF356222
<i>Kabatana newberryi</i>	<i>Eucyclogobius newberryi</i>	EF202572
<i>Kabatana seriola</i>	<i>Seriola quinqueradiata</i>	AJ295322
<i>Loma acerinae</i>	<i>Gymnocephalus cernuus</i>	AJ252951
<i>Loma embiotocia</i>	<i>Cymatogaster aggregate</i>	AF320310
<i>Loma salmonae</i>	<i>Oncorhynchus tshawytscha</i>	U78736
<i>Loma</i> sp.	<i>Encelyopus cimbrius</i>	AF104081
<i>Microgemma caulleryi</i>	<i>Hyperoplus lanceolatus</i>	AY033054
<i>Microgemma tincae</i>	<i>Symphodus tinca</i>	AY651319
<i>Microgemma vivaresi</i>	<i>Taurulus bubalis</i>	AJ252952
<i>Microsporidium cypselurus</i>	<i>Cypselurus pinnatibarbus japonicus</i>	AJ300706
<i>Microsporidium prosopium</i>	<i>Prosopium williamsoni</i>	AF151529
<i>Microsporidium</i> sp. GHB1	<i>Sparus aurata</i>	AJ295324
<i>Microsporidium</i> sp. RSB1	<i>Pagrus major</i>	AJ295323
<i>Microsporidium</i> sp. STF	<i>Salmo trutta fario</i>	AY140647
<i>Microsporidium</i> MYX1	<i>Takifugu rubripes</i>	AJ295329
<i>Myosporidium merluccius</i>	<i>Merluccius</i> sp.	AY530532
<i>Nucleospora salmonis</i>	<i>Oncorhynchus tshawytscha</i>	U78176
<i>Ovipleistophora mirandellae</i>	<i>Gymnocephalus cernuus</i>	AF356223
<i>Ovipleistophora ovariae</i>	<i>Notemigonus crysoleucas</i>	AJ252955
<i>Pleistophora ehrenbaumi</i>	<i>Anarhichas lupus</i>	AF044392
<i>Pleistophora finsterrensis</i>	<i>Micromesistius poutassou</i>	AF044393
<i>Pleistophora hippoglossoides</i>	<i>Hippoglossoides platessoides</i>	AJ252953
<i>Pleistophora typicalis</i>	<i>Myoxocephalus scorpius</i>	AF044387
<i>Pleistophora</i> sp. 1	<i>Glyptocephalus cynoglossus</i>	AF044394
<i>Pleistophora</i> sp. 2	<i>Zeugopterus punctatus</i>	AF044389
<i>Pleistophora</i> sp. 3	<i>Taurulus bubalis</i>	AF044390
<i>Pseudoloma neurophilia</i>	<i>Danio rerio</i>	AF322654
<i>Spraguea americana</i>	<i>Lophius americanus</i>	AF056014
<i>Spraguea lophii</i> (1)	<i>Lophius piscatorius</i>	AF104086
<i>Spraguea lophii</i> (2)	<i>Lophius piscatorius</i>	AF033197
<i>Spraguea</i> sp.	<i>Lophius litulon</i>	AY465876
<i>Tetramicra brevifilum</i>	<i>Scophthalmus maximus</i>	AF364303

outgroup. Sequences were aligned as described by Azevedo *et al.* (2006). Alignment using Clustal W (Thompson *et al.* 1994), in MEGA 4 software (Tamura *et al.* 2007), with an opening gap penalty of 10 and a gap extension penalty of 4 was done for both pairwise and multiple alignments. Subsequent phylogenetic and molecular evolutionary analyses were conducted using MEGA 4, with the 42 rDNA sequences for microsporidian species and the outgroup species selected. Distance estimation was carried out using the Kimura-2 parameters model distance matrix for transitions and transversions. For the phylogenetic tree reconstructions, maximum parsimony analysis was conducted using the close neighbour interchange (CNI) heuristic option with a

search factor of 2 and random initial trees addition of 2000 replicates. Bootstrap values were calculated over 100 replicates.

RESULTS

Macroscopical and light microscopical observations

Some spherical to ellipsoidal whitish cysts (xenomas) were macroscopically observed adherent to the internal wall of the coelomatic cavity of the teleost fish near the anal region. These xenomas with a variable number (up to 7) could reach dimensions of up to ~0.8 mm (Fig. 1A). In semi-thin section, the thick xenoma wall showed a lucent area surrounded by a

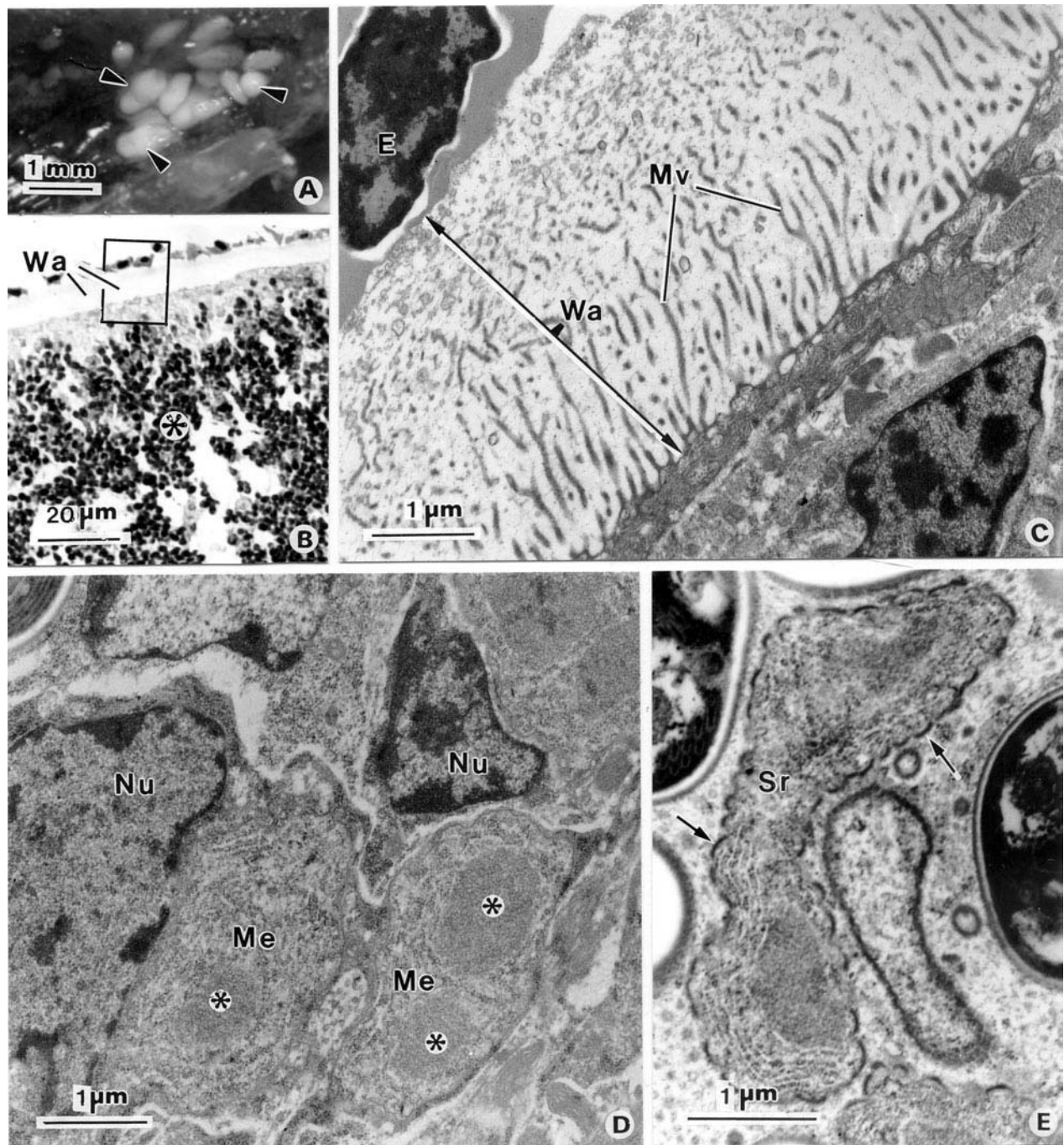


Fig. 1. (A–E) Light and transmission electron micrographs of the microsporidian *Potaspora morhaphis* n. gen., n. sp. (A) Some xenomas (arrowheads) on the abdominal cavity. (B) Semi-thin section of the xenoma periphery, showing the xenoma wall (Wa) and the matrix of the xenoma containing developmental stages including spores (*). The boxed area is enlarged in the figure C. (C) Ultrathin section of the xenoma wall (Wa) showing numerous filiform and anastomosed microvilli-like structures (Mv) projected toward the periphery and, externally, an erythrocyte nucleus (E) in contact with the wall. (D) Ultrathin section of the internal periphery of the xenoma, showing several host cell nuclei (Nu) and a dividing meront (Me), showing some nuclei (*), in direct contact with the host-cell cytoplasm. (E) Ultrathin section of a sporogonial plasmodium in division (Sr) showing the wall formation by a gradual deposition of the dense material on the membrane (arrows).

layer of cells and inside was filled with numerous spores and other developmental stages (Fig. 1B). After rupture of the xenoma wall, the free spores were easily identified as belonging to the phylum Microsporidia (Fig. 4).

Ultrastructural observations

Xenoma. The xenoma wall was formed by numerous filiform and anastomosed microvilli-like structures, with a regular diameter, projected from the

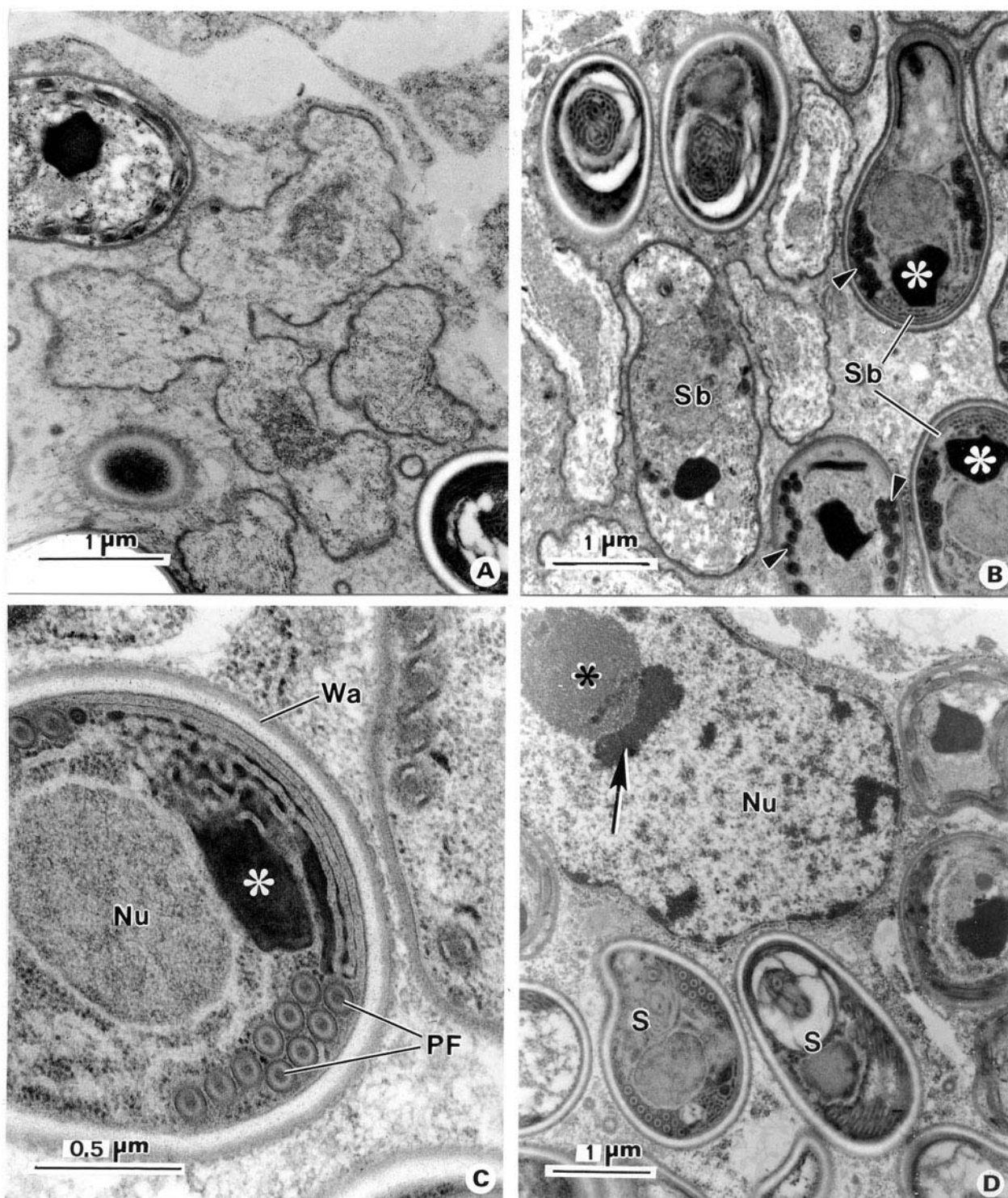


Fig. 2. (A–D) Late sporogonic development of the microsporidian *Potasporea morhaphis* n. gen., n. sp. (A) Sporogonial plasmodium in division giving rise to 4 sporoblasts. (B) Some sporoblasts (Sb) in different developmental stages showing a dense globule (*) that gradually decreases in density and the polar filament in differentiation (arrowheads). (C) Detail of an immature spore showing the dense globule (*) strongly associated to the polar filament formation (PF). Nucleus (Nu). (D) Ultrastructure of a host cell showing the nucleus (Nu) and the nucleolus (*) with peripheral nucleolar heterochromatin (arrow) surrounding the nucleolus. The mature spores (S) are contained in the cytoplasm of the host cell.

surface toward the periphery. The microvilli were intermingled by an amorphous, finely granular material. In favourable sections the microvilli were 4–5 µm long (Fig. 1C). Some zones towards the

apical region of the microvilli were in contact with an external layer of erythrocytes (Fig. 1B, C). The asynchronous development was characterized by several merogonic and early sporogonic stages

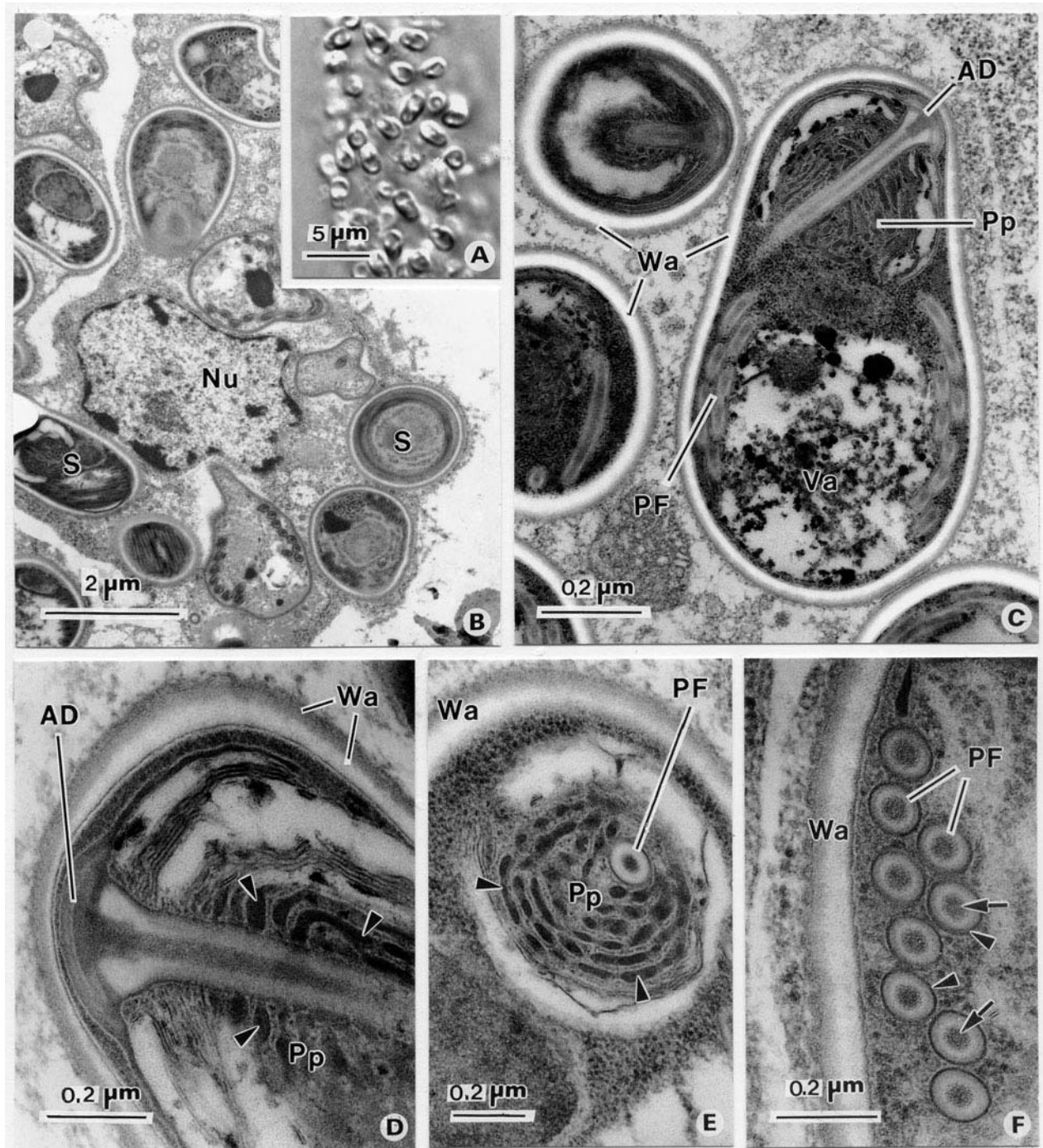


Fig. 3. (A–F) Morphological and ultrastructural details of the microsporidian *Potaspora morhaphis* n. gen., n. sp. (A) Several isolated mature spores observed by DIC microscopy. (B) Some spores (S) in different stages of development in close contact with the cytoplasm of the host cell that shows the (Nu). (C) Ultrathin longitudinal and two transverse sections of a spore showing the typical microsporidian structures and organelles. Wa, wall; AD, anchoring disc; Pp, polaroplast; PF, polar filament; Va, vacuole. (D) Ultrastructural detail of the apical region of a spore showing anchoring disc (AD) in close contact with the wall (Wa) and the lamellar region of the polaroplast (Pp) containing dense material (arrowheads). (E) Ultrastructural detail of a transverse section of a spore showing the lamellar region of the polaroplast (Pp) containing dense material (arrowheads), the polar filament (PF) and the wall (Wa). (F) Ultrastructural detail of the wall (Wa), the polar filament coils (PF) showing the external membrane (arrowheads), as well as a central dense mass (arrows).

predominantly along the xenoma periphery, while immature and mature spores were more internally localized in the centre of the xenoma (Fig. 1B). Internally, the matrix of the xenoma possessed

numerous host cells, their nuclei showing a prominent nucleolus, and great mass of peripheral heterochromatin (Figs 1D and 2D). Inside each host cell, the parasite was always in direct contact with the

cytoplasm of host cells, without any surrounding membrane and frequently in the same stage of the developmental life cycle (Fig. 3B).

Description of the development stages

Meronts. These cells grow into multinucleate plasmodia. They appeared in ultrathin sections as round to elliptical uninucleated or binucleated cells always with the nuclei unpaired. In these cells, the chromatin was homogeneous in contrast to the nuclei of the host cells in which the chromatin was organized in dense masses. Their cytoplasm possessed numerous free ribosomes and was uniformly granular and poorly endowed with cytoplasmatic organelles (Fig. 1D). Meronts divided by binary fission and transformed into sporonts (Figs 1E and 2A).

Sporonts. The transition from merogony to sporogony is characterized by the acquisition of a thick and dense cell coat located on the outer surface of the plasmalemma (Figs 1E and 2A). Early on, the discontinuous coat of the sporogony stages appeared to be formed by isolated patches (Fig. 1E). They were rounded and uninucleated cells and in their cytoplasm several well-developed cisternae of rough endoplasmatic reticulum and small vesicles were observed. Before the sporont transformed in uninucleate sporoblasts they divided again by multiple fission giving rise to 4 sporoblasts (Figs 1E and 2A).

Sporoblasts. The sporoblasts do not have the capacity to divide further and gradually differentiate the organelles typical of the spores, composed of an anchorage disc, polaroplast, polar filament and posterior vacuole. In the sporoplasm a very electron-dense irregularly-shaped globule that persists until sporogenesis is concluded, was frequently observed (Fig. 2B). This structure is associated with the reticular body present in the sporoplasm during differentiation of the spores and later appears to be immersed into a posterior vacuole (Fig. 2C).

Systematic position

Phylum Microsporidia Balbiani, 1882; Class Haplophasea Sprague, Becnel and Hazard, 1992; Family Tetramicridae Matthews and Matthews, 1980.

Description of the genus

Name: *Potasporea* n. gen.

Diagnosis: Xenoma formation has several nuclei and the plasmalemma differentiates numerous filiform and anastomosed microvilli-like structures projected externally. In all developmental stages the nuclei are monokaryotic and develop in direct

contact with host cell cytoplasm. The merogony stages are binucleated and divide by binary fission. Each meront differentiates into a sporont by a gradual development of a thick electron-dense coat. The sporont divides by multiple fission into 4 sporoblasts. In this stage a very electron-dense irregular-shaped body differentiates. Monomorphic spores containing polaroplast with 2 distinct kinds of lamellae.

Description of the species

Name: *Potasporea morhaphis* n. gen., n. sp.

Type host: *Potamorhaphis guianensis* Schomburgk, 1843 (Teleostei, Belonidae).

Type Locality: Estuarine region of the Amazon river (01°11'S and 47°18'W) near the city of Belém (Pará State), Brazil.

Location in the host: Xenoma in the coelomatic cavity near the anal region.

Prevalence of infection: Twelve of 30 (40%).

Type specimens: One slide containing mature free spores and another with semi-thin sections of tissues containing spores and different developmental stages of hapantotype were deposited in the International Protozoan Type Slide Collection at Smithsonian Institution Washington, DC. 20560, USA with acquisition number (USNM 1113817). The histological semi-thin sections containing different developmental stages were deposited at the laboratory of the senior author.

Etymology: The genus name is the prefix from the name of the host genus and the specific name is derived from the suffix of the host genus name.

Description of the spores: Pyriform spores measuring $2.8 \pm 0.3 \times 1.5 \pm 0.2 \mu\text{m}$ and containing all the typical characteristic structures of the Microsporidia (Figs 3A, C and 4). The spore wall was about 125 (114–131) nm thick ($n=30$), except for the anterior end where the anchoring disc contacted with the wall, which was $\sim 50\text{--}70$ nm thick (Fig. 3C, D). The spore wall consisted of an electron-lucent endospore and an electron-dense exospore each with just about the same thickness (Fig. 3C, D, E, F). The exospore was externally surrounded by a thin irregular layer of granular material (Fig. 3D).

The anchoring disc is located in the apical region of the spore in an eccentric position in relation to the spore axis, giving the spore bilateral asymmetry (Fig. 3C, D). The anterior part of the polar filament (FP) (manubrium) measured about 145 (140–149) nm ($n=25$) and the angle of tilt anterior PF to the spore axis was $\sim 45^\circ$ (Fig. 3C, D). The PF was iso-filar arranged into 9–10 (rarely 11) coils in 2 layers and, when sectioned transversally, the PF exhibited concentric layers (Fig. 3F). The polaroplast (Pp) has 2 distinct lamellae folded around the PF. In the anterior zone the lamellae were without a lumen and were irregularly packed with a lucent space between them, while in the posterior lamellae the

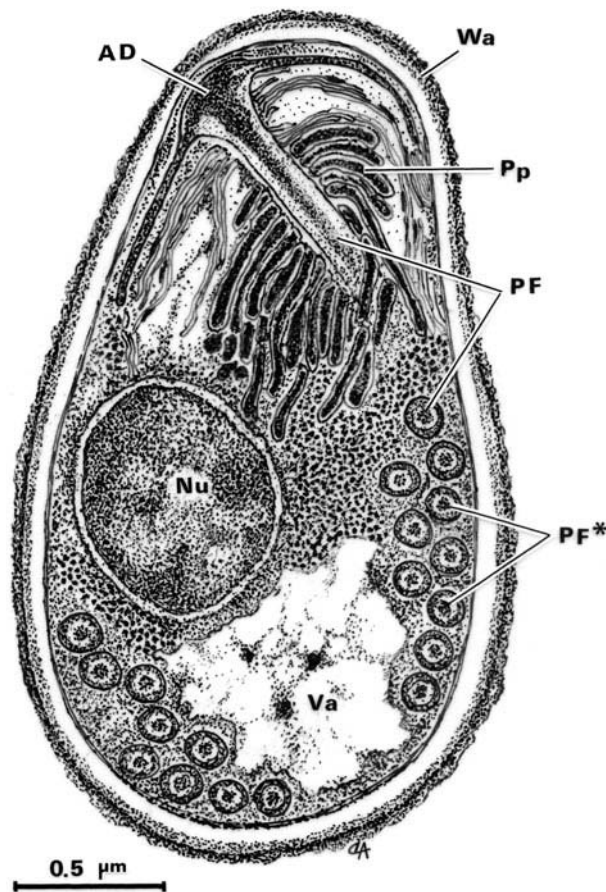


Fig. 4. Semi-schematic drawing of a spore of *Potaspora morhaphis* n. g., n. sp. showing specific characters, such as spore shape and dimensions, spore wall (Wa), polaroplast (Pp), anchoring disc (AD), polar filament (PF) coils (PF*), nucleus (Nu) and vacuole (Va).

lumen was filled with electron-dense material approximately 35–40 nm thick (Fig. 3D,E). The nucleus, containing a moderately uniform nucleoplasm and surrounded by numerous ribosomes, was situated laterally between the polaroplast and the posterior vacuole. The posterior vacuole, situated at the basal part of the spore between the PF coils, was irregular and contained some masses of dense material (Fig. 3C).

Molecular analysis

Two bands of approximately 1.4 kb and 1.1 kb were obtained after amplification of the microsporidian genomic DNA. The primers used were V1f-1492r and HG4F-HG4R, respectively. The sequences were assembled and the resulting consensus DNA sequence of the complete SSU rRNA, ITS, and the 5'-end of the LSU rRNA gene was 1826 bp in length. This sequence with a GC content of 47% was deposited in GenBank (Accession number EU534408). In total, 42 SSU rDNA sequences, including those with the highest BLAST scores, were aligned with the *Potaspora morhaphis* SSU rDNA sequence.

Only sequences belonging to species parasitizing fishes were included in the final analyses (Table 1). *Trachipleistophora hominis* found in muscle of humans, some *Pleistophora* spp. found in crustacean species and several *Dictyocoela* spp. parasitizing amphipods were excluded. The length of the aligned sequences used for phylogenetic analysis was 1527 bases after trimming the 3' end. Before phylogenetic analysis, only those sites which could be unambiguously aligned among all Microsporidia and outgroups were used, resulting in an alignment of 1321 bases long.

Based on pairwise comparisons among the SSU rDNA sequences, the maximal similarity was observed with *Microgemma tincae*, *Microgemma caulleryi* and *Tetramicra brevifilum* species, 87.3%, 87.2% and 87.2%, respectively (Table 2). Phylogenetic analyses using maximum parsimony placed *Potaspora morhaphis* clustered with the sequences of the *Kabatana* (AF356222, AJ295322, EF202572), *Microgemma* (AJ252952, AY651319, AY033054), *Spraguea* (AF104086, AF033197, AY465876, AF056014), *Tetramicra* (AF364303) genera and *Microsporidium* (AJ295323, AJ295324) collective group. This clade has 72% bootstrap support. Only *Spraguea* (68% bootstrap) clade suggested monophyly (Fig. 5). Neighbour-joining and maximum likelihood analyses resulted in identical tree topology.

DISCUSSION

Ultrastructural studies

The ultrastructural organization of the xenoma, as well as aspects of the developmental stages described in the present study, showed that all structures were typically from Phylum Microsporidia, Class Haplophasea and family Tetramicridae (Lom and Dyková, 1992; Larsson, 1999; Lom and Nilsen, 2003).

Of at least 156 fish microsporidian species distributed among 17 genera (Azevedo and Matos, 2003; Lom and Nilsen, 2003; Baquero *et al.* 2005), only 12 develop xenoma. These formations are a characteristic consequence of the host cell defence to the parasite development having features specific to the genus and species (Lom and Nilsen, 2003). Among these, the xenoma wall, of only 4 genera (*Ichthyosporidium*, *Tetramicra*, *Microfilum* and *Amazonspora*), possesses a structure characterized by numerous anastomosed microvilli-like structures, which could partially resemble the xenoma wall of the parasite reported by us. However, some ultrastructural aspects of the developmental stages of those genera are very distinct. In *Ichthyosporidium*, the xenoma wall presents microvilli-like ramified projections irregularly intermingled in the wall, but this parasite has the nuclei organized as a diplokaryon during all sporogonic stages and the polar filament (up to 46 coils) is the largest of the microsporidian

Table 2. Comparison of some SSU rDNA sequences: percentage of identity (top diagonal) and pairwise distance (bottom diagonal) obtained by Kimura-2 parameter analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(1) <i>Potasporea morhaphis</i>	—	86.8	86.8	86.8	86.8	86.4	85.9	87.3	87.2	87.2	85.2	85.2	85.2	86.0
(2) <i>Spraguea lophii</i> (1)	0.132	—	100	98.2	100	99.6	95.3	98.2	98.9	98.9	91.0	91.0	91.0	96.4
(3) <i>Spraguea</i> sp. <i>Lophius litulon</i>	0.132	0.000	—	98.2	100	99.6	95.3	98.2	98.9	98.9	91.0	91.0	91.0	96.4
(4) <i>Microgemma vivax</i>	0.132	0.018	0.018	—	98.2	97.9	94.1	98.6	98.2	98.2	90.6	90.6	90.6	95.3
(5) <i>Spraguea lophii</i> (2)	0.132	0.000	0.000	0.018	—	99.6	95.3	98.2	98.9	98.9	91.0	91.0	91.0	96.4
(6) <i>Spraguea americana</i>	0.136	0.004	0.004	0.021	0.004	—	94.9	97.9	97.9	98.6	98.6	91.6	91.6	96.0
(7) <i>Kabatana takedai</i>	0.141	0.047	0.047	0.059	0.047	0.051	—	94.1	94.9	94.9	90.2	90.2	90.2	96.4
(8) <i>Microgemma tincae</i>	0.127	0.018	0.014	0.014	0.018	0.021	0.059	—	98.2	98.2	90.6	90.6	90.6	95.3
(9) <i>Tetramicra brevifilum</i>	0.128	0.011	0.011	0.018	0.011	0.014	0.051	0.018	—	100	91.6	91.6	91.6	96.0
(10) <i>Microgemma caulleryi</i>	0.128	0.011	0.011	0.018	0.011	0.014	0.051	0.018	0.000	—	91.6	91.6	91.6	96.0
(11) <i>Microsporidium</i> sp. RSB1	0.148	0.090	0.090	0.094	0.090	0.094	0.098	0.094	0.094	0.094	—	100	100	89.8
(12) <i>Kabatana seriola</i>	0.148	0.090	0.090	0.094	0.090	0.094	0.098	0.094	0.094	0.094	0.000	—	100	89.8
(13) <i>Microsporidium</i> GHB	0.148	0.090	0.090	0.094	0.090	0.094	0.098	0.094	0.094	0.094	0.000	0.000	—	89.8
(14) <i>Kabatana newberryi</i>	0.140	0.036	0.036	0.047	0.036	0.040	0.036	0.047	0.040	0.040	0.102	0.102	0.102	—

group (Sprague and Vernick, 1974; Casal and Azevedo, 1995). In *Microfilum*, the xenoma wall was described as a very dense region covered by numerous apparently disorganized and ramified microvilli. This parasite gives rise to a spore characterized by a manubrium inserted on a laterally offset anchoring disc and extruding into a short non-coiled polar filament, which was very different from those of the present study (Faye *et al.* 1991). A xenoma wall including a microvillous surface layer formed by anastomosed elongated cytoplasmic processes have been described in the genus *Tetramicra* (Matthews and Matthews, 1980). Meronts located within a vacuole in the host cytoplasm and spores with conspicuous posterosomes surrounded by a membrane and located inside the posterior vacuole.

Recently in an Amazonian fish, a new genus and species (*Amazonspora hassar*) having a xenoma, strongly encapsulated, consisting of numerous anastomosed microvilli-like projections penetrating the 1–3 first layers of collagen fibres was described. Up to approximately 22 juxtaposed crossed layers of collagen fibres were observed (Azevedo and Matos, 2003).

The presence of dense globules of unknown nature in the sporoplasm was also seen in other fish-infecting Microsporidia. Several electron-dense inclusion bodies, sometimes very large, measuring up to 1.38 μm in diameter, were described in sporoblasts and spores of *Tetramicra brevifilum* species (Matthews and Matthews, 1980). In *Kabatana arthuri* (Lom *et al.* 1999) and *K. takedai* (Lom *et al.* 2001) a very similar globule was reported, while in *Loma acerinae* (Lom and Pekkarinen, 1999) 1–3 homogeneous dense globules occupying all the space of the posterior vacuole were observed. In our observations a large inclusion consisting of reticular material like that reported in *Ichthyosporidium giganteum*, was also found (Sprague and Vernick, 1974; Casal and Azevedo, 1995).

The polaroplast of *Potasporea morhaphis* has a bipartite structure comprising the anterior region having folds with a lamellar organization and the posterior region with larger lamellae (cisternae) with dense contents. A similar organization was reported by Lom *et al.* (1999) in the species *Kabatana arthuri* which infects the trunk muscles of fishes from the South-East Asia freshwater fish, *Pangasius sutchi*, as well as in *Kabatana takedai* (Lom *et al.* 2001). The polaroplast of the microsporidian, *Spraguea americana*, found in the nervous tissues of the Japanese anglerfish *Lophius litulon* (Freeman *et al.* 2004) has a similar organization.

Phylogenetic relationships

The availability, in the public databases, of sequences from different species belonging to the phylum Microsporidia makes the SSU rRNA gene

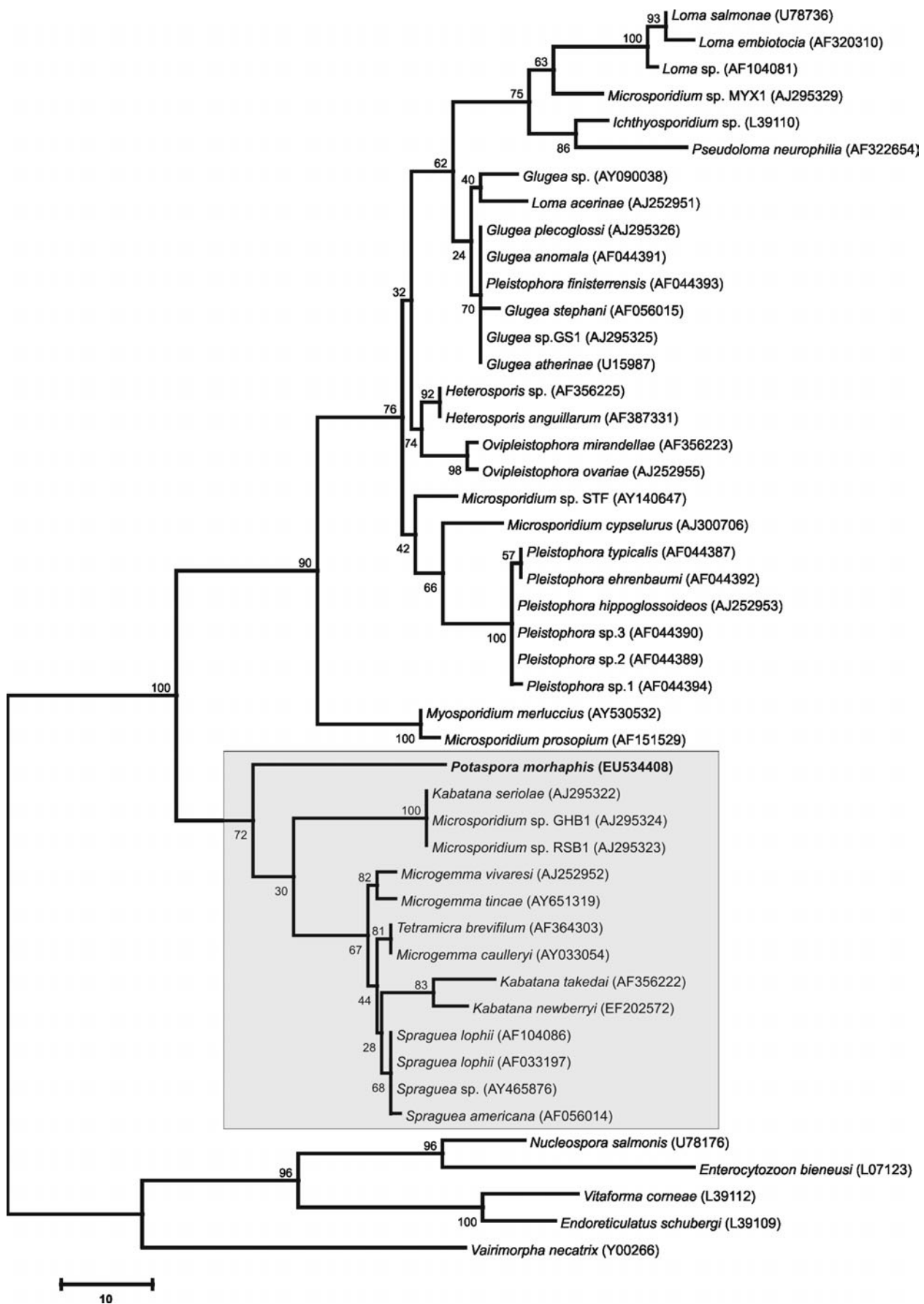


Fig. 5. Parsimony tree of SSU rDNA sequences to compare *Potaspora morhaphis* with selected sequences from other fish-infecting Microsporidia. The analysis was conducted using 1321 aligned nucleotide positions of the highest

the most suitable not only for development of diagnostic tools and species identification, but also for molecular characterization of new parasites through phylogenetic studies (Weiss and Vossbrinck, 1999; Lom and Nilsen, 2003).

In these studies we can see that there is 72% bootstrap support for a clade composed of microsporidian belonging to the *Kabatana* (Lom *et al.* 1999, 2001; McGourty *et al.* 2007), *Microgemma* (Cheney *et al.* 2000; Leiro *et al.* 2000; Mansour *et al.* 2005), *Spraguea* (Freeman *et al.* 2004), *Tetramicra* (Leiro *et al.* 2000) genera, 2 unclassified species of *Microsporidium* group (Bell *et al.* 2001) and the microsporidian reported in this study. This result is in concordance with cladograms previously obtained by Lom and Nilsen (2003) and designated as group IV. Our SSU rDNA sequence analysis also shows that *Potaspora* n. gen. does not have any sister taxa and the lineage is distantly related to the other species examined. Comparing SSU rRNA gene sequences between *P. morhaphis* with species *Microgemma caulleryi* and *Tetramicra brevifilum* (clade with bootstrap 81%) the genetic distances are 12.8% for both species. The smallest genetic distance was observed with the species *Microgemma tincae* (12.7%) but the SSU rRNA was not completely sequenced. The bootstrap support for this species and another *Microgemma vivaresi* is 81%. On the other hand, *K. takedai* and *K. newberryi* group in a clade with 83% bootstrap and all *Spraguea* species are clustered in the clade with 68% bootstrap.

Conclusion

When comparing the xenoma wall of the parasite described here with those fish Microsporidia which form xenoma some structural differences were found, such as the organization of the microvilli-like structures. In addition, the ultrastructural organization of the polaroplast and the presence of a dense globule were the most evident differences found compared with other mature spores of previously described species. Concerning this last aspect, the only exception is the spore of *Kabatana* genus which presents some similarities. However, they were found to parasitize only the muscle fishes and they do not develop inside of xenomas (Lom *et al.* 1999, 2001; McGourty *et al.* 2007). As concerns molecular biology, the most parsimonious cladogram has shown that *Potaspora morhaphis* is placed in the same group as the *Kabatana*, *Microgemma*, *Spraguea* and *Tetramicra* genera, does not have any sister taxa

and has the lowest percentage identity within the group.

So, our results suggest that this parasite does not fit into any of the known fish microsporidian genera, and for these reasons we propose a new genus *Potaspora* and a new species, *Potaspora morhaphis*.

This work was partially supported by the Eng^o. A. Almeida Foundation (Porto, Portugal), Ph.D. grant from 'CESPU' (G. Casal), 'CNPq' and 'CAPES' – Brazil. We would like to thank the iconographic work of Mr João Carvalheiro. We would like to thank the anonymous reviewers for their helpful suggestions and comments.

REFERENCES

- Azevedo, C. and Matos, E. (2002). Fine structure of a new species, *Loma myrophis* (Phylum Microsporidia), parasite of the Amazonian fish *Myrophis platyrhynchus* (Teleostei, Ophichthidae). *European Journal of Protistology* **37**, 445–452.
- Azevedo, C. and Matos, E. (2003). *Amazonspora hassar* n. gen. and n. sp. (phylum Microsporidia, fam Glugeidae), a parasite of the Amazonian teleost *Hassar orestis* (fam. Doradidae). *Journal of Parasitology* **89**, 336–341.
- Azevedo, C., Balseiro, P., Casal, G., Gestal, C., Aranguren, R., Stokes, N. A., Carnegie, R. B., Novoa, N., Bureson, E. M. and Figueras, A. (2006). Ultrastructural and molecular characterization of *Haplosporidium montforti* n. sp., parasite of the European abalone *Haliotis tuberculata*. *Journal of Invertebrate Pathology* **92**, 23–32.
- Baquero, E., Rubio, M., Moura, I. N. S., Pieniazek, J. and Jordana, R. (2005). *Myosporidium merluccius* n. g., n. sp. infecting muscle of commercial hake (*Merluccius* sp.) from fisheries near Namibia. *The Journal of Eukaryotic Microbiology* **52**, 476–483.
- Bell, A. S., Aoki, T. and Yokoyama, H. (2001). Phylogenetic relationships among Microsporidia based on rDNA sequence data, with particular reference to fish-infecting *Microsporidium* Balbiani 1884 species. *The Journal of Eukaryotic Microbiology* **48**, 258–265.
- Casal, G. and Azevedo, C. (1995). New ultrastructural data on the microsporidian *Ichthyosporidium giganteum* infecting the marine teleostean fish *Ctenolabrus rupestris*. *Journal of Fish Diseases* **18**, 191–194.
- Cheney, S. A., Lafranchi-Tristem, N. J. and Canning, E. U. (2000). Phylogenetic relationships of *Pleistophora*-like Microsporidia based on small subunit ribosomal DNA sequences and implications for the source of *Trachipleistophora hominis* infections. *The Journal of Eukaryotic Microbiology* **47**, 280–287.
- Faye, N., Toguebaye, B. S. and Bouix, G. (1991). *Microfilum lutjani* n. g. n. sp. (Protozoa, Microsporida), a gill parasite of the golden African snapper *Lutjanus fulgens* (Valenciennes, 1830) (Teleost

BLAST score microsporidian sequences and 4 more microsporidian sequences as outgroup. The bar indicates the equivalence between the distance and the number of changes. The numbers on the branches indicate bootstrap support from 100 replicates. *Potaspora morhaphis* is placed within group IV (Lom and Nilsen, 2003) (highlighted box), which includes the sequences of the genera *Kabatana*, *Microgemma*, *Spraguea*, *Tetramicra*, and *Microsporidium*.

- Lutjanidae): Developmental cycle and ultrastructure. *Journal of Protozoology* **38**, 30–40.
- Freeman, M. A., Yokoyama, H. and Ogawa, K.** (2004). A microsporidian parasite of the genus *Spraguea* in the nervous tissues of the Japanese anglerfish *Lophius litulon*. *Folia Parasitologica* **51**, 167–176.
- Gatehouse, H. S. and Malone, L. A.** (1998). The ribosomal RNA gene region of *Nosema apis* (Microspora): DNA sequence for small and large subunit rRNA genes and evidence of a large tandem repeat unit size. *Journal of Invertebrate Pathology* **71**, 97–105.
- Larsson, J. I. R.** (1999). Identification of Microsporidia. *Acta Protozoologica* **38**, 161–197.
- Leiro, J., Siso, M. I. G., Parama, A., Ubeira, F. M. and Sanmartin, M. L.** (2000). RFLP analysis of PCR-amplified small subunit ribosomal DNA of three fish microsporidian species. *Parasitology* **120**, 113–119.
- Lom, J.** (2002). A catalogue of described genera and species of microsporidians parasitic in fish. *Systematic Parasitology* **53**, 81–99.
- Lom, J. and Dyková, I.** (1992). Microsporidia (Phylum Microspora Sprague, 1977). In *Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Sciences* (ed. Lom, J. and Dyková, I.), Vol. 26, pp. 125–157. Elsevier, Amsterdam.
- Lom, J., Dyková, I. and Tonguthai, K.** (1999). *Kabataia* gen. n., new genus proposed for *Microsporidium* spp. infecting trunk muscles of fishes. *Diseases of Aquatic Organisms* **38**, 39–46.
- Lom, J. and Nilsen, F.** (2003). Fish Microsporidia: fine structural diversity and phylogeny. *International Journal for Parasitology* **33**, 107–127.
- Lom, J., Nilsen, F. and Urawa, S.** (2001). Redescription of *Microsporidium takedai* (Awakura, 1974) as *Kabatana takedai* (Awakura, 1974) comb. n. *Diseases of Aquatic Organisms* **44**, 223–230.
- Lom, J. and Pekkarinen, M.** (1999). Ultrastructural observations on *Loma acerinae* (Jirovec, 1930) comb. nov. (Phylum Microsporidia). *Acta Protozoologica* **38**, 61–74.
- Mansour, L., Prensier, G., Jemaa, S. B., Hassine, O. K. B., Méténier, G., Vivarès, C. P. and Cornillot, E.** (2005). Description of a xenoma-inducing microsporidian, *Microgemma tincae* n. sp., a parasite of the teleost fish *Symphodus tinca* from Tunisian coasts. *Diseases of Aquatic Organisms* **65**, 217–226.
- Matos, E. and Azevedo, C.** (2004). Ultrastructural description of *Microsporidium brevirostris* sp. n., parasite of the teleostean *Brachyhyopomus brevirostris* (Hypopomidae) from the Amazon River. *Acta Protozoologica* **43**, 261–267.
- Matos, E., Corral, L. and Azevedo, C.** (2003). Ultrastructural details of the xenoma of *Loma myrophis* (phylum Microsporidia) and extrusion of the polar tube during autoinfection. *Diseases of Aquatic Organisms* **54**, 203–207.
- Matthews, R. A. and Matthews, B. F.** (1980). Cell and tissue reactions of turbot *Scophthalmus maximus* (L.) to *Tetramicra brevifilum* gen. n., sp. n. (Microspora). *Journal of Fish Diseases* **3**, 495–515.
- Nilsen, F.** (2000). Small subunit ribosomal DNA phylogeny of Microsporidia with particular reference to genera that infect fish. *Journal of Parasitology* **86**, 128–133.
- McGourty, K. R., Kinzger, A. P., Hendrickson, G. L., Goldsmith, G. L., Casal, G. and Azevedo, C.** (2007). A new microsporidian infecting the musculature of the endangered tidewater goby (Gobiidae). *Journal of Parasitology* **93**, 655–660.
- Sprague, V., Becnel, J. J. and Hazard, E. I.** (1992). Taxonomy of phylum Microspora. *Critical Reviews in Microbiology* **18**, 285–395.
- Sprague, V. and Vernick, S.** (1974). Fine structure of the cyst and some sporulation stages of *Ichthyosporidium* (Microsporidia). *Journal of Protozoology* **21**, 667–677.
- Tamura, K., Dudley, J., Nei, M. and Kumar, S.** (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.
- Thompson, J. D., Higgins, D. G. and Gilson, T. J.** (1994). Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.
- Vossbrinck, C. R., Baker, M. D., Didier, E. S., Debrunner-Vossbrinck, B. A. and Shadduck, J. A.** (1993). Ribosomal DNA sequences of *Encephalitozoon hellem* and *Encephalitozoon cuniculi*: species identification and phylogenetic construction. *The Journal Eukaryotic of Microbiology* **40**, 354–362.
- Weiss, L. and Vossbrinck, C.** (1999). Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the Microsporidia. In *The Microsporidia and Microsporidiosis* (ed. Wittner, M. and Weiss, L.), pp. 129–171. American Society of Microbiology, Washington, DC.

Capítulo 3

MORPHOLOGICAL AND GENETICAL DESCRIPTION OF *LOMA PSITTACA* SP. N. ISOLATED FROM THE AMAZONIAN FISH SPECIES *COLOMESUS PSITTACUS*

Parasitology Research (2009) *in press*

Graça Casal, Edilson Matos, M. Leonor Teles-Grilo & Carlos Azevedo

Morphological and genetical description of *Loma psittaca* sp. n. isolated from the Amazonian fish species *Colomesus psittacus*

Graça Casal · Edilson Matos · M. Leonor Teles-Grilo · Carlos Azevedo

Received: 2 April 2009 / Accepted: 19 June 2009
© Springer-Verlag 2009

Abstract A previously unrecognised fish-infecting microsporidia (*Loma psittaca* n. sp.), found adherent to the intestinal mucosa of the freshwater puffer fish *Colomesus psittacus* (Teleostei, Tetraodontidae) from lower Amazon River, was described based on light and transmission electron microscope and phylogenetic analysis. The whitish xenoma was completely filled by numerous spores, including several developmental stages of the parasite. In all of these stages, the nuclei were monokaryotic. The merogonial plasmodium divided by binary fission and the sporont gave rise to disporoblastic ovoid spores measuring $4.2 \pm 0.4 \times 2.8 \pm 0.4 \mu\text{m}$. In mature spores, the polar filament was

arranged in 10–11 (rarely 12) coils in one row in turn of posterior vacuole. The polaroplast had two distinct regions around the manubrium. The polyribosomes were organised in coiled tapes. The small subunit rRNA gene was sequenced and maximum parsimony analysis placed the microsporidian described here in the clade that includes the genera *Ichthyosporidium*, *Loma* and *Pseudoloma*. Based on differences from previously described microsporidians, such as ultrastructural characteristics of the xenoma, developmental stages including the spore and phylogenetic analysis supported the recognition of a new species, herein named *L. psittaca* n. sp.

G. Casal · C. Azevedo (✉)
Department of Cell Biology, Institute of Biomedical Sciences,
University of Porto (ICBAS/UP),
Lg. A. Salazar no. 2,
4099-003 Porto, Portugal
e-mail: azevedoc@icbas.up.pt

G. Casal · C. Azevedo
Laboratory of Pathology,
Centre for Marine Environmental Research (CIIMAR/UP),
4050-123 Porto, Portugal

G. Casal
Departamento de Ciências,
Instituto Superior de Ciências da Saúde–Norte,
Gandra, Portugal

E. Matos
Carlos Azevedo Research Laboratory,
Federal Rural University of Amazonia,
Belém (Pará), Brazil

M. L. Teles-Grilo
Laboratory of Molecular Genetics,
Institute of Biomedical Sciences,
University of Porto (ICBAS/UP),
Porto, Portugal

Introduction

The members of the phylum Microsporidia Balbiani, 1882 are widespread, minute, obligatory intracellular parasites found in most invertebrate phyla and in vertebrates, with the majority of species in insects and fish (Lom and Dyková 1992; Sprague et al. 1992; Larsson 1999; Lom 2002). Presently, there are at least 144 available genera (Larsson 1999), 18 of them occurring in teleost fishes from the different geographic areas and habitat (Azevedo and Matos 2003; Lom and Nilsen 2003; Baquero et al. 2005; Casal et al. 2008), and some of them are recognised as serious pathogens for their hosts. Fishes are hosts to 156 recorded species of microsporidia, 11 species belonging to the genus *Loma* Morrison and Sprague, 1981 and the other eight parasitoses were classified as *Loma* spp. (Lom 2002). One of them, *Loma myrophis*, was found in the subepithelial tissues of the fish gut *Myrophis platyrhynchus* from Amazonian fauna (Azevedo and Matos 2002; Matos et al. 2003). About those from South America, particularly from the Amazon River where lives a diverse assemblage of

Published online: 11 July 2009

 Springer

several hundred species of fishes, little is known. Recently, some other microsporidiosis were described in Amazonian fishes: *Amazonspora hassar* was found in the gill of the teleost *Hassar orestis* (Azevedo and Matos 2003), *Microsporidium brevirostris* in the skeletal muscle of the abdominal cavity of the fish *Brachyhypopomus brevirostris* (Matos and Azevedo 2004), and *Potasporea morhaphis* adherent to the wall of coelomic cavity of the freshwater fish, *Potamorhaphis guianensis* (Casal et al. 2008).

Ultrastructurally, the genus *Loma* is characterised to form xenoma, the nuclei to be unpaired during all stages of development and the sporogony to be polysporoblastic within parasitophorous vacuole bound with host cell-derived membrane (Morrison and Sprague 1981; Lom and Pekkarinen 1999; Lom 2002). Presently, there is little information about the origin of vacuole formed during the sporogony of *Loma* species (Matthews et al. 2001). Small subunit (SSU) ribosomal DNA (rDNA) sequence comparison is a well-recognised technique for providing valuable information about phylogenetic relationships (Hillis and Dixon 1991). Only for three *Loma* species was the SSU rDNA gene sequenced: *Loma embiotocia* in shiner perch *Cymatogaster aggregata* (Shaw et al. 1997), *Loma salmonae* found in *Oncorhynchus mykiss* (Docker et al. 1997) and *Loma acerinae* (Cheney et al. 2000). Phylogenetic analysis using SSU rDNA gene show evidences that *Loma* spp. do not comprise a monophyletic group, being placed in the same clade with the genera *Ichthyosporidium* and *Pseudoloma* (Lom and Nilsen 2003). Sometimes, the phylogenetic trees do not support traditional taxonomic schemes (Sprague et al. 1992). Important morphological characters presented by those genera, such as the number of nuclei per spores and the presence of a parasitophorous vacuole or sporophorous vesicle, are not in concordance with molecular data.

In this paper, we describe a new species of a microsporidian based on morphological and ultrastructural observations. Phylogenetic relationships comparing the *Loma psittaca* SSU rRNA gene with those of other fish-infecting microsporidian species were also done. The morphological characteristics and taxonomic position are discussed.

Materials and methods

Fish, location of infection and prevalence

Thirty specimens of freshwater teleost puffer fish *Colomesus psittacus* Bloch and Schneider, 1801 (Teleostei, Tetraodontidae) (Brazilian common name “baiacá”) were collected from the estuarine region of the Amazon River (02°14' S, 48°57' W) near the city of Cametá (Pará State),

Fig. 1 Light and transmission electron micrographs of the microsporidian *L. psittaca* n. sp. parasite of *Colomesus psittacus*. 1 A group of fresh spores observed in DIC. Scale bar, 10 µm. 2 An isolated fresh mature spore observed in DIC. Scale bar, 10 µm. 3 Semi-thin section of the xenoma showing the wall (W) and the matrix of the xenoma containing numerous spores. Scale bar, 50 µm. 4 Semi-thin section of the xenoma periphery showing the wall (W) and the matrix containing developmental stages (asterisk) and numerous spores. Scale bar, 10 µm. 5 Ultrathin section of a xenoma showing the wall formed by several fibroblast layers (Fb). The matrix shows some spores (Sp). Scale bar, 5 µm. 6 Ultrathin section of some spores (Sp) sectioned at different levels showing the internal organisation. Scale bar, 1 µm. 7 Ultrastructural details of the spore apical zone showing the spore wall (Wa), the anchoring disc (AD) and the polar filament sections (PF) of which the anterior part was surrounded by two types of polaroplast lamellae (Pp). Several polyribosomes organised in long tapes (arrows) are observed. Scale bar, 0.5 µm. 8 Ultrastructural details of the polyribosomes arranged in long coiled tapes (arrows). The wall (Wa) and some transverse section of the polar filament (PF) are also observed. Scale bar, 0.2 µm. 9 Ultrastructural details of some transverse sections of the polar filaments (PF) containing some internal concentric layers. The spore wall (Wa) was composed by two layers of different densities (arrowheads). Scale bar, 0.2 µm

Brazil. The specimens were anaesthetised by MS 222 (Sandoz Lab.) and later measured (8–12 cm in length). Infection was determined by the presence of xenomas located in the intestinal mucosa. The prevalence of infection was 30% (nine fishes in 30 examined) in both sexes.

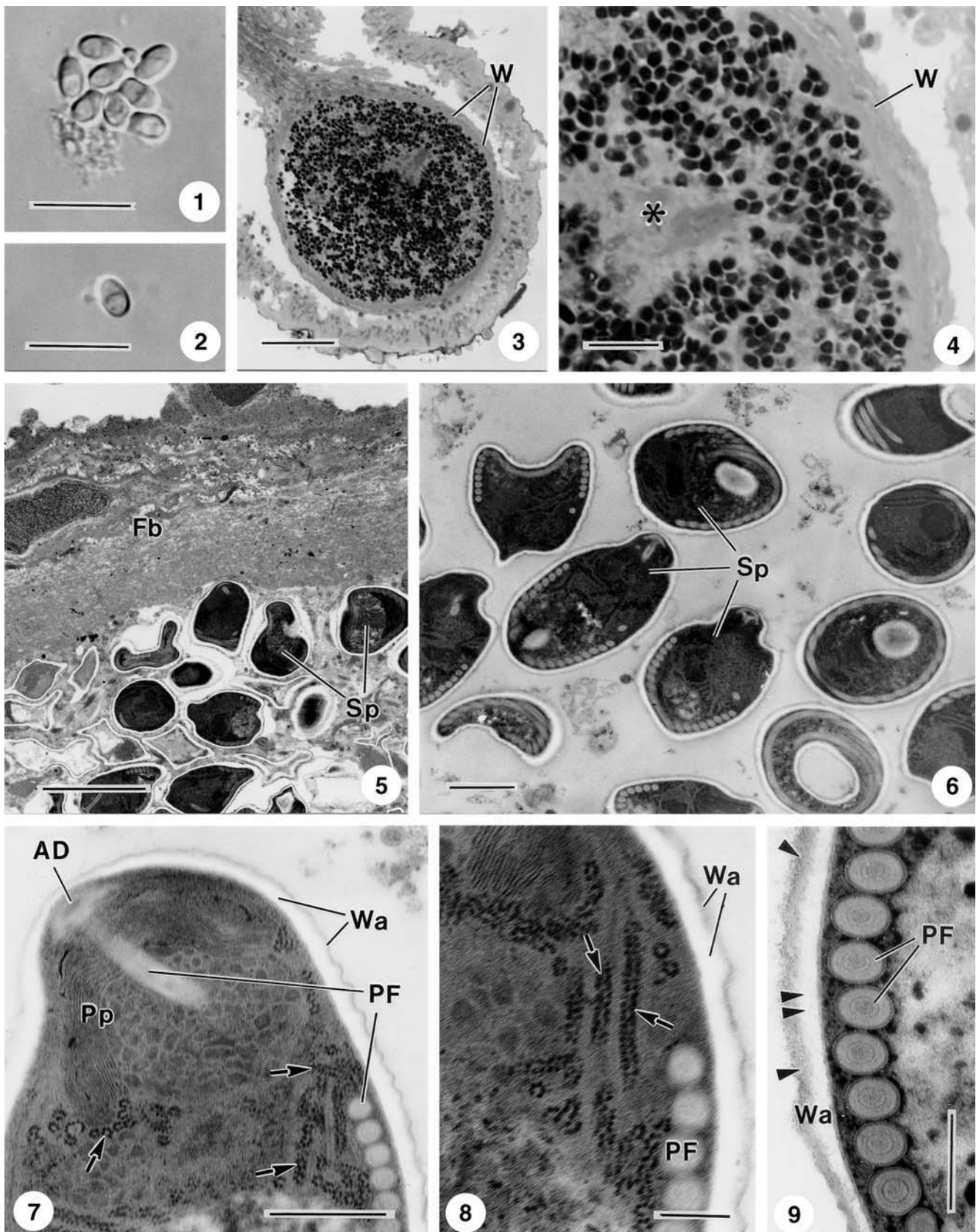
Light and transmission electron microscopy

For light microscopy, smears of xenoma and free spores were observed directly without fixation or stain by a light microscope equipped with Nomarski interference contrast [differential interference contrast (DIC)] optics.

For ultrastructural studies, the xenomas were excised and fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4°C for 24 h. After washing overnight in the same buffer at 4°C and post-fixed in 2% osmium tetroxide in the same buffer and temperature for 3 h, the fragments were dehydrated through a graded ethanol ascending series, followed by propylene oxide (three changes of 2 h each) and embedded in Epon (12 h in each change). Semi-thin sections were stained with methylene blue-Azur II and observed by DIC optics. Ultrathin sections were contrasted with aqueous uranyl acetate and lead citrate and observed with a JEOL 100CXII TEM, operated at 60 kV.

DNA isolation and PCR amplification

Several xenomas were dissected from fishes following homogenisation to isolate the spores and then were stored in 80% ethanol at 4°C. The genomic DNA of about 5×10^6 spores was extracted using a GenElute™ Mammalian



Genomic DNA Miniprep kit (Sigma) according to the manufacturer's instructions for animal tissue protocol, except for the incubation time. The DNA was stored in 50 µl of TE buffer at -20°C until use. The DNA concentration was estimated with the QubitTM Fluorometer (Invitrogen). The majority of the region coding for the SSU rRNA gene was amplified by polymerase chain reaction (PCR) using the primers V1f (5' CACCAGGTT GATTCTGCC 3') and 1492r (5' GGTTACCTTGTTAC GACTT 3') (Vossbrinck et al. 1993; Nilsen 2000). PCR was carried out in 50 µl reactions using 10 pmol of each primer, 10 nmol of each dNTP, 2 mM of MgCl_2 , 5 µl 10X *Taq* polymerase buffer, 1.25 U *Taq* DNA polymerase (Invitrogen products) and 3 µl of the genomic DNA. The reactions were run on Hybaid PxT Thermocycler (Thermo Electron Corporation, Milford, MA, USA). The amplification programme consisted of 94°C denaturation for 5 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min. A final elongation step was performed at 72°C for 10 min. Aliquots (5 µl) of PCR products were visualised with ethidium bromide staining after running on a 1% agarose gel.

DNA sequencing

PCR product for the SSU rRNA gene has an approximate size of 1,400 bp. It was cleaned using the MinElute PCR purification kit (Qiagen) and then three purified PCR products were sequenced in both directions. Sequencing was done using BigDye terminator v1.1 of Applied Biosystems kit, and the sequence reactions were run on an ABI3700 DNA analyser Perkin-Elmer, Applied Biosystems, Stabvida, Co., Oeiras, Portugal).

Distance and phylogenetic analysis

To evaluate the relationship of *L. psittaca* to other microsporidians, a homology search was performed using BLAST programme (Altschul et al. 1990). We used 44 rDNA sequences belonging to the microsporidians parasitising fish species. The sequence and NCBI accession number data obtained from GenBank are the following: *Aspalatospora milevae* (EF990668); *Glugea anomala* (AF044391); *Glugea atherinae* (U15987); *Glugea plecoglossi* (AJ295326); *Glugea stephani* (AF056015); *Glugea*

Table 1 Comparative measurements (in µm) from *Loma* spp.

<i>Loma</i> sp.	Host and local infection	Habitat countries	Spore shape	Spore		Polar filament		References
				Length	Width	Coils	Row	
<i>L. branchialis</i> (= <i>L. morhua</i>)	<i>Melanogrammus aeglefinus</i> Gill filaments	Marine Boreo-Artic	Ellipsoid / ovoid	4.2 6	2.0 4	16–17 16–19	isofilar	(Morrison and Sprague 1981)
<i>L. salmonae</i>	<i>Oncorhynchus mykiss</i> Gill filaments	Freshwater Several countries	Pyriform/ ellipsoidal	3.7 4.4	2.2 2.3	12–14 14–17		(Putz et al. 1965)
<i>L. fontinalis</i>	<i>Salvelinus fontinalis</i> Gill lamellae	Freshwater Canada	–			12–14		(Morrison and Sprague 1983)
<i>L. dimorpha</i>	<i>Gobius niger</i> (and others species) Connective tissue of digestive tract	Marine France and Spain	Ovoid/ ellipsoidal	4.5	1.8–2.0	13–15	Isofilar	(Loubès et al. 1984)
<i>L. diplodae</i>	<i>Diplodus sargus</i> Vessels of the gill filaments	Marine France	Ovoid	4.17	2.22	17–18		Bekhti and Bouix 1985)
<i>L. trichiuri</i>	<i>Trichurus savaia</i> Gill filaments	Marine India	Pyriform	3.0	2.0	–		(Sandeep and Kalavati 1985)
<i>L. camerounensis</i>	<i>Oerochromis niloticus</i> Oesophagus to intestine	Freshwater Cameroon	Ovoid	3.96	2.16	11–12		(Fomena et al. 1992)
<i>L. boopsi</i>	<i>Boops boops</i> Liver and digestive tract	Marine Senegal	Ovoid	3.7	2.4	12–14 16–18	Isofilar	(Faye et al. 1995)
<i>L. embiotocia</i>	<i>Cymatogaster aggregate</i> Gills	Marine Canada	Ovoid	4.8	2.6	14–18		(Shaw et al. 1997)
<i>L. acerinae</i>	<i>Gymnocaphtalus cernuus</i> Intestine wall	Freshwater Czech Republic	Ellipsoidal	4.64	2.19	11–23	Isofilar	(Lom and Pekkarinen 1999)
<i>L. myrophis</i>	<i>Myrophis platyrhynchus</i> Subepithelial gut tissue	Freshwater Brazil	Ellipsoidal	4.06	1.61	13–14	Isofilar	(Azevedo and Matos 2002)
<i>L. psittaca</i> n. sp.	<i>Colomesus psittacus</i> Intestinal wall	Freshwater Brazil	Ovoid	4.2	2.8	11–12	Isofilar	This study

sp. GS1 (AJ295325); *Glugea* sp. (AY090038); *Heterosporis anguillarum* (AF387331); *Heterosporis* sp. PF (AF356225); *Ichthyosporidium* sp. (L39110); *Kabatana takedai* (AF356222); *Kabatana newberryi* (EF202572); *Kabatana seriola* (AJ295322); *Loma acerinae* (AJ252951); *Loma embiotocia* (AF320310); *L. salmonae* (U78736); *Loma* sp. (AF104081); *Microgemma caulleryi* (AY033054); *Microgemma tincae* (AY651319); *Microgemma vivaresi* (AJ252952); *Microsporidium cypselurus* (AJ300706); *Microsporidium prosopium* (AF151529); *Microsporidium* GHB1 (AJ295324); *Microsporidium* sp. RSB1 (AJ295323); *Microsporidium* sp. STF (AY140647); *Microsporidium* MYX1 (AJ295329); *Myosporidium merlucius* (AY530532); *Nucleospora salmonis* (U78176); *Ovipleistophora mirandellae* (AF356223); *Ovipleistophora ovariae* (AJ252955); *Pleistophora ehrenbaumi* (AF044392); *Pleistophora finisterrensis* (AF044393); *Pleistophora hippoglossoides* (AJ252953); *Pleistophora typicalis* (AF044387); *Pleistophora* sp. 1 (AF044394); *Pleistophora* sp. 2 (AF044389); *Pleistophora* sp. 3 (AF044390); *Potaspora morhaphis* (EU534408); *Pseudoloma neurophilia* (AF322654); *Spraguea americana* (AF056014); *Spraguea lophii* (1) (AF104086); *S. lophii* (2) (AF033197); *Spraguea* sp. (AY465876); *Tetramicra brevifilum* (AF364303). *Endoreticulatus schubergi* (L39109); *Enterocytozoon bienersi* (L07123); *Vairimorpha necatrix* (Y00266) and *Vittaforma corneae* (L39112) were used as outgroup. Sequences were aligned as described by Casal et al. (2008). Alignment was done through Clustal W (Thompson et al. 1994) in MEGA 4 software (Tamura et al. 2007), with an opening gap penalty of 10 and a gap extension penalty of 4 for both pairwise and multiple alignments. Subsequent phylogenetic and molecular evolutionary analyses were conducted using MEGA 4, with the 44 rDNA sequences for microsporidian species and the outgroup species selected. Distance estimation was carried out using the Kimura-2 parameter model distance matrix for transitions and transversions. For the phylogenetic tree reconstructions, maximum parsimony analysis was conducted using the close neighbour interchange heuristic option with a search factor 2 and random initial trees addition of 2,000 replicates. Bootstrap values were calculated over 100 replicates.

Results

Some spherical whitish xenomas were macroscopically observed adherent to the intestinal mucosa of the fish. After rupture of the xenoma wall, free spores (1 and 2 in Fig. 1) were easily microscopically observed and identified as belonging to the phylum Microsporidia. These xenomas with up to ~310 µm diameter, filled with numerous spores

and different developmental stages, contained a thick xenoma wall formed by several juxtaposed fibroblast layers (3 and 4 in Fig. 1).

Description of *L. psittaca* n. sp.

Systematic position (Figs. 1 and 2)

Phylum Microsporidia Balbiani, 1882

Class Haplophorea Sprague, Becnel and Hazard, 1992

Order Glugeida Issi, 1986

Family Glugeidae Thélohan, 1892

Genus *Loma* Morrison and Sprague, 1981

Species: *L. psittaca* n. sp.

Description of the species

Type host: *C. psittacus* Bloch and Schneider, 1801 (Teleostei, Tetraodontidae) (Brazilian common name “baiacú”).

Type locality: Estuarine region of the Amazon River (02° 14' S, 48°57' W) near the city of Cametá (Pará State), Brazil.

Pathogenicity: The whitish cysts (xenoma) wall was formed by several juxtaposed collagen layers intermingled with some fibroblasts, but no other tissue reactions were observed and no clinical signs were detected.

Location in the host: Xenoma in the intestinal mucosa.

Prevalence of infection: Nine of 30 (30%).

Type specimens: One glass slide containing mature free spores and others with semi-thin sections of tissues containing spores and different developmental stages of hapantotype were deposited in the International Protozoan Type Slide Collection at Smithsonian Institution Washington, DC, 20560, USA, with acquisition number USNM 1123998. The histological semi-thin sections containing different developmental stages were deposited at the laboratory of the senior author.

Etymology: The specific name is derived from the generic name of the host species.

Description of the spores

Ovoid spores measuring $4.2 \pm 0.4 \times 2.8 \pm 0.4$ µm ($n = 30$) contained all typical characteristic structures of the Microsporidia (1, 2, 6 and 7 in Fig. 1). The spore wall was about 87 nm thick, except for the anterior end where the central zone of the anchoring disc contacted with the wall, which was about 20–35 nm thick consisting of an electron-lucent

endospore and a thin electron-dense exospore (7–9 in Fig. 1). The anchoring disc is located in the apical region of the spore in an eccentric position in relation to the spore axis, giving a bilateral asymmetry (6 and 7 in Fig. 1). The anterior part of the polar filament (PF) (manubrium) measured about 125 (118–131) nm in diameter and the angle of tilt anterior PF to the spore axis was $\sim 48^\circ$ (7 in Fig. 1). The PF was isofilar arranged into 10–11 (rarely 12) coils in one row, and when sectioned transversally, it had 80–90 nm in diameter and exhibited three concentric layers (9 in Fig. 1). The last coil measured ~ 60 nm in diameter (9 in Fig. 1). The polaroplast had two distinct lamellar structures folded around the PF. In the anterior zone, the compacted lamellae was without lumen, whilst in the posterior lamellae, the lumen was filled with electron-dense material (7 in Fig. 1). The nucleus, containing a moderately uniform nucleoplasm, was surrounded by numerous polyribosomes forming coiled tapes (7 and 8 in Fig. 1). The posterior vacuole, situated at the basal part of the spore between the PF coils, was irregular and contained light material (7 in Fig. 1).

Developmental stages

Developmental stages with asynchronous distribution and a hypertrophic nucleus centrally positioned were observed (5 in Fig. 1). In the cytoplasm xenoma, it was possible to see many mitochondria surrounding the parasites (10–12 in Fig. 2).

Meronts

They appeared in ultrathin sections as round to elliptical uninucleated or binucleated cells with the unpaired nuclei. These nuclei presented homogeneous chromatin without apparent nucleolus. The cytoplasm possessing numerous free ribosomes was uniformly granular and poorly endowed with cytoplasmic organelles (10 in Fig. 2). Meronts divided by multiple fissions and transformed into sporonts (10 and 12 in Fig. 2).

Sporonts

These cells were characterised by a gradual acquisition of a thick and dense discontinuous cell coat formed by isolated patches located on the outer surface of the plasmalemma (12 in Fig. 2). The multinucleated sporogonial plasmodia had several cisternae of RER surrounding the nucleus. Between the sporont and host cytoplasm, a small space appeared, growing up until transforming into parasitophorous vacuole (PV) (membrane lining the vacuole originated by host cell). The cytoplasm of the host cell in close contact with the sporogony vacuole gradually accumulated a great quantity of electron-dense material (11 and 12 in Fig. 2). Later,

Fig. 2 Ultrastructural aspects of some developmental stages of *L. psittaca* n. sp. parasite of *C. psittacus*. 10 A dividing meront (*Mr*) located amongst mature spores (*Sp*). Scale bar, 2 μ m. 11 A sporogonial plasmodium in division showing the wall formation by deposition of dense material around the plasmalemma (arrowheads). Scale bar, 2 μ m. 12 Some early sporoblasts (*Sb*) located amongst dividing meront (*Mr*) and mature spores (*Sp*). A dense granular substance (arrowheads) was interposed between early sporoblasts. Scale bar, 2 μ m. 13 Some free mid sporoblasts (*Sb*) located in the parasitophorous vacuole space (asterisk) containing some dense granular substances (arrowheads) and tubular appendages (arrows). Scale bar, 2 μ m. 14 Some late sporoblasts (*Sb*) in the parasitophorous vacuole (asterisk) containing some dense granular substances (arrowheads). Scale bar, 1 μ m

this material appears to be transferred to PV space, and simultaneously, the sporont divided into sporoblast cells.

Sporoblasts

The sporoblasts gradually differentiate the typical organelles of the spores and became with irregular contours (13 and 14 in Fig. 2). Sporoplasm became dense and the endospore (internal portion of the wall) became more evident. Simultaneously, inside the PV space, the mass of electron-dense material dispersed between the sporoblasts seemed to dissipate into tubular structures (13 in Fig. 2).

Molecular analysis

Conserved SSU rDNA primers V1f /1492r permitted to amplify a fragment with approximately 1.4 kb. After sequencing both strains, a sequence 1,260 bp in length corresponding to the almost SSU rRNA gene was obtained. This sequence with a GC content of 55.5% was deposited in GenBank (accession number FJ843104). Blast search confirmed that it belongs to 16S rDNA and bears the closest similarity to other microsporidians that have fish species as a host. Forty-four SSU rDNA sequences were aligned with the *L. psittaca* SSU rDNA sequence. The length of the aligned sequences used for phylogenetic analysis was 1,459 bases after trimming the 3' end. Before phylogenetic analysis, only those sites which could be unambiguously aligned amongst all microsporidians and outgroups were used, resulting in an alignment of 1,339 bases long.

Based on GenBank BLAST searches of the SSU rRNA gene, *L. acerinae* (AJ252951) is the most similar species (96.9% of identity), whereas *G. anomala* and *G. atherinae* species had 96% and *P. finisterrensis*, *G. plecoglossi* and *Glugea* sp. GS1 had 95.6%. The distances observed between *L. psittaca* and the other previously described *Loma* species were higher than 10%: *Loma* sp. (12.7%), *L. salmonae* (13.1%) and *L. embiotocia* (14.6%; Table 2). The maximum parsimony phylogenetic analyses of the SSU rRNA showed that *L. acerinae* is a sister species to *L.*

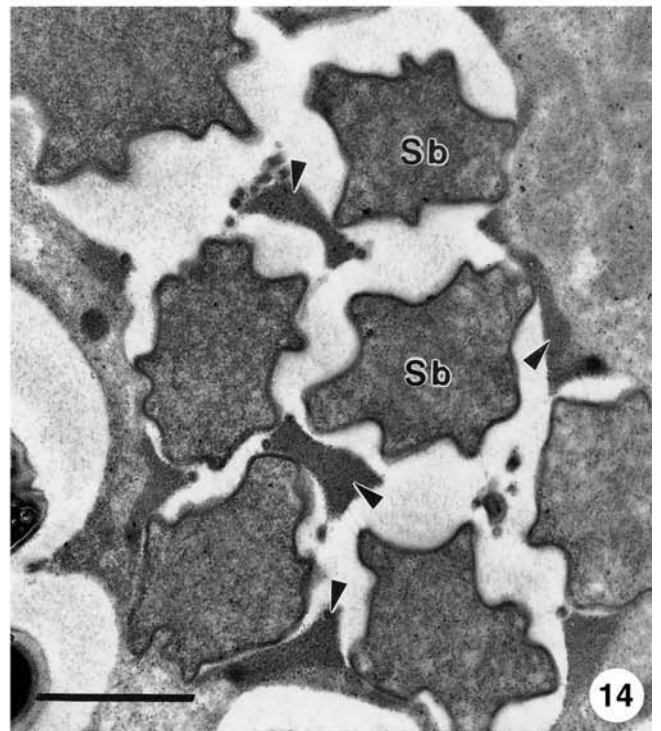
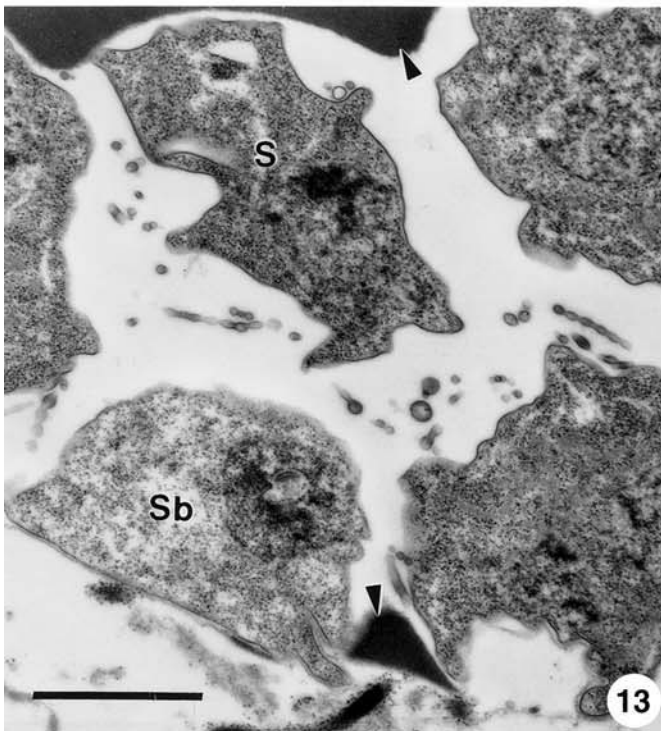
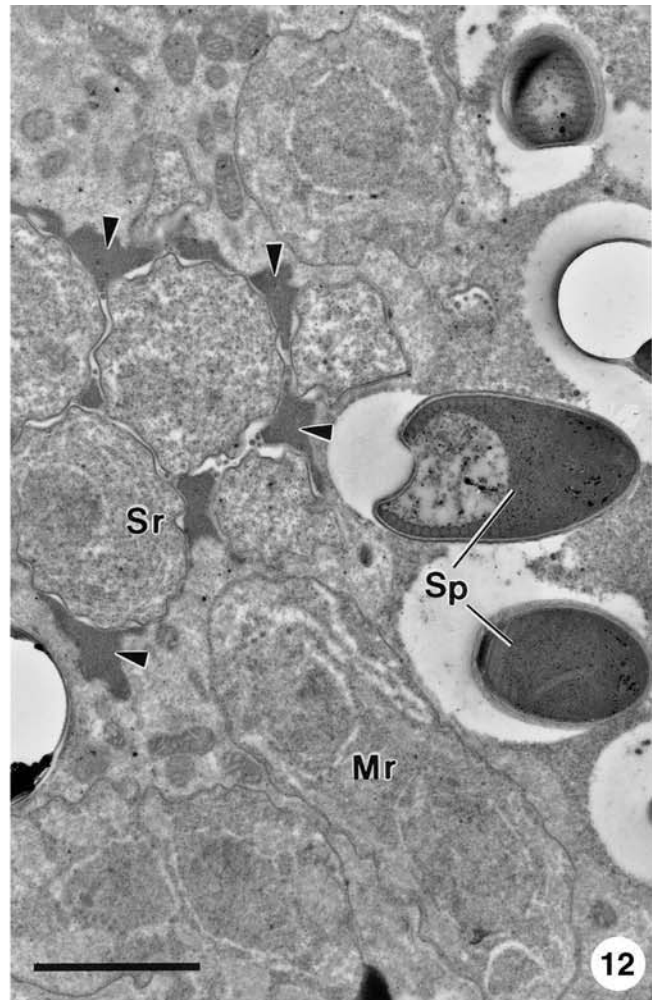
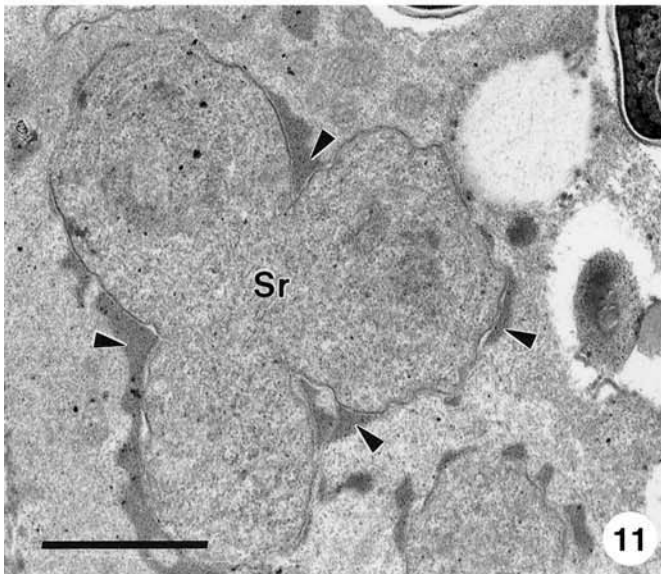
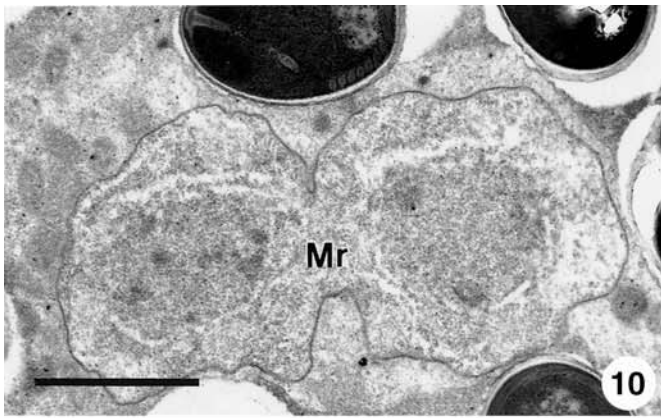


Table 2 Comparison of some SSU rDNA sequences: percentage of identity (top diagonal) and pairwise distance (bottom diagonal) obtained by Kimura-2 parameter analysis

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>Loma psittaca</i> n. sp.	–	96.9	96.0	96.0	95.6	95.6	95.6	95.2	94.4	89.6	89.6	89.2	87.3	86.9	85.4
<i>Loma acerinae</i>	0.031	–	97.3	97.3	96.5	96.5	96.5	96.5	96.5	90.6	89.7	91.0	88.8	88.3	86.9
<i>Glugea atherinae</i>	0.040	0.027	–	100	98.8	98.8	98.8	99.2	97.3	92.7	90.1	91.5	89.6	89.2	87.8
<i>Glugea anomala</i>	0.040	0.027	0.000	–	98.8	98.8	98.8	99.2	97.3	92.7	90.1	91.5	89.6	89.2	87.8
<i>Pleistophora finisterrensis</i>	0.044	0.035	0.012	0.012	–	100	100	98.1	96.5	93.6	89.6	91.0	89.6	90.5	89.2
<i>Glugea plecoglossi</i>	0.044	0.035	0.012	0.012	0.000	–	100	98.1	96.5	93.6	89.6	91.0	89.6	90.5	89.2
<i>Glugea</i> sp. GS1	0.044	0.035	0.012	0.012	0.000	0.000	–	98.1	96.5	93.6	89.6	91.0	89.6	90.5	89.2
<i>Glugea stephani</i>	0.048	0.035	0.008	0.008	0.019	0.019	0.019	–	96.5	91.9	89.6	91.0	88.8	88.3	86.9
<i>Glugea</i> sp.	0.056	0.035	0.027	0.027	0.035	0.035	0.035	0.035	–	90.6	90.1	90.0	87.8	87.4	86.0
<i>Microsporidium</i> sp. MX1	0.104	0.094	0.073	0.073	0.064	0.064	0.064	0.081	0.094	–	91.9	92.8	92.7	93.1	91.8
<i>Pseudoloma neurophilia</i>	0.104	0.103	0.099	0.099	0.104	0.104	0.104	0.104	0.099	0.081	–	93.6	87.8	88.3	86.9
<i>Ichthyosporidium</i> sp.	0.108	0.090	0.085	0.085	0.090	0.090	0.090	0.090	0.090	0.072	0.064	–	90.6	90.2	88.8
<i>Loma</i> sp.	0.127	0.112	0.104	0.104	0.104	0.104	0.104	0.112	0.122	0.073	0.122	0.094	–	98.4	97.3
<i>Loma salmonae</i>	0.131	0.117	0.108	0.108	0.095	0.095	0.095	0.117	0.126	0.069	0.117	0.098	0.016	–	98.8
<i>Loma embiotocia</i>	0.146	0.131	0.122	0.122	0.108	0.108	0.108	0.131	0.140	0.082	0.131	0.112	0.027	0.012	–

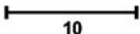
psittaca, supported by 78% bootstrap. Both are clustered in a group together with *Ichthyosporidium*, *Loma*, *Pseudoloma* genera and the *Microsporidium* sp. MX1. However, this clade is poorly supported with a bootstrap lower than 50%. The most parsimonious trees suggested paraphyly for *Loma* species (Fig. 3).

Discussion

The light and ultrastructural observation of the xenoma, developmental stages as well as spore morphology described in the present study, showed all structures typical of the parasites belonging to the phylum Microsporidia (Lom and Dyková 1992; Larsson 1999; Lom and Nilsen 2003).

The fishes represented at least 156 species is one of the largest group parasitised by microsporidians. They were found in different geographic area, habitat and local of infection (Lom 2002). The parasite described in the present work is the second occurrence in teleost fish belonging to the family Tetraodontidae. Ogawa and Yokoyama (1998) found in the intestine of the tiger puffer fish, *Takifugu rubripes*, from a mariculture in Japan, another microsporidian, but it was not classified. Comparing the morphology and ultrastructural aspects of the developmental stages of the parasite here described with microsporidian fish previously characterised, it seemed similar to *Loma* spp. (Lom and Dyková 1992; Lom and Nilsen 2003).

Presently, there are 11 *Loma* species and they were reported in the gills and digestive tract of the fresh and marine fishes (Lom 2002; Table 1). The species type *Loma branchialis* was found in the gills of Atlantic cod (Morrison and Sprague 1981), likely as *L. salmonae* in several salmonids species and from different regions (Putz et al. 1965), *Loma fontinalis* (Morrison and Sprague 1983), *Loma dimorpha* found in different hosts (Loubès 1984), *Loma trichiuri* (Sandeep and Kalavati 1985) and *L. embiotocia* (Shaw et al. 1997). Parasitising the intestine, oesophagus or liver, five species were reported: one in Europe, *Loma diplodae* found in *Diplodus sargus* (Bekhti and Bouix 1985); *Loma boopsi* and *Loma camerounensis* identified in African fishes, *Boops boops* from Senegal (Faye et al. 1995) and in the tilapia species *Oreochromis niloticus* from the Cameroon (Fomena et al. 1992), respectively; *L. acerinae* (Lom and Pekkarinen 1999) in the freshwater *Gymnocaphtalus cernuus* from Czech Republic. Finally, *L. myrophis* found in the Amazonian fish *M. platyrhynchus* was described by Azevedo and Matos (2002). Concerning the habitat, shape and size of the mature spores and the number of polar filament coils, *L. psittaca* did not seem similar with other previously described species. Comparing with the species from the same geographic area, *L. myrophis* found also in intestinal tissue of an Amazonian freshwater fish pre-



sented some differences, mainly in the shape and dimensions of spores, being 67% and 39.7% (relationship width/length) in *L. psittaca* and *L. myrophis*, respectively.

Ultrastructural studies

Comparing *L. psittaca* n. sp. with the other *Loma* species, we saw some ultrastructural similarities, namely the developmental stage aspects. Small xenomas with a centrally located hypertrophic host cell nucleus were observed also in *L. branchialis* (Morrison and Sprague 1981), *L. acerinae* (Lom and Pekkarinen 1999) and *L. myrophis* (Azevedo and Matos 2002). In these *Loma* species, like in some *Glugea* species, it was possible to see in the episporontal space electrodense masses that differentiate several tubular appendages. Curiously, in the genus *Loma*, the origin of the episporontal space is not consensus. It has been described for some species by coalescence of host cell vesicles (PV) (Morrison and Sprague 1981, 1983; Lom and Pekkarinen 1999; Azevedo and Matos 2002), whilst in others, apparently episporontal space has been originated from blisters at the surface of the parasite cell (Bekhti and Bouix 1985; Fomena et al. 1992; Faye et al. 1995).

Phylogenetic analysis

Phylogenetic analysis using the SSU rRNA sequences of fish microsporidian suggested that the parasite found in the puffer fish of the Amazonian fauna, *L. psittaca* n. sp., is a sister species of *L. acerinae*. The most parsimonious tree was supported by 78% bootstrap. All previous phylogenetic trees obtained by parsimony and likelihood maximum presented a similar topology (Docker et al. 1997; Lom and Nilsen 2003; Casal et al. 2008), clustering the almost *Loma* species together with *Ichthyosporidium* sp., *P. neurophilia* and *Microsporidium* sp. MX1 in the group I defined by Lom and Nilsen (2003). The same trees also show that the *Loma* species are a paraphyletic group placing *L. acerinae* and *L. psittaca* in a basal position of the group I or alternatively must be considered an outgroup microsporidian of group I, as suggested by Lom and Nilsen (2003). In this study, the genetic distances (Kimura 2-parameter methods) also show that there are some similarity in SSU rRNA sequences with the species belonging to group II, namely with *G. atherinae*, *G. anomala*, *G. plecoglossi*, *G. stephani*, *Glugea* sp. GS1 and *P. finisterrensis* (last one probably needing to change taxonomic group). The diagnosis of *Glugea* and *Loma* genera presents many similarities that have been confirmed by phylogenetic analysis. Definitely, the morphological and ultrastructural aspects of *L. psittaca* do not accommodate within the genera *Glugea* (Canning et al. 1982), characterised by large xenomas

with a retractile wall and by the presence of a RER cistern surrounding the meronts during developmental stages.

Based on all these morphological and ultrastructural organisation and host specificity described in the present work and comparing them with those of fish microsporidia, which form xenoma, we have found some ultrastructural differences. On the other hand, the genetic data allowed the diagnosis of other fish-infecting microsporidian, supporting the description of a new species. Lom and Nilsen (2003) have reported that a new genus to accommodate *L. acerinae* and in this case *L. psittaca* also would be created. At the moment, we did not find significant ultrastructural differences that justify the creation of a new genus.

Acknowledgements This work partially supported by the Eng. A. Almeida Foundation (Porto, Portugal), PhD grant from “CESPU” (G. Casal), “CNPq” and “CAPES”–Brazil. We would like to thank the iconographic work of Joana Carvalheiro and João Carvalheiro. We assure that this work complies with the current laws of our countries where this was performed.

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Parasitol* 215:403–410
- Azevedo C, Matos E (2002) Fine structure of a new species, *Loma myrophis* (phylum Microsporidia), parasite of the Amazonian fish *Myrophis platyrhynchus* (Teleostei, Ophichthidae). *Eur J Protistol* 37:445–452
- Azevedo C, Matos E (2003) *Amazonspora hassar* n. gen. and n. sp. (phylum Microsporidia, fam Glugeidae), a parasite of the Amazonian teleost *Hassar orestis* (fam. Doradidae). *J Parasitol* 89:336–341
- Baquero E, Rubio M, Moura INS, Pieniazek J, Jordana R (2005) *Myosporidium merluccius* n. g., n. sp. infecting muscle of commercial hake (*Merluccius* sp.) from fisheries near Namibia. *J Eukaryot Microbiol* 52:476–483
- Bekhti M, Bouix G (1985) *Loma salmonae* (Putz, Hoffmann et Dunbar, 1965) et *Loma diplodae* n. sp., microsporidies parasites de branchies de poissons téléostéens: implantation et données ultrastructurales. *Protistologica* 21:47–59
- Canning EU, Lom J, Nicholas JP (1982) Genus *Glugea* Thélohan, 1891 (Phylum Microspora): redescription of the type species *Glugea anomala* (Moniez, 1887) and recognition of its sporogonic development within sporophorous vesicles (pansporoblastic membranes). *Protistologica* 18:193–210
- Casal G, Matos E, Teles-Grilo ML, Azevedo C (2008) A new microsporidian parasite, *Potasporea morhaphis* n. gen., n. sp. (Microsporidia) infecting the Teleostean fish, *Potamorhaphis guianensis* from the River Amazon. Morphological, ultrastructural and molecular characterization. *Parasitology* 135:1053–1064
- Cheney SA, Lafranchi-Tristem NJ, Canning EU (2000) Phylogenetic relationships of *Pleistophora*-like Microsporidia based on small subunit ribosomal DNA sequences and implications for the source of *Trachipleistophora hominis* infections. *J Eukaryot Microbiol* 47:280–287
- Docker MF, Devlin RH, Richard J, Kent ML (1997) Sensitive and specific polymerase chain reaction assay for detection of *Loma salmonae* (Microsporea). *Dis Aquat Org* 29:41–48

- Faye N, Toguebaye BS, Bouix G (1995) On the cytology and development of *Loma boopsi* n. sp. (Microsporida, Glugeidae), parasite of *Boops boops* (Pisces, Teleostei, Sparidae) from the coasts of Senegal. Arch Protistenkd 146:85–93
- Fomena A, Coste F, Bouix G (1992) *Loma camerounensis* sp. nov. (Protozoa: Microsporida) a parasite of *Oreochromis niloticus* Linnaeus, 1757 (Teleost: Cichlidae) in fish-rearing ponds in Melen, Yaoundé, Cameroon. Parasitol Res 78:201–208
- Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66:411–453
- Larsson JIR (1999) Identification of Microsporidia. Acta Protozool 38:161–197
- Lom J (2002) A catalogue of described genera and species of microsporidians parasitic in fish. Syst Parasitol 53:81–99
- Lom J, Dyková I (1992) Protozoan parasites of fishes. Elsevier, Amsterdam, p 315
- Lom J, Nilsen F (2003) Fish microsporidia: fine structural diversity and phylogeny. Int J Parasitol 33:107–127
- Lom J, Pekkarinen M (1999) Ultrastructural observations on *Loma acerinae* (Jirovec, 1930) comb. nov. (phylum Microsporida). Acta Protozool 38:61–74
- Loubès C, Maurand J, Gasc C, Buron I, Barral J (1984) Étude ultrastructurale de *Loma dimorpha* n. sp., microsporidie parasite de poissons Gobiidae languedociens. Protistologica 20:579–589
- Matos E, Azevedo C (2004) Ultrastructural description of *Microsporidium brevirostris* sp. n., parasite of the teleostean *Brachyhypopomus brevirostris* (Hypopomidae) from the Amazon River. Acta Protozool 43:261–267
- Matos E, Corral L, Azevedo C (2003) Ultrastructural details of the xenoma of *Loma myrophis* (phylum Microsporida) and extrusion of the polar tube during autoinfection. Dis Aquat Org 54:203–207
- Matthews JL, Brown AMV, Larison K, Bishop-Stewart JK, Rogers P, Kent ML (2001) *Pseudoloma neurophilia* n. g., n. sp., a new microsporidium from the central nervous system of the zebrafish (*Danio rerio*). J Eukaryot Microbiol 48:227–233
- Morrison CM, Sprague V (1981) Electron microscopical study of a new genus and new species of microsporidia in the gills of Atlantic cod *Gadus morhua* L. J Fish Dis 4:15–32
- Morrison CM, Sprague V (1983) *Loma salmonae* (Putz, Hoffman and Dunbar, 1965) in the rainbow trout, *Salmo gairdneri* Richardson, and *L. fontinalis* sp. nov. (Microsporida) in the brook trout, *Salvelinus fontinalis* (Mitchill). J Fish Dis 6:345–353
- Nilsen F (2000) Small subunit ribosomal DNA phylogeny of microsporidia with particular reference to genera that infect fish. J Parasitol 86:128–133
- Ogawa K, Yokoyama H (1998) Parasitic diseases of cultured marine fish in Japan. Fish Pathol 33:303–309
- Putz RE, Hoffman GL, Dunbar CE (1965) Two new species of *Pleistophora* (Microsporida) from North America fish with a synopsis of Microsporida of freshwater and euryhaline fishes. J Protozool 12:228–236
- Sandeep BV, Kalavati C (1985) A new microsporidian, *Loma trichiuri* n. sp., from the gill of a marine fish, *Trichiurus salva* Cuv. (Trichiuridae). Indian J Parasitol 9:257–259
- Shaw RW, Kent ML, Docker MF, Brown AMV, Devlin RH, Adamson ML (1997) A new species of *Loma* (Microsporea) in shiner perch (*Cymatogaster aggregata*). J Parasitol 83:296–301
- Sprague V, Becnel JJ, Hazard EI (1992) Taxonomy of phylum Microsporida. Crit Rev Microbiol 18:285–395
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
- Thompson JD, Higgins DG, Gilson TJ (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
- Vossbrinck CR, Baker MD, Didier ES, Debrunner-Vossbrinck BA, Shadduck JA (1993) Ribosomal DNA sequences of *Encephalitozoon hellem* and *Encephalitozoon cuniculi*: species identification and phylogenetic construction. J Eukaryot Microbiol 40:354–362

Capítulo 4

**ULTRASTRUCTURAL AND MOLECULAR CHARACTERIZATION OF A NEW
MICROSPORIDIUM PARASITE FROM THE AMAZONIAN FISH,
GYMNORHAMPHICHTHYS RONDONI (RHAMPHICHTHYIDAE)**

Journal of Parasitology (2009) em revisão

Graça Casal, Edilson Matos, M. Leonor Teles-Grilo & Carlos Azevedo

ABSTRACT

A new species of a microsporidium identified as *Microsporidium rondoni* n. sp. found in the freshwater teleost *Gymnorhamphichthys rondoni* collected on lower Amazon River were described based on light, ultrastructural and phylogenetic studies. This parasite develops in the skeletal muscle of the abdominal cavity forming whitish like-cysts containing numerous spores. Mature spores, lightly pyriform to ellipsoidal with rounded ends, measuring $4.25 \pm 0.38 \times 2.37 \pm 0.42 \mu\text{m}$ (n= 30) were observed. The spore wall which measured about 102 nm was composed of two layers with approximately the same thickness. The isofilar polar filament was coiled with 9-10 (rarely 8) turns. The posterior vacuole appeared as a pale area, occupying about 1/3 of the spore length, contained a spherical posterosome composed of granular material, denser at the periphery. The myofibrils located near the spores appeared to be in advanced degradation. Molecular analysis of the rRNA genes, including the ITS region, and phylogenetic analyses using maximum parsimony, maximum likelihood and Bayesian Inference were performed. The ultrastructural characteristics of the spores and phylogenetic data strongly suggested that it is a new species, related to *Kabatana*, *Microgemma*, *Potasporea*, *Spraguea* and *Tetramicra*. We provisionally placed this new species from Amazonian fauna in the collective group *Microsporidium*.

INTRODUCTION

Microsporidia (phylum Microsporidia) are intracellular parasites that occur in almost all taxonomic groups (Canning and Lom 1986; Sprague et al., 1992; Larsson 1999; Lom 2002), and are best known to cause diseases in commercially important fish hosts (Lom and Dyková, 1992; Lom, 2002; Lom and Nilsen, 2003). Microsporidian species, simultaneously parasitizing freshwater and marine fishes from different geographic areas, are included into 17 genera assigned among about 150 genera of Microsporidia (Lom, 2002; Lom and Nilsen, 2003; Azevedo and Matos, 2003; Baquero et al., 2005; Casal et al., 2008).

Presently Microsporidia contain about 156 species and two of them were identified as new genera and new species in the freshwater fishes from the Amazon fauna: *Amazonspora hassar* which occurs in the gills of *Hassar orestis* (Azevedo and Matos, 2003) and *Potasporea morhaphis* in the coelomic cavity of *Potamorhaphis guianensis* (Casal et al., 2008). Other two microsporidia from the same region were described: *Loma myrophis* parasitizing the sub-epithelial gut tissues of *Myrophis platyrhynchus* (Azevedo and Matos, 2002) and *Microsporidium brevirostris* in the skeletal muscle adjacent to the abdominal cavity of the teleost fish *Brachyhypopomus brevirostris* (family Hipopomidae) (Matos and Azevedo, 2004). The last species and the microsporidium described in the present report from *Gymnorhamphichthys rondoni* (fam. Rhamphichthyidae) were the first reference of microsporidiosis in teleost knifefishes (Order Gymnotiformes). Phylogenetic studies based on the molecular analysis of the rRNA genes have been a powerful tool in the identification of new genus and species, as well as in grouping in family taxa (Weiss and Vossbrinck, 1999; Vossbrinck and Debrunner-Vossbrinck, 2005). Presently, there are several SSU rRNA sequences available in the Genbank, corresponding to around 44 fish-microsporidian species. According Lom and Nilsen (2003), fish microsporidia are clustered in five groups and only some of the genera are monophyletic.

Herein, we describe some light microscopic, morphological and ultrastructural features of a new microsporidian species found in a fish from the Amazon River. The molecular characterization and phylogenetic relationships for the SSU rRNA gene were also performed, as well as an analysis of the pathological effects of the spores in the muscle.

MATERIALS AND METHODS

Fish, location of infection and prevalence

Several irregular whitish aggregated of spores (cyst-like structures), located in the skeletal muscles of the internal wall of the ventral abdominal cavity, were removed from the freshwater fish *Gymnorhamphichthys rondoni* (fam. Rhamphichthyidae) (Brazilian common name: Itui transparente). The fish were collected in the lower Amazonian region (01° 46' S / 47° 26' W), near Irituia city, Pará State, Brazil. The fish (12-25 cm long) were taken alive to the laboratory, where they were anaesthetized with MS 222 (Sandoz Laboratories), and necropsied. For measurements fresh isolated spores were observed in the Nomarski differential interference – contrast (DIC) optics. The prevalence of infection was 36% (18 fishes in 50 examined).

Electron microscopy

For transmission electron microscopy (TEM), small fragments of the infected tissues were fixed in 3% glutaraldehyde with 0.2 M sodium cacodylate buffer (pH 7.2) for 12 h at 4 °C, washed overnight in the same buffer at 4 °C and post-fixed in 2% OsO₄ buffered in the same solution for 3 h at same temperature. After dehydration in an ascending ethanol series and propylene oxide, the fragments were embedded in Epon. The semithin sections were stained with blue methylene-Azure II for light microscopy. The ultrathin sections were contrasted with both aqueous uranyl acetate and lead citrate and observed with JEOL 100CXII TEM operated at 60 kV.

DNA isolation and PCR amplification

Several cysts dissected from fishes, were homogenized to isolate the spores and subsequently stored in 80% ethanol at 4 °C. The genomic DNA of about 5×10^6 spores was extracted using a GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma) following the manufacturer instructions for animal tissues, except for the incubation time (12 h). The DNA was stored in 50 µl of TE buffer at – 20 °C until further use. Further The DNA concentration was estimated with the Qubit™ Fluorometer (Invitrogen). The majority of the region coding the small subunit (SSU) rRNA gene was amplified by PCR using the primers V1f (5'CACCAGGTTGATTCTGCC3') and 1492r (5'GGTTACCTTGTTACGAC TT3') (Vossbrinck et al., 1993; Nilsen, 2000). To amplify the 3'-end of the SSU, internal transcribed spacer (ITS) and 5'-end of the large subunit (LSU) rRNA gene, HG4F (5'GCGGCTTAATTTGACTCAAC) and HG4R (5'TCTCCTTGGTCCGTGTTTCAA) primers

were used (Gatehouse and Malone, 1998). PCR was carried out in 50 µl reactions using 10 pmol of each primer, 10 nmol of each dNTP, 2 mM of MgCl₂, 5 µl 10X *Taq* polymerase buffer, 1.25 units *Taq* DNA polymerase (Invitrogen products), and 3 µl of the genomic DNA. The reactions were run on Hybaid PxE Thermocycler (Thermo Electron Corporation, Milford, MA). The amplification program consisted of 94 °C denaturation for 5 min, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min. A final elongation step was performed at 72 °C for 10 min. 5 µl aliquots of the PCR products were electrophoresed through a 1% agarose 1x Tris-acetate-EDTA buffer (TAE) gel stained with ethidium bromide.

DNA cloning and sequencing

The PCR product for the SSU gene with an approximate size of 1400 bp was excised from the agarose gel and purified with NucleoSpin Extract II (Macherey-Nagel). The DNA was cloned into a pGEM-T Easy Vector System II (Promega) following the manufacturer instructions. JM109 Competent cells, high efficiency (Promega) were transformed and 2 positive clones selected. The plasmid DNA isolation were carried out with a NucleoSpin Plasmid (Macherey-Nagel) according to the manufacturer manual. Cloning was confirmed by digestion with the restriction enzyme *EcoRI* (Promega) and through sequencing with the universal sequencing primers T7 forward / SP6. For the ITS region, a PCR product of about 1100 bp was sequenced directly, after cleaning. The sequencing reactions were done using BigDye Terminator v1.1 kit (Applied Biosystems) and were run on an ABI3700 DNA analyzer (Perkin-Elmer, Applied Biosystems, Stabvida, Co., Oeiras, Portugal).

Distance and phylogenetic analysis

Previously, the various forward and reverse sequence segments were aligned manually with ClustalW (Thompson et al., 1994) in MEGA 4 software and ambiguous bases were clarified using corresponding ABI chromatograms. To evaluate the relationship of *Microsporidium rondoni* to other microsporidia, a homology search was performed using BLAST (NCBI). We used 45 rDNA sequences belonging to the microsporidia having fish as hosts. The sequence and NCBI accession number data obtained from GenBank are as follows: *Aspalatospora milevae* (EF990668); *Glugea anomala* (AF044391); *Glugea atherinae* (U15987); *Glugea plecoglossi* (AJ295326); *Glugea stephani* (AF056015); *Glugea* sp. GS1 (AJ295325); *Glugea* sp. (AY090038); *Heterosporis anguillarum* (AF387331); *Heterosporis* sp. PF (AF356225); *Ichthyosporidium* sp. (L39110); *Kabatana takedai* (AF356222); *Kabatana newberryi* (EF202572); *Kabatana seriola* (AJ295322);

Kabatana sp. (EU682928); *Loma acerinae* (AJ252951); *Loma embiotocia* (AF320310); *Loma salmonae* (U78736); *Loma* sp. (AF104081); *Microgemma caulleryi* (AY033054); *Microgemma tincae* (AY651319); *Microgemma vivaresi* (AJ252952); *Microsporidium cypselurus* (AJ300706); *Microsporidium prosopium* (AF151529); *Microsporidium* GHB1 (AJ295324); *Microsporidium* sp. RSB1 (AJ295323); *Microsporidium* sp. STF (AY140647); *Microsporidium* MYX1 (AJ295329); *Myosporidium merlucius* (AY530532); *Nucleospora salmonis* (U78176); *Ovipleistophora mirandellae* (AF356223); *Ovipleistophora ovariae* (AJ252955); *Pleistophora ehrenbaumi* (AF044392); *Pleistophora finisterrensis* (AF044393); *Pleistophora hippoglossoideos* (AJ252953); *Pleistophora typicalis* (AF044387); *Pleistophora* sp. 1 (AF044394); *Pleistophora* sp. 2 (AF044389); *Pleistophora* sp. 3 (AF044390); *Potaspora morhaphis* (EU534408); *Pseudoloma neurophilia* (AF322654); *Spraguea americana* (AF056014); *Spraguea lophii* (1) (AF104086); *Spraguea lophii* (2) (AF033197); *Spraguea* sp. (AY465876); *Tetramicra brevifilum* (AF364303). *Endoreticulatus schubergi* (L39109), *Enterocytozoon bieneusi* (L07123), *Vairimorpha necatrix* (Y00266) and *Vittaforma corneae* (L39112) were used as outgroup.

The alignment was performed with ClustalW in MEGA 4 software (Tamura et al., 2007), with an opening gap penalty of 10 and a gap extension penalty of 4 for both pairwise and multiple alignments. Subsequent phylogenetic and molecular evolutionary analyses were conducted using MEGA 4, with the 45 rDNA sequences for microsporidian species and the outgroup species selected. Distance estimation was carried out using the Kimura-2 parameters model distance matrix for transitions and transversions. For the phylogenetic tree reconstructions, the maximum parsimony analysis was performed using the close neighbour interchange heuristic option with a search factor of 2 and random initial trees addition of 2000 replicates. Clade support was assessed with bootstrapping of 100 replicates.

Maximum likelihood (ML) and Bayesian Inferences (BI) analysis were performed on the Phylogeny.fr platform (Dereeper et al., 2008) and sequences were aligned with ClustalW. The ambiguous regions (i. e. containing gaps and/or poorly aligned) were subsequently removed with Gblocks using the default parameters. The ML method was implemented in the PhyML program (v3.0 aLRT) (Guindon et al., 2005). The GTR substitution model was selected assuming an estimated proportion of invariant sites (of 0.282) and 4 gamma-distributed rate categories to account for rate heterogeneity across sites. The gamma shape parameter was estimated directly from the data (gamma = 1.386). Reliability for the internal branch was assessed using the bootstrapping method (100 bootstrap replicates).

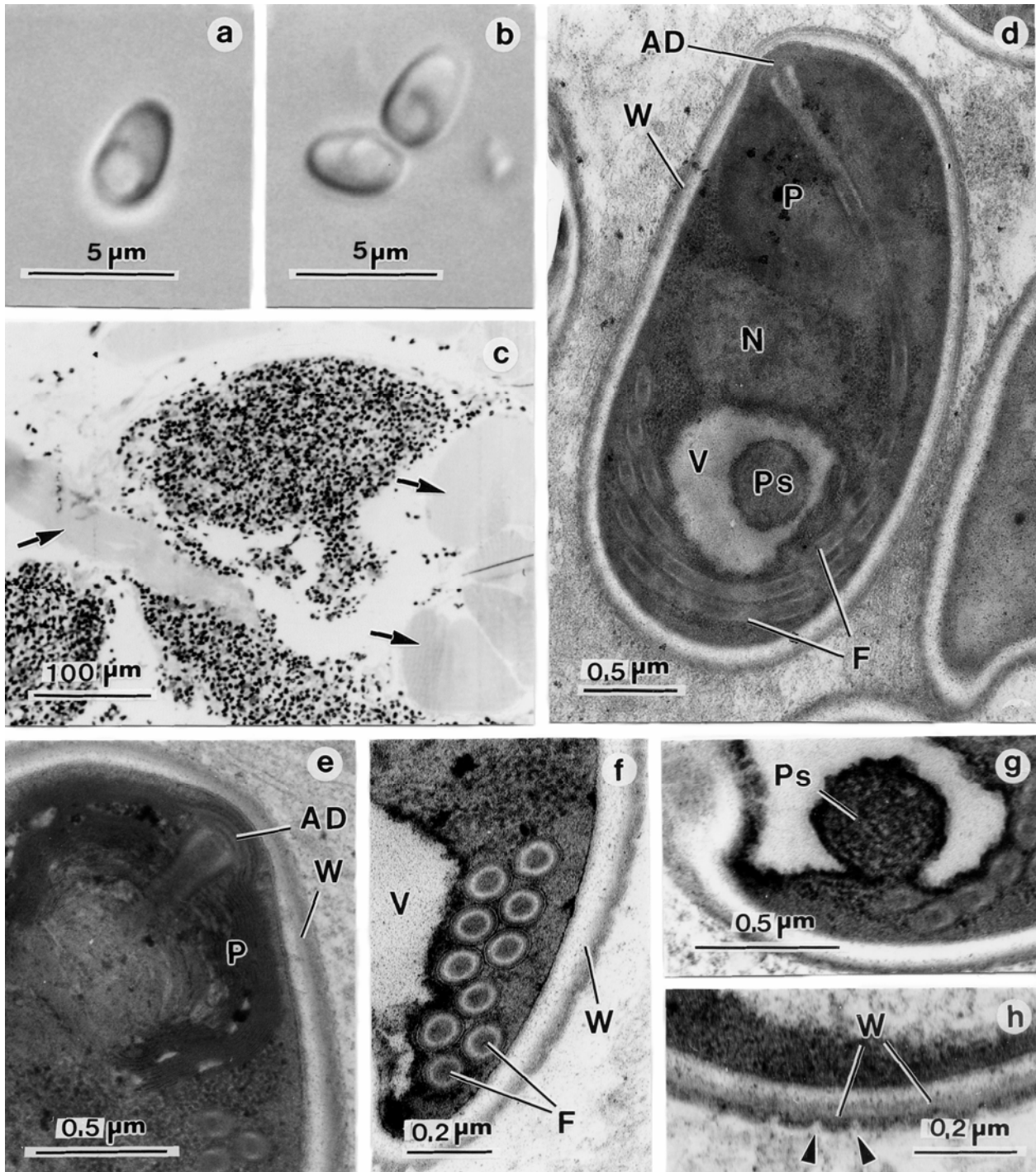


Figure 1 Light and transmission electron micrographs of *Microsporidium rondoni* n. sp. infecting the muscle fibres of the teleost fish *Gymnorhamphichthys rondoni*. (a, b) Fresh spores released from the muscle observed in DIC, showing the pyriforme to ellipsoidal shape and their prominent posterior vacuole. (c) Semithin section of whitish patches containing numerous spores, located among muscle fibres (arrows). (d) Longitudinal section of a spore, showing the wall (W), anchoring disc (AD), different sections of the polar filament (F), polaroplast (P) and the nucleus (N). The posterior vacuole (V) contains a posterosome (Ps). (e) Detail of the anterior region of a spore showing the wall (W) composed of two evident layers (exospore and endospore), anchoring disc (AD) and polaroplast (P). (f) A packed of double layer coils of the polar filament (F) with 10 turns between the wall (W) and the vacuole (V). (g) Detail of a posterosome (Ps) composed by a granular matrix and surrounded by denser material. (h) Transverse

section of the spore wall (W) showing the external region of the exospore containing incisions distributed regularly on the spore surface (arrowheads).

BI was performed with the MrBayes program (v3.1.2) (Ronquist and Huelsenbeck, 2003) with the following parameters: the standard (4 by 4) model of nucleotide substitution was used, the number of substitution types = 6 and rates variation across sites was fixed to "invgamma". Probability distributions were generated using Markov Chain Monte Carlo methods. Four chains were run for 10^5 generations, sampling every 100 generations, with the first 100 sampled trees discarded as "burn-in". Finally, a 50% majority rule consensus tree was constructed. Both the trees were built with the TreeDyn program.

DESCRIPTION

Microsporidium rondoni n. sp.

(Figs. 1-3)

General diagnosis: Isolated and grouped whitish like-cysts in the skeletal muscle of the abdominal cavity (Figs 1a, b). This parasite does not develop xenomas and spores in direct contact with the myofibrils (Fig. 1c).

Description of the spores: Monomorphic, uninucleated mature spores, lightly pyriform to ellipsoidal with rounded ends; $4.25 \pm 0.38 \mu\text{m}$ long and $2.37 \pm 0.42 \mu\text{m}$ wide ($n = 30$) (Figs 1a, b). Nucleus in a central position between the apical polaroplast and the posterior vacuole (Figs 1d, 3). Polaroplast lamellate, bipartite with the elements of distal position somewhat expanded (Fig. 1e). Isofilar polar filament, formed by 3 concentric layers of membranes (Fig. 1f), with 115 (110-121) nm ($n = 50$) in diameter, an angle of tilt of about 45° (42-47) ($n = 10$) (Fig. 1e) and posteriorly arranged in a packed double layered coils with 9-10 (rarely 8) turns (Figs 1d, f). Posterior vacuole with 1/3 of the spore length, contained generally 1-2 conspicuous inclusions - the posterosome, consists of a central granular mass surrounded by amorphous and irregular material, denser at the periphery (Figs 1d, g). Spore wall about 102 (95-110) nm thick ($n = 50$) composed of two layers: an electron dense exospore of ~ 27 nm width and an electron lucent endospore, both with approximately the same thickness (Figs 1d-h). Light incisions distributed regularly on the exospore (Fig. 1h). Spores inside of the sporophorous vesicles were never observed.

Histopathology: Whitish elongated cysts-like structures containing numerous spores were observed in contact with the myofibrils of the internal wall of the abdominal cavity. The infected muscle showed degradation characterized by the disorganization of the

myofibrils (Figs 2a-c). The spores located within the cytoplasm of the host cells were in close contact with the nuclei (Figs 1d, 2a-c) and its cytoplasm appeared partially destroyed (Figs 1d, 2a, b). Phagocytic cells appearing to ingest mature spores were frequently observed near the muscle fibres (Figs 2c, d).

Molecular characterization and phylogeny: Two bands of approximately 1.4 kb and 1.1 kb were obtained after amplification of the microsporidian genomic DNA with the primers V1f-1492r and HG4F-HG4R, respectively. All the sequences obtained were aligned, and the sequence consensus corresponding to the complete SSU rRNA gene, ITS and partial LSU rRNA gene was 1914 bp in length, with a GC content of 43.7%. The sequence was deposited in the Genbank database under the accession number FJ843105. BAST analysis was performed and the highest alignment excluded all the microsporidian SSU rRNA sequences that do not parasite fish species. Then the 3-end of SSU rRNA gene was trimmed, it resulted in an alignment with 1536 bp. The SSU rRNA gene of *Microsporidium rondoni* shows some nucleotidic insertions that allows the classification of this species of others microsporidians: A 13 bp insert from position 779 and a 13 bp at the position 1057 that are common to *Kabatana takedai*. Before the phylogenetic analysis, only those sites which could be unambiguously aligned among all microsporidia and outgroups were used, resulting in an alignment of 1402 bp.

BLAST analysis of the *Microsporidium rondoni* sequence showed that *Kabatana takedai* (AF356222) and *Kabatana* sp. (EU682928) had the highest score, followed by three *Spraguea* spp. sequences. Based on pairwise comparisons among the SSU rDNA sequences, the maximal similarity (Kimura 2-parameter) of *Microsporidium rondoni* with the species of the same clade is for the *Spraguea* (96.4 – 96.8%), *Microgemma* and *Tetramicra* (95.6 – 96.0 %) genera. A longest range of percentage of identity for *Kabatana* species (88.2 – 95.2%) was also observed (Table 1). Maximum parsimony phylogenetic analyses of the SSU rRNA gene strongly supported a clade (bootstrap 91%) where cluster containing *Kabatana*, *Microgemma*, *Potasporea*, *Spraguea*, *Tetramicra* genera and some species of the collective group *Microsporidium* (Fig. 4). Within this clade, the new microsporidium forms a sister taxa with *Spraguea* and *Microgemma* species. After BLAST search we also found a partial SSU rDNA for *Aspalatosporea milevae* (EF990668) that showed a 93.9% identify to *M. rondoni*. With the aim to clarify the phylogenetic position of this new species, the Bayesian inference and maximum likelihood phylogenetic analyses were also performed, confirming similar topology trees (Fig. 5).

Taxonomic summary

Type host: *Gymnorhamphichthys rondoni* (Miranda-Ribeiro, 1920) (Teleostei: Rhamphichthyidae) with 12-25 cm of the length in average.

Type locality: lower Amazon River (01° 46' S / 47° 26' W) near Irituia city, Pará State, Brazil.

Site of infection: skeletal muscle of the internal abdominal cavity.

Prevalence of infection: eighteen of 50 (36%) with no statistical difference between sexes.

Type material: One glass slide with semithin sections containing mature spores of the hapantotype were deposited in the International Protozoan Type Slide Collection at the Smithsonian Institution, Washington D.C. 20560 (USNM no. 1123996).

Etymology: the specific name "*rondoni*" derives from the species epithet of the host species *G. rondoni*.

Remarks

Of the 17 microsporidian genera found in teleost fishes, only *Heterosporis*, *Kabatana*, *Pleistophora* and the collective group *Microsporidium* have affinity to the myocytes of the skeletal muscle and some induce serious pathological changes (Dyková and Lom, 2000). The genera *Heterosporis*, *Kabatana*, *Ovipleistophora* and *Pleistophora* are characterized by the incapacity to develop structures known as xenomas which confer good conditions for parasite development and simultaneously minimize the proliferation of the parasite to other organs / tissues of the host (Lom, 2002; Lom and Nilsen, 2003).

Based on the spore's morphological data (shape, dimensions), ultrastructural aspects of the internal organization, with special evidence for the anchoring disc, polaroplast, polar filament coils surrounding the posterior vacuole, the organization of the posterosome, as well as lack of sporophorous vesicles differentiation, the site of infection, and absence of xenoma formation, the microsporidium described here seems to be similar, at least in part, to the genus *Kabatana* (Lom et al., 1999, 2000, 2001; McGourty et al., 2007).

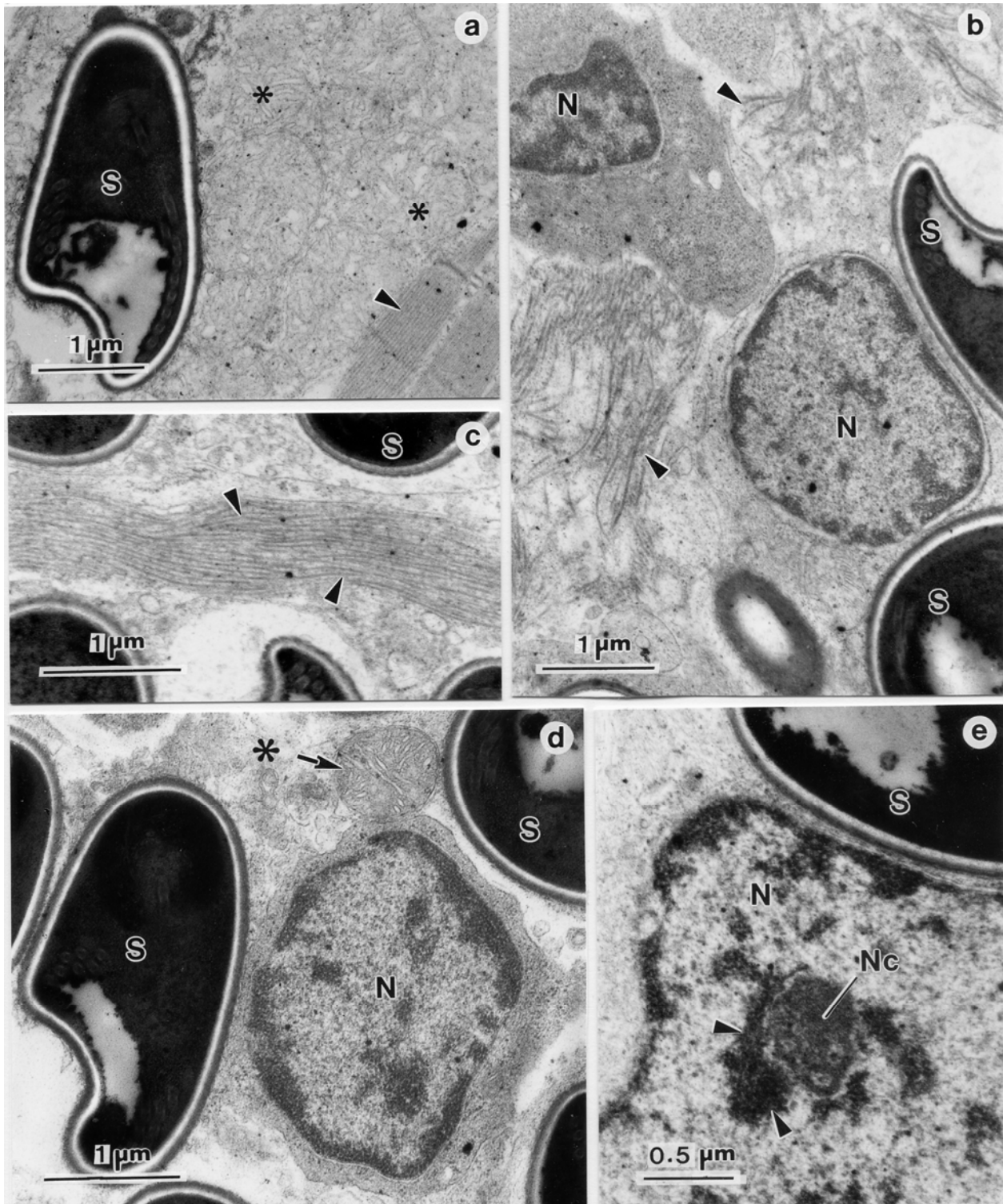


Figure 2 Transmission electron micrographs of *Microsporidium rondoni* n. sp. infecting the muscle of the teleost fish *G. rondoni*. (a) A spore (S) apparently located within the sarcoplasm containing some mitochondria (*) and evident muscle fibres showing normal myofibrils (arrowhead). (b) Some spores (S) in contact with phagocyte cells, each with a nucleus (N), showing among them numerous disorganized myofibrils (arrowheads). (c) Numerous disorganized myofibrils (arrowheads) in contact with spores. (d) Aspect of a phagocyte with a nucleus (N) located several spores (S) that seemed to have a disorganized cytoplasm (*) except for the mitochondria (arrow). (e) Detail of a spore (S) in close contact with a

nucleus (N) of a phagocyte. The nucleus contains a nucleolus (Nc) surrounded by some dense masses of perinucleolar chromatin (arrowheads).

The presence of one or more dense globules, posterosomes, which lie inside the posterior vacuole, can be observed in the spores of genus *Kabatana* (Lom et al., 1999, 2001; McGourty et al., 2007), as well as in the *Tetramicra brevifilum* (Matthews and Matthews, 1980). Another ultrastructural characteristic common to the all *Kabatana* species are small depressions regularly distributed in all surfaces of the external spore's wall (Egusa, 1982; Lom et al., 1999, 2001; McGourty et al., 2007). This differentiation has been reported in microsporidian species of host fishes, such as genera *Spraguea* (Loubès et al., 1979; Freeman et al., 2004). Moreover, for the genus *Amazonspora*, although it was not been reported directly it can be observed in the microphotographs the small fields of the exospore (Azevedo and Matos, 2003).

The location of infection is another characteristic that must be considered. Apparently, the species within a genus often show tissue or organ specify. All *Microgemma* spp. infect the liver, *Spraguea* spp., the ganglion cells of the nervous tissues, *Kabatana* spp. the skeletal

muscular fibres, *Pleistophora* spp. skeletal and smooth muscles and almost *Loma* spp. infect primarily gill filaments. Most of the microsporidia that infect the muscles could inflict heavy damage on the surrounding muscle cell. Moreover, the enzymatic action induced by the presence of parasites belonging to the genera *Kabatana* and *Pleistophora* is clearly present, and is similar to the one observed in members of the myxozoan genus *Kudoa* (Lom et al., 1999). The presence of the *Kudoa* spores in direct contact with the muscle fibres has been suggested to be the reason for the liquefaction of the muscles (Moran et al., 1999).

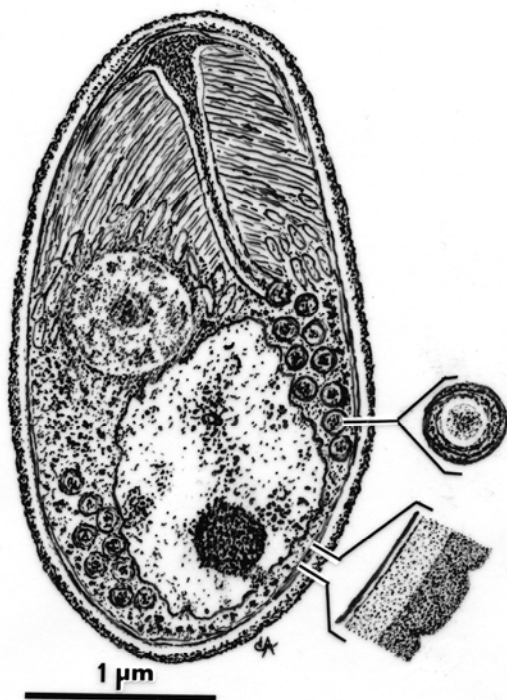


Figure 3 Schematic drawing of a longitudinal section of a spore of *Microsporidium rondoni* n. sp., showing all typical structures described in the text. Details of transverse sections of the polar filament and spore wall are represented.

Table 1 Comparison of some SSU rDNA sequences: percentage of identity (top diagonal) and pairwise distance (bottom diagonal) obtained by Kimura-2 parameter analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
(1) <i>Microsporidium rondoni</i>	-	96.8	96.8	96.8	96.4	96.0	95.6	95.6	95.6	95.2	94.7	93.9	93.9	88.2	88.2	88.2	83.9
(2) <i>Spraguea</i> sp.	0.032	-	100	100	99.6	99.2	98.8	98.8	98.8	96.8	96.4	95.6	95.6	89.5	89.5	89.5	85.3
(3) <i>Spraguea lophii</i> (1)	0.032	0.000	-	100	99.6	99.2	98.8	98.8	98.8	96.8	96.4	95.6	95.6	89.5	89.5	89.5	85.3
(4) <i>Spraguea lophii</i> (2)	0.032	0.000	0.000	-	99.6	99.2	98.8	98.8	98.8	96.8	96.4	95.6	95.6	89.5	89.5	89.5	85.3
(5) <i>Spraguea americana</i>	0.036	0.004	0.004	0.004	-	98.8	98.4	98.4	98.4	96.4	96.0	95.2	95.2	89.1	89.1	89.1	84.9
(6) <i>Microgemma tincae</i>	0.040	0.008	0.008	0.008	0.012	-	99.6	99.2	99.2	96.8	96.4	95.6	95.6	89.5	89.5	89.5	85.8
(7) <i>Microgemma vivaresi</i>	0.044	0.012	0.012	0.012	0.016	0.004	-	98.8	98.8	96.4	96.0	95.2	95.2	89.1	89.1	89.1	85.3
(8) <i>Microgemma caulleryi</i>	0.044	0.012	0.012	0.012	0.016	0.008	0.012	-	100	96.4	96.0	95.2	95.2	89.1	89.1	89.1	85.8
(9) <i>Tetramicra brevifilum</i>	0.044	0.012	0.012	0.012	0.016	0.008	0.012	0.000	-	96.4	96.0	95.2	95.2	89.1	89.1	89.1	85.2
(10) <i>Kabatana</i> sp.	0.048	0.032	0.032	0.032	0.036	0.032	0.036	0.036	0.036	-	99.6	96.8	97.2	89.5	89.5	89.5	85.3
(11) <i>Kabatana newberryi</i>	0.053	0.036	0.036	0.036	0.040	0.036	0.040	0.040	0.040	0.004	-	96.4	96.8	88.0	88.0	88.0	84.8
(12) <i>Aspalatospora milevae</i>	0.061	0.044	0.044	0.044	0.048	0.044	0.048	0.048	0.048	0.032	0.036	-	96.8	90.4	90.4	90.4	87.7
(13) <i>Kabatana takedai</i>	0.061	0.044	0.044	0.044	0.048	0.044	0.048	0.048	0.048	0.028	0.032	0.032	-	90.4	90.4	90.4	86.1
(14) <i>Kabatana seriola</i>	0.118	0.105	0.105	0.105	0.109	0.105	0.109	0.109	0.109	0.105	0.110	0.096	0.096	-	100	100	85.3
(15) <i>Microsporidium</i> sp. GHB1	0.118	0.105	0.105	0.105	0.109	0.105	0.109	0.109	0.109	0.105	0.110	0.096	0.096	0.000	-	100	85.3
(16) <i>Microsporidium</i> sp. RSB1	0.118	0.105	0.105	0.105	0.109	0.105	0.109	0.109	0.109	0.105	0.110	0.096	0.096	0.000	0.000	-	85.3
(17) <i>Potasporea morhaphis</i>	0.161	0.147	0.147	0.147	0.151	0.142	0.147	0.142	0.142	0.147	0.152	0.123	0.139	0.147	0.147	0.147	-

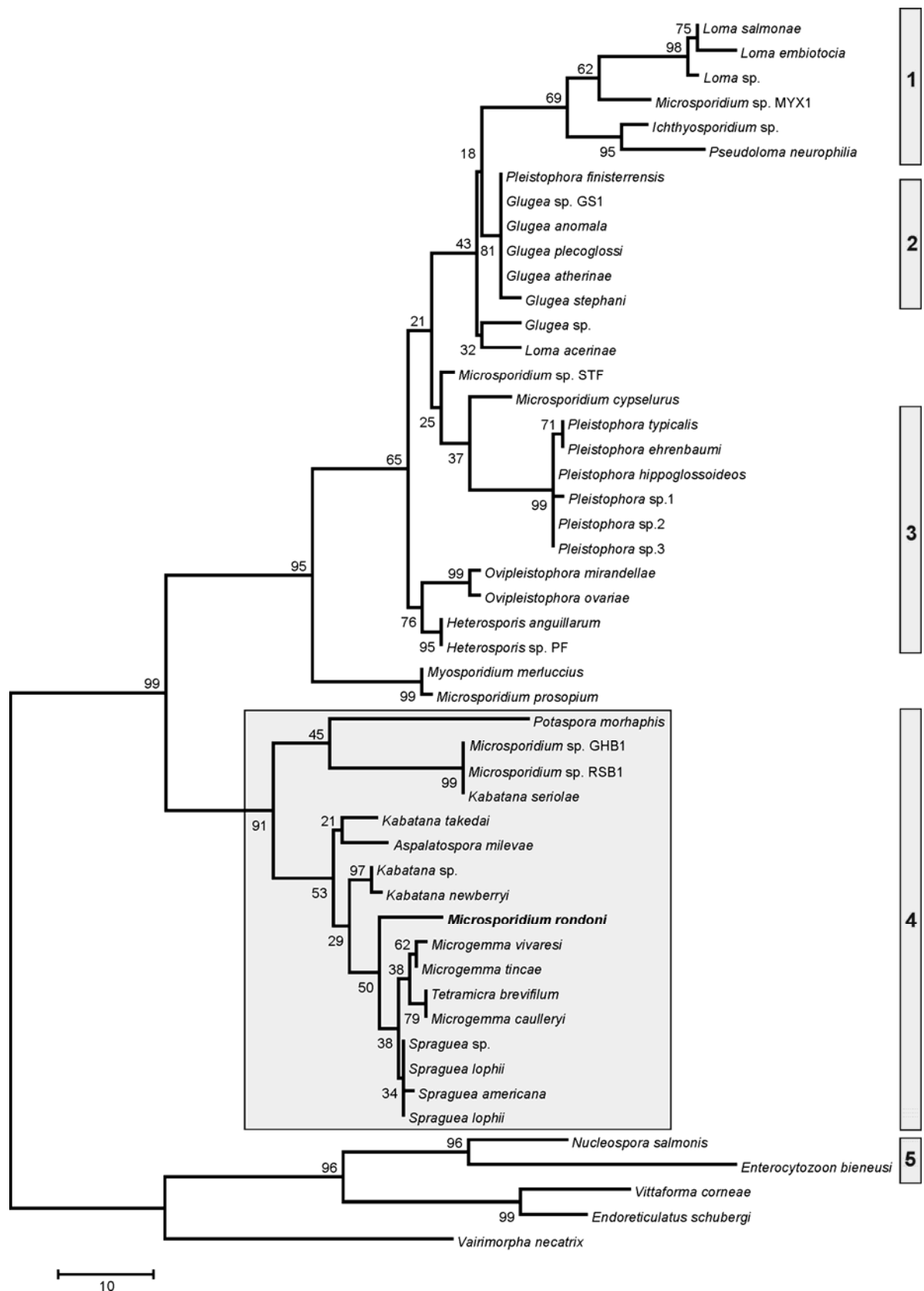


Figure 4 The maximum parsimony tree of SSU rDNA sequences of *Microsporidium rondoni* n. sp. and other selected microsporidia. The numbers on the branches are bootstrap confidence levels on 100 replicates. GenBank accession numbers are in parentheses after the species names and the scale is given under the tree. *Microsporidium rondoni* places within the group 4 (Lom and Nilsen, 2003) (highlighted box), include the sequences of the genera *Kabatana* *Microgemma*, *Potaspora*, *Spraguea*, *Tetramicra*, and *Microsporidium*.

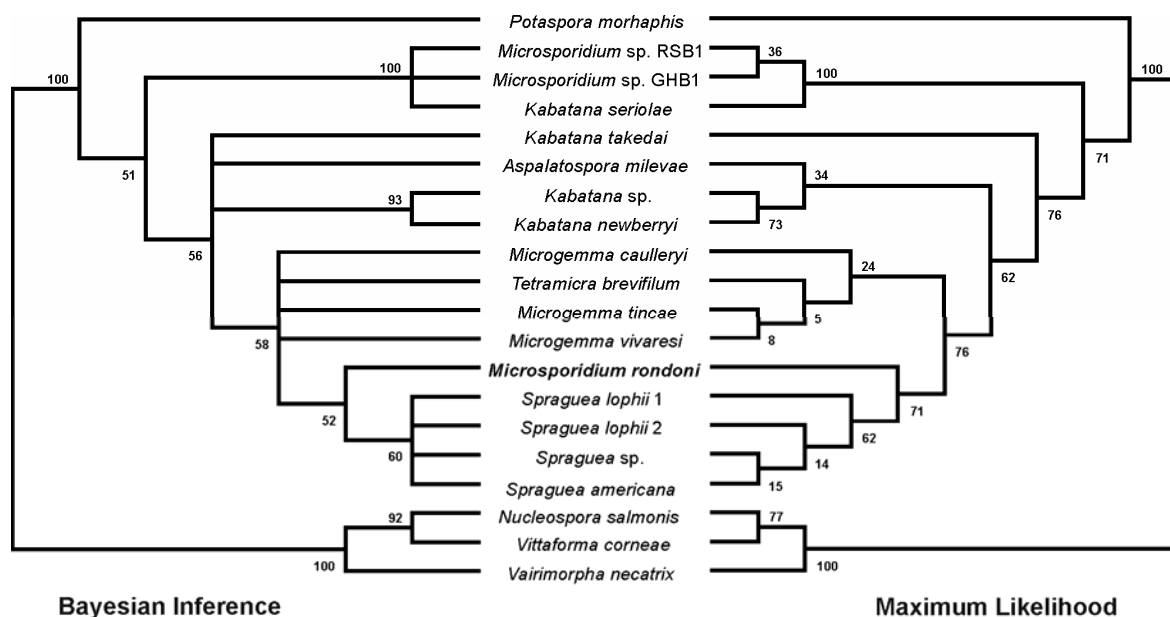


Figure 5 Phylogenetic tree based on Bayesian inference and maximum likelihood analysis of SSU rDNA sequences for both *Microsporidium rondoni* n. sp. and of microsporidia positioned in the same clade (Fig. 4 - group IV) provided identical topology.

Four *Kabatana* species were reported to parasite trunk musculature of freshwater and marine fishes from distinct geographic areas. In Thailand, *K. arthuri* was found in catfish *Pangasius sutchi* (Lom et al., 1990, 1999, 2000), in the Japan yellowtail *Seriola quinqueradiata* is parasitized by *K. seriola* (Egusa, 1982) whereas *K. takedai* was found in the heart, trunk and other muscles of freshwater salmonids in Japan and eastern Russia (Lom et al., 2001). Recently, *K. newberryi* was reported in two different gobies species. In tidewater goby *Eucyclogobius newberryi* in coastal lagoons in Northern California (McGourty et al., 2007) and in two-spotted goby *Gobiusculus flavescens* caught in the Swedish Gullmarsfjord (Barber et al., 2009).

Comparing our results with previously described *Kabatana* spp, we found some morphological differences on the spores, mainly on the number and the arrangements of the polar filaments coils. Both species, *M. rondoni* and *K. newberryi* spores have similar number of coils (9-10), however, *M. rondoni* has typically the coils organized in 2 rows, while *K. newberryi* has 1 or 2 rows. On the other hand the spores of *M. rondoni* are longer than those of *K. newberryi* (McGourty et al., 2007; Barber et al., 2009).

Phylogenetic analysis by MP and ML methods, as well as Bayesian Inferences using SSU rDNA are in concordance with previous cladograms (Lom and Nilsen, 2003; Casal et al., 2008; Barber et al., 2009). The parasite described here is placed in clade (MP: 91% bootstrap) composed of microsporidia belonging to *Kabatana*, *Microgemma*, *Potaspora*,

Spraguea, *Tetramicra* genera, 2 unclassified species of the *Microsporidium* group and *Aspalatospora milevae* [Mladineo and Lovy - *Aspalatospora milevae* n. g., n. sp.: xenoma-forming microsporidian from the intestine of the Atlantic bluefin tuna (*Thunnus thynnus*) - personal communication]. Like Matthews et al. (2001) we tried to identify signature sequences. We found two regions in the SSU rDNA sequence of *M. rondoni* similar to that of *Kabatana takedai*. This kind of analysis has been encouraged the characterization of the new species (Lom and Nilsen, 2003).

All methods provide evidences that *Kabatana* species are a paraphyletic group. The exception is *K. newberryi* (parasite of a goby species from Pacific coast, USA) and *Kabatana* sp. (parasite of a goby species from Atlantic coast, Sweden) considered to be of the same species (Barber et al., 2009). MP analysis, clusters *Aspalatospora milevae* in a sister taxa with *K. takedai*. Nevertheless, the bootstrap (21%) for this clade is poorly supported. The species *K. seriolae* is the most genetically distinct (11.8%) and forms a stable clade (bootstrap 100%), together with two *Microsporidium* spp. (Bell et al., 2001). MP methods, *M. rondoni* occupies a basal position (bootstrap 50%) clustered with all *Microgemma* spp., *Spraguea* spp. and *Tetramicra brevifilum*. Using phylogenetic analyses by ML method (bootstrap 71%) and BI (bootstrap 52%), *M. rondoni* is included with *Spraguea* spp. in the same clade.

In conclusion, morphological, ultrastructural and molecular analyses in the present study demonstrated that this microsporidium is a new species belonging to the group 4. This parasite probably belongs to the genus *Kabatana*. As there is not adequate information on their developmental stages to assign this species to a specific genus, we provisionally placed it in the collective group *Microsporidium* Balbiani, 1884.

ACKNOWLEDGMENTS

Work partially supported by the Eng^o. A. Almeida Foundation (Porto, Portugal), PhD grant from “CESPU” (G. Casal), “CNPq” and “CAPES” - Brazil. We would like to thank the iconographic work of Joana Carvalheiro and João Carvalheiro. This work comply with the current laws of the countries in where they were performed.

LITERATURE CITED

- AZEVEDO, C., AND E. MATOS. 2002. Fine structure of a new species, *Loma myrophis* (Phylum Microsporidia), parasite of the Amazonian fish *Myrophis platyrhynchus* (Teleostei, Ophichthidae). *European Journal of Protistology* **37**: 445-452.
- _____, AND _____. 2003. *Amazonspora hassar* n. gen. and n. sp. (phylum Microsporidia, fam. Glugeidae), a parasite of the Amazonian teleost *Hassar orestis* (fam. Doradidae). *Journal of Parasitology* **89**: 336-341.
- BAQUERO, E., M. RUBIO, I. N. S. MOURA, J. PIENIAZEK, AND R. JORDANA. 2005. *Myosporidium merluccius* n. g., n. sp. infecting muscle of commercial hake (*Merluccius* sp.) from fisheries near Namibia. *Journal of Eukaryotic Microbiology* **52**: 476-483.
- BARBER, I., A. J. DAVIES, J. E. IRONSIDE, E. FORSGREN, AND T. AMUNDSEN. 2009. First record of a *Kabatana* sp. microsporidium infecting fish in the Atlantic Ocean. *Diseases of Aquatic Organisms* **83**: 145-152.
- BELL, A. S., T. AOKI, AND H. YOKOYAMA. 2001. Phylogenetic relationships among Microsporidia based on rDNA sequence data, with particular reference to fish-infecting *Microsporidium* Balbiani, 1884 species. *Journal of Eukaryotic Microbiology* **48**: 258-265.
- CANNING, E. U., AND J. LOM. 1986. *The Microsporidia of Vertebrates*, Academic Press, London.
- CASAL, G., E. MATOS, M. L. TELES-GRILLO, AND C. AZEVEDO. 2008. A new microsporidian parasite, *Potaspora morhaphis* n. gen., n. sp. (Microsporidia) infecting the teleostean fish, *Potamorhaphis guianensis* from the River Amazon. Morphological, ultrastructural and molecular characterization. *Parasitology* **135**: 1053-1064.
- DEREEPER A., V. GUIGNON, G. BLANC, S. AUDIC, S. BUFFET, F. CHEVENET, J. F. DUFAYARD, S. GUINDON, V. LEFORT, M. LESCOT, J. M. CLAVERIE, AND O. GASCUEL. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* **36**: 465-469.
- DYKOVÁ, I., AND J. LOM. 2000. Histopathology of *Kabatana arthuri* (Microspora) infection in sutchi catfish, *Pangasius sutchi*. *Folia Parasitologica* **47**: 161-166.
- EGUSA, S. 1982. A microsporidian species from yellowtail juveniles, *Seriola quinqueradiata*, with 'Beko' disease. *Fish Pathology* **16**: 187-192.
- FREEMAN, M. A., H. YOKOYAMA, AND K. OGAWA. 2004. A microsporidian parasite of the genus *Spraguea* in the nervous tissues of the Japanese anglerfish *Lophius litulon*. *Folia Parasitologica* **51**: 167-176.
- GATEHOUSE, H. S., AND L. A. MALONE. 1998. The ribosomal RNA gene region of *Nosema apis* (Microspora): DNA sequence for small and large subunit rRNA genes and evidence of a large tandem repeat unit size. *Journal of Invertebrate Pathology* **71**: 97-105.
- GUINDON, S., F. LETHIEC, P. DUROX, AND O. GASCUEL. 2005. PHYML online – a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Research* **33**: 557-559.
- LARSSON, J. I. R. 1999. Identification of Microsporidia. *Acta Protozoologica* **38**: 161-197.
- LOM, J. 2002. A catalogue of described genera and species of microsporidians parasitic in fish. *Systematic Parasitology* **53**: 81-99.

- _____, AND I. DYKOVÁ. 1992. Microsporidia (Phylum Microspora Sprague, 1977). In *Protozoan Parasites of Fishes*, J. Lom, and I. Dyková (eds.). *Developments in Aquaculture and Fisheries Science*, Elsevier, Amsterdam. vol. 26, p. 125-157.
- _____, _____, AND F. SHAHAROM. 1990. *Microsporidium arthuri* n. sp., parasite of *Pangasius sutchi* (Pangasiidae, Siluroidea) in South-East Asia. *Diseases of Aquatic Organisms* **8**: 65-67.
- _____, _____, AND K. TONGUTHAI. 1999. *Kabataia* gen. n., new genus proposed for *Microsporidium* spp. infecting trunk muscles of fishes. *Diseases of Aquatic Organisms* **38**: 39-46.
- _____, _____, AND _____. 2000. *Kabatana* gen. n., new name for the microsporidian genus *Kabataia* Lom, Dyková and Tonguthai, 1999. *Folia Parasitologica* **47**: 78.
- _____, AND F. NILSEN. 2003. Fish microsporidia: fine structural diversity and phylogeny. *International Journal for Parasitology* **33**: 107-127.
- _____, _____, AND S. URAWA. 2001. Redescription of *Microsporidium takedai* (Awakura, 1974) as *Kabatana takedai* (Awakura, 1974) comb. n. *Diseases of Aquatic Organisms* **44**: 223-230.
- LOUBÉS, C., J. MAURAND, AND R. ORMIÈRES. 1979. Étude ultrastructurale de *Spraguea lophii* (Doflein, 1898), microsporidie parasite de la Baudroie: essai d'interpretation du dimorphisme sporal. *Protistologica* **15**: 43-54.
- MATOS, E., AND C. AZEVEDO. 2004. Ultrastructural description of *Microsporidium brevirostris* sp. n., parasite of the teleostean *Brachyhypopomus brevirostris* (Hypopomidae) from the Amazon River. *Acta Protozoologica* **43**: 261-267.
- MATTHEWS, J. L., A. M. V. BROWN, K. LARISON, J. K. BISHOP-STEWART, P. ROGERS, AND M. L. KENT. 2001. *Pseudoloma neurophilia* n. g., n. sp., a new microsporidium from the central nervous system of the zebrafish (*Danio rerio*). *Journal of Eukaryotic Microbiology* **48**: 227-233.
- MATTHEWS, R. A., AND B. F. MATTHEWS. 1980. Cell and tissue reactions of turbot *Scophthalmus maximus* (L.) to *Tetramicra brevifilum* gen. n., sp. n. (Microspora). *Journal of Fish Diseases* **3**: 495-515.
- MCGOURTY, K. R., A. P. KINZGER, G. L. HENDRICKSON, G. H. GOLDSMITH, G. CASAL, AND C. AZEVEDO. 2007. A new microsporidian infecting the musculature of the endangered tidewater goby (Gobiidae). *Journal of Parasitology* **93**: 655-660.
- MORAN, J. D. W., D. J. WHITAKER, AND M. L. KENT. 1999. A review of the myxosporean genus *Kudoa* Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture* **172**: 163-196.
- NILSEN, F. 2000. Small subunit ribosomal DNA phylogeny of Microsporidia with particular reference to genera that infect fish. *Journal of Parasitology* **86**: 128-133.
- RONQUIST, F. AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.
- SPRAGUE, V., J. J. BECNEL, AND E. I. HAZARD. 1992. Taxonomy of phylum Microspora. *Critical Review of Microbiology* **18**: 285-395.
- TAMURA, K., J. DUDLEY, M. NEI, AND S. KUMAR. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology Evolution* **24**: 1596-1599.

- THOMPSON, J. D., D. G. HIGGINS, AND T. J. GILSON. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673-4680.
- VOSSBRINCK, C. R., M. D. BAKER, E. S. DIDIER, B. A. DEBRUNNER-VOSSBRINCK, AND J. A. SHADDUCK. 1993. Ribosomal DNA sequences of *Encephalitozoon hellem* and *Encephalitozoon cuniculi*: species identification and phylogenetic construction. *Journal of Eukaryotic Microbiology* **40**: 354-362.
- VOSSBRINCK, C. R., AND B. A. DEBRUNNER-VOSSBRINCK. 2005. Molecular phylogeny of the Microsporidia: ecological, ultrastructural and taxonomic considerations. *Folia Parasitologica* **52**: 131-142.
- WEISS, L., AND C. VOSSBRINCK. 1999. Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the Microsporidia. *In* The Microsporidia and Microsporidiosis, M. Wittner, and L. Weiss (eds.). American Society of Microbiology, Washington, DC, p. 129-171.

Capítulo 5

**FINE STRUCTURE AND PHYLOGENY OF A NEW SPECIES,
SPRAGUEA GASTROPHYSUS (PHYLUM, MICROSPORIIDIA), A PARASITE OF THE
ANGLERFISH *LOPHIUS GASTROPHYSUS* (TELEOSTEI, LOPHIIDAE) FROM BRAZIL**

European Journal of Protistology (2009) submetido

Graça Casal, Sérgio S. Clemente, Patrícia Matos, Marcelo Knoff,
Edilson Matos & Carlos Azevedo

ABSTRACT

The ultrastructure of the fish-infecting Microsporidium *Spraguea gastrophysus* n. sp., found in the dorsal ganglia and kidney of the anglerfish, *Lophius gastrophysus* (fam. Lophiidae), collected on the Brazilian Atlantic coast is described. The parasite develops several groups of whitish xenomas up to 3.1 x 1.8 mm. Inside, there is a hypertrophic host cell surrounded by a hypertrophic cytoplasm containing some intermingled life cycle stages, which consist mainly of mature spores, and several developmental stages with unpaired nuclei. Monomorphic spores are ellipsoidal, lightly curved and measure about $3.35 \pm 0.45 \times 1.71 \pm 0.36 \mu\text{m}$ (n = 50). Polar filament is isofilar with expended base attached to the anchoring disc, constricting abruptly, and then tapering to form 5 - 6 coils in a single row. Polaroplast with two distinct kinds of lamellae is located in the apical portion of the spore occupying one-third of the total volume of the spore. It is composed by an anterior portion that consists of a tightly patched lamellar and regularly spaced, whereas the posterior one is larger, spaced and irregularly organized. Nucleus occupies a central zone of the spores where several polyribosomes are present. The posterior vacuole occupying one-quarter of the volume of the spore contained a voluminous spherical and granular posterosome measuring up to $\sim 0.65 \mu\text{m}$ in diameter. Ultrastructural morphology of the spores and the molecular characterization of the SSU rRNA gene suggest the generic assignment to the genus *Spraguea* and the name the parasite as a new microsporidian species, *Spraguea gastrophysus* n. sp.

Keywords: *Spraguea gastrophysus* n. sp.; Parasite; Microsporidia; Ultrastructure; Phylogeny; *Lophius gastrophysus*

INTRODUCTION

The anglerfish of the genus *Lophius* occurring in different geographic areas are represented by five species (*L. piscatorius*, *L. budegassa*, *L. americanus*, *L. litulon* and *L. gastrophysus*). These species contain fish-infecting microsporidians which are mainly located in the nervous tissues. This parasite was first reported from the spinal ganglia of *L. piscatorius* Linnaeus, 1758 previously classified as genus *Glugea* and later identify as belong to *G. lophii* (Doflein 1898). More detailed morphological studies developed by Mrázek (1899) reinforced that this parasite belongs to this genus. However, this parasite was subsequently transferred to the genus *Nosema*, as *N. lophii* (Pace 1908), name posteriorly confirmed by Weissenberg (1909, 1911a, b, c). Later, Vávra and Sprague in a footnote published in the Weissenberg' paper (1976) refer for the first time the name "*Spraguea* n. gen." and simultaneously transfer *Glugea lophii* to *Spraguea lophii* (Doflein, 1898) Weissenberg, 1976 as type species.

The first ultrastructural data of *S. lophii*, parasite of the European anglerfish *L. budegassa* and *L. piscatorius* both having dimorphic spores, were carried by Loubès et al. (1979). On the other hand, in the spinal and cranial ganglia of American anglerfish, *L. americanus*, caught from the northeast Atlantic coast of the USA were also described the presence of microsporidian spores (Takvorian and Cali 1986). Considering some ultrastructural differences in *L. americanus*, mainly having monomorphic spore type, relatively to previously described dimorphic spore occurring in *L. budegassa* and *L. piscatorius* from Europe, the microsporidian found in *L. americanus* was included in the genus *Glugea*, as *G. americanus* (Takvorian and Cali 1986). However, some recent molecular results based on the SSU rRNA genes sequences suggested that this species would be transferred to the genus *Spraguea*, as *S. americana* (Lom and Nilsen 2003; Nilsen 2000; Pomport-Castillon et al. 2000). More recently, on the basis of ultrastructural and molecular studies, xenomas containing monomorphic microsporidian parasite identified as *Spraguea americana* was found in the nervous tissues of the Japanese anglerfish *Lophius litulon* (Freeman et al. 2004).

The only reference to the presence of a similar microsporidian from the South America anglerfish *Lophius gastrophysus* that was collected in the Brazilian and Venezuelan coasts was reported by Jakowska and Nigrelli (1958, 1959) and Jakowska (1964, 1966), however, with no microscopical images or drawings. Relatively to the Brazilian fauna two new genera *Amazonspora* (Azevedo and Matos 2003) and *Potasporea* (Casal et al. 2008) were identified. There are also information for another three parasitosis, *Loma myrophis*

(Azevedo and Matos 2002) and *Microsporidium brevirostris* (Matos and Azevedo 2004), all of them found in Amazonian fishes.

In the present work, we describe a new microsporidian species on the basis of the ultrastructural morphology of the spores, with special emphasis to host and tissues specificity and molecular characterization of the SSU rRNA gene.

MATERIALS AND METHODS

Light and electron microscopy

Thirty six adult specimens of the marine anglerfish, *Lophius gastrophysus* Miranda-Ribeiro, 1915 (Teleostei, Lophiidae) (Brazilian common name “peixe-sapo pescador”) (27 - 68 cm long; 0.550 – 5.600 gr weight) were collected from the Atlantic coast of “Cabo Frio” (22° 50'S /42° 03'W), State of Rio de Janeiro, Brazil. The fishes were lightly anesthetised with MS 222 (Sandoz Laboratories), transported to the laboratory (UFF - Niterói), dissected and the infected tissues, containing several whitish cysts (cyst-like plasmodia) were removed from the peripheral muscles of the internal abdominal cavity in contact with the dorsal nerves and kidney, and examined by a light microscope equipped with Nomarski interference-contrast (DIC) optics.

For ultrastructural studies, small fragments of the parasitized tissues containing xenoma were excised and fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4 °C for 12 h. After rinsed overnight in the same buffer at 4 °C and post-fixed in 2.0 % osmium tetroxide in the same buffer for 3 h at 4 °C, the fragments were dehydrated through an ascending ethanol series, followed by propylene oxide and embedded in Epon. Semithin sections were stained with methylene blue-Azur II and observed by DIC optics. Ultrathin sections were double stained with aqueous uranyl acetate and lead citrate and observed under a transmission electron microscope (TEM) JEOL 100CXII operated at 60 kV.

DNA isolation, PCR amplification and DNA sequencing

Several cysts were dissected from fishes, were homogenized to isolate the spores that were consequently stored in 80% ethanol at 4 °C. The genomic DNA of about 6×10^6 spores was extracted using a GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma) following the manufacturer instructions for animal tissue, except for the incubation

time. The DNA was stored in 50 µl of TE buffer at - 20°C until further used. The majority of the region coding for the small subunit (SSU) rRNA gene was amplified by PCR using the primers V1f (5'CACCAGGTTGATTCTGCC3') and 1492r (5'GGTTACCTTGTTACGAC TT3') (Nilsen, 2000; Vossbrinck et al. 1993). To amplify the 3'-end of the SSU, internal transcribed spacer (ITS) and 5'-end of the large subunit (LSU) rRNA gene, HG4F (5'GCGGCTTAATTTGACTCAAC) and HG4R (5'TCTCCTTGGTCCGTGTTTCAA) primers were used (Gatehouse and Malone 1998). PCR was carried out in 50 µl reactions using 10 pmol of each primer, 10 nmol of each dNTP, 2 mM of MgCl₂, 5 µl 10 X *Taq* polymerase buffer, 1.25 units *Taq* DNA polymerase (Invitrogen products), and 3 µl of the genomic DNA. The reactions were run on Hybaid PxE Thermocycler (Thermo Electron Corporation, Milford, MA). The amplification program consisted of 94 °C denaturation for 5 min, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min. A final elongation step was performed at 72 °C for 10 min. 5 µl aliquots PCR products were visualized with ethidium bromide staining after running on a 1% agarose gel. PCR products for the SSU gene and ITS region have approximate sizes of 1400 bp and 1100 bp, respectively. These were cleaned using the NucleoSpin Extract II (Macherey-Nagel) and then three purified PCR products were sequenced in both directions. The sequencing reactions were done using BigDye Terminator v1.1 kit (Applied Biosystems) and were run on an ABI3700 DNA analyzer (Perkin-Elmer, Applied Biosystems, Stabvida, Co., Oeiras, Portugal).

Distance and phylogenetic analysis

Previously, the various forward and reverse sequence segments were aligned manually with ClustalW (Thompson et al. 1994) in MEGA 4 software and ambiguous bases were clarified using corresponding ABI chromatograms. To evaluate the relationship of *Spraguea gastrophysus* n. sp. to other Microsporidia, we have used the 35 rDNA sequences that have a fish as host. The sequence and NCBI accession number data obtained from GenBank (Table 1). The corresponding sequences and GenBank/NCBI accession number of *Vairimorpha necatrix* (Y00266) and *Vittaforma corneae* (L39112) were used as the outgroup.

Sequences were aligned as described by Casal et al. (2008). The alignment was performed through the use of Clustal W (Thompson et al. 1994) in MEGA 4 software (Tamura et al. 2007), with an opening gap penalty of 10 and a gap extension penalty of 4 for both pairwise and multiple alignments. Subsequent phylogenetic and molecular evolutionary analyses were conducted using MEGA 4, with the sequences for

microsporidian species and the outgroup species selected. Distance estimation was carried out using the Kimura-2 parameters model distance matrix for transitions and transversions. For the phylogenetic tree reconstructions, the maximum parsimony analysis was performed using the close neighbour interchange (CNI) heuristic option with a search factor of 2 and random initial trees addition of 2000 replicates. Bootstrap values were calculated over 100 replicates.

<i>Aspalatospora milevae</i>	(EF990668)	<i>Microsporidium</i> sp. RSB1	(AJ295323)
<i>Glugea anomala</i>	(AF044391)	<i>Myosporidium merluccius</i>	(AY530532)
<i>Glugea atherinae</i>	(U15987)	<i>Nucleospora salmonis</i>	(U78176)
<i>Glugea plecoglossi</i>	(AJ295326)	<i>Ovipleistophora mirandellae</i>	(AF356223)
<i>Glugea stephani</i>	(AF056015)	<i>Ovipleistophora ovariae</i>	(AJ252955)
<i>Heterosporis anguillarum</i>	(AF387331)	<i>Pleistophora ehrenbaumi</i>	(AF044392)
<i>Heterosporis</i> sp. PF	(AF356225)	<i>Pleistophora finisterrensis</i>	(AF044393)
<i>Ichthyosporidium</i> sp.	(L39110)	<i>Pleistophora hippoglossoides</i>	(AJ252953)
<i>Kabatana takedai</i>	(AF356222)	<i>Pleistophora typicalis</i>	(AF044387)
<i>Kabatana newberryi</i> (1)	(EF202572)	<i>Potasporea morhaphis</i>	(EU534408)
<i>Kabatana newberryi</i> (2)	(EU682928)	<i>Pseudoloma neurophilia</i>	(AF322654)
<i>Kabatana seriola</i>	(AJ295322)	<i>Spraguea americana</i> (1)	(AF056014)
<i>Loma embiotocia</i>	(AF320310)	<i>Spraguea americana</i> (2)	(AY465876)
<i>Loma salmonae</i>	(U78736)	<i>Spraguea lophii</i> (1)	(AF104086)
<i>Microgemma caulleryi</i>	(AY033054)	<i>Spraguea lophii</i> (2)	(AF033197)
<i>Microgemma tincae</i>	(AY651319)	<i>Spraguea lophii</i> (3)	(AF056013)
<i>Microgemma vivaresi</i>	(AJ252952)	<i>Tetramicra brevifilum</i>	(AF364303)
<i>Microsporidium</i> GHB1	(AJ295324)		

Table 1 GenBank accession numbers for 35 SSU rDNA sequences from some microsporidian fishes.

RESULTS

Large whitish cysts (up to 3.1 x 1.8 mm long) and several small cysts (xenomas) were observed macroscopically in the abdominal cavity in closed contact with the internal abdominal muscle near the dorsal ganglia of the anglerfish, *Lophius gastrophysus* (Fig. 1). Similar groups of smaller cysts were observed in kidney. After dissection and rupture of both types of xenomas, it was observed that they had numerous ellipsoidal spores (some thousands) identified as belonging to the phylum Microsporidia (Fig. 2, inset). The xenomas seen in semithin sections had an irregular form and contained several groups of juxtaposed cysts (Fig. 3). At high magnification, it was observed that the xenomas were formed by a wall encircling a hypertrophic cell with a central hypertrophic nucleus surrounded by numerous spores in contact with the cytoplasm of the hypertrophic host

cell. Different life cycle stages of the microsporidian were observed intermingled among the spores in the matrix of the xenoma (Figs 3, 4).

Diagnosis

Phylum Microsporidia Balbiani, 1882

Class Haplosporidia Sprague, Becnel and Hazard, 1992

Family Spragueidae Vávra and Sprague, 1976

Genus *Spraguea* Vávra and Sprague, 1976

Species *Spraguea gastrophysus* n. sp.

Description of the species

Name: *Spraguea gastrophysus* n. sp.

Type host: *Lophius gastrophysus* Miranda-Ribeiro, 1915 (Teleostei, Lophiidae).

Type locality: Atlantic coast of Cabo Frio (22° 50'S /42° 03'W), State of Rio de Janeiro, Brazil.

Location in the host: Xenoma in the dorsal muscle of the internal abdominal cavity and kidney.

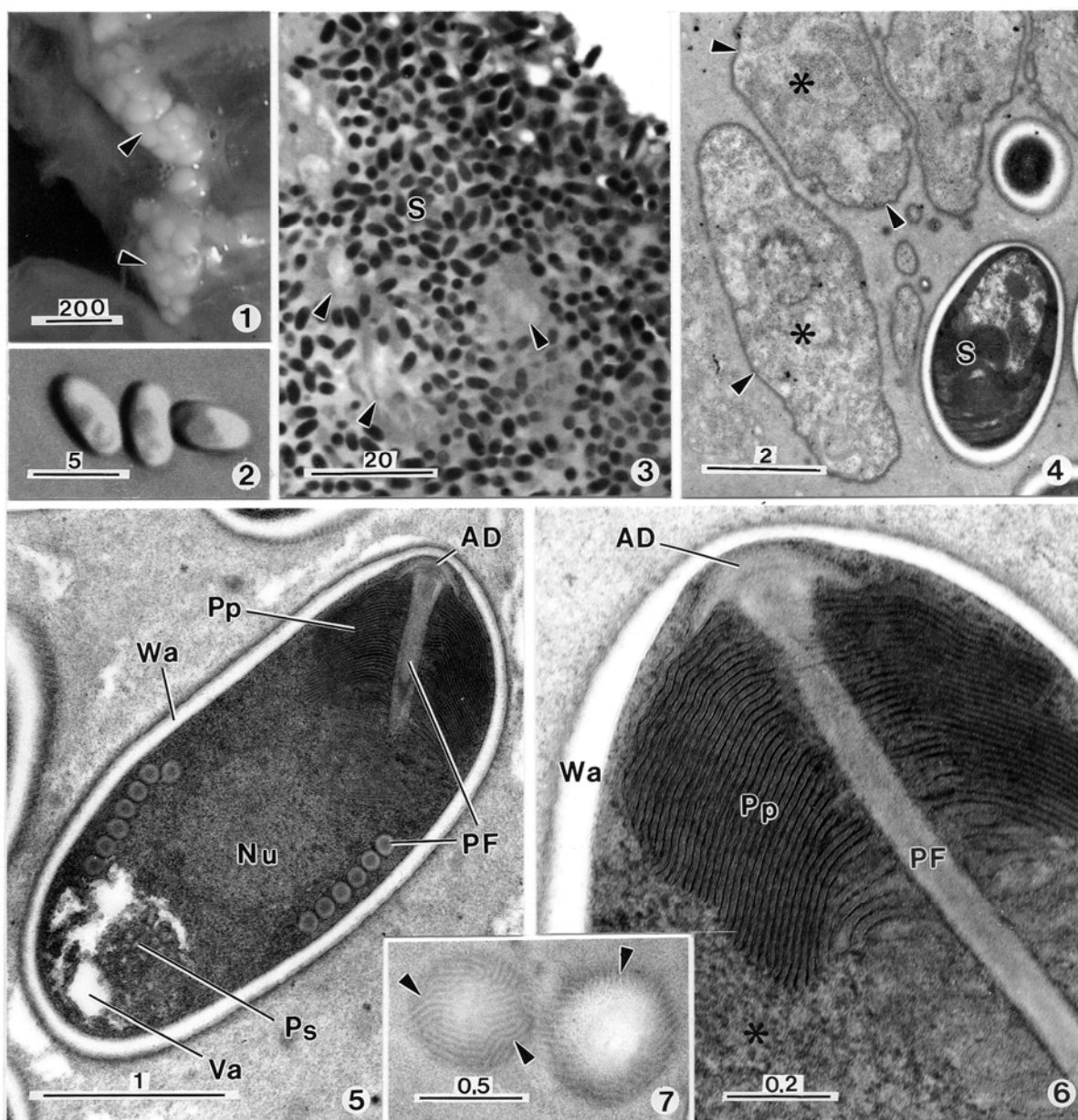
Prevalence of infection: Twenty of 36 examined (55.5%) with similar rates in both sexes.

Type specimens: One glass slide with a semithin section of a xenoma containing different developmental stages, mainly mature spores of hapantotype were deposited in the International Protozoan Type Slide Collection at Smithsonian Institution Washington, DC. 20560, USA, with the acquisition number (USNM).

Etymology: The specific epithet “*gastrophysus*”, is derived from the specific epithet of the host species.

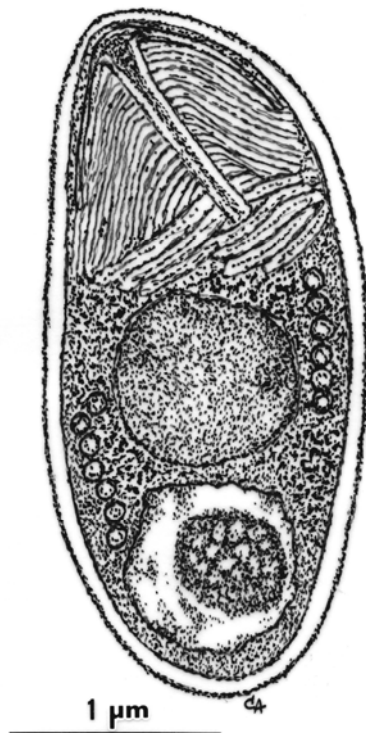
Description of the spore:

Ellipsoidal spores measuring $3.35 \pm 0.45 \mu\text{m} \times 1.71 \pm 0.36 \mu\text{m}$ ($n = 50$) (Fig. 2), and containing all the typical characteristics of the Microsporidia (Figs 5, 8) were observed in the two types of xenomas. The spore wall was 75.3 ± 2.9 ($n = 20$) nm in thickness and consisted of a thin electron-dense exospore with 18.2 ± 2.2 ($n = 20$) nm and a thick electron-lucent endospore with 60.6 ± 3.8 ($n = 20$) nm of thickness (Fig. 6). The spore wall was thinner than the wall (~ 45 nm thick) over the sub-apical positioned anchoring disc.



Figures 1-7. Light and electron micrographs of the xenomas, developmental stages and spores of *Spraguea gastrophysus* n. sp. parasite of the peripheral muscle of the internal abdominal cavity of the teleost *Lophius gastrophysus* (Scale bars in µm). **1.** Some grouped xenoma (arrowheads) were observed in DIC. **2.** Fresh spores observed in DIC. **3.** Semithin section of the periphery of a xenoma showing numerous spores (S) and some other developmental stages (arrowheads). **4.** A group of sporoblasts (*) showing an electron dense wall (arrowheads) and spores (S). **5.** A mature spore longitudinally sectioned showing the wall (Wa), anchoring disc (AD), polaroplast (Pp), polar filament (PF), nucleus (Nu), posterosome (Ps) and posterior vacuole (Va). **6.** Ultrastructural detail of the anterior portion of a mature spore with special evidence of the polaroplast (Pp) organization, spore wall (Wa), anchoring disc (AD), polar filament (manubrium) (PF) and numerous ribosomes (arrowheads). **7.** Tangential section of the spore surface showing the external ornamentation seeming a fingerprints (arrowheads).

The external surface of the exospore is ornamented with numerous fingerprints uniformly distributed at the periphery of the spore wall (Fig. 7). The anchoring disc was in close contact with the internal apical portion of the spore wall (Figs 5, 6). The anchoring disc is located in the apical region of the spore in an eccentric position in relation to the spore axis in continuity with the anterior part of the polar filament (PF) (manubrium) (Figs 5, 6). The anterior part of the PF measured 120.2 ± 5.1 nm ($n = 20$) and was passing through the polaroplast with an angle of tilt of $\sim 30^\circ$. The PF was isofilar, measuring 100–110 nm in diameter, arranged into 5 – 6 coils in one row (Fig. 5). The polaroplast consisted of a



complex membranous system with two distinct kinds of lamellae. The anterior group of lamellae was closely packed and parallel lamellae (~ 12 nm between the folds) and the posterior was larger spaced lamellae irregularly organized (Fig. 6). The spores were monomorphic uninucleate and the nucleus occupied a position between the apical polaroplast and the basal vacuole (Fig. 5). The posterior vacuole occupied about one-quarter of the total volume of the spore (Fig. 5) and contained a spherical electron dense posterosome, measuring about 0.65 nm.

Figure 8. Schematic drawing of a spore of *Spraguea gastrophysus* n. sp., showing all typical specific structures of the microsporidian spore.

Small subunit rDNA and phylogenetic analysis

1824 bp sequence (GC content 46.8%) representing the partial SSU, complete ITS and partial LSU rDNA of the parasite was successfully amplified and deposited in GenBank with the accession number (GQ868443). A Blast search of the GenBank database with the sequence obtained from *Spraguea gastrophysus* detected close matches to other microsporidian rRNA sequences, namely with all *Spraguea* spp. sequences. Previously all microsporidian sequences that have a fish as host were aligned and the most parsimonious tree showed that *Spraguea gastrophysus* is grouped with all *Spraguea* spp. A second alignment with 35 selected sequences, including all from the group 4 designed by Nilsen and Lom (2003) was done. The 5-end and 3-end SSU rDNA were trimmed, resulting in the alignment with 1448 bp. Before the phylogenetic analysis was performed

	Length of SSU used in the analysis	Gaps	Insertions	Transitions	Transversions
<i>Spraguea lophii</i> (1) AF104086	1287	7	5	7	6
<i>Spraguea lophii</i> (2) AF033197	1324	0	13	4	11
<i>Spraguea lophii</i> (3) AF056013	1175	0	9	6	6
<i>Spraguea americana</i> (1) AF056014	1174	2	10	4	6
<i>Spraguea americana</i> (2) AY465876	1206	0	4	1	1

Table 2 Comparative analysis of all *Spraguea* spp. sequences with the obtained in this study from of *Lophius gastrophysus*. Included are the SSU rDNA length, the number the gaps, insertions, transitions and transversions.

Host species Parasite	Country (region) Host tissues	Spore dimensions (in μm)	Wall thick (in nm) Exospore / Endospore	Polar filament coils	Spore surface ornamentation	Spore dimorphism	References
<i>Lophius piscatorius</i> <i>Spraguea lophii</i>	France (Atlantic coast)	3.5× 1.5	-	5 - 6	+	+	Loubès et al., 1979
<i>L. budegassa</i> <i>S. lophii</i>	France (Mediterranean)	4× 1.25	-	3 – 4	-	+	Loubès et al., 1979
<i>L. americanus</i> <i>S. americana</i>	USA (Atlantic coast)	2.8 × 1.5	- 12.5 / 70	6 - 9	+	-	Takvorian and Cali, 1986
<i>L. litulon</i> <i>S. americana</i>	Japan	3.4 × 1.8	- ~30 / ~65	5 – 8	+	-	Freemann et al., 2004
<i>L. gastrophysus</i> <i>S. gastrophysus</i> n. sp.	Brasil (Atlantic coast)	3.35 × 1.71	~ 75 ~18 / ~60	5 - 6	+	-	Present study

Table 3 Comparative measurements (in μm) from *Spraguea* spp.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(1) <i>Spraguea gastrophysus</i> n. sp.		99.2	99.2	99.2	98.9	98.9	97.7	97.3	96.5	96.5	95.3	94.5	92.9	92.9
(2) <i>Spraguea lophii</i> AF104086	0.008		100	100	99.6	99.6	98.5	98.1	97.3	97.3	96.1	95.3	93.7	93.7
(3) <i>Spraguea lophii</i> AF033197	0.008	0.000		100	99.6	99.6	98.5	98.1	97.3	97.3	96.1	95.3	93.7	93.7
(4) <i>Spraguea americana</i> AY465876	0.008	0.000	0.000		99.6	99.6	98.5	98.1	97.3	97.3	96.1	95.3	93.7	93.7
(5) <i>Spraguea lophii</i> AF056013	0.011	0.004	0.004	0.004		100	98.1	97.7	96.9	96.9	95.7	94.9	93.3	93.3
(6) <i>Spraguea americana</i> AF056014	0.011	0.004	0.004	0.004	0.000		98.1	97.7	96.9	96.9	95.7	94.9	93.3	93.3
(7) <i>Microgemma tincae</i> AY651319	0.023	0.015	0.015	0.015	0.019	0.019		99.6	98.5	98.5	96.9	96.1	94.5	94.5
(8) <i>Microgemma vivaresi</i> AJ252952	0.027	0.019	0.019	0.019	0.023	0.023	0.004		98.1	98.1	96.5	95.7	94.1	94.1
(9) <i>Microgemma caulleryi</i> AY033054	0.035	0.027	0.027	0.027	0.031	0.031	0.015	0.019		100	95.7	95.3	93.3	93.7
(10) <i>Tetramicra brevifilum</i> AF364303	0.035	0.027	0.027	0.027	0.031	0.031	0.015	0.019	0.000		95.7	95.3	93.3	93.7
(11) <i>Kabatana newberryi</i> EU682928	0.047	0.039	0.039	0.039	0.043	0.043	0.031	0.035	0.043	0.043		99.2	96.9	96.9
(12) <i>Kabatana newberryi</i> EF202572	0.055	0.047	0.047	0.047	0.051	0.051	0.039	0.043	0.047	0.047	0.008		96.1	96.9
(13) <i>Kabatana takedai</i> AF356222	0.071	0.063	0.063	0.063	0.067	0.067	0.055	0.059	0.067	0.067	0.031	0.039		96.1
(14) <i>Aspalatospora milevae</i> EF990668	0.071	0.063	0.063	0.063	0.067	0.067	0.055	0.059	0.063	0.063	0.031	0.031	0.039	

Table 4 Comparison of some SSU rDNA sequences: percentage of identity (top diagonal) and pairwise distance (bottom diagonal) obtained by Kimura-2 parameter analysis.

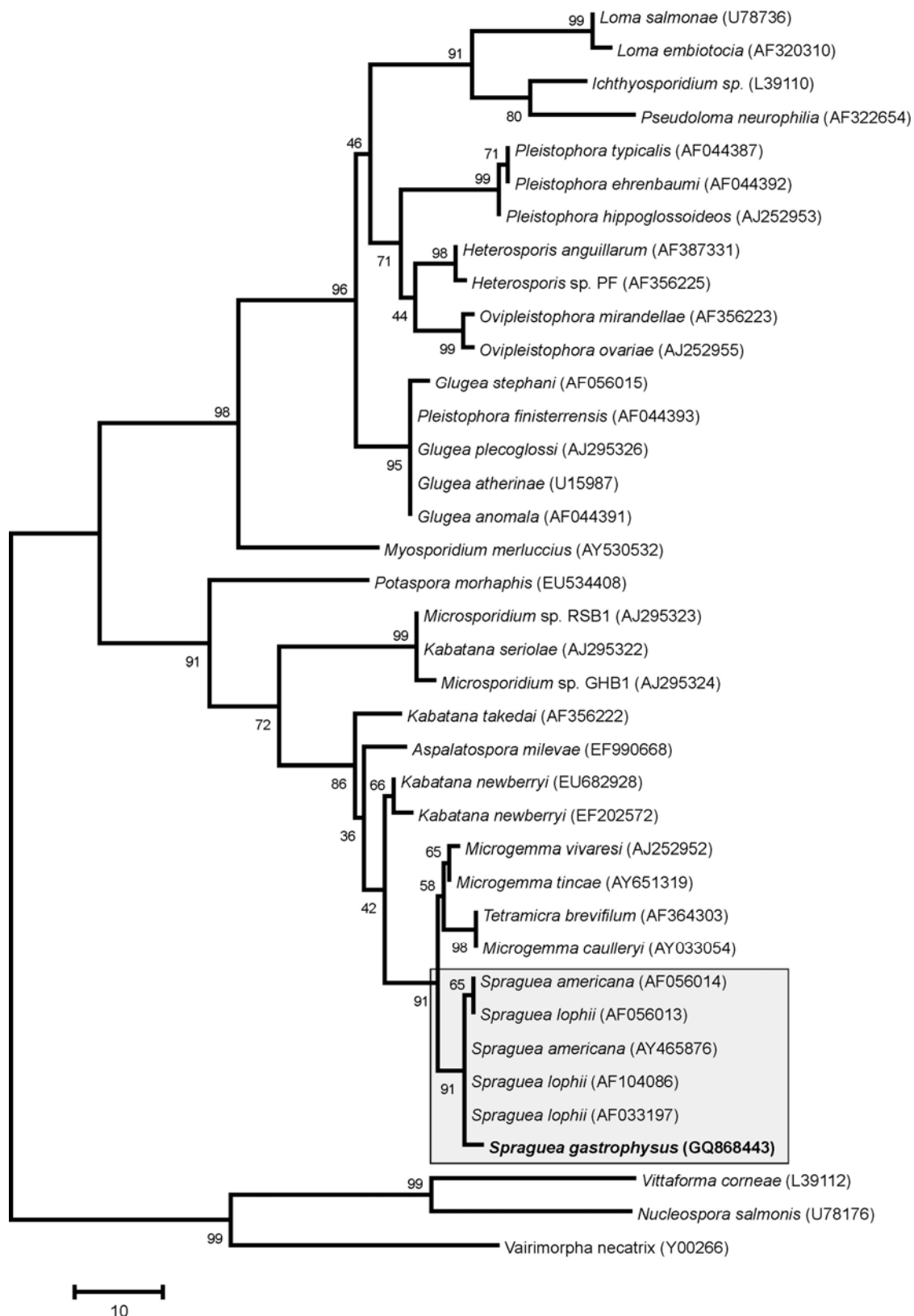


Figure 9. The maximum parsimony tree of SSU rDNA sequences of *Spraguea gastrophysus* n. sp. and other selected microsporidian species. The numbers on the branches are bootstrap confidence levels on 100 replicates. GenBank accession numbers are in parentheses after the species names and the scale is given under the tree. *Spraguea gastrophysus* clusters with all other *Spraguea* spp. (highlighted box).

only those sites which could be unambiguously aligned among all microsporidia and outgroup were used, resulting in an alignment of 1371 bases long.

By the Kimura-2-parameter model the pairwise distance shown that the sequences with more affinities were all belonging to the *Spraguea* genus with a percentage of identity of 99.2% (*S. lophii* AF104086, *S. lophii* AF033197, *S. americana* AY465876) and 98.9% (*S. lophii* AF056013, *S. americana* AF056014). The final phylogenetic tree was built to the maximum parsimony and it shown that all *Spraguea* spp. are a monophyletic group with 91% of bootstrap. The same % of bootstrap was found for the clade composes by all *Spraguea* spp, *Microgemma* spp. and genus *Tetramicra* with a only species. Upon analysis of the sequences, a small number of gaps, insertions, transitions and transversions were found (Table 2).

Discussion

The parasite described in this paper presents all typical morphology and characters of the phylum Microsporidia (Cali and Takvorian 1999; Larsson 1999; Lom and Dyková 1992). Among 17 genera infecting fish, 12 of these produce xenomas: *Amazonspora* Azevedo and Matos 2003; *Glugea* Thélohan, 1891; *Ichthyosporidium* Caullery and Mesnil, 1905; *Loma* Morrison and Sprague, 1981; *Microfilum* Faye, Toguebaye and Bouix, 1991; *Microgemma* Ralphs and Matthews, 1986; *Myosporidium* Baquero, Rubio, Moura, Pieniazek and Jordana, 2005; *Neonosemoides* Faye, Toguebaye and Bouix, 1996; *Pseudoloma* Matthews, Brown, Larison, Bishop-Stewart, Rogers and Kent, 2001; *Potasporea* Casal, Matos, Teles-Grilo and Azevedo, 2008; *Spraguea* Vávra and Sprague, 1976 and *Tetramicra* Matthews and Matthews, 1980.

Among these microsporidians, the *Spraguea* genus is a typical case of close relationship of parasite, host specificity and the local of infection. All infections by *Spraguea* spp. are confined to the hosts belonging to the *Lophius* genus from different geographic areas, like Europe, America and Japan. Presently, it is known that the five species (*L. piscatorius*, *L. budegassa*, *L. americanus*, *L. litulon* and *L. gastrophysus*) are parasitized with *Spraguea* spp. and all in the nervous tissues. They have been localized in the spinal nerves of the vertebral column, trigeminal nerves, vagal nerves or on the medulla oblongata region of the hind brain (Jakowska 1964; Takvorian and Cali 1986; Weissenberg 1911c, 1976). One exception to the parasite-host specificity was observed in the anglerfish *Lophius budegassa*. In Spain, the microsporidian *Tetramicra brevifilum* was also found in musculature and hepatocytes of this lophii fish (Maíllo et al. 1998). This species, which is phylogenetically close to the *Spraguea* spp., frequently parasite the connective tissues of

the musculature of *Scophthalmus maximus* (Matthews and Matthews 1980). Some details like the absence of a conspicuous inclusion body inside the posterior vacuole and the presence of ornamentation outside of the exospore exclude the possibility of the parasite described here belonging to the genus *Tetramicra*.

Irrefutably the parasite described in this study is morphological and phylogenetically close to two *Spraguea* species previously reported in the different anglerfish species. *Spraguea lophii* was described in *Lophius piscatorius* and *L. budegassa* in the European Atlantic coasts and Mediterranean coasts (Loubès et al. 1979) and *Spraguea americana*, first as *Glugea americanus*, in the USA Atlantic coast (*L. americanus*) (Takvorian and Cali 1986) and later in *L. litulon* from Japanese coast (Freeman et al. 2004). The molecular data has shown that the *Glugea americanus* sequences are close to all others *Spraguea lophii* sequences and consequently it was transferred to the genus *Spraguea* and renamed as *S. americana* (Pomport-Castillon et al. 2000; Nilsen 2000; Lom and Nilsen 2003).

The morphology of the spores show several morphological similarities when compared with the uninuclear spores sequence of the *Spraguea* genus, except for the thickness of the two layers of the wall (exospore and endospore). The spore wall found in *Lophius gastrophysus* is thicker than that of other species (Table 3).

For some genera, such as *Amazonspora* (Azevedo and Matos 2003), *Kabatana* (Lom et al. 1999; 2001; McGourty et al. 2007) and *Spraguea* (Loubès et al. 1979; Freeman et al., 2004) the fingerprint-like structures of the external surface of the exospore wall are a morphological characteristic common at all species. Usually, the external ornamentation is regularly distributed and the elevations on the surface of the mature spores, when they are observed in tangential section, present a hexagonal fingerprint-like shape (genus *Amazonspora*) or a tubular shape (genera *Kabatana* and *Spraguea*).

The most parsimonious phylogenetic tree (Fig. 9) clustered all *Spraguea* infections sequences in same clade (bootstrap 91%) and this cladogram had a similar topology to the previous described trees (Casal et al. 2008; Freeman et al. 2004; Lom and Nilsen 2003). Comparison of the SSU rDNA sequence of *Spraguea gastrophysus* with all the others known sequences from *Spraguea* infections from Europe, Japan and America showed that the genetic distances range from 0.8 to 1.1% (Table 4). Whereas, the genetic distance between all previously reported *Spraguea* infections is equal or lower than 0.4%. These data are in accordance with that obtained by Freeman et al. (2004) and it suggests that the microsporidia found in anglerfish *Lophius gastrophysus* from South America is the most phylogenetically distanced of the three *Spraguea* species.

According the original description by Loubés et al. (1979) the genus *Spraguea* is a dimorphic microsporidian that produces two types of spores in two distinct developmental sequences. In one sequence, is characterized by all stages having unpaired nuclei and polysporoblastic sporogony that produce uninucleate spores whereas the other present diplokarya and disporoblastic sporogonic stages that give rise to slender curved diplokaryotic spores. Curiously, the spore dimorphism of the genus and species type is in contradiction with all others ultrastructural descriptions. Apparently, this is an exclusive characteristic of the *Spraguea* infections from *Lophius piscatorius* and *L. budegassa* from European species because the infections from American and Japanese species only produces uninucleated spores. Definitely, as recommended by Lom (2002), Lom and Nilsen (2003) and Freeman et al. (2004) the genus diagnosis need to be redescribed since the exceptions must not be a general characteristic of the genus.

Considering the morphological and molecular data, as well as the host specificity, we believe that this microorganism represents a new species that should be included in the genus *Spraguea* with the name *Spraguea gastrophysus* n. sp.

ACKNOWLEDGEMENTS

This work was partially supported by Eng^o. A. Almeida Foundation, Porto, Portugal, PhD grant from “CESPU” (G. Casal), “CNPq” and “CAPES”, Brazil. We would like the technical assistance of J. Carneiro (ICBAS/UP) and Nilza Felizardo (UFF). This work is original and complies with the current laws of the countries in which it has been performed.

REFERENCES

- Azevedo, C., Matos, E., 2002. Fine structure of a new species, *Loma myrophis* (Phylum Microsporidia), parasite of the Amazonian fish *Myrophis phatyrhynchus* (Teleostei, Ophichthidae). *Europ. J. Protistol.* 37, 445-452.
- Azevedo, C., Matos, E., 2003. *Amazonspora hassar* n. gen. and n. sp. (Phylum Microsporidia, fam. Glugeidae), a parasite of the Amazonian teleost *Hassar orestis* (fam. Doradidae). *J. Parasitol.* 89, 336-341.
- Cali, A., Takvorian, P.U., 1999. Developmental morphology and life cycles of the Microsporidia. In *The Microsporidia and Microsporidiosis* (ed. Wittner, M. and. Weiss, L.), pp. 85-128, American Society of Microbiology, Washington, DC.
- Casal G., Matos E., Teles-Grilo M.L., Azevedo C., 2008. A new microsporidian parasite, *Potasporea morhaphis* n. gen., n. sp. (Microsporidia) infecting the teleostean fish, *Potamorhaphis guianensis* from the River Amazon. Morphological, ultrastructural and molecular characterization. *Parasitology* 135, 1053-1064.

- Döflein, F., 1898. Studien zur Naturgeschichte der Protozoen. III. Ueber Myxosporidien. Zool. Jahrb. Anat. 11, 281-350.
- Freeman, M.A., Yokoyama, H., Ogawa, K., 2004. A microsporidian parasite of the genus *Spraguea* in the nervous tissues of the Japanese anglerfish *Lophius litulon*. Folia Parasitol. 51, 167-176.
- Gatehouse, H.S., Malone, L.A., 1998. The ribosomal RNA gene region of *Nosema apis* (Microspora): DNA sequence for small and large subunit rRNA genes and evidence of a large tandem repeat unit size. J. Invert. Pathol. 71, 97-105.
- Jakowska, S., 1964. Infecção microsporídea das células nervosas numa população de peixes marinhos, *Lophius americanus*. Annual 2nd Congress Latin- American Zoology (S. Paulo, Brazil, 1962). 1: 265-273 (In Portuguese).
- Jakowska, S., 1966. Infection with neurotropic microsporidians in South American *Lophius*. Trans. Am. Microsc. Soc. 85: 161-162.
- Jakowska, S., Nigrelli, R.F., 1958. Preliminary biochemical studies on neurotropic microsporidil *Glugea*-cyst in the American anglerfish. J. Protozool. 5 (Suppl.): 16.
- Jakowska, S., Nigrelli, R.F., 1959. Nosematiasis in the American anglerfish. J. Protozool., 6 (Suppl.), 7.
- Larsson, J.I.R. 1999. Identification of Microsporidia. Acta Protozool. 38, 161-197.
- Lom, J. 2002. A catalogue of described genera and species of microsporidians parasitic in fish. Syst. Parasitol. 53, 81-99.
- Lom, J., Dyková, I., 1992. Microsporidia (Phylum Microspora Sprague, 1977). In *Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Sciences* (ed. Lom, J. and Dyková, I.), vol 26, pp. 125-157. Elsevier, Amsterdam.
- Lom, J., Nilsen, F., 2003. Fish microsporidia: fine structural diversity and phylogeny. Int. J. Parasitol. 33, 107-127.
- Lom, J., Dyková, I., Tonguthai K., 1999. *Kabataia* gen. n., new genus proposed for *Microsporidium* spp. infecting trunk muscles of fishes. Dis. Aquat. Org. 38, 39-46.
- Lom, J., Nilsen, F., Urawa, S., 2001. Redescription of *Microsporidium takedai* (Awakura, 1974) as *Kabatana takedai* (Awakura, 1974) comb. n. Dis. Aquat. Org. 44, 223-230.
- Loubès, C., Maurand, J., Ormières, R., 1979. Étude ultrastructurale de *Spraguea lophii* (Doflein, 1898, microsporidie parasite de la Baudroie: essai d'interprétation du dimorphisme sporal. Protistologica 15, 43-54.
- Maíllo, P.A., Amigó, J.M., Baena, R., Salvadó, H., Gracia, M.P., 1998. *Tetramicra brevifilum* (Matthews & Matthews, 1980) (Microsporida: Tetramicriidae) in a new fish host, *Lophius budegassa* (Spinola, 1807) in Spain. Parasitol. Res. 84, 208-212.
- Matos, E., Azevedo, C., 2004. Ultrastructural description of *Microsporidium brevirostris* sp. n., parasite of the teleostean *Brachyhypopomus brevirostris* (Hypopomidae) from the Amazon River. Acta Protozool. 43, 261-267.
- Matthews, R.A., Matthews, B.F., 1980. Cell and tissue reactions of turbot *Scophthalmus maximus* (L.) to *Tetramicra brevifilum* gen. n., sp. n. (Microsporida). J. Fish Dis. 3, 495-515.

- McGourty, K.R., Kinzger, A.P., Hendrickson, G.L., Goldsmith, G.L., Casal, G., Azevedo, C., 2007. A new microsporidian infecting the musculature of the endangered tidewater goby (Gobiidae). *J. Parasitol.* **93**, 655-660.
- Mrázek, A., 1899. Sporozoenstudien II. *Glugea lophii* Döflein. Sitzungsber. Böhm. Ges. Wiss. Mathnaturwiss. Cl., Prag. 34, 1-8.
- Nilsen, F., 2000. Small subunit ribosomal DNA phylogeny of microsporidia with particular reference to genera that infect fish. *J. Parasitol.* **86**, 128-133.
- Pace, D., 1908. Parasiten und Pseudoparasiten der Nervenzelle. Vorläufige Mitteilungen über vergleichende Parasitologie des Nervensystems. *Z. Hyg. Infektionskr.* **60**, 62-74.
- Pomport-Castillon, C., De Jonckheere, J.F., Romestand, B., 2000. Ribosomal DNA sequences of *Glugea anomala*, *G. stephani*, *G. americanus* and *Spraguea lophii* (Microsporidia): phylogenetic reconstruction. *Dis. Aquat. Org.* **40**, 125-129.
- Takvorian, P.M., Cali, A., 1986. The ultrastructure of spores (Protozoa: Microsporidia) from *Lophius americanus*, the angler fish. *J. Protozool.* **33**, 570-575.
- Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596-1599.
- Thompson, J.D., Higgins, D.G., Gilson, T.J., 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* **22**, 4673-4680.
- Vossbrinck, C.R., Baker, M.D., Didier, E.S., Debrunner-Vossbrinck, B.A., Shadduck, J.A., 1993. Ribosomal DNA sequences of *Encephalitozoon hellem* and *Encephalitozoon cuniculi*: species identification and phylogenetic construction. *J. Eukaryot. Microbiol.* **40**, 354-362.
- Weissenberg, R., 1909. Beiträge zur Kenntnis von *Glugea lophii* Döflein. I. Über den Sitz und die Verbreitung der Mikrosporidien-Zysten am Nervensystem von *Lophius piscatorius* und *budegassa*. Sitzungsber. Ges. Naturforsch. Freunde Berlin **9**, 557-565.
- Weissenberg, R., 1911a. Über einige Mikrosporidien aus Fischen (*Nosema lophii* Döflein, *Glugea anomala* Moniez, *Glugea hertwigii* nov. spec.). Sitzungsber. Ges. Naturforsch. Freunde Berlin. **8**, 344-357.
- Weissenberg, R., 1911b. Beiträge zur Kenntnis von *Glugea lophii* Döflein. II. Über den Bau der Zysten und die Beziehungen zwischen Parasit und Wirtsgewebe. Sitzungsber. Ges. Naturforsch. Freunde Berlin. **3**, 149-157.
- Weissenberg, R., 1911c. Über Mikrosporidien aus dem Nervensystem von Fischen (*Glugea lophii* Döflein) und die Hypertrophie der befallenen Ganglienzellen. *Arch. Mikrosk. Anat.* **78**, 383-421.
- Weissenberg, R., 1976. Microsporidian interactions with the host cell. In *Comparative Pathobiology*, (ed. Bulla L. A. and Cheng, T. C.), Volume 1, pp. 203-237, New York and London, Plenum Press.

PARTE III

MIXOSPORIDIOSES

Capítulo 6

**ULTRASTRUCTURAL DATA ON THE SPORE OF *MYXOBOLUS MACULATUS* N. SP.
(PHYLUM MYXOZOA), PARASITE FROM THE AMAZONIAN FISH
METYNNIS MACULATUS (TELEOSTEI)**

Diseases of Aquatic Organisms (2002) **51**: 107–112

Graça Casal, Edilson Matos & Carlos Azevedo

Ultrastructural data on the spore of *Myxobolus maculatus* n. sp. (phylum Myxozoa), parasite from the Amazonian fish *Metynnis maculatus* (Teleostei)

G. Casal^{1,2}, E. Matos³, C. Azevedo^{2,4,*}

¹Department of Biological Sciences, High Institute of Health Sciences, 4580 Paredes, Portugal

²CIIMAR—Centre for Marine and Environmental Research, University of Oporto, 4150-180 Porto, Portugal

³Laboratory of Animal Biology, Faculty of Agricultural Sciences, Belém, Brazil

⁴Department of Cell Biology, Institute of Biomedical Sciences, University of Oporto, 4099-003 Porto, Portugal

ABSTRACT: Light and electron microscopy studies of a myxosporean, parasitic in the intertubular interstitial tissue of the kidney of the freshwater teleost fish *Metynnis maculatus* Kner, 1860 (Characidae) from the lower Amazon River (Brazil), are described. We observed polysporic histozoic plasmodia delimited by a double membrane and with several pinocytotic channels and containing several life cycle stages, including mature spores. The spore body was of pyriform shape and was 21.0 µm long, 8.9 µm wide and 7.5 µm thick. Elongated-pyriform polar capsules were of equal size (12.7 × 3.2 µm) and contained a polar filament with 14 or 15 coils. The spore features fit those of the genus *Myxobolus*. Densification of the capsular primordium matrix, which increased in density from the inner core outwards, differentiating at the periphery into small microfilaments measuring 45 nm each, and tubuli arranged in aggregates and dispersed within the capsular matrix of the mature spores, are described. Based on the morphological differences and specificity of the host, we propose the creation of a new species named *Myxobolus maculatus* n. sp.

KEY WORDS: Ultrastructure · Parasite · Myxosporidian · *Myxobolus maculatus* · Amazonian fish

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The genus *Myxobolus* Bütschli, 1882 (family Myxobolidae), is the largest myxosporean group, and its members are important pathogens of freshwater and marine fishes in several geographical areas. The morphology and ultrastructure of myxosporean species have been widely studied (Landsberg & Lom 1991, Lom & Dyková 1992). However, in Brazilian host species, few have been described and, with the exception of 1 ultrastructural study (Casal et al. 1996), only light microscopy descrip-

tions are available (Walliker 1969, Kent & Hoffman 1984, Molnár & Békési 1993, Gioia & Cordeiro 1996, Molnár et al. 1998). In this paper we present light and electron microscopical data of a new myxosporidian species, *M. maculatus* n. sp., found in the teleost fish, *Metynnis maculatus* collected from the Amazon River. Some peculiar ultrastructural aspects of the structure of the plasmodium and developmental stages of the capsulogenesis are described and discussed.

MATERIALS AND METHODS

A parasite found in the kidney of the freshwater teleost *Metynnis maculatus* Kner, 1860 (family Characidae), known by the Brazilian common name 'pacú', was investigated. The specimens were collected peri-

*Corresponding author.

Present address: Department of Cell Biology, Institute of Biomedical Sciences, University of Oporto, Lg. Professor Abel Salazar no. 2, 4099-003 Porto, Portugal.

E-mail: azevedoc@icbas.up.pt

odically during the year 2000 from the estuarine region of the Amazon River, near Belém, Brazil. Plasmodia with mature spores were examined in fresh mounts with a light microscope equipped with Nomarski differential interference-contrast optics. For TEM, small parasitized fragments were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH = 7.4) at 4°C for 6 h, then washed with the same buffer overnight and post-fixed in 2% OsO₄ buffered with 0.2 M sodium cacodylate for 2 h at the same temperature. The fragments were dehydrated in an ascending ethanol and propylene oxide series and then embedded in Epon. Semithin sections were stained with methylene blue and photographed under the light microscope (DIC). Ultrathin sections, cut with a diamond knife, were stained with both aqueous uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM operated at 60 Kv.

RESULTS

Several foci of infection, plasmodia of approximately 150 µm diameter localized in the intertubular interstitial tissue of the kidney, were observed. The infected kidney presented cellular and nuclear hypertrophy accompanied by morphological changes, such as organelle disorganization and cytoplasm vacuolization (Fig. 1). Sporogenic stages released into the renal interstitium due to basement membrane rupture were frequently observed (Fig. 7). Asynchronous histozoic plasmodia containing several life-cycle developmental stages of the parasite (generative cells, sporogenic stages and mature spores) were observed (Figs. 1 & 3). The plasmodia were delimited by 2 membranes, the innermost being continuous with a distinct zone of pinocytic channels (Fig. 3: inset). The external plasmodial membrane was slightly separated from the endothelial cells and possessed a sinuous outline with some papillary buds (Fig. 3).

Sporogenesis

Pansporoblast formation followed the well-known pattern of a sporogenic cell developing into 2 spores within a pericyte. Morphogenesis of capsulogenic cells corresponded to that of most myxosporeans, yet some specific features were found. Capsulogenesis began with a club-shaped formation that posteriorly changed to a globular structure, the capsular primordium, which extended into an external tube (Figs. 4 & 6). Early in development, the distal end of the external tube was located below the future discharge channel leading through the shell valve, and was sealed by an electron-

dense structure (Fig. 6). Inside the capsular primordium, before the inversion of the external tube, the matrix was composed of a fine dense granular structure. This was organized in concentric layers that gradually densified from the inner core outwards, being differentiated into fine microfilaments at the periphery. These microfilaments measured about 45 ± 5 nm in diameter and were arranged in a continuous row (Figs. 4 & 5). Simultaneously, the polar filament differentiated at the external tubule and then invaginated and coiled inside the matrix (Fig. 7). In mature spores, the matrix became denser and numerous electrolucent aggregates of tubuli, arranged in bundles around the polar filament, were observed (Fig. 8).

Spore characteristics

Fresh mature spores were of pyriform shape, tapering anteriorly to a slightly knob-like end, and measured $\sim 21.0 \times \sim 8.9$ µm in anterior view (Figs. 2 & 9). The spore wall was thin and smooth, comprising 2 equal valves joined by a sutural ridge. No mucus envelope was observed at the surface of the spore (Fig. 2). Internally, 2 capsulogenic cells, located side by side, contained prominent polar capsules (PCs) of elongated pyriform shape and equal size, measuring $\sim 12.7 \times \sim 3.2$ µm (Figs. 2 & 9). The PCs occupied approximately two-thirds of the total spore length. Inside the PCs, a polar filament displayed 14 or 15 coils perpendicular or slightly oblique to the longitudinal axis (Figs. 2 & 7). No intercapsular appendix was present (Fig. 2). At the posterior pole of the spore, a binucleated sporoplasm contained numerous electron-dense vesicles, sporoplasmosomes, glycogen granules and an extensive system of rough endoplasmic reticulum cisternae (Fig. 7). Fresh mature spores and ultrathin sections demonstrated the existence of a large iodophilous vacuole in the sporogenic cell measuring approximately 4.5 µm in diameter (Fig. 2).

Diagnosis

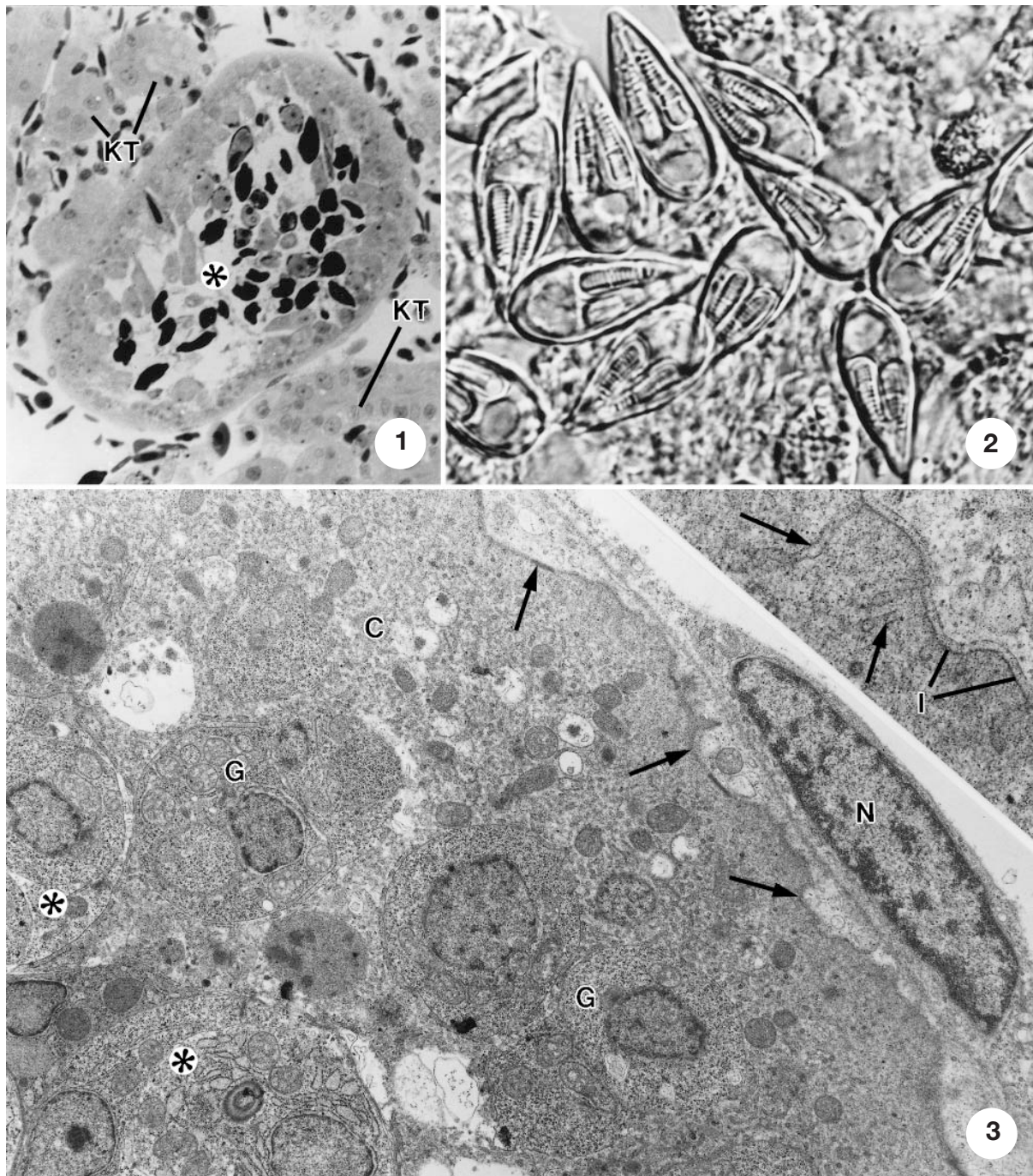
Host: teleost fish, *Metynnis maculatus* Kner, 1860 (family Characidae).

Locality: estuarine region of the Amazon river (01° 11' 30" S, 47° 18' 54" W) near Belém, Brazil.

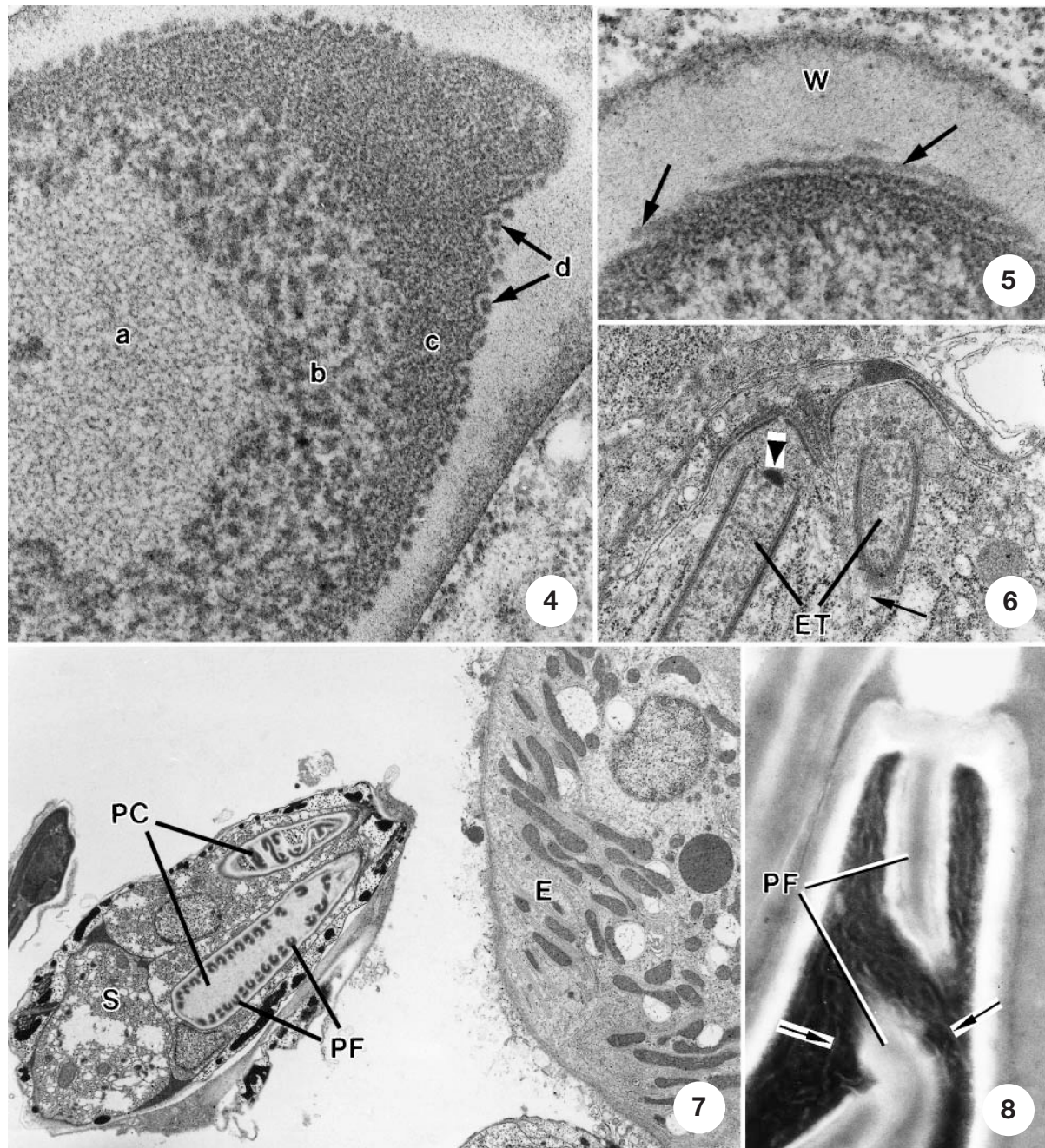
Site of infection: spores were located in the kidney.

Prevalence and intensity: 12 out of 30 (40%).

Fresh spore measurements (n = 40): length = 21.0 (19.7 to 23.0) µm, width = 8.9 (7.9 to 9.5) µm, thickness = 7.5 (7.2 to 7.9) µm; polar capsules: length = 12.7 (11.8 to 13.8) µm, width = 3.2 (3.0 to 3.6) µm; number of polar filament turns = 14 to 15.



Figs. 1–3. *Myxobolus maculatus* n. sp. Life cycle stages of the parasite in the kidney of *Metynnis maculatus*. Fig. 1. Semithin section showing plasmodium (*) in the intertubular interstitial tissue of the kidney (KT). Fig. 2. Fresh mature spores observed with differential interference-contrast (Nomarski). Fig. 3. Plasmodium delimited by double membrane (arrows) showing much cytoplasmic degradation (C) and different life cycle stages, such as generative cells (G) and sporogonic stages (*); Outside the plasmodium an endothelial cell nucleus (N) is visible. Inset ($\times 8800$) shows detail of the plasmodium wall with membranes, the innermost (I) of which is in direct contact with the pinocytic channels (arrows). (Scale bars: 1 = $\times 500$; 2 = $\times 1525$; 3 = $\times 32\,000$)



Figs. 4–8. *Myxobolus maculatus* n. sp. Life cycle stages of the parasite in the kidney of *Metynnis maculatus*. Fig. 4. Transverse section of a capsular primordium showing the matrix with different degrees of densification (a,b,c), and the periphery differentiated into fine microfilaments (d). Fig. 5. Detail of a capsular primordium in tangential section showing some microfilaments (arrows) near the capsular primordium wall (W). Fig. 6. Anterior pole of 2 capsulogenic cells, each with an external tubule (ET) surrounded by microtubules (arrow) and sealed by an electron-dense structure (arrowhead). Fig. 7. Immature spore localized in the interstitial tissue showing the polar capsules (PC) with polar filaments (PF) coiled inside, sporoplasm (S) and epithelial cells (E) of the uninfected kidney tubule. Fig. 8. Detail of a densified mature polar capsule, showing numerous tubuli organized into aggregates (arrows) and associated with the polar filament (PF). (Scale bars: 4 = $\times 40\,000$; 5 = $\times 50\,400$; 6 = $\times 20\,800$; 7 = $\times 4480$; 8 = $\times 40\,000$)

Specimens deposited: slides with holotype were deposited in the International Protozoan Type Slides Collection at the Smithsonian Institution, Washington, DC 20560, USA (USNM #1002151) and in the collection of the senior author.

Etymology: the specific name is derived from the name of the host species ('*maculatus*').

DISCUSSION

The mature spores obtained from *Metynnis maculatus* revealed morphological similarities to those of the genus *Myxobolus* Bütschli, 1882. Comparison of the plasmodium wall and sporogenesis of the cycle life stages of this species to those of other *Myxobolus* spp. also revealed morphologic and ultrastructural similarities (Lom & Puytorac 1965, Dessler & Paterson 1978, Current et al. 1979, Lom & Dyková 1992).

The plasmodial wall presented an organization typical for histozoic *Myxobolus* species, with a double membrane and pinocytic channels region extending

into the ectoplasmic zone of the plasmodium (Dessler & Paterson 1978, Current et al. 1979). All *Myxobolus* species give rise to histozoic plasmodia, except for one species, *M. coneii*, that is found in the lumen of bile ducts in the liver of *Pseudocaranx dentex* (Lom & Dyková 1994). Usually, myxosporidian species that parasitize renal tubules are coelozoic and do not belong to the genus *Myxobolus* (Lom 1969, Dessler et al. 1983, Lom & Dyková 1985). In the present work we describe a new histozoic species found in the inter-tubular interstitial tissue of the kidney. Unfortunately, studies of other *Myxobolus* species reported to parasitize the same organ make but few references to the ultrastructural morphology of the plasmodia (Lom & Dyková 1992).

The ultrastructural process of capsulogenesis differentiation has been well documented in several myxosporidian genera (Lom & Puytorac 1965, Lom 1969, Current et al. 1979, Dessler et al. 1983). The structure of the capsular matrix, i.e. immature spores formed by concentric layers and differentiated at the periphery into only 1 row of microfilaments, presents some differences with the one species (*Thelohanellus nikolskii*) described by Dessler et al. (1983), who described a microfilamentous girdle surrounding the capsular matrix and speculated that it was probably connected with the contractions required for external tubule inversion (Dessler et al. 1983). In mature spores, bundles of tubuli in the capsular matrix have already been referred to in some genera, such as *Sphaeromyxa* (Lom 1969) and *Henneguya* (Rocha et al. 1992), but never in *Myxobolus* species. This suggests that these tubules probably have an important function in the extrusion of the polar filament.

The pathological changes in the renal tissue such as degeneration and vacuolisation of the renal epithelial cells associated with the presence of the parasites *Sphaerospora* spp. (Lom & Dyková 1985), are similar to those observed in *M. maculatus* in the present study.

There are at least 444 species belonging to this genus (Landsberg & Lom 1991), and most of the early species descriptions are vague, presenting only line drawings of the spores. At present there are 16 *Myxobolus* species described in Amazonian fishes (Walliker 1969, Kent & Hoffman 1984, Molnár & Békési 1993, Casal et al. 1996, Gioia & Cordeiro 1996, Molnár et al. 1998). The Brazilian species—*M. cunhai* (Penido 1927), microspores of *M. serrasalmi* (Walliker 1969) and *M. braziliensis* (Casal et al. 1996)—all have a similar body shape, but are smaller than the species described here. Only *M. inaequus* (Kent & Hoffman 1984) is of similar size, however its oval, unequal polar capsules and its infestation of other host species all exclude the possibility of it being the same species as that described herein.

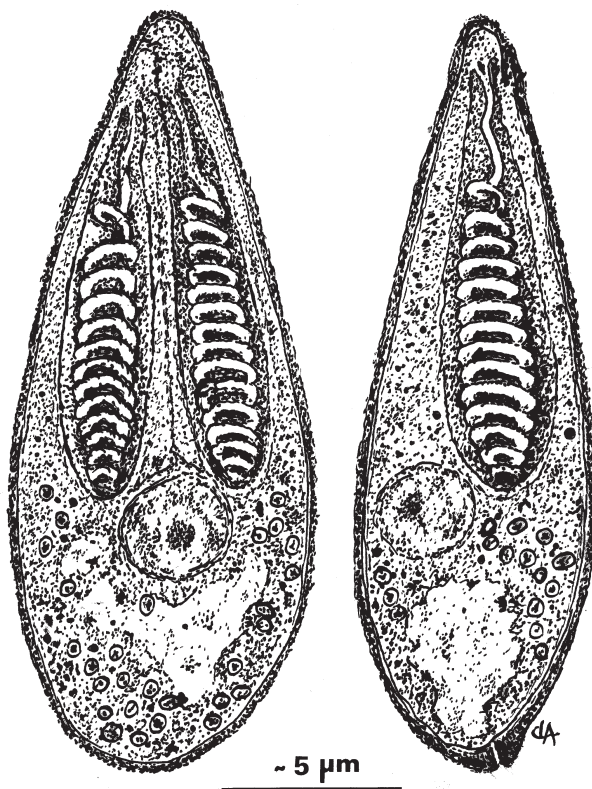


Fig. 9. *Myxobolus maculatus* n. sp. Schematic drawings of morphology of a spore in anterior (left) and lateral (right) view as described in 'Results' and illustrated in Figs. 2 & 7

Among *Myxobolus* spp. from other geographic localities, some present a similar body shape, such as *M. koi* and *M. funduli* (Kudo 1919), *M. procerus* (Kudo 1934), *M. neurophilus* and *M. scleroperca* (Guilford 1963), *M. punctatus* (Ray-Chaudhuri & Chakravarty 1970), *M. pharyngeus* (Parker et al. 1971), and *M. maruliensis* (Sarkar et al. 1985), but all are smaller in size. Comparison of the spores of 3 species—*M. magnasherus* (Cone & Anderson 1977), *M. ovoidalis* (Fantham 1930) and *M. squamaphilus* (Molnár 1997)—revealed similar sizes to that in our study, but all were oval in shape compared to the pyriform shape in our study.

Comparison of our results with those for other *Myxobolus* species revealed some significant differences, mainly in size and body shape of the spores as well as host-specificity and ultrastructural details, suggesting that this parasite (*M. maculatus*) is a new species.

Acknowledgements. This work was partially supported by a grant from the Engenheiro Antônio Almeida Foundation, Porto, Portugal. We would like to thank the iconographic work of Mr. João Carvalheiro. The electron microscopy assistance provided by Mrs. Laura Corral is gratefully acknowledged.

LITERATURE CITED

- Casal G, Matos E, Azevedo C (1996) Ultrastructural data on the life cycle stages of *Myxobolus braziliensis* n. sp., parasite of an Amazonian fish. *Eur J Protistol* 32:123–127
- Cone DK, Anderson RC (1977) Myxosporidian parasites of pumpkinseed (*Lepomis gibbosus* L.) from Ontario. *J Parasitol* 63:657–666
- Current WL, Janovy J Jr, Knight SA (1979) *Myxosoma funduli* Kudo (Myxosporidia) in *Fundulus kansae*: ultrastructure of the plasmodium wall and of sporogenesis. *J Protozool* 26:574–583
- Desser SS, Paterson WB (1978) Ultrastructural and cytochemical observations on sporogenesis of *Myxobolus* sp. (Myxosporidia: Myxobolidae) from the common shiner *Notropis cornutus*. *J Protozool* 25:314–326
- Desser SS, Molnár K, Weller I (1983) Ultrastructure of sporogenesis of *Thelohanellus nikolskii* Akhmerov, 1955 (Myxozoa: Myxosporidia) from the common carp, *Cyprinus carpio*. *J Parasitol* 69:504–518
- Fantham HB (1930) Some parasitic protozoa found in South Africa. *S Afr J Sci* 27:376–390
- Gioia I, Cordeiro NS (1996) Brazilian myxosporidians' checklist (Myxozoa). *Acta Protozool* 35:137–149
- Guilford HG (1963) New species of Myxosporidia found in percid fishes from Green Bay (Lake Michigan). *J Parasitol* 49:474–478
- Kent ML, Hoffman GL (1984) Two new species of Myxozoa, *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the brain of a South American knife fish, *Eigemannia virescens* (V.). *J Protozool* 31:91–94
- Kudo RR (1919) Studies on Myxosporidia. III. *Biol Monogr* 5:241–503
- Kudo RR (1934) Studies on some protozoan parasites of fishes of Illinois. III. *Biol Monogr* 13:1–41
- Landsberg JH, Lom J (1991) Taxonomy of the genera of the *Myxobolus/Myxosoma* group (Myxobolidae: Myxosporidia): current listing of species and revision of synonyms. *Syst Parasitol* 18:165–186
- Lom J (1969) Notes on the ultrastructure and sporoblast development in fish parasitizing myxosporidian of the genus *Sphaeromyxa*. *Z Zellforsch* 97:416–437
- Lom J, Dyková I (1985) *Hoferellus cyprini* Doflein, 1898 from carp kidney: a well established myxosporidian species or a sequence in the developmental cycle of *Sphaeromyxa renicola* Dyková and Lom, 1982? *Protistologica* 21:195–206
- Lom J, Dyková I (1992) Myxosporidia (phylum Myxozoa). In: Lom J, Dyková I (eds) *Protozoan parasites of fishes. Developments in aquaculture and fisheries science*, Vol 26. Elsevier, Amsterdam, p 159–235
- Lom J, Dyková I (1994) Studies on protozoan parasites of Australian fishes. III. Species of the genus *Myxobolus* Bütschli, 1882. *Eur J Protistol* 30:431–439
- Lom J, Puytorac P (1965) Studies on the myxosporidian ultrastructure and polar capsule development. *Protistologica* 1:53–65
- Molnár K (1997) *Myxobolus squamaphilus* sp. n. (Myxozoa: Myxosporidia), a common parasite of the scales of bream (*Abramis brama* L.). *Acta Protozoologica* 36:221–226
- Molnár K, Békési L (1993) Description of a new *Myxobolus* species, *M. colossomatis* n. sp. from the teleost *Colossoma macropomum* of the Amazon River basin. *J Appl Ichthyol* 9:57–63
- Molnár K, Ranzani-Paiva MJ, Eiras JC, Rodrigues EL (1998) *Myxobolus macroplasmoidalis* sp. n. (Myxozoa: Myxosporidia), a parasite of the abdominal cavity of the characid teleost, *Salminus maxillosus*. *Acta Protozoologica* 37:241–245
- Parker JD, Spall RD, Warner MC (1971) Two new Myxosporidia, *Henneguya gambusi* sp. n. and *Myxosoma pharyngeus* sp. n., in the mosquitofish, *Gambusia affinis* (Baird and Girard). *J Parasitol* 57:1297–1301
- Penido JCN (1927) Quelques nouvelles myxosporidies parasites des poissons d'eau douce du Brésil. *CR Séances Soc Biol* 97:850–852
- Ray-Chaudhuri S, Chakravarty MM (1970) Studies on Myxosporidia (Protozoa, Sporozoa) from the food fishes of Bengal. I. Three new species from *Ophicephalus punctatus* Bloch. *Acta Protozool* 8:167–175
- Rocha E, Matos E, Azevedo C (1992) *Henneguya amazonica* n. sp. (Myxozoa, Myxobolidae), parasitizing the gills of *Crenicichla lepidota* Heckel, 1840 (Teleostei, Cichlidae) from Amazon river. *Eur J Protistol* 28:273–278
- Sarkar NK, Mazumder SK, Pramanik A (1985) Observations on 4 new species of Myxosporidia (Myxozoa) from channid (ophicephalid) fishes of west Bengal, India. *Arch Protistenkd* 130:289–296
- Walliker D (1969) Myxosporidia of some Brazilian freshwater fishes. *J Parasitol* 55:942–948

Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: July 15, 2001; Accepted: February 18, 2002
Proofs received from author(s): August 9, 2002

Capítulo 7

**LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE MYXOSPOREAN,
HENNEGUYA FRIDERICI N. SP. FROM THE AMAZONIAN TELEOSTEAN FISH,
*LEPORINUS FRIDERICI***

Parasitology (2003) **126**: 313-319

Graça Casal, Edilson Matos & Carlos Azevedo

Light and electron microscopic study of the myxosporean, *Henneguya friderici* n. sp. from the Amazonian teleostean fish, *Leporinus friderici*

G. CASAL^{1,4}, E. MATOS² and C. AZEVEDO^{3,4*}

¹ Department of Biological Sciences, High Institute of Health Sciences, 4580 Paredes, Portugal

² Laboratory of Animal Biology, Faculty of Agricultural Sciences, Belém, Brazil

³ Department of Cell Biology, Institute of Biomedical Sciences, University of Oporto, 4099-003 Porto, Portugal

⁴ CIIMAR-Centre for Marine and Environmental Research, University of Oporto, 4150-180 Porto, Portugal

(Received 1 July 2002; revised 6 November 2002; accepted 20 November 2002)

SUMMARY

A new histozoic species of myxosporean was found to infect the gill filaments, gut, kidney and liver of the freshwater teleost *Leporinus friderici*, collected from the estuarine region of the Amazon, near the city of Belém, Brazil. The plasmodia show asynchronous development, at any one time composed of mature spores and all sporogonic stages. The ellipsoidal spore body, measuring 10.4 µm long and 5.7 µm wide, consists of 2 equal shell valves adhering together along the straight suture line. Each valve has a caudal process measuring 23.3 µm in length. There are 2 symmetric polar capsules, without intercapsular appendix, measuring 5.0 µm × 2.1 µm, and each has a polar filament with 7–8 coils. In general, ultrastructural details of sporoblast and spore development are in agreement with previously described myxosporeans. Some ultrastructural aspects such as cellular alterations of the pericyte in the different organs infected and characterization of the sporoplasmosomes during the sporoplasm maturation are described. This parasite was studied under light and electron microscope and compared with others species of the genus *Henneguya*, considering also host specificity. From our observations we propose the creation of a new species, *Henneguya friderici* n. sp.

Key words: ultrastructure, Myxozoa, *Henneguya friderici* n. sp., parasite, Amazonian fish.

INTRODUCTION

Since the first description of *Henneguya* Thélohan, 1892 (Lom & Dyková, 1992), the second largest genus of Myxobolidae family, many species have been reported, mainly parasitizing freshwater fishes throughout the world. In total 27 species have been described from Brazilian fauna, by light microscopy photos and diagrammatic illustrations (Walliker, 1969; Kent & Hoffman, 1984; Gióia & Cordeiro, 1996; Eiras, 2002). More recently, ultrastructural studies on developmental life-cycle stages and on mature spores, supported the classification of 8 of those species (Rocha, Matos & Azevedo, 1992; Azevedo & Matos, 1995, 1996, 2002, 2003; Azevedo, Corral & Matos, 1997; Casal, Matos & Azevedo, 1997; Vita *et al.* 2003).

In the present paper, we report light and electron microscopical data on the sporogenesis and mature spores of a new parasite, designated herein as *Henneguya friderici* n. sp., infecting several organs of a teleost fish of some economical importance from the river Amazon.

* Corresponding author: Department of Cell Biology, Institute of Biomedical Sciences, University of Oporto, Lg. Prof. Abel Salazar no. 2, P-4099-003 Porto, Portugal. Fax: +351.22.206.2232/33. E-mail: azevedoc@icbas.up.pt; gcasal@excite.com

MATERIALS AND METHODS

Fish, location of infection and prevalence

Several infected adult specimens of the freshwater teleost *Leporinus friderici* Bloch, 1794 (Teleostei, Anostomidae) (Brazilian common name 'aracú'), were collected from the estuarine region of the River Amazon (01° 11' 30" S/47° 18' 54" W) near the city of Belém, Brazil. The prevalence of infection was 30% (9 fishes in 30 examined) in both sexes. The fishes were dissected and the infected gills, gut, kidney and liver containing numerous cyst-like plasmodia were removed and examined by a light microscope equipped with Nomarski interference-contrast (DIC) optics.

Electron microscopy

For ultrastructural studies, small fragments of the parasitized tissues were excised and fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4 °C for 5 h. After washing in the same buffer and post-fixation in 2.0% osmium tetroxide in the same buffer both for 2 h at 4 °C, the fragments were dehydrated through a graded ethanol series, followed by propylene oxide and embedded in Epon. Ultra-thin sections were contrasted with aqueous uranyl acetate and lead citrate and observed with

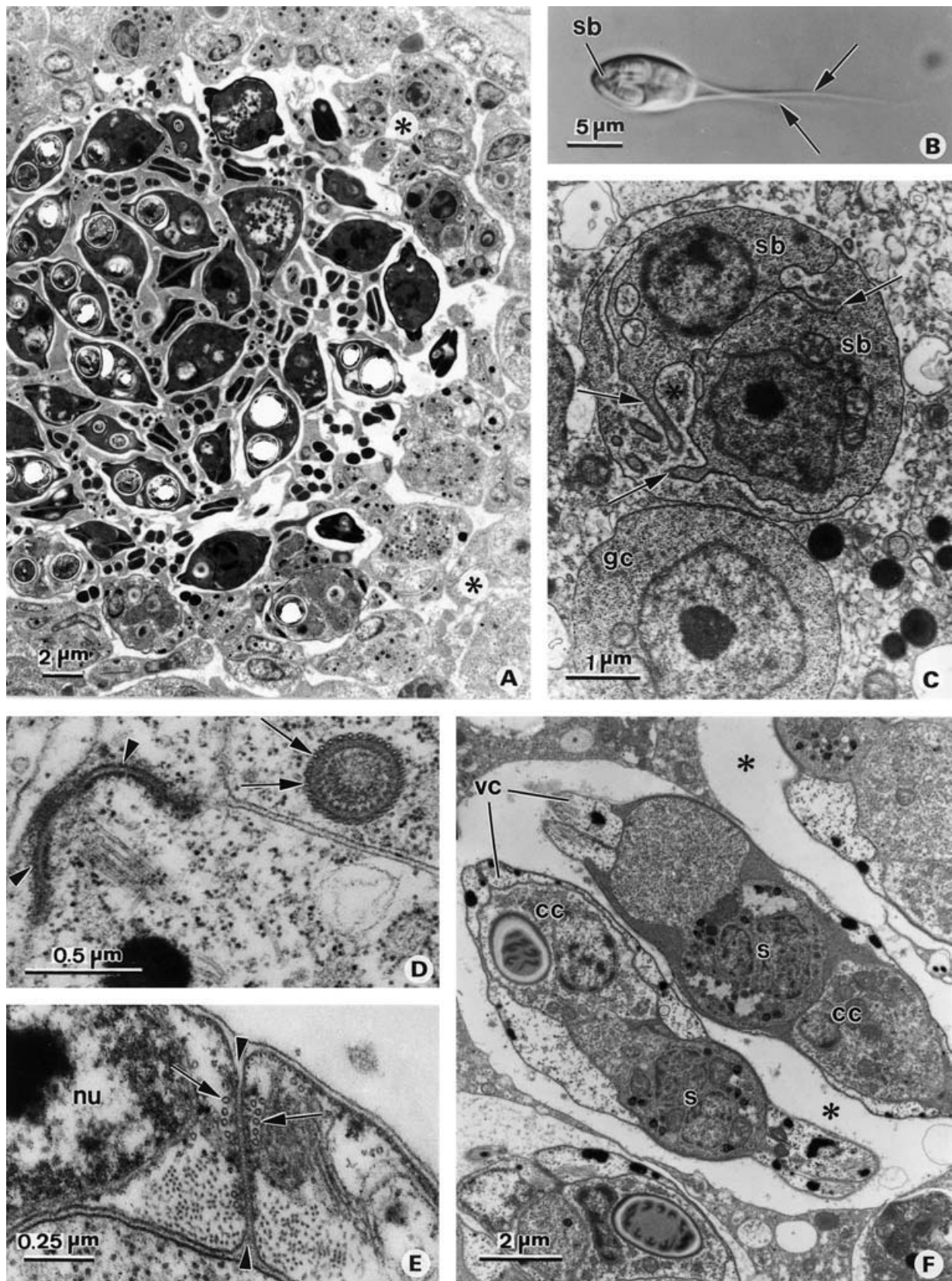


Fig. 1. (A) Ultra-thin section of a plasmodium of *Henneguya friderici* localized in *Leporinus friderici* gill filaments showing sporogonic stages (*) and mature spores in the central zone. (B) Isolated mature spore observed by Nomarski differential interference contrast photomicrography. Note the spore body (sb) and the bifurcated tail (arrows). (C–F) Transmission electron microscopy images of *H. friderici* infecting the gut showing different sporogonic stages of the life-cycle. (C) Ultra-thin section showing 1 generative cell isolated (gc) and 2 sporoblast cells (sb) into the pericyte (*). Note several pseudopodia simultaneously projected to both cells (arrows). (D) Details of the 2 capsulogenic cells showing increased membrane density (arrowheads) in the discharge channel region and several

a transmission electron microscope (TEM) JEOL 100CXII operated at 60 kV.

For scanning electron microscopy (SEM), isolated spores removed from mature plasmodia, were fixed as described above. Then, the material was dehydrated in ethanol, gold coated and examined in a JEOL 35 SEM operated at 15 kV.

RESULTS

Systematic position

Phylum Myxozoa, Class Myxosporidia, Order Bivalvulida, and Family Myxobolidae, according to the classification proposed by Lom & Noble (1984).

Description of the species

Henneguya friderici n. sp.

Type host: *Leporinus friderici* Bloch, 1794 (Teleostei, Anostomidae).

Host size: 15 cm of the length in average.

Type locality: estuarine region of the river Amazon (01° 11' 30" S/47° 18' 54" W), near Belém (Pará), Brazil.

Location in the host: histozoic infecting several organs, such as gills, gut, kidney and liver.

Prevalence and intensity: 9 out of 30 adult fishes were parasitized and in equal % in both sexes.

Type specimens: 2 slides containing mature spores of the holotypes were deposited in the International Protozoan Type Slide Collection at Smithsonian Institution Washington, DC. 20560, USA with acquisition number (USNM n° 1007181). The histological semi-thin sections showing varied developmental stages were deposited at the laboratory of the senior author.

Etymology: the specific name is derived from the name of the host species ('friderici').

Description of spores: for description of the mature spores scanning electron microscopy (SEM) (Fig. 2A), light microscopy (DIC) (Fig. 1B) and a schematic drawing (Fig. 3) were used. Variability in shape and size of the spores on the different organs was not observed and the measurements were done with plasmodia obtained from the gut tissue. The spores were ellipsoidal with a total length 33.8 (28.7–39.3) μm ($n=25$), body length 10.4 (9.6–11.8) μm ($n=25$), body width (frontal view) 5.7 (4.8–6.6) μm ($n=25$) and body thickness (side view) 4.9 (4.6–5.2) μm ($n=25$). The valves, symmetric and thin, are each prolonged by a caudal process 23.3 (19.1–28.7) μm ($n=25$) long. The spores presented neither a mucous envelope nor particular details on

the surface when observed by SEM (Fig. 2A). Elongated polar capsules localized in the anterior pole of the spore were of equal size, measuring 4.98 (4.25–5.90) μm in length ($n=25$) by 2.14 (1.59–2.62) μm ($n=25$) in width and there is not an intercapsular appendix. Inside the polar capsules, the polar filament is coiled 7–8 turns obliquely to the longitudinal axis (Fig. 2B).

Ultrastructural observations

Several organs of the freshwater teleost, *L. friderici*, were found to be parasitized by a myxosporidian of the genus *Henneguya* Thélohan, 1892. Whitish and round-shaped polysporic plasmodia, measuring about 0.5–1.0 mm, indicated an asynchronous development. These were composed of vegetative nuclei and different sporogenesis stages, such as generative cells and early sporogonic stages predominantly along the plasmodium periphery, while immature and mature spores were more internally localized in the centre (Fig. 1A). Pansporoblast formation was disporoblastic (gives rise 2 spores), typical of the myxobolids and comprised envelopment by a pericyte capsulogenesis, valvogenesis and sporoplasm maturation (Fig. 1F).

At the beginning of the sporogenesis phase, 2 morphologically identical rounded generative cells were frequently observed in narrow association. Initially both cells projected numerous pseudopodia inside the cytoplasm of another cell and later this association finished with the envelopment of one of them, the sporogonic cell by the pericyte (Fig. 1C). The sporogonic cell divided several times giving rise to a disporic pansporoblast stage (Fig. 1F) composed by valvogenic, capsulogenic and sporoplasm cells that later gave rise to 2 mature spores (Figs 1B and 2A, B).

Frequently, several longitudinally oriented microtubules were found in the cytoplasm of capsulogenic and valvogenic cells, surrounding the external tube (Fig. 1D) and giving form to the valve and caudal process (Fig. 1E) respectively.

The binucleate sporoplasm cell, which contained an iodophilous vacuole, mitochondria, an extensive system of cisternae of endoplasmic reticulum and numerous electron-dense vesicles, sporoplasmosomes, was located in the spore posterior pole (Figs 1F and 2E, F). A single membrane limits the sporoplasmosomes and during the early sporogonic stages they appeared as a spherical body. After sporoplasm maturation, they changed their form and appeared like a teardrop, approximately 190–210 nm in diameter

microtubules bundle-oriented to the external tubule in transverse section (arrows). (E) Detail of a sutural line (arrowheads) of adjoining valvogenic cells with several associated microtubules (arrows). Nucleus of a valvogenic cell (nu). (F) Two immature spores lie in a vacuole in the pericyte (*) showing cellular differentiation, into valvogenic cells (vc), capsulogenic cells (cc) and sporoplasm binucleated (s).

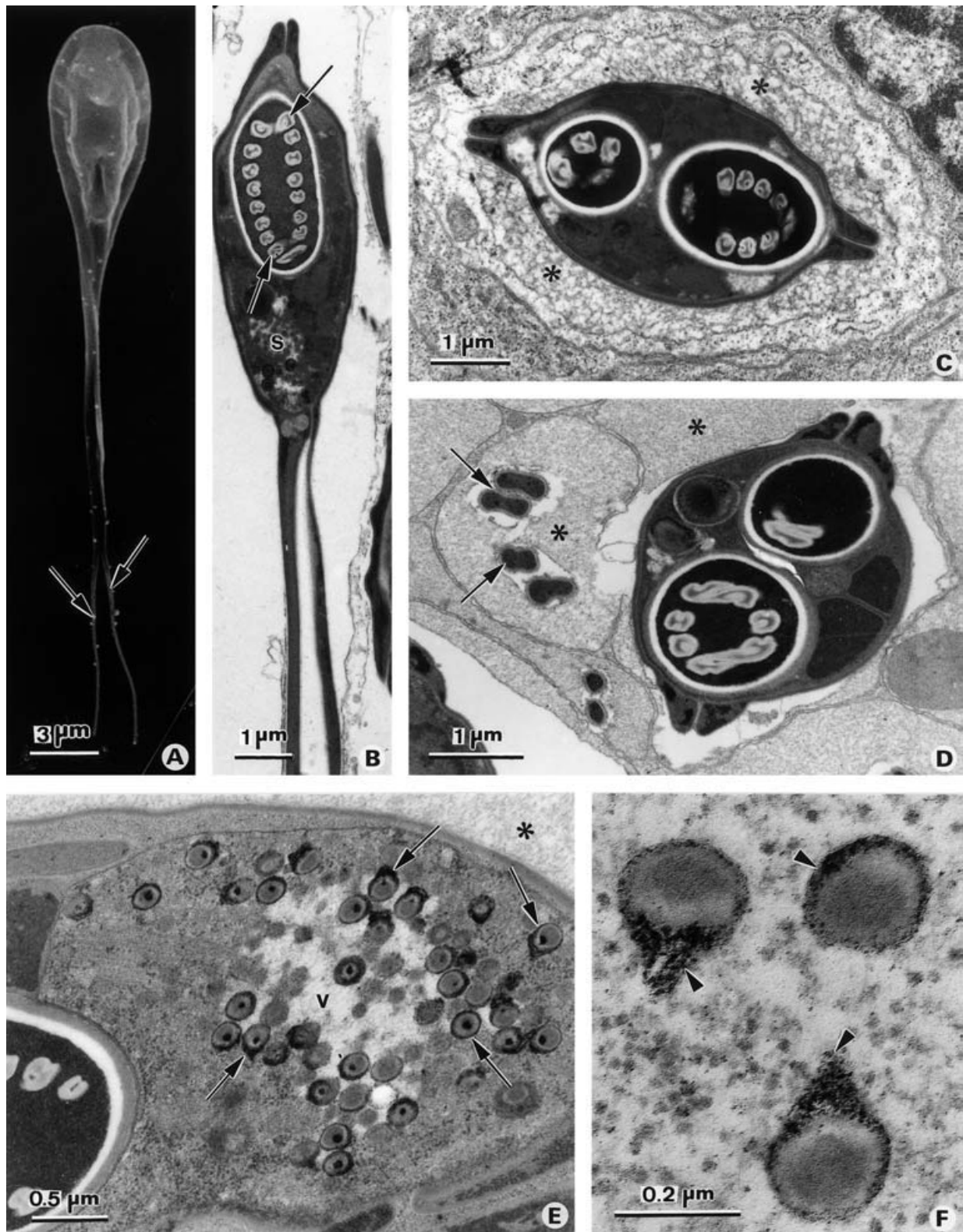


Fig. 2. (A–F) Electron micrographs of *Leporinus friderici* showing different organs infected by *Henneguya friderici*. (A) Scanning electron microscopy image of a mature spore showing 2 individual tail projections (arrows). (B–F) Transmission electron microscopy images of *H. friderici* in late sporogonic stages of the life-cycle. (B) Mature spore in lateral section showing a polar capsule in an anterior position containing 7–8 coils of the polar filament (arrows) and the sporoplasm (S) with sporoplasmosomes. (C) Transverse section of the mature spore in kidney showing 2 polar capsules side-by-side and a flocculent material surrounding the spore (*). (D) Mature spores found in the liver cut in different transverse sections showing 2 pairs of the caudal process (arrows) and the cytoplasmic preservation of the pericyte cell (*). (E) Ultra-thin section of the sporoplasm cell showing numerous sporoplasmosomes, with a characteristic form (arrows) near the iodophilous vacuole (v). Pericyte cell (*). (F) Detail of 3 sporoplasmosomes resembling the teardrop with an unusual electron density externally (arrowheads).

Table 1. Comparative measurements of the spore from the Brazilian species of the genus *Henneguya* that present morphological similarities

Species	Host	TL	BL	BW	TaL	PCL	PCW	FC	References
<i>H. leporini</i>	<i>Leporinus mormyrops</i>	28–33	13–15	5.0	15–18	—	—	—	Nemeczek (1926)
<i>H. fonsecai</i>	<i>Leporinus copelandi</i>	23–27	10–12	4.5–5.0	13–15	—	—	—	Guimarães (1931)
<i>H. santae</i>	<i>Tetragodonopterus santae</i>	21.0	9.6	5.3	11.2	2.9	—	—	Guimarães & Bergamin (1934)
<i>H. visceralis</i>	<i>Electrophorus electricus</i>	22–24	11–12	5.0–6.5	11–12	6.5–8	2	—	Jakowska & Nigrelli (1953)
<i>H. electrica</i>	<i>Electrophorus electricus</i>	35–39	11–13	6–8	24–27	5–7	2	—	Jakowska & Nigrelli (1953)
<i>H. adherens</i>	<i>Acestrorhynchus falcatus</i>	32.3	12.4	5.8	20.5	3.1	1.2	3–4	Azevedo & Matos (1995)
<i>H. malabarica</i>	<i>Hoplias malabaricus</i>	28.3	12.6	4.8	17.1	3.7	1.8	6–7	Azevedo & Matos (1996)
<i>H. friderici</i>	<i>Leporinus friderici</i>	33.8	10.4	5.7	23.3	5.0	2.1	7–8	Present study

(Abbreviations: TL, total length; BL, body length; BW, body width; TaL, tail length; PCL, polar capsule length; PCW, polar capsule width; FC, number of the polar coil.)

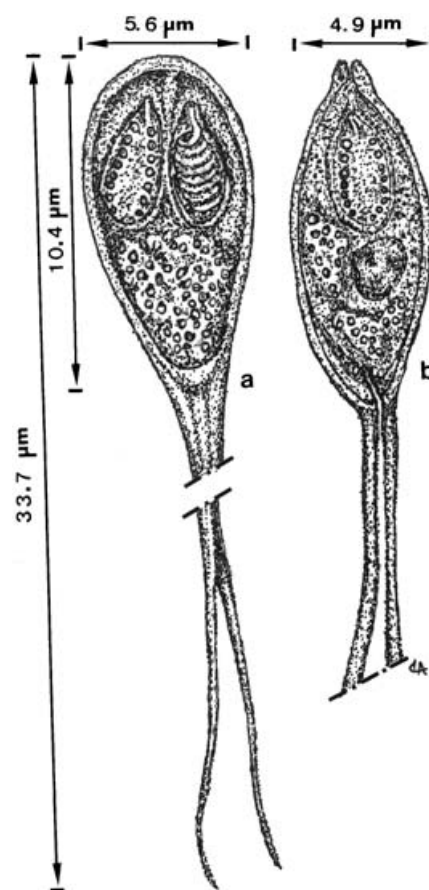


Fig. 3. Schematic drawing in 2 longitudinal sections, frontal (a) and lateral (b) view, of the spores *Henneguya friderici* n. sp.

(Fig. 2E, F). At the periphery, there was unusual deposited material with high electron density and which was uniformly distributed except on the peaked side of the vesicle where it was accumulated in greater quantity. Internally, many have a central dense dot (Fig. 2E) and in the protuberance region there was a small channel differentiated with strong electron density (Fig. 2E, F).

In the different organs, heterogeneous ultrastructural aspects during sporogenesis concerning the pericyte, were observed. In the kidney infection pericyte cellular integrity persisted until late sporogenesis, characterized by organelle preservation. Simultaneously, flocculent material appeared between mature spores and pericyte, that was gradually compressed in later sporogenesis stages (Fig. 2C). In other organs, all pericyte cytoplasm was occupied by thinly granular material as seen in the liver (Fig. 2D) and, in an opposite situation, early degeneration of the enveloping cell in the gut infection was also observed (Fig. 1E).

DISCUSSION

Sporogenesis of this parasite presents many similarities with other species previously described, such as *H. psorospermica* (Lom & Puytorac, 1965),

H. exilis (Current & Janovy, 1977), *H. adiposa* (Current, 1979) and *H. amazonica* (Rocha *et al.* 1992). Although the differentiation and maturation process of the sporoplasm cell has been well studied, little is known about the nature and function of the electron-dense sporoplasmosomes, typical of these parasites. They have been in the sporoplasm of numerous myxosporidia and their morphological characteristics, such as form, size and inner organization have no relationship with genus or family (Lom *et al.* 1989; Lom & Dyková, 1992). In the studies of freshwater fish parasites from the River Amazon belonging to the genus *Henneguya* (Rocha *et al.* 1992; Azevedo & Matos, 2002; Vita *et al.* 2003) and *Myxobolus* (Casal, Matos & Azevedo, 1996) the sporoplasmosomes have been ultrastructurally characterized. The form and size heterogeneity of these vesicles show that it is possible to use this cellular structure as an ultrastructural parameter to differentiate parasites of the same genus, mainly when they are diagnosed in hosts from the same hydrological basin. In this case, an atypical electron-dense material surrounding the vesicle and forming a protuberance has never been described before.

The ultrastructural aspects of the enveloping cell during sporogenesis have been occasionally described as a gradual and generalized degeneration. Sometimes microfibril-forming regions (Current & Janovy, 1977) or long microfilaments resembling myosin filaments (Casal *et al.* 1997) in the cytoplasmic space have been described. In this study we found some heterogeneity in the morphological aspects of the pericyte in the different organs infected, from a relative organelle preservation in liver tissue to an extensive degradation, as verified in the gut infection. This fact seemed indicative of the existence of some adaptation of the pericyte to the host infected tissue.

The morphological and ultrastructural aspects of the mature spores, as well as the host specificity and localization of the infection with this parasite, were compared with other myxosporidian species of the genus *Henneguya*, from different geographical areas mainly, with those that have Brazilian freshwater fishes as host (Table 1).

Among the more than 100 non-Brazilian species described in the literature (see the revision papers Kostoingue *et al.* 2001 and Eiras, 2002), 7 species, *H. lagodoni* (Hall & Iversen, 1967), *H. shaharini* (Shariff, 1982), *H. latesi* (Haldar, Das & Sharma, 1983), *H. mystusia* (Sarkar, 1985), *H. laterocapsulata* and *H. suprbranchiae* (Landsberg, 1987) and *H. mbourensis* (Kpatcha *et al.* 1997) present approximately the same body size. Although all have a different body shape not many of those species were ultrastructurally characterized.

Brazilian species have mostly been described by light microscopy or simply by schematic drawings

(Walliker, 1969; Kent & Hoffman, 1984; Gióia & Cordeiro, 1996) and some of them show morphological similarities. The species *H. leporini* (see Nemeček, 1926) and *H. fonscae* (see Guimarães, 1931) parasitize fishes of the same genus *Leporinus*, but although they were caught in rivers with a distinct geographical localization, they are similar in total size and body shape, respectively. Of 3 other species with similar body shape, *H. santae* (Guimarães & Bergamin, 1934) and *H. visceralis* (Jakowska & Nigrelli, 1953) present a smaller size, while *H. electrica* (Jakowska & Nigrelli, 1953) is longer.

During the last 10 years, this group of parasites has been studied at the ultrastructural level, resulting in the description of 8 new Brazilian *Henneguya* species, all in different hosts (Rocha *et al.* 1992; Azevedo & Matos, 1995, 1996, 2002, 2003; Azevedo *et al.* 1997; Casal *et al.* 1997; Vita *et al.* 2002). Of these, only the spores of *H. adherens* (Azevedo & Matos, 1995) and *H. malabarica* (Azevedo & Matos, 1996) resemble in size the parasite studied by us in *L. friderici*. However, *H. friderici* lacks a sheath around the 2 tails and differs in the arrangement of the polar filament coil.

After a comparative study based on the morphological and ultrastructural differences with *Henneguya* species previously described, we conclude that *Henneguya friderici* is a new species.

This work was partially supported by a grant from the Eng° António Almeida Foundation, Porto, Portugal. We would like to thank the iconographic work of Mr João Carneiro. The technical assistance provided by Mrs Laura Corral is gratefully acknowledged.

REFERENCES

- AZEVEDO, C. & MATOS, E. (1995). *Henneguya adherens* n. sp. (Myxozoa, Myxosporidia), parasite of the Amazonian fish, *Acestrorhynchus falcatus*. *Journal of Eukaryotic Microbiology* **42**, 515–518.
- AZEVEDO, C. & MATOS, E. (1996). *Henneguya malabarica* sp. nov. (Myxozoa, Myxobolidae) in the Amazonian fish *Hoplias malabaricus*. *Parasitology Research* **82**, 222–224.
- AZEVEDO, C. & MATOS, E. (2002). Fine structure of the Myxosporidian, *Henneguya curimata* n. sp., parasite of the Amazonian fish, *Curimata inornata* (Teleostei, Curimatidae). *Journal of Eukaryotic Microbiology* **49**, 197–200.
- AZEVEDO, C. & MATOS, E. (2003). Fine structure of *Henneguya pilosa* sp. n. (Myxozoa: Myxosporidia), parasite of *Serrasalmus altuvei* (Characidae) in Brazil. *Folia Parasitologica* **50**, 35–40.
- AZEVEDO, C., CORRAL, L. & MATOS, E. (1997). Light and ultrastructural data on *Henneguya testicularis* n. sp. (Myxozoa, Myxobolidae), a parasite from the testis of the Amazonian fish *Moenkhausia oligolepis*. *Systematic Parasitology* **37**, 111–114.
- CASAL, G., MATOS, E. & AZEVEDO, C. (1996). Ultrastructural data on the life cycle stages of *Myxobolus braziliensis*

- n. sp. parasite of an Amazonian fish. *European Journal of Protistology* **32**, 123–127.
- CASAL, G., MATOS, E. & AZEVEDO, C. (1997). Some ultrastructural aspects of *Henneguya striolata* sp. nov. (Myxozoa, Myxosporea), a parasite of the Amazonian fish *Serrasalmus striolatus*. *Parasitology Research* **83**, 93–95.
- CURRENT, W. L. (1979). *Henneguya adiposa* Minchew (Myxosporidia) in the channel catfish: ultrastructure of the plasmodium wall and sporogenesis. *Journal of Protozoology* **26**, 209–217.
- CURRENT, W. L. & JANOVY, J. J. (1977). Sporogenesis in *Henneguya exilis* infecting the channel catfish: an ultrastructural study. *Protistologica* **13**, 157–167.
- EIRAS, J. C. (2002). Synopsis of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa: Myxosporea: Myxobolidae). *Systematic Parasitology* **52**, 43–54.
- GIOIA, I. & CORDEIRO, N. S. (1996). Brazilian Myxosporidians' check-list (Myxozoa). *Acta Protozoologica* **35**, 137–149.
- GUIMARÃES, J. R. A. (1931). Mixosporídeos da ictiofauna brasileira. Ph.D. thesis, Faculdade de Medicina de São Paulo, Brazil.
- GUIMARÃES, J. R. A. & BERGAMIN, F. (1934). *Henneguya santae* sp. n. Um novo mixosporídeo parasito de *Tetragopterus* sp. *Revista de Industria Animal* **2**, 110–113.
- HALDAR, D. P., DAS, M. K. & SHARMA, B. K. (1983). Studies on protozoan parasites from fishes. Four new species of the genera *Henneguya* Thélohan, 1892, *Thelohanellus* Kudo, 1933, and *Myxobolus* Bütschli, 1892. *Archive für Protistenkunde* **127**, 283–296.
- HALL, D. L. & IVERSEN, E. S. (1967). *Henneguya lagodoni*, a new species of myxosporidian parasitizing the pinfish, *Lagodon rhomboides*. *Bulletin of Marine Science* **17**, 274–279.
- JAKOWSKA, S. & NIGRELLI, R. F. (1953). The pathology of myxosporidiosis in the electric eel, *Electrophorus electricus* (Linnaeus), caused by *Henneguya visceralis* and *E. electrica* spp. nov. *Zoologica* **38**, 183–191.
- KENT, M. L. & HOFFMAN, G. L. (1984). Two new species of Myxozoa, *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the brain of a South American knife fish, *Eigemannia virescens* (V.). *Journal of Protozoology* **31**, 91–94.
- KOSTOŃGUE, B., DIEBAKATE, C., FAYE, N. & TOGUEBAYE, B. S. (2001). Presence of Myxosporidea (Myxozoa: Myxosporea) of the genus *Henneguya* Thélohan, 1892 in freshwater fishes from Chad (Central Africa). *Acta Protozoologica* **40**, 117–123.
- KPATCHA, T. K., FAYE, N., DIEBAKATE, C., FALL, N. & TOGUEBAYE, B. S. (1997). Nouvelles espèces d'*Henneguya* Thélohan, 1895 (Myxozoa, Myxosporea) parasites des poissons marins du Sénégal: Étude en microscopie photonique et électronique. *Annales des Sciences Naturelles Zoologie* **18**, 81–91.
- LANDSBERG, J. H. (1987). Myxosporean parasites of the catfish, *Clarias lazera* (Valenciennes). *Systematic Parasitology* **9**, 73–81.
- LOM, J. & DYKOVÁ, I. (1992). Myxosporidia (phylum Myxozoa). In *Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science, Vol. 26* (ed. Lom, J. & Dyková, I.), pp. 159–235. Elsevier, Amsterdam.
- LOM, J. & NOBLE, E. R. (1984). Revised classification of the class Myxosporea Bütschli, 1881. *Folia Parasitologica* **31**, 193–205.
- LOM, J. & PUYTORAC, P. (1965). Studies on the myxosporidian ultrastructure and polar capsule development. *Protistologica* **1**, 53–65.
- LOM, J., FEIST, S. W., DYKOVÁ, I. & KEPR, T. (1989). Brain myxoboliasis of bullhead, *Cottus gobio* L., due to *Myxobolus jiroveci* sp. nov.: light and electron microscope observations. *Journal of Fish Diseases* **12**, 15–27.
- NEMECZEK, A. (1926). Beiträge zur Kenntnis der Myxosporidienfauna Brasiliens. *Archive für Protistenkunde* **54**, 137–150.
- ROCHA, E., MATOS, E. & AZEVEDO, C. (1992). *Henneguya amazonica* n. sp. (Myxozoa, Myxobolidae), parasitizing the gills of *Crenicichla lepidota* Heckel, 1840 (Teleostei, Cichlidae) from Amazon river. *European Journal of Protistology* **28**, 273–278.
- SARKAR, N. K. (1985). Myxosporidian *Henneguya mystusia* sp. n. (Myxozoa, Myxosporea), from the gills of a fresh water teleost fish *Mystus* sp. *Acta Protozoologica* **24**, 55–58.
- SHARIFF, M. (1982). *Henneguya shaharini* sp. nov. (Protozoa: Myxozoa), a parasite of marble goby, *Oxyeleotris marmoratus* (Bleeker). *Journal of Fish Diseases* **5**, 37–45.
- VITA, P., CORRAL, L., MATOS, E. & AZEVEDO, C. (2003). Ultrastructural aspects of the myxosporean *Henneguya astyanax* n. sp. (Myxozoa: Myxobolidae), a parasite of the Amazonian teleost *Astyanax keithi* (Characidae). *Diseases of Aquatic Organisms* **53**, 55–60.
- WALLIKER, D. (1969). Myxosporidea of some Brazilian freshwater fishes. *Journal of Parasitology* **55**, 942–948.

Capítulo 8

A NEW MYXOZOAN PARASITE FROM THE AMAZONIAN FISH *METYNNIS ARGENTEUS* (TELEOSTEI, CHARACIDAE): LIGHT AND ELECTRON MICROSCOPE OBSERVATIONS

Journal of Parasitology (2006) **92**: 817-821

Graça Casal, Edilson Matos & Carlos Azevedo

A NEW MYXOZOAN PARASITE FROM THE AMAZONIAN FISH *METYNNIS ARGENTEUS* (TELEOSTEI, CHARACIDAE): LIGHT AND ELECTRON MICROSCOPE OBSERVATIONS

Graça Casal, Edilson Matos*, and Carlos Azevedo†

Department of Sciences, High Institute of Health Sciences, 4585-116 Gandra, Portugal. e-mail: azevedoc@icbas.up.pt

ABSTRACT: *Myxobolus metynnis* n. sp. (Phylum Myxozoa) is described in the connective subcutaneous tissues of the orbicular region of the fish, *Metynnis argenteus* (Characidae), collected in the lower Amazon River, near the city of Peixe Boi, Pará State, Brazil. Polysporic, histozoic plasmodia were delimited by a double membrane with numerous microvilli on the peripheral cytoplasm. Several life-cycle stages, including mature spores, were observed. An envelope formed by numerous fine and anastomosed microfibrils was observed at the spore surface. The spore body presented an ellipsoidal shape and was about 13.1 µm long, 7.8 µm wide, and 3.9 µm thick. Elongated-pyriform polar capsules were of equal size, measuring 5.2 µm in length, 3.2 µm in width, and possessing a polar filament with 8–9 turns around the longitudinal axis. The binucleated sporoplasm contained a vacuole and numerous sporoplasmosomes. These were circular in cross-section, showing an adherent eccentric, dense structure, with a half-crescent section. Based on the morphological differences and host specificity, we propose that the parasite is a new species named *Myxobolus metynnis* n. sp.

The Myxosporidia of the Myxozoa is an assemblage of more than 1,500 species. They have been reported from different geographic areas, by morphological and ultrastructural studies, mainly as fish parasites (Lom and Dyková, 1992). Among them, species of *Myxobolus* Bütschli, 1882 (Myxobolidae), is the largest group and includes a number of important pathogens of freshwater and marine fishes (Lom and Dyková, 1992; Eiras et al., 2005). Some of the species of *Myxobolus* are considered highly pathogenic to their hosts (Longshaw et al., 2003). In Brazilian fishes, few myxozoans have been described and, with the exception of 4 ultrastructural studies (Casal et al., 1996, 2002; Azevedo et al., 2002; Tajdari et al., 2005), only light microscopy descriptions and diagrammatic drawings are available (Penido, 1927; Pinto, 1928; Walliker, 1969; Kent and Hoffman, 1984; Molnár and Békési, 1993; Gioia and Cordeiro, 1996; Molnár et al., 1998; Adriano et al., 2002; Cellere et al., 2002).

In the present study, we describe light and electron ultrastructural features of a new myxosporidian found in the connective subcutaneous tissues of the orbicular region of a teleost fish collected from the Amazon river.

MATERIALS AND METHODS

The freshwater teleost, *Metynnis argenteus* Aht, 1923 (Characidae) ("Piaba Chata"), was collected in the estuarine region of the Amazon River (01°11'30"S, 47°18'54"W) near the city of Peixe Boi (Pará State), Brazil. Immediately after collection, 50 fish were transported alive to the laboratory, where they were anesthetized, killed, and necropsied.

Plasmodia with mature spores were examined in fresh mounts under a light microscope (LM) equipped with Nomarski interference-contrast differential (DIC) optics. For transmission electron microscopy (TEM), small fragments of host tissue were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) at 4 °C for 12 hr, then washed overnight with the same buffer at 4 °C and postfixed in 2% osmium tetroxide (OsO₄) buffered with sodium cacodylate for 2 hr at the same temperature. The fragments were dehydrated in an ascending ethanol and propylene oxide series (3 × 2 hr in each change) and embedded in Epon (10–12 hr in each change). Semithin sections were stained with

methylene blue. The ultrathin sections, cut with a diamond knife, were contrasted with both aqueous uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM operated at 60 kV.

DESCRIPTION

Myxobolus metynnis n. sp.

(Figs. 1–10)

Plasmodia and vegetative stages: Cyst white, ellipsoidal to spherical, up to 350 µm in diameter (Fig. 1). Plasmodial membrane is covered with fine projections or microvilli (Figs. 3, 4) and surrounded by a continuous layer of several fibroblasts intermingled with concentric collagen fiber layers (Fig. 5). Plasmodia polysporic, with asynchronous development, early sporogonic stages present in the external layer of the plasmodium; developing spores and mature spores are located more internally (Fig. 3).

Mature spores: Spores typical of *Myxobolus* spp. Mature spores are ellipsoidal shaped in frontal view, 13.1 (12.9–13.5) µm (n = 50) in length, 7.8 (7.5–8.3) µm (n = 30) in width, and 3.9 (3.4–4.5) µm (n = 14) in thickness (Figs. 2, 10). Elongate-pyriform polar capsules are of equal size, 5.2 (5.0–5.5) µm (n = 25) in length and 3.2 (3.0–3.6) µm (n = 12) in width. Eight to 9 filament coils are slightly oblique to the longitudinal axis (Figs. 6, 7). Intercapsule appendix is absent.

Taxonomic summary

Type host: Teleost fish, *Metynnis argenteus* Aht, 1923 (Characidae).

Type locality: Estuarine region of the Amazon River (01°11'30"S, 47°18'54"W) near the city of Peixe Boi (Pará State), Brazil.

Site of infection: Spores are located in the connective subcutaneous tissues of the orbicular region.

Prevalence of infection: Thirteen of 50 (26%).

Etymology: The specific name is derived from the name of the host species ("metynnis").

Type specimens: One slide containing mature spores of the syntypes were deposited in the International Protozoan Type Slide Collection at the Smithsonian Institution, Washington, D.C. 20560, USA, with acquisition number (USNM 1086177). The histological, semithin sections, showing varied developmental stages, were deposited at the laboratory of the senior author (C.A.).

Ultrastructural studies

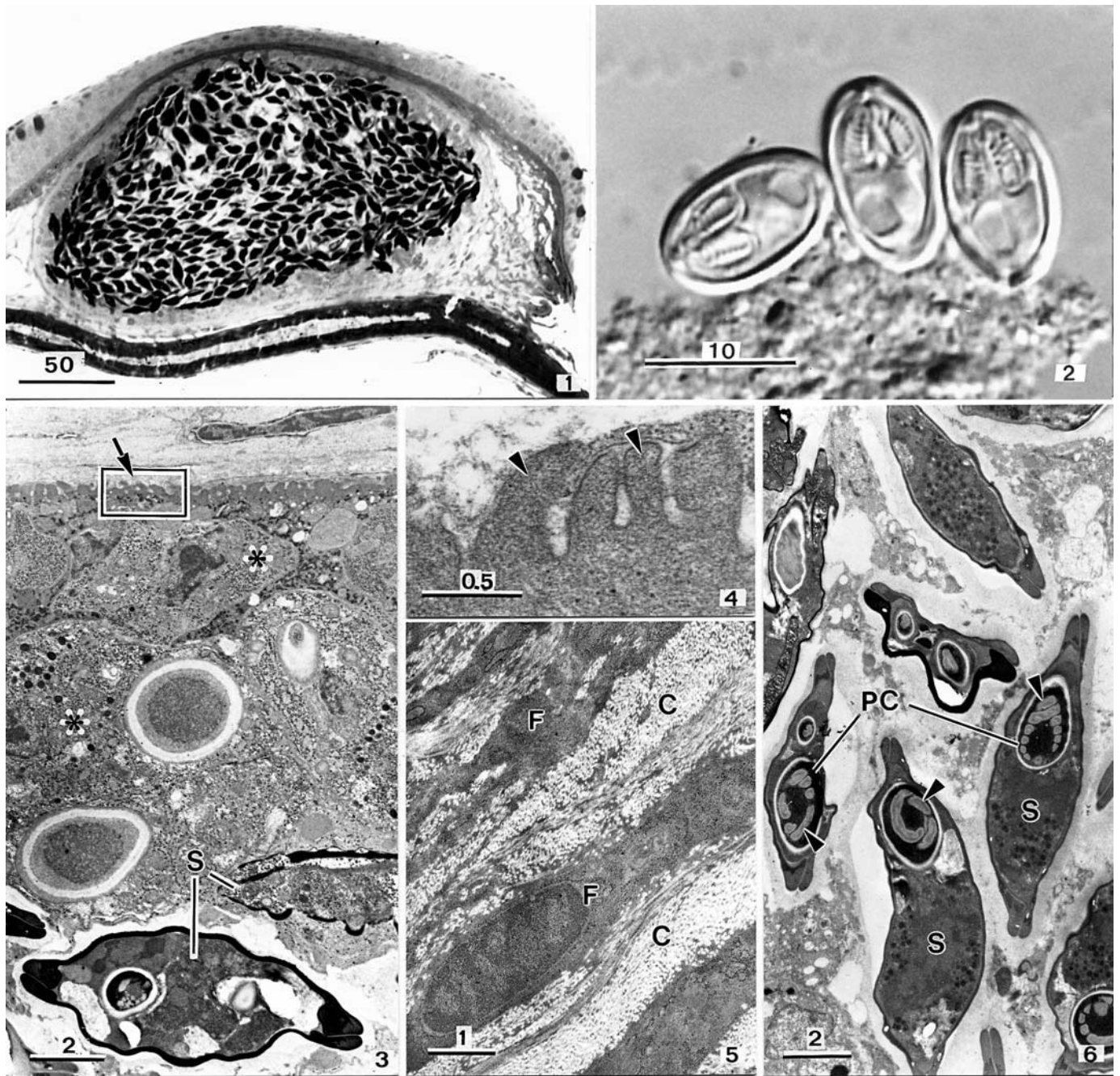
The spore wall was thin and smooth, comprising 2 unequal valves kept close by a sutural ridge. The spore wall was surrounded by a very fine network of irregular and complex anastomosed microfibrils projecting from the surface of the spore wall toward the surrounding spaces (Fig. 8). Internally, 2 capsulogenic cells, located side by side, contained a prominent polar capsule (PC). The PCs presented a very elongated, pyriform shape and were of equal size. The PC occupied approximately 2/5 of the total spore length. Inside of them, a coiled polar filament with 8–9 turns can be observed. These coils showed a slightly oblique position around the longitudinal axis (Figs. 6, 7).

The sporoplasm cell, located in the posterior pole of the spore, con-

Received 28 September 2005; revised 26 January 2006; accepted 27 January 2006.

* Carlos Azevedo Research Laboratory, Federal Rural University of the Amazonia, 66.000 Belém (Pará), Brazil.

† To whom correspondence should be addressed. Department of Cell Biology, Institute of Biomedical Sciences, University of Porto (ICBAS/UP) and Laboratory of Protoparasitology, University of Porto (CIIMAR/UP), Lg. A. Salazar no. 2, 4099-003 Porto, Portugal.

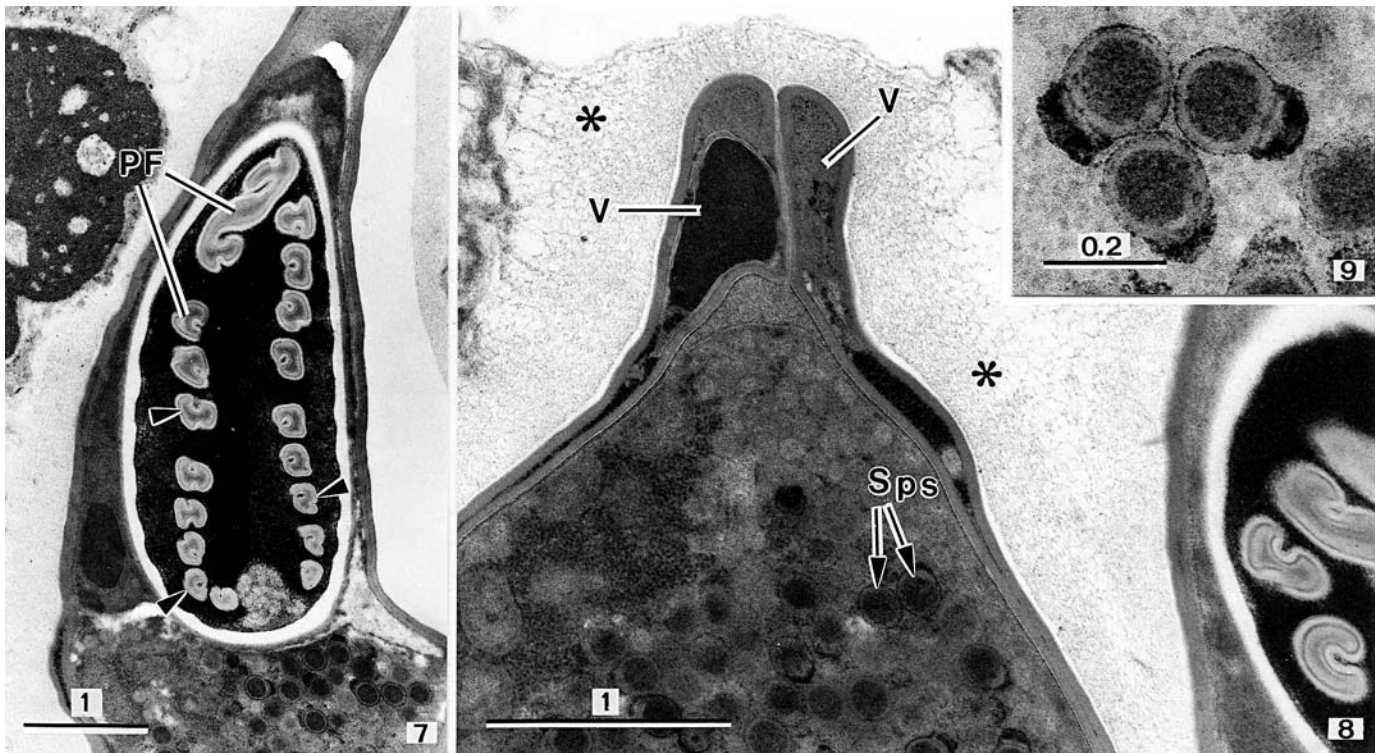


FIGURES 1–6. Light and transmission electron microscopy (TEM) aspects of *Myxobolus metynnis* n. sp., a parasite located in the connective subcutaneous tissues of the orbicular region of the freshwater fish *Metynnis argenteus*. (1) Semithin section of a cyst containing numerous spores. (2) Some isolated spores observed with interference-contrast differential (DIC) optics. (3) Ultrathin section of the periphery of a plasmodium showing different life-cycle stages (*) including spores (S). The boxed area (arrow) is enlarged in Figure 4. (4) Ultrastructural details of the periphery of the plasmodium, showing the organization of the microvilli (arrowheads). (5) Ultrastructural detail of the external periphery of a plasmodium showing several fibroblasts (F), some of them surrounded by numerous collagen fibres (C). (6) Ultrathin section of some spores sectioned at different levels showing the transverse sections of the sporoplasm (S), polar capsules (PC), and polar filament sections (arrowheads).

tained 2 nuclei, surrounded by an irregularly dense matrix, plus an iodophilous vacuole, mitochondria, an extensive endoplasmic reticulum, numerous electron-dense vesicles, and sporoplasmosomes (Sps) (Figs. 6, 8). At high magnification, the Sps appeared as spherical, double-membrane-bound bodies of about 180–200 nm in diameter, containing a massive dense core separated by narrow, lucent, circular space. Each Sps contained an eccentric, dense structure with a half-crescent section in close contact with the membrane of the sporoplasmosome (Fig. 9).

DISCUSSION

There are at least 744 species belonging to *Myxobolus* (Eiras et al., 2005), and most of the early descriptions are vague because they only present line drawings of the spores. Nineteen species of *Myxobolus* have been described in Brazilian fishes before the new one reported here (Kudo, 1920; Nemeček,



FIGURES 7–9. Some ultrastructural aspects of the spore details of *Myxobolus metynnis* n. sp. (7) Ultrathin, longitudinal section of a polar capsule showing the polar filament (PF) in different transverse sections (arrowheads). (8) Ultrathin section of the posterior pole of the spore showing the 2 unequal valves (V) and, inside the sporoplasm, several sporoplasmosomes (Sps). Externally, numerous anastomosed microfibrils were observed adhering to the valves (*). (9) Ultrastructural detail of 3 sporoplasmosomes, each with an eccentric, dense structure with a half-crescent section located in a matrix formed by granular masses.

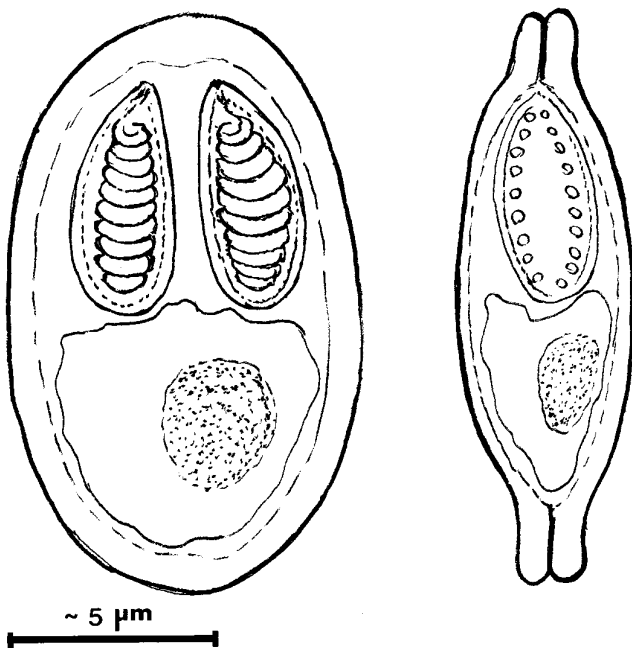


FIGURE 10. *Myxobolus metynnis* n. sp. Schematic drawing of the morphology of a spore in valvar (left side) and sutural (right side) view as described in the text and illustrated in the figures.

1926; Penido, 1927; Pinto, 1928; Walliker, 1969; Kent and Hoffman, 1984; Molnár and Békési, 1993; Casal et al., 1996; Gioia and Cordeiro, 1996; Molnár et al., 1998; Adriano et al., 2002; Azevedo et al., 2002; Casal et al., 2002; Cellere et al., 2002; Tajdari et al., 2005); a comparison of several characteristics of these 20 species is presented in Table I. When contrasting *M. metynnis* with other species found in Brazil, we only found similarities with *M. serrasalmi* (Walliker, 1969) and *M. noguchii* (Pinto, 1928). The macrospore of *M. serrasalmi* (Walliker, 1969) was equal in body shape to the species described here, but its spore was longer, whereas the spore of *M. noguchii* (Pinto, 1928) presented a similar size and shape to our species. Nevertheless, in all 3 species, a different host and different site of infection, exclude the possibility of these organisms belonging to the same species.

Among *Myxobolus* spp. that do not parasitize South American fishes (Eiras et al., 2005), only 3 species present similar body size, equal polar capsules, equal number of polar filament turns, and no intercapsular process, i.e., *M. attui* (Sarkar, 1985), *M. benineusis* (Sakiti et al., 1991), and *M. dechtiari* (Cone and Anderson, 1977). However, the host and site of infection are different in all of them.

In general, the ultrastructural aspects that concern the plasmodium wall, sporogenesis development, and mature spores of the parasite found in *Metynnis argenteus*, show all the characteristics of *Myxobolus* Bütschli, 1882 (Myxobolidae) (Lom and Puytorac, 1965; Desser and Paterson, 1978; Current et al., 1979; Lom and Dyková, 1992).

TABLE 1. Morphological comparison of the spores of different *Myxobolus* species reported in Brazilian fishes.*

<i>Myxobolus</i> species	TL	BW	PCL	PCW	PFC	Host	Site of infection	References
<i>M. inaequalis</i>	5.2	3.3	Unequal	—	—	<i>Piramatana blochii</i>	Skin of head	Kudo 1920
<i>M. lutei</i>	10	7	—	—	—	<i>Girardinus jamuaris</i>	Testis	Kudo 1920
<i>M. chondrophilus</i>	6	4.5	3	—	—	<i>Sardinella anchovia</i>	Gills	Nemeczek 1926
<i>M. associatus</i>	15	10	7	—	—	<i>Leporinus mormyrops</i>	Kidney	Nemeczek 1926
<i>M. pygocentris</i>	15–16	9–11	9–11	3–4	—	<i>Pygocentris piraya</i>	Intestinal contents	Penido 1927
<i>M. cunhai</i>	9–11	4–6	Unequal	—	—	<i>Pygocentris piraya</i>	Intestinal contents	Penido 1927
<i>M. noguchii</i>	13.6	8.5	6.8	2.2	—	<i>Serrasalmo spilopleura</i>	Gills?	Pinto 1928
<i>M. stokesi</i>	8.5	5.3	3.4	1.7	—	<i>Pimelodella</i> sp.	Subcutaneous tissues	Pinto 1928
<i>M. kudoii</i>	8.5–8.9	6.5–7.3	3.5–4.2	1.3–2	—	<i>Nematognatha</i> sp.	Skin	Walliker 1969
<i>M. serrasalmi</i>	12.5–18	7–10	6–9	2.5–4	—	<i>Serrasalmus rhombeus</i>	Spleen, kidney, liver	Walliker 1969
	7–9.5	3.5–5	5–7.5	1–2	—			
<i>M. inaequus</i>	19.8	8.6	11.8	—	—	<i>Eigemannia virescens</i>	Brain	Kent and Hoffman 1984
			4.8					
<i>M. colossomatis</i>	11.8	6.9	6.0	2.1	7–8	<i>Colossoma macropomum</i>	Connective tissues	Molnár and Békési 1993
<i>M. braziliensis</i>	10.2	5.3	5.3	1.4	9–11	<i>Bunocephalus coracoides</i>	Gills	Casal et al. 1996
<i>M. macropylasmodialis</i>	11.0	8.5	4.5	2.8	6	<i>Salminus maxillosus</i>	Abdominal cavity	Molnár et al. 1998
<i>M. porofilus</i>	5.7	4.8	1.6	1.1	3	<i>Prochilodus lineatus</i>	Visceral cavity	Adriano et al. 2002
<i>M. desaequalis</i>	18.3	11.2	11.2	4.9	11–12	<i>Apteronotus albifrons</i>	Gills	Azevedo et al. 2002
			4.6	2.8	4–5			
<i>M. maculatus</i>	21.0	8.9	12.7	3.2	14–15	<i>Metynnis maculatus</i>	Kidney	Casal et al. 2002
<i>M. absomus</i>	15.7	10.2	6.4	3.6	5	<i>Pimelodus maculatus</i>	Opercular cavity	Cellere et al. 2002
			4.2	2.5	3			
<i>M. testicularis</i>	8.6	7.2	3.5	1.7	5–6	<i>Hemiodopsis microlepis</i>	Testis	Tajdari et al. 2005
<i>M. metynnis</i>	13.1	7.8	5.2	2.3	8–9	<i>Metynnis argenteus</i>	Connective subcutaneous tissues	Present study

* Abbreviations: TL: total length; BW: body width; PCL: polar-capsule length; PCW: polar-capsule width; PFC: polar-filament coils; —: no data.

The most obvious ultrastructural difference between the previously described species and *M. metynnis* was in the organization of the sporoplasmosomes. Unfortunately, the original description of the earliest *Myxobolus* sp. did not present ultrastructural details, making it impossible to establish a comparison with the results found for this parasite.

Sporoplasmosomes (Sps) exhibiting great diversity in size and structure located in the binucleated sporoplasm is a common feature in myxosporidians. The Sps appeared in different *Myxobolus* spp. as vesicular, single or double membrane-bound bodies (Lom et al., 1989; Casal et al., 1996). In our observations, the Sps showed an electron-dense deposit, with a half-crescent shape, outside the single-membrane-bound bodies, which presented some similarities with the Sps previously described in *M. desaequalis*. However, the latter species is not comparable with the present data, in spite of containing 2 strikingly unequal PCs (Azevedo et al., 2002).

No *Myxobolus* species has previously been reported from the host, *Metynnis argenteus*, which has a wide distribution in all Amazonian regions. In addition, the connective subcutaneous tissues of the orbicular region were not previously reported as a site of infection for *Myxobolus* species in Brazilian fishes. The pathology associated with this parasite appears, especially, during the later stages, when some signs of lyses in the host cells were observed, corresponding to the higher mortality period. The parasite described here is the second report of a *Myxobolus* spp. in a species of the Characidae from the Amazon region. Thus, *M. maculatus* was reported in the kidney of *Metynnis maculatus* and showed major morphometric and ultrastructural differences compared with our observations (Casal et al., 2002).

ACKNOWLEDGMENTS

This work was partially supported by the Ant3nio Almeida Foundation (Porto, Portugal). We would like to thank the iconographic work of Jo3o Carvalheiro and Jessica Tajdari for helping in the English revision.

LITERATURE CITED

- ADRIANO, E. A., S. ARANA, P. S. CECCARELLI, AND N. S. CORDEIRO. 2002. Light and scanning electron microscopy of *Myxobolus porofilus* sp. n. (Myxosporidia: Myxobolidae) infecting the visceral cavity of *Prochilodus lineatus* (Pisces: Characiformes: Prochilodontidae) cultivated in Brazil. *Folia Parasitologica* **49**: 259–262.
- AZEVEDO, C., L. CORRAL, AND E. MATOS. 2002. *Myxobolus desaequalis* n. sp. (Myxozoa, Myxosporidia), parasite of the Amazonian freshwater fish, *Apteronotus albifrons* (Teleostei, Apteronotidae). *Journal of Eukaryotic Microbiology* **49**: 485–488.
- CASAL, G., E. MATOS, AND C. AZEVEDO. 1996. Ultrastructural data on the life cycle stages of *Myxobolus braziliensis* n. sp., parasite of an Amazonian fish. *European Journal of Protistology* **32**: 123–127.
- , ———, AND ———. 2002. Ultrastructural data on the spore of *Myxobolus maculatus* n. sp. (phylum Myxozoa), parasite from the Amazonian fish *Metynnis maculatus* (Teleostei). *Diseases of Aquatic Organisms* **51**: 137–149.
- CELLERE, E. F., N. S. CORDEIRO, AND E. ADRIANO. 2002. *Myxobolus absonus* sp. n. (Myxozoa: Myxosporidia) parasitizing *Pimelodus maculatus* (Siluriformes: Pimelodidae), a South American freshwater fish. *Memorias do Instituto Oswaldo Cruz* **97**: 79–80.
- CONE, D. K., AND R. C. ANDERSON. 1977. Myxosporidian parasites of pumpkinseed (*Lepomis gibbosus* L.) from Ontario. *Journal of Parasitology* **63**: 657–666.
- CURRENT, W. L., J. JANOVY, JR., AND S. A. KNIGHT. 1979. *Myxosoma funduli* Kudo (Myxosporidia) in *Fundulus kansae*: Ultrastructure of the plasmodium wall and of sporogenesis. *Journal of Protozoology* **26**: 574–583.
- DESSER, S. S., AND W. S. PATERSON. 1978. Ultrastructural and cytochemical observations on sporogenesis of *Myxobolus* sp. (Myxosporidia: Myxobolidae) from the common shiner *Notropis cornutus*. *Journal of Protozoology* **25**: 314–326.
- EIRAS, J. C., K. MOLNÁR, AND Y. S. LU. 2005. Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporidia: Myxobolidae). *Systematic Parasitology* **61**: 1–46.
- GIOIA, I., AND N. S. CORDEIRO. 1996. Brazilian myxosporidians, checklist (Myxozoa). *Acta Protozoologica* **35**: 137–149.
- KENT, M. L., AND G. L. HOFFMAN. 1984. Two new species of Myxozoa, *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the brain of a South American knife fish, *Eigemannia virescens* (V.). *Journal of Protozoology* **31**: 91–94.
- KUDO, R. R. 1920. Studies on Myxosporidia. III. Biological Monographs **5**: 1–265.
- LOM, J., AND P. DE PUYTORAC. 1965. Studies on the myxosporidian ultrastructure and polar capsule development. *Protistologica* **1**: 53–65.
- , AND I. DYKOVÁ. 1992. Myxosporidia (Phylum Myxozoa). In *Protozoan parasites of fishes: Developments in aquaculture and fisheries science*, Vol. 26, J. Lom and I. Dyková (eds.). Elsevier, Amsterdam, The Netherlands, p. 159–235.
- , S. W. FEIST, I. DYKOVÁ, AND T. KEPR. 1989. Brain myxoboliasis of bullhead, *Cottus gobio* L., due to *Myxobolus jiroveci* sp. nov.: Light and electron microscope observations. *Journal of Fish Diseases* **12**: 15–27.
- LONGSHAW, M., P. FREAR, AND S. W. FEIST. 2003. *Myxobolus buckei* sp. n. (Myxozoa), a new pathogenic parasite from the spinal column of three cyprinid fishes from the United Kingdom. *Folia Parasitologica* **50**: 251–162.
- MOLNÁR, K., AND L. BÉKÉSI. 1993. Description of a new *Myxobolus* species, *M. colossomatis* n. sp. from the teleost *Colossoma macropomum* of the Amazon River basin. *Journal of Applied Ichthyology* **9**: 57–63.
- , M. J. RANZANI-PAIVA, J. C. EIRAS, AND E. L. RODRIGUES. 1998. *Myxobolus macroplasmoidalis* sp. n. (Myxozoa: Myxosporidia), a parasite of the abdominal cavity of the characid teleost, *Salminus maxillosus*. *Acta Protozoologica* **37**: 241–245.
- NEMECZEK, A. 1926. Beiträge zur Kenntnis der Myxosporidienfauna Brasiliens. *Archiv für Protistenkunde* **54**: 137–150.
- PENIDO, J. C. N. 1927. Quelques nouvelles Myxosporidies parasites des poissons d'eau douce du Brésil. *Conte Rendue de la Société de Biologie (Paris)* **97**: 850–852.
- PINTO, C. 1928. *Myxobolus noguchii*, *M. stokesi*, *Henneguya iheringi*, espécies novas de Myxosporidios de peixes de água doce do Brasil. *Boletim de Biologia* **12**: 41–43.
- SAKITI, N., E. BLANC, A. MARQUES, AND G. BOUIX. 1991. Myxosporidies (Myxozoa, Myxosporidia) du genre *Myxobolus* Bütschli, 1882 parasites de poissons cichlidae du lac Nokoué au Bénin (Afrique de l'Ouest). *Journal of African Zoology* **105**: 173–186.
- SARKAR, N. K. 1985. Some coelozoic myxosporidians (Myxozoa: Myxosporidia) of anabantid fishes of West Bengal, India. *Acta Protozoologica* **24**: 17–180.
- TAJDARI, J., E. MATOS, I. MENDONÇA, AND C. AZEVEDO. 2005. Ultrastructural morphology of *Myxobolus testicularis* sp. n., parasite of the testis of *Hemiodopsis microlepis* (Teleostei: Hemiodontidae) from the NE of Brazil. *Acta Protozoologica* **44**: 377–384.
- WALLIKER, D. 1969. Myxosporidia of some Brazilian freshwater fishes. *Journal of Parasitology* **55**: 942–948.

Capítulo 9

**ULTRASTRUCTURAL DESCRIPTION OF *CERATOMYXA TENUISPORA*
(MYXOZOA), A PARASITE OF THE MARINE FISH *APHANOPUS CARBO*
(TRICHIURIDAE), FROM THE ATLANTIC COAST OF
MADEIRA ISLAND (PORTUGAL)**

Folia Parasitologica (2007) **54**: 165-171

Graça Casal, Graça Costa & Carlos Azevedo

Ultrastructural description of *Ceratomyxa tenuispora* (Myxozoa), a parasite of the marine fish *Aphanopus carbo* (Trichiuridae), from the Atlantic coast of Madeira Island (Portugal)

Graça Casal^{1,2,3}, Graça Costa⁴ and Carlos Azevedo^{1,2}

¹Department of Cell Biology, Institute of Biomedical Sciences (ICBAS/UP), University of Porto, Lg. A. Salazar no. 2, P-4099-003 Porto, Portugal;

²Laboratory of Pathology, CIIMAR/UP, University of Porto, Rua dos Bragas no. 117, P-4050-123 Porto, Portugal;

³Department of Sciences, High Institute of Health Sciences, P-4585-116 Gandra, Portugal;

⁴Centre for Macaronesian Studies (CEM), University of Madeira, P-9000-390 Funchal, Portugal

Key words: Myxozoa, *Ceratomyxa tenuispora*, parasite, marine fish, *Aphanopus carbo*, ultrastructure

Abstract. The first ultrastructural description of *Ceratomyxa tenuispora* Kabata, 1960 (Myxozoa, Bivalvulida) from Madeira Island (Portugal), a parasite found in the gall bladder of the commercially important black-scabbard fish, *Aphanopus carbo* Lowe is presented. This parasite possesses spherical to ellipsoidal disporous trophozoites. Spores have a central crescent-shaped body averaging 11.0 µm in length, 28.5 µm in thickness and 12.1 µm in width. The valves have two long opposite lateral processes (ribbon-like structures or tails), each averaging 173 µm in length. The total thickness of the spore averages 375 µm. The spore has two sub-spherical polar capsules (~5.2 × 4.1 µm), each with a polar filament with 7 to 8 coils. Some ultrastructural aspects of the sporogonic stages are described. The trophozoites develop without contact with epithelial cells. The cytoplasmic membrane has numerous evenly distributed external slender projections about 0.3 to 0.7 µm long. The sporogenesis produces two spores without pansporoblast formation. In the matrix of the capsular primordium, microtubules with an unusual organisation were observed. A binucleate sporoplasm that contains several sporoplasmosomes and dense bodies fills the spore cavity and extends to the tails without penetrating them.

The genus *Ceratomyxa* Thélohan, 1892 is one of the largest genera of Myxosporidia (phylum Myxozoa), which includes about 172 species, mostly parasites of marine fish (Lom and Dyková 2006). Most of them are coelozoic, rarely histozoic. They have a world-wide distribution and cause severe infections, mainly of the digestive tract organs (for revision see Lom and Dyková 1992, Eiras 2006). Only some species were ultrastructurally studied, such as *C. shasta* (Yamamoto and Sanders 1979), *C. globulifera* (Desportes and Théodorides 1982), *Ceratomyxa* sp. hyperparasitized with the microsporidian *Nosema ceratomyxae* (Diamant and Paperna 1989), *C. labracis* and *C. diplodae* (Alvarez-Pellitero and Sitjà-Bobadilla 1993, Sitjà-Bobadilla and Alvarez-Pellitero 1993a), *C. sparusaurati* (Sitjà-Bobadilla et al. 1995, Palenzuela et al. 1997), *C. drepanopsettae* (Morrison et al. 1996), and *C. protopsettae* (Cho et al. 2004).

Ceratomyxa tenuispora Kabata, 1960 was described from the black-scabbard fish, *Aphanopus carbo* Lowe based only on a schematic drawing (Kabata 1960). Later, this parasite was reported from the same fish host, a commercially important fish species, collected from deep-waters around Madeira Island (Costa et al. 1996). The present paper details the ultrastructure of the spores and some sporogonic stages of the life cycle of *Ceratomyxa tenuispora* are described.

MATERIALS AND METHODS

One hundred and one specimens of black-scabbard fish, *Aphanopus carbo* Lowe (Teleostei, Trichiuridae) (specimens from 100 to 130 cm long), were collected at depths of 600–1,200 meters and at 5 to 10 miles off the North Atlantic coast of Madeira Island (33°07'–32°02'N, 16°16'–17°16'W). Immediately after capture, fish were placed on ice and brought to the laboratory for parasitological examination. After necropsy, the gall bladders were removed and examined for infections with myxosporeans by light microscopy (LM). Wet smears revealed myxosporean spores and other life-cycle stages in the bile. Fresh isolated mature spores were observed using differential interference contrast (DIC) (Nomarski) optics. For transmission electron microscopy (TEM), small fragments of parasitized gall bladders as well as bile fluid were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 10h at 4°C, concentrated in agar and then washed overnight in the same buffer at 4°C. The samples were postfixed with 2% osmium tetroxide in the same buffer for 2h at 4°C, dehydrated through an ascending ethanol and propylene oxide series, and embedded in Epon. Semithin sections for LM observations were stained with methylene blue-Azur II. Ultrathin sections were double-stained with uranyl acetate and lead citrate before observation in a JEOL 100 CXII TEM, operated at 60 kV.

Address for correspondence: G. Casal, Department of Cell Biology, Institute of Biomedical Sciences, University of Porto, Lg. A. Salazar no. 2, P-4099-003 Porto, Portugal. Phone: ++351 222 062 200; Fax: ++351 222 062 232/33; E-mail: gcasal@icbas.up.pt; azevedoc@icbas.up.pt

RESULTS

Disporous plasmodia of spherical to ellipsoidal shape (Fig. 1) and spores (Fig. 2) were found floating in the bile of several fish (7 out of 12). The body of the isolated mature spores was $11.0 \pm 0.9 \mu\text{m}$ ($n = 15$) long by $28.5 \pm 1.2 \mu\text{m}$ ($n = 12$) thick and $12.1 \pm 1.1 \mu\text{m}$ wide in sutural view. The spore body was crescent-shaped in front view with a convex anterior end and a flattened posterior one (Fig. 2). Two polar capsules were equal, sub-spherical to pyramidal, and measured $5.2 \pm 0.3 \mu\text{m} \times 4.1 \pm 0.4 \mu\text{m}$ ($n = 10$) (Fig. 2). The spore had two smooth symmetrical valves that were prolonged by two fine long, opposite lateral processes (ribbon-like projections or tails) which tapered gradually towards the tips, each one of $173.2 \pm 6.3 \mu\text{m}$ ($n = 30$) in length. Total dimension of the tailed spore was $375.5 \pm 17.1 \mu\text{m}$ ($n = 15$), ranking the spore as one of the largest among myxosporeans (Fig. 2).

Ultrastructural observations

The earliest stages observed were uninucleate cells measuring $12.7\text{--}19.5 \mu\text{m}$ in diameter, whose nuclei presented a prominent nucleolus. Rounded trophozoites were both free-floating or joined to each other beads-like, and their cytoplasm contained a variable number of secondary cells. Neither contact with epithelial cells of the gall bladder nor pansporoblast formation was observed (Fig. 3). The cytoplasm of the primary cell contained mitochondria, scattered ribosomes and several membranous structures similar to small vesicles with a matrix of heterogeneous content (Figs. 3, 4). Cytoplasmic membrane of the primary cells contained, at the periphery, several external slender projections basally attached to the surface. These had a uniform shape and variable length ranging from 0.3 to $0.7 \mu\text{m}$, and were evenly distributed throughout the plasmodia surface (Figs. 3, 4).

Immature spores were easily identified in early sporoblasts by their valvogenic, capsulogenic and sporoplasmic cells (Fig. 5). Valvogenic cells, which were joined in suture line by a continuous septate junction, occupied an external position relative to other sporogenic cells (Figs. 5, 8, 9, 13). Their external plasmalemma was smooth and dense and the internal one was thin and showed an irregular outline in close contact with other inner spore cells (Figs. 10, 13, 15). During early sporogenic phase, the valvogenic cells gradually differentiated to form two long tails surrounding the spore body. Initially those extensions of the valvogenic cells were dilated (Fig. 5) and later they modified to ribbon-shaped (Figs. 8, 10, 11, 14). The cytoplasm of the valvogenic cells in mature spores appeared to contain only small vesicles (Figs. 5, 10, 11, 14).

During the early differentiation of the capsulogenic cells, the spherical capsular primordium was prolonged by the external tube. Before the inversion of the external

tube, microtubules with an unusual organisation were observed in the matrix of the capsular primordium. A larger aggregate with hundreds of microtubules was grouped in several bundles and these were arranged in different orientations (Figs. 6, 7).

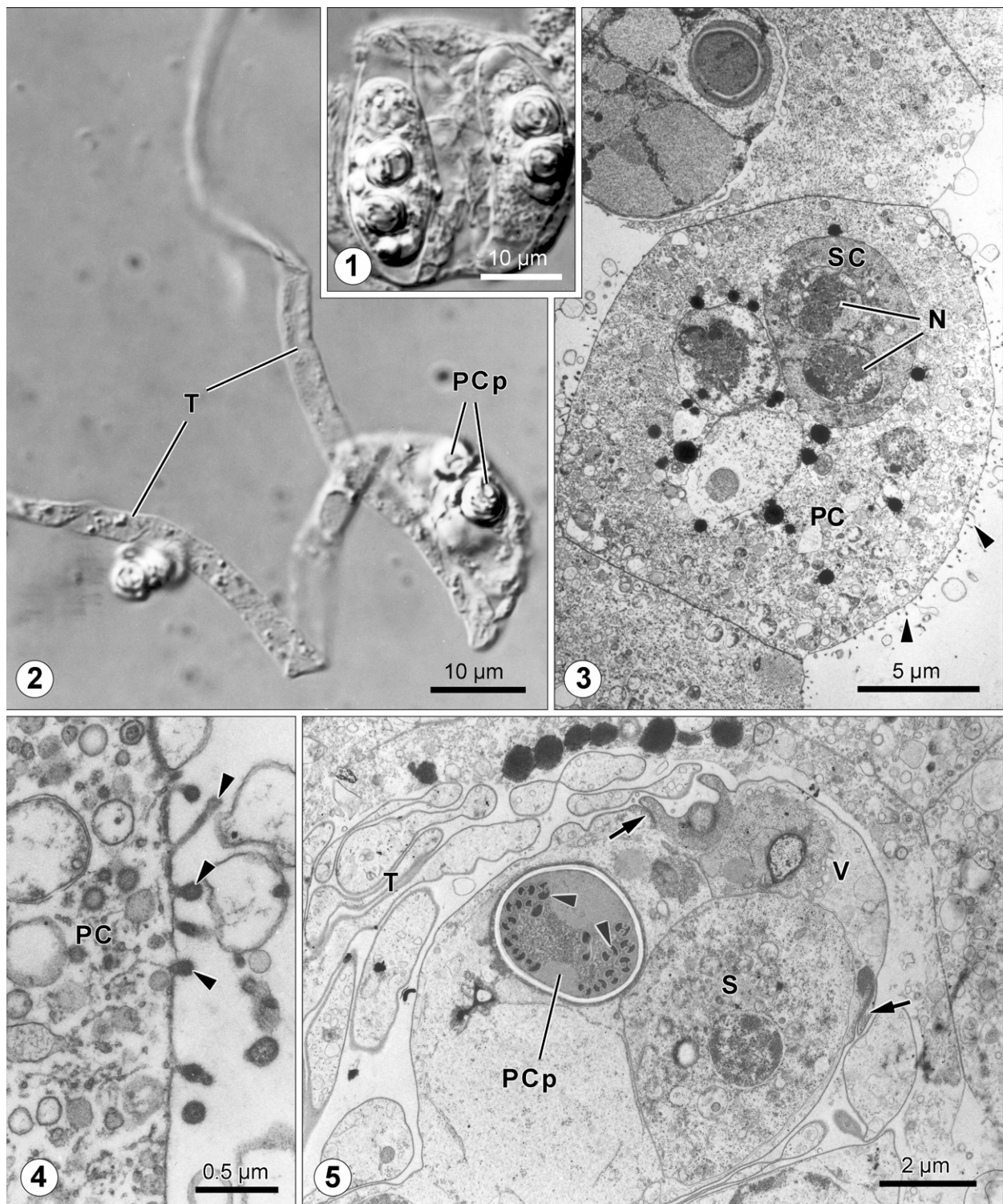
In mature spores the nuclei persisted until completion of the capsulogenesis (Fig. 8) and the polar capsules had a polar filament with a basal straight central shaft and coils of 7 to 8 turns (Figs. 5, 8, 12). Polar capsules consisted of a thin electron-dense external wall and a thicker and lighter inner one (Figs. 12, 13). The apical channel for the polar filament discharge showed close contact with valves (Figs. 12, 13).

The sporoplasm was located in the posterior end of the spore and the cytoplasm contained two nuclei close to each other, mitochondria, ribosomes, cisternae of endoplasmic reticulum and several sporoplasmosomes. These spherical-shaped vesicles were membrane-bounded and contained an electron-dense and homogeneous matrix (Figs. 10, 14, 15). The sporoplasm filled the spore cavity and extended to the tails, without penetrating them (Figs. 8, 10, 15). A schematic drawing of the spore, based on LM and TEM observations, is shown in Fig. 16.

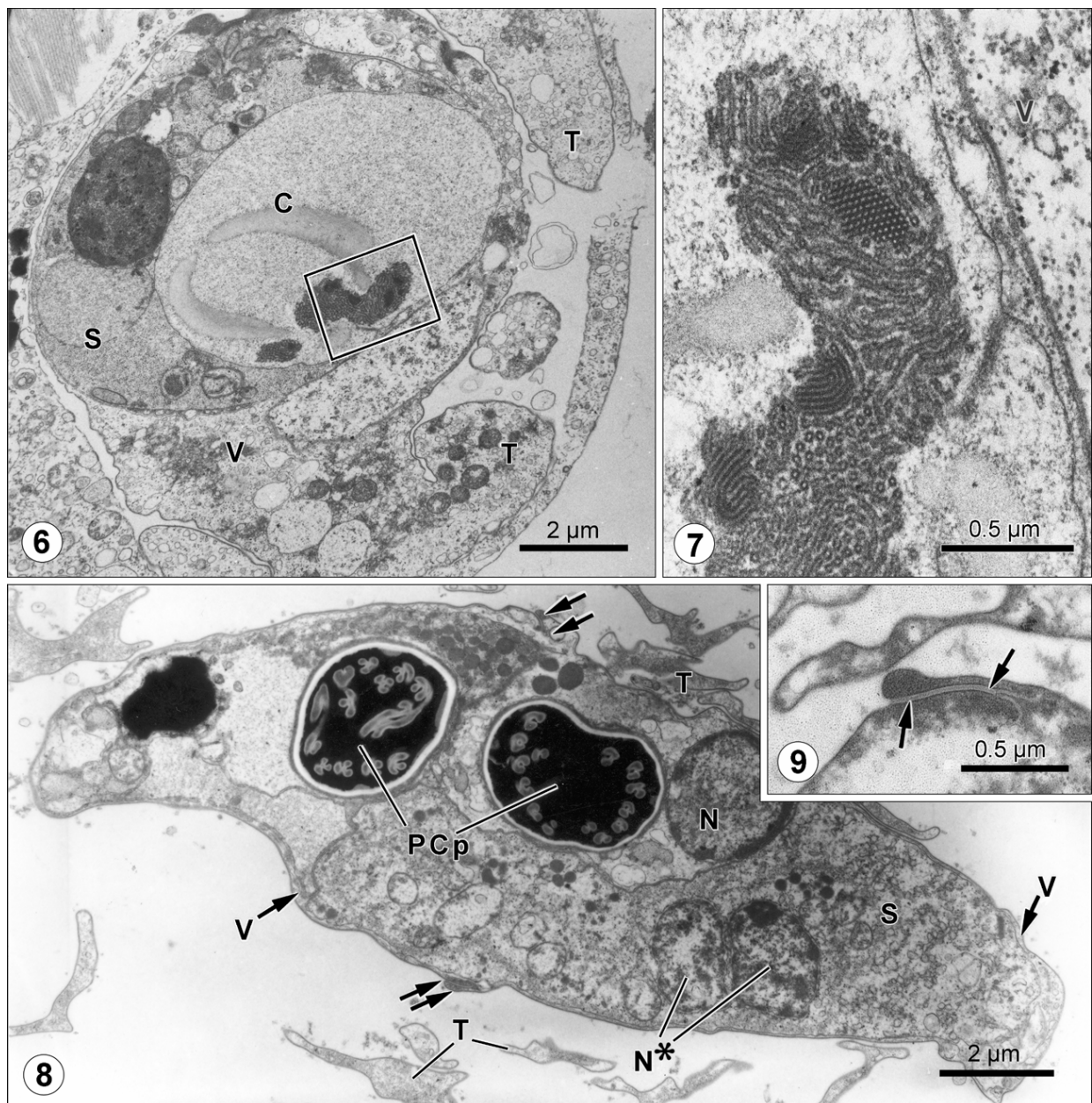
DISCUSSION

The ultrastructural observations of the sporogenesis and consequently of the spores of *Ceratomyxa tenuispora* Kabata, 1960 showed several similarities to species from the family Ceratomyxidae Doflein, 1899 (Lom and Dyková 1992). This species is ultrastructurally described for the first time in the present study.

Cytoplasmic extensions of the outer plasmodial membrane have usually been described in coelozoic parasites of various genera found in gall bladder and they are closely correlated with the type of nutrition (Sitjà-Bobadilla and Alvarez-Pellitero 1993b). *Ceratomyxa protopsettae* trophozoites are attached to the epithelial cells by short or long finger-like projections (Cho et al. 2004); ramified microvillus-like projections of different sizes were described in *Zschokkella icterica* (Diamant and Paperna 1992). Long finger-like pseudopodial projections reaching a length of about $5 \mu\text{m}$ were described in *Zschokkella mugilis* (Sitjà-Bobadilla and Alvarez-Pellitero 1993b). A similar interaction was observed in *Myxidium trachinorum*, which contacts the epithelium of the gall bladder through two to three filose processes (Canning et al. 1999). At one end of some primary cells, rhizoid-like projections were observed in *Ceratomyxa sparusaurati* (Sitjà-Bobadilla et al. 1995). Unlike in this last species, in *C. tenuispora* we did not see any contact with epithelial cells of the gall bladder and the shape and distribution of the cytoplasmic projections found in *C. tenuispora* show some ultrastructural differences.



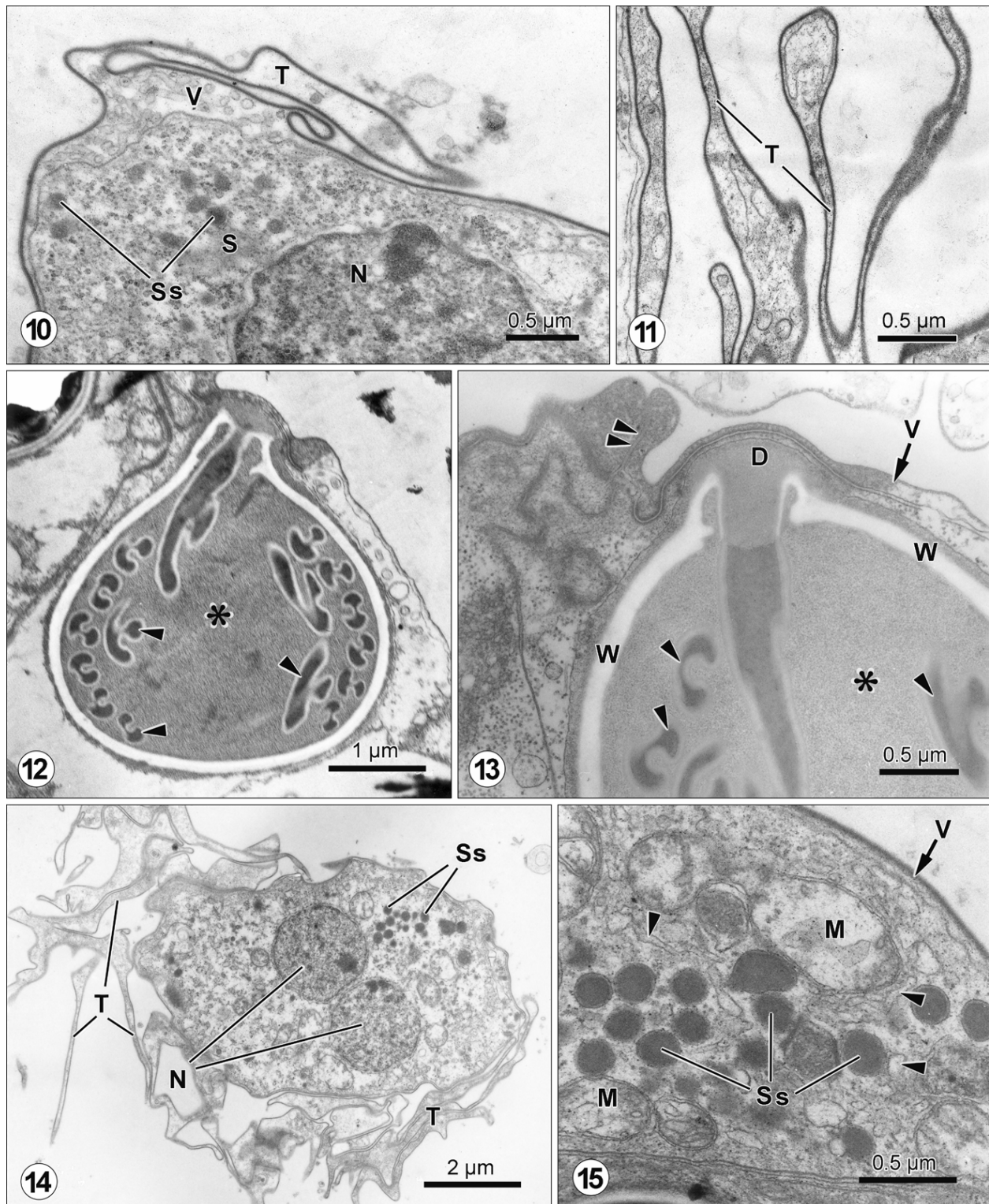
Figs. 1–5. *Ceratomyxa tenuispora*, DIC (Figs. 1, 2) and transmission electron micrographs (Figs. 3–5). **Fig. 1.** Fresh disporous plasmodium. **Fig. 2.** Free mature spore showing two polar capsules (PCp) and two long tapering lateral opposite tails (T). **Fig. 3.** A secondary cell (SC) with two nuclei (N) within a primary cell (PC) containing several slender projections (arrowheads). **Fig. 4.** Detail of the periphery of the primary cell (PC) showing several slender projections (arrowheads). **Fig. 5.** Spore showing a polar capsule (PCp) with different sections of the polar filament (arrowheads), a sporoplasm (S) and valvogenic cells (V) with the suture line (arrows). At the periphery, several sections of tails (T) can be observed.



Figs. 6–9. Transmission electron micrographs of *Ceratomyxa tenuispora*. **Fig. 6.** A spore showing the valves (V), several sections of their two tails (T), the capsular primordium (C) containing a well-organized cluster of microtubules (boxed area) and the sporoplasm (S). **Fig. 7.** Detail of the cluster of microtubules (boxed area in Fig. 6). At the periphery, valvogenic cell (V). **Fig. 8.** Longitudinal section of a spore showing one nucleus (N) of a capsulogenic cell, two polar capsules (PCp), and the binucleate (N^*) sporoplasm (S). Valvogenic cells (V) are reduced to a thin layer in close contact with internal cells. Sutures (double arrows) between the valves can be seen. Some tail sections (T) can be seen at the periphery of the spore. **Fig. 9.** Detail of the continuous septate junction of the suture line.

Ultrastructural description concerning the polar capsule differentiation referred to the presence of unusual structures inside the capsular primordium, such as globule of electron-dense material (Lom 1969), concentric structure (Lom et al. 1989) or the differentiation of the microfilament-like structures (Casal et al. 2002). Bundles of tubuli in the capsular matrix were reported in the polar capsules of mature spores in some genera, such as *Sphaeromyxa* (Lom 1969), *Henneguya* (Rocha et al.

1992) and *Myxobolus* (Casal et al. 2002). In the cytoplasm of the capsulogenic cells, microtubules are regularly seen around the external tube interpreted to provide the mechanic force needed for its inversion into the capsular primordium (Current 1979). We reported the presence of several microtubular bundles inside the capsular matrix before the inversion of the external tube which apparently also contributes to that.



Figs. 10–15. Transmission electron micrographs of *Ceratomyxa tenuispora*. **Fig. 10.** Detail of valve (V), its tail (T) and the sporoplasm (S) with a nucleus (N) and some sporoplasmosomes (Ss). **Fig. 11.** Sections of spore tail (T) showing an electron-lucid matrix. **Fig. 12.** Longitudinal section of a polar capsule with electron-dense matrix (*), showing the polar filament sectioned at different levels (arrowheads). **Fig. 13.** Detail of the apical region of a polar capsule showing the wall (W), the channel for filament discharge (D), and some polar filament sections (arrowheads) within the capsular matrix (*). Externally, the valve (V) and the suture (double arrowhead). **Fig. 14.** Sporoplasm showing the two nuclei (N), several sporoplasmosomes (Ss) and several sections of the tails (T). **Fig. 15.** Detail of the sporoplasm showing several sporoplasmosomes (Ss), mitochondria (M) and some cisternae of endoplasmic reticulum (arrowheads). Externally, a surrounding valve (V) can be seen.

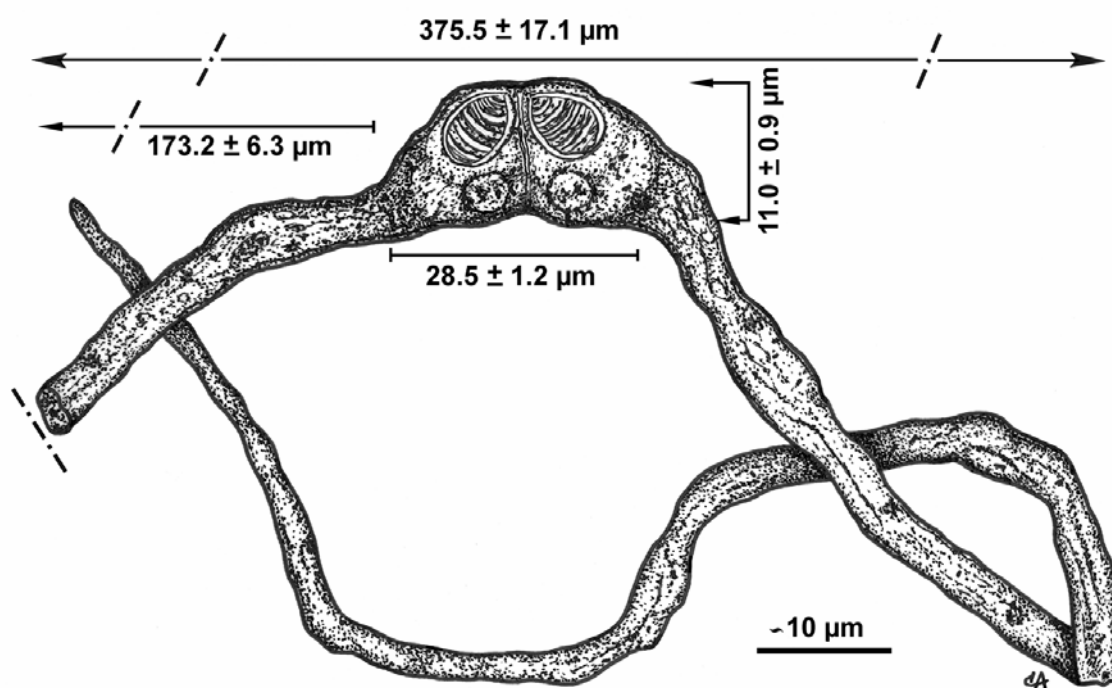


Fig. 16. Schematic drawing of *Ceratomyxa tenuispora* showing the spore morphology, with special emphasis on the two long tapering opposite lateral tails.

Table 1. *Ceratomyxa tenuispora*, comparison of spore characteristics between original description of Kabata (1960) and the present specimens (measurements in µm).

	Original description	Present material
Host and organ	<i>Aphanopus carbo</i> , gall bladder	
Geographical location	Scotland (United Kingdom)	North Atlantic coast of Madeira Island (Portugal)
Spore body	crescent-shaped with convex anterior end and flattened posterior end	
– length	8.7 (8.4–9.8)	11.0 ± 0.9
– width	–	12.1 ± 1.1
– thickness	–	28.5 ± 1.2
Total thickness	387 (308–504)	375.5 ± 17.1
Polar capsules	two equal, sub-spherical to pyramidal	
– length	6.4 (5.6–7.0)	5.2 ± 0.3
– width	–	4.1 ± 0.4
Polar filament	–	coils of 7 to 8 turns
Sporoplasm	slightly granular with vacuoles	binucleate

More than 30 species of *Ceratomyxa* with long lateral processes have been described in different hosts and geographic areas (Sitjà-Bobadilla and Alvarez-Pellitero 1993a). *Ceratomyxa tenuispora* is the second longest of this *Ceratomyxa* spp. group. These processes have not been ultrastructurally described except for *C. labracis* (Sitjà-Bobadilla and Alvarez-Pellitero 1993a), showing similar ultrastructural aspects to those described in this study.

Since the description of *C. tenuispora* based on a drawing obtained from LM observations by Kabata (1960) and a later report by Costa et al. (1996), no other reports of this species have been published. The mor-

phological and morphometrical characteristics of the spore seem to be the same, except for total thickness, the present specimens being somewhat smaller than *C. tenuispora* studied previously (Kabata 1960, Costa et al. 1996). Also, the parasite was collected from the same host species (*Aphanopus carbo*) and the same organ (gall bladder) (Table 1).

Acknowledgements. This work was supported by the António de Almeida Foundation-Porto-Portugal and a CESPU (Cooperativa de Ensino Superior, Politécnico e Universitário) PhD grant. We would like to thank Mr. João Carvalheiro for technical assistance and to Dr. Victor Ferreira for helping in the English revision. The helpful suggestions and comments of the reviewers are greatly appreciated.

REFERENCES

- ALVAREZ-PELLITERO P., SITJÀ-BOBADILLA A. 1993: Pathology of Myxosporea in marine fish culture. *Dis. Aquat. Org.* 17: 229–238.
- CANNING E.U., CURRY A., ANDERSON C.L., OKAMURA B. 1999: Ultrastructure of *Myxidium trachinorum* sp. nov. from the gallbladder of the lesser weever fish *Echiichthys vipera*. *Parasitol. Res.* 85: 910–919.
- CASAL G., MATOS E., AZEVEDO C. 2002: Ultrastructural data of the spore of *Myxobolus maculatus* n. sp. (Phylum Myxozoa), parasite from the Amazonian fish *Metynnis maculatus* Kner, 1860 (Teleostei). *Dis. Aquat. Org.* 51: 107–112.
- CHO J.B., KWON S.R., KIM S.K., NAM Y.K., KIM K.H. 2004: Ultrastructure and development of *Ceratomyxa protopsettae* Fujita, 1923 (Myxosporea) in the gallbladder of cultured olive flounder, *Paralichthys olivaceus*. *Acta Protozool.* 43: 241–250.
- COSTA G., EIRAS J.C., CHUBB J., MACKENZIE K., BERLAND B. 1996: Parasites of the black scabbard fish, *Aphanopus carbo* Lowe, 1839 from Madeira. *Bull. Eur. Assoc. Fish Pathol.* 16: 13–16.
- CURRENT W.L. 1979: *Henneguya adiposa* Minchew (Myxosporidia) in the channel catfish: ultrastructure of the plasmodium wall and sporogenesis. *J. Protozool.* 26: 209–217.
- DESPORTES I., THEODORIDES J. 1982: Données ultrastructurales sur la sporogénèse de deux myxosporidies rapportées aux genres *Leptotheca* et *Ceratomyxa* parasites de *Merluccius merluccius* (L.) (Téléostéen Merlucciidae). *Protistologica* 18: 533–557.
- DIAMANT A., PAPERNA I. 1989: Cytopathology of *Ceratomyxa* sp. (Myxosporea) hyperparasitized with the microsporidian *Nosema ceratomyxae*. *Dis. Aquat. Org.* 6: 75–79.
- DIAMANT A., PAPERNA I. 1992: *Zschokkella icterica* sp. nov. (Myxozoa, Myxosporea), a pathogen of wild rabbitfish *Siganus luridus* (Ruppell, 1829) from the Red Sea. *Eur. J. Protistol.* 28: 71–78.
- EIRAS J.C. 2006: Synopsis of the species of the genus *Ceratomyxa* Thélohan, 1892 (Myxozoa: Myxosporea: Ceratomyxidae). *Syst. Parasitol.* 65: 49–71.
- KABATA Z. 1960: On two myxosporidian parasites of marine fishes, including one new species (*Ceratomyxa tenuispora*). *Ann. Mag. Nat. Hist.* 13: 305–306.
- LOM J. 1969: Notes on the ultrastructure and sporoblast development in fish parasitizing myxosporidian of the genus *Sphaeromyxa*. *Z. Zellforsch.* 97: 416–437.
- LOM J., DYKOVÁ I. 1992: Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science. Vol. 26. Elsevier, Amsterdam, 315 pp.
- LOM J., DYKOVÁ I. 2006: Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol.* 53: 1–36.
- LOM J., FEIST S.W., DYKOVÁ I., KEPR T. 1989: Brain myxoboliasis of bullhead, *Cottus gobio* L., due to *Myxobolus jiroveci* sp. nov.: light and electron microscope observations. *J. Fish Dis.* 12: 15–27.
- MORRISON C.M., MARTELL D.J., LEGGIARDO C., O'NEIL D. 1996: *Ceratomyxa drepanopsettae* in the gallbladder of Atlantic halibut, *Hippoglossus hippoglossus*, from the Northwest Atlantic Ocean. *Folia Parasitol.* 43: 20–36.
- PALENZUELA O., SITJÀ-BOBADILLA A., ALVAREZ-PELLITERO P. 1997: *Ceratomyxa sparusaurati* (Protozoa: Myxosporea) infections in cultured gilthead sea bream *Sparus aurata* (Pisces: Teleostei) from Spain: aspects of the host-parasite relationship. *Parasitol. Res.* 83: 539–548.
- ROCHA E., MATOS E., AZEVEDO C. 1992: *Henneguya amazonica* n. sp. (Myxozoa, Myxobolidae), parasitizing the gills of *Crenicichla lepidota* Heckel, 1840 (Teleostei, Cichlidae) from Amazon river. *Eur. J. Protistol.* 28: 273–278.
- SITJÀ-BOBADILLA A., ALVAREZ-PELLITERO P. 1993a: Light and electron microscopical description of *Ceratomyxa labracis* n. sp. and a redescription of *C. diplodae* (Myxosporea: Bivalvulida) from wild and cultured Mediterranean sea bass *Dicentrarchus labrax* (L.) (Teleostei: Serranidae). *Syst. Parasitol.* 26: 215–223.
- SITJÀ-BOBADILLA A., ALVAREZ-PELLITERO P. 1993b: *Zschokkella mugilis* n. sp. (Myxosporea: Bivalvulida) from mullets (Teleostei: Mugilidae) of Mediterranean waters: light and electron microscopic description. *J. Eukaryot. Microbiol.* 40: 755–764.
- SITJÀ-BOBADILLA A., PALENZUELA O., ALVAREZ-PELLITERO P. 1995: *Ceratomyxa sparusaurati* n. sp. (Myxosporea: Bivalvulida), a new parasite from cultured gilthead seabream (*Sparus aurata* L.) (Teleostei: Sparidae): light and electron microscopic description. *J. Eukaryot. Microbiol.* 42: 529–539.
- YAMAMOTO T., SANDERS J.E. 1979: Light and electron microscopic observations of sporogenesis in the *Ceratomyxa shasta* (Noble, 1950). *J. Fish Dis.* 2: 411–428.

Received 22 May 2006

Accepted 6 June 2007

Capítulo 10

**A NEW SPECIES OF MYXOZOA, *HENNEGUYA RONDONI* N. SP. (MYXOZOA),
FROM THE PERIPHERAL NERVOUS SYSTEM OF THE AMAZONIAN FISH,
GYMNORHAMPHICHTHYS RONDONI (TELEOSTEI)**

The Journal Eukaryotic Microbiology (2008) **55**: 229-234

Carlos Azevedo, Graça Casal, Patrícia Matos & Edilson Matos

A New Species of Myxozoa, *Henneguya rondoni* n. sp. (Myxozoa), from the Peripheral Nervous System of the Amazonian Fish, *Gymnorhamphichthys rondoni* (Teleostei)

CARLOS AZEVEDO,^{a,b} GRAÇA CASAL,^{a,b,c} PATRICIA MATOS^d and EDILSON MATOS^e

^aDepartment of Cell Biology, Institute of Biomedical Sciences, University of Porto (ICBAS/UP),
Lg. A. Salazar no. 2, P-4099-003 Porto, Portugal, and

^bLaboratory of Pathology, Centre for Marine and Environmental Research (CHIMAR/UP), P-4050-123 Porto, Portugal, and

^cDepartment of Sciences, High Institute of Health Sciences, P-4585-116 Gandra, Portugal, and

^dLaboratory of Aquatic Animals, Federal University of Pará, 66000 Belém, Brazil, and

^eCarlos Azevedo Research Laboratory, Federal Rural University of Amazonia, 66000 Belém, Brazil

ABSTRACT. *Henneguya rondoni* n. sp. found in the peripheral lateral nerves located below the two lateral lines of the fish *Gymnorhamphichthys rondoni* (Teleostei, Rhamphichthyidae) from the Amazon river is described using light and electron microscopy. Spherical to ellipsoid cysts measuring up to 110 µm in length contained only immature and mature spores located in close contact with the myelin sheaths of the nervous fibres. Ellipsoidal spores measured 17.7 (16.9–18.1)-µm long, 3.6 (3.0–3.9)-µm wide, and 2.5 (2.2–2.8)-µm ($n = 25$) thick. The spore body measuring 7.0 (6.8–7.3)-µm long was formed by two equal symmetric valves, each with an equal tapering tail 10.7 (10.3–11.0) µm in length. The tails were composed of an internal dense material surrounded by an external homogeneous sheath of hyaline substance. The valves surrounded two equal pyriform polar capsules measuring 2.5 (2.2–2.8)-µm long and 0.85 (0.79–0.88)-µm ($n = 25$) wide and a binucleated sporoplasm cell containing globular sporoplasmosomes 0.38 (0.33–0.42) µm ($n = 25$) in diam. with an internal eccentric dense structure with half-crescent section. Each polar capsule contains an anisofilar polar filament with 6–7 turns obliquely to the long axis. The matrix of the polar capsule was dense and the wall filled with a hyaline substance. The spores differed from those of previously described species. Based on the ultrastructural morphology of the spore and specificity to the host species, we propose a new species name *H. rondoni* n. sp.

Key Words. Amazon river, myxosporean, parasite, spore, ultrastructure.

THE class Myxosporidia Bütschli, 1881 comprises more than 2,180 available species (Lom and Dyková 2006) of which actually about 2,160 were found in fish. Certainly, a large number of species still remain to be discovered (Lom and Dyková 2006), in particular in Brazil, where an extremely high number of fish species live (about 8,000 species) (Cellere, Cordeiro, and Adriano 2002). Among the myxosporidians, the genus *Henneguya* Thélohan, 1892 with 204 species described is one of the largest of the family Myxobolidae (Lom and Dyková 2006). Some of these species have been reported as important pathogens in freshwater fish (Kent et al. 2001; Lom and Dyková 2006).

Very few South American myxosporidian species have been described in detail. Most descriptions are in particular from Brazil, where species are only illustrated by light microscopy (LM) and diagrammatic drawings (Cordeiro et al. 1984; Cunha and Fonseca 1918; Eiras 2002; Eiras, Pavanelli, and Takemoto 2004; Eiras et al. 2004; Gioia and Cordeiro 1996; Gioia, Cordeiro, and Artigas 1986; Kent and Hoffman 1984; Martins and de Souza 1997; Martins et al. 1999; Nemeczek 1926; Walliker 1969). Recently, some species of the genus *Henneguya* were described on the basis of the ultrastructural data (Azevedo, Corral, and Matos 1997; Azevedo and Matos 1995, 1996; Barassa, Cordeiro, and Arana 2003; Casal, Matos, and Azevedo 1997; Matos, Tajdari, and Azevedo 2005; Vita et al. 2003). None has been reported from peripheral nervous fibres of a fish. During a parasitological survey of Amazonian fish, a myxozoan parasite was discovered in the peripheral nervous system of a fish, and it is this isolate that we describe as a new species in this report.

MATERIALS AND METHODS

The teleost *Gymnorhamphichthys rondoni* (Teleostei, Rhamphichthyidae) (Brazilian common name “Ituí transparente”) was collected from the Amazon river near the beach of Irituia, State of Pará, Brazil (01°46'S/47°26'W). After collection, 30 fish (14–19-cm long) were transported live to the laboratory, where their behaviour was studied. For microscopic study they were anaesthetized with MS 222 (Sandoz Laboratories), killed, and necropsied.

Smears of small portions of the fresh peripheral nervous fibres of the lateral lines of the body containing cysts were prepared for observation by LM using Nomarski differential interference contrast (DIC) optics. For transmission electron microscopy (TEM), small fragments of the parasitized tissues were fixed in 5% (v/v) glutaraldehyde buffered in 0.2 M sodium cacodylate (pH 7.2) at 4 °C for 24 h, washed overnight in the same buffer at 4 °C, and post-fixed in 2% (w/v) osmium tetroxide with the same buffer and at the same temperature for 3 h. After dehydration in an ascending ethanol series followed by propylene oxide (three changes of 2 h), the parasitized fragments were embedded in Epon. Semithin sections, were stained with methylene blue-Azure II for LM and ultrathin sections were double contrasted with uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM (Japan) operated at 60 kV.

RESULTS

Some cysts, containing only immature and mature spores, were observed exclusively among the peripheral nervous fibres located in both longitudinal medial lines on the lateral body of specimens with disturbances in their behaviour. No other tissue or organ contained visible parasites. Only infected fish exhibited lethargy and sudden, short disturbances in their movements and no parasites were found in specimens with apparently normal behaviour. Ten of 30 (33.3%) of the fish contained spherical to ellipsoid cysts up to 110-µm long (Fig. 1, 2).

Corresponding Author: C. Azevedo, Department of Cell Biology, Institute of Biomedical Sciences, University of Porto (ICBAS/UP), Lg. A. Salazar no. 2, P-4099-003 Porto, Portugal—Telephone number: +351 22 206 22 00; FAX number: +351 22 206 22 32/33; e-mail: azevedo@icbas.up.pt

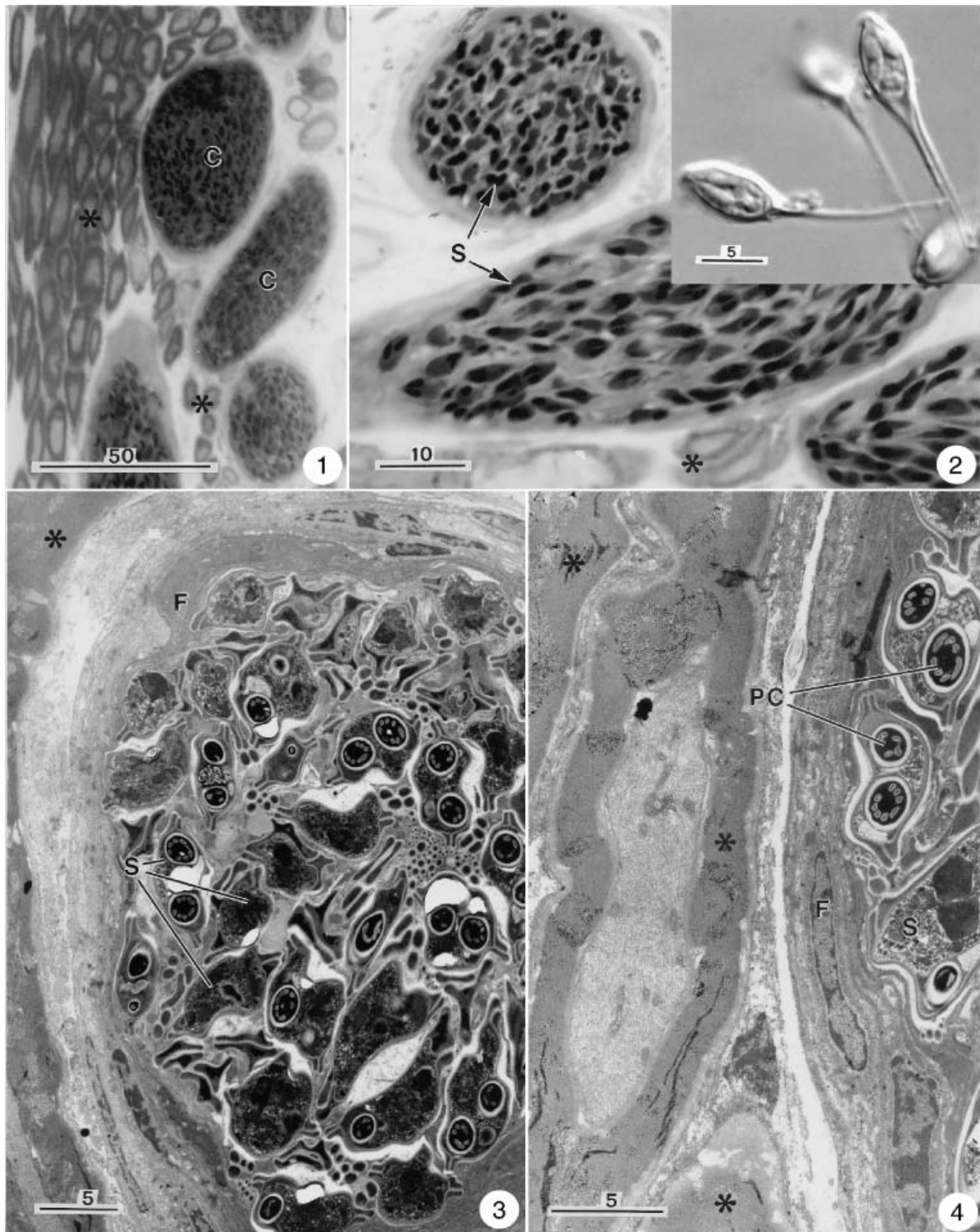


Fig. 1–4. Light and transmission electron micrographs of the myxosporean *Henneguya rondoni* n. sp. from peripheral nervous fibres of the lateral lines of the fish body of *Gymnorhamphichthys rondoni*. (Scale bars in μm). 1. Semithin section showing some cysts (C) filled by numerous spores in contact with numerous bundles of peripheral nervous fibres (*). 2. Semithin section of the cysts (C) containing spores (S). *Inset*. Free spores observed in DIC. 3. Ultrathin section of a cyst in close contact with the nervous fibres (*). The cyst wall shows some fibroblasts (F) surrounding the internal spores (S). 4. Ultrastructural detail of the periphery of a cyst containing some fibroblasts (F) in contact with the myelin sheaths of nervous fibres (*). Two spores show the transverse section at the polar capsule (PC) level.

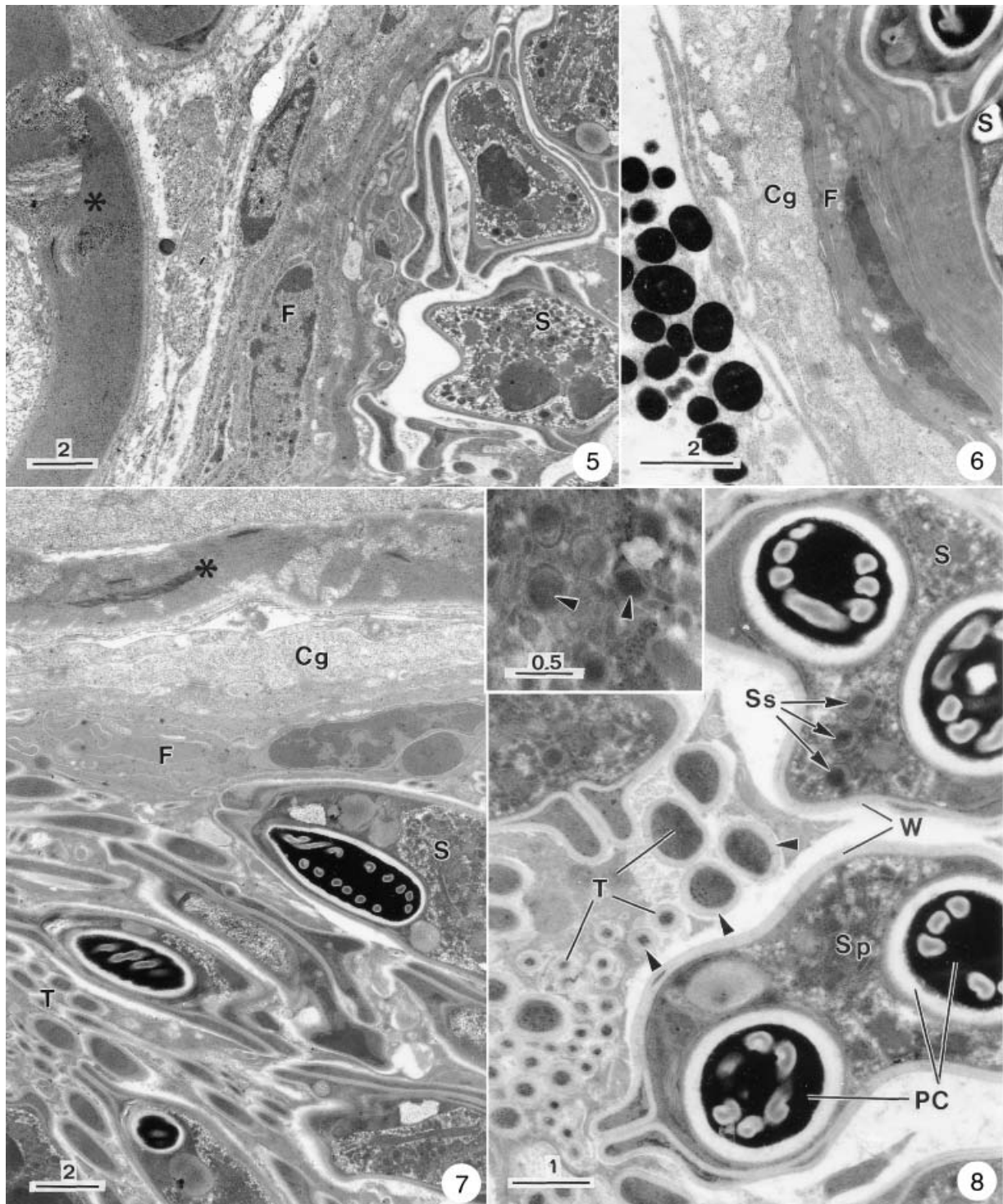


Fig. 5–8. Transmission electron micrographs of the spores of the myxosporean *Henneguya rondoni* n. sp. from peripheral nervous fibres of the lateral lines of the fish body of *Gymnorhamphichthys rondoni*. (Scale bars in μm). 5. Ultrastructural details of the contact zone of the nervous fibres (*) with the periphery of the cyst formed by the fibroblast (F) layer. Internally the cyst contains numerous spores (S). 6. Ultrastructural aspects of a group of dense bodies located near the cyst wall (CW) where it is possible to observe numerous collagen fibres (Cg) and fibroblasts (F). 7. Ultrastructural aspect of the contact zone among the nervous fibres (*) and the cyst, showing numerous collagen fibres (Cg), fibroblasts (F), and spores sectioned at the different levels. Several aspects of the tail (T) and polar capsule are observed. 8. Details of some ultrastructural aspects of the spores showing the spore wall (W), polar capsules (PC), sporoplasm (Sp), sporoplasmosomes (Ss) (details in inset – arrowheads), and transverse sections of the tails (T) sectioned at the different levels. The tails show the internal dense zone surrounded by a sheath of an adherent hyaline substance (arrowheads).

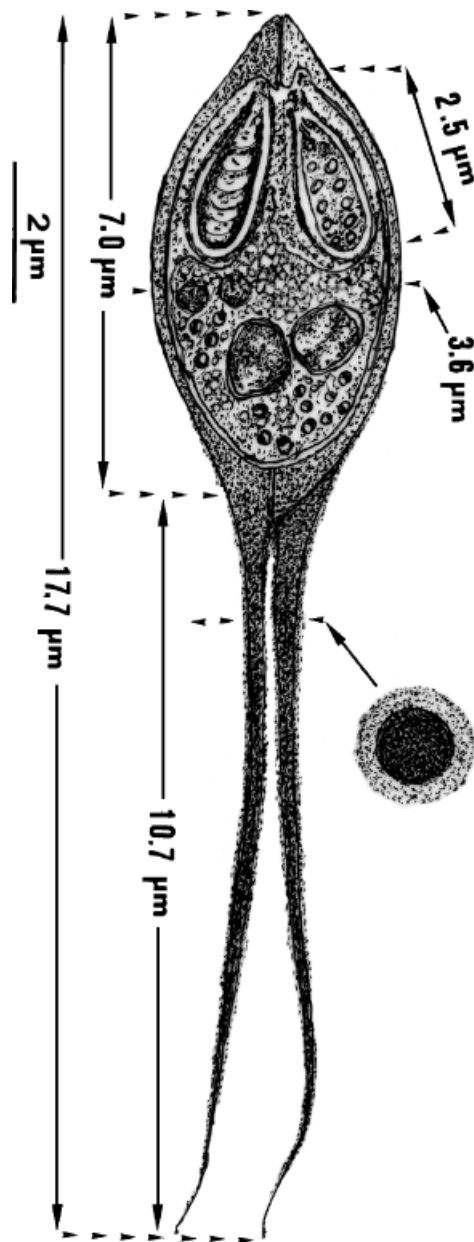


Fig. 9. Schematic drawing of a spore of *Henneguya rondoni* n. sp., parasite of *Gymnorhamphichthys rondoni*, showing species-specific characters, such as the spore shape and size, the two equal polar capsules with six to seven polar filament coils, and the binucleated sporoplasm with sporoplasmosomes.

Henneguya rondoni n. sp. (Fig. 1–9)

Vegetative stages. Spherical to ellipsoidal cysts, measuring up to 110-µm across, were located among the nervous fibres near the two lateral lines of the fish body (Fig. 1–4). The cyst wall was formed by numerous collagen fibril layers and some fibroblasts (Fig. 3). Deposits of dense material were scattered throughout the parasitized tissues near the cysts (Fig. 6).

Description. Spores with the morphological characters of the genus *Henneguya* Thélohan, 1892 were observed. The ellipsoidal spore body formed by two shell valves, each with one tapering tail and enclosing two pyriform polar capsules and a binucleated sporoplasm with sporoplasmosomes inside the cysts. Mature

spores had a total length of 17.7 (16.9–18.1) µm, width 3.6 (3.0–3.9) µm, and thickness 2.5 (2.2–2.8) µm ($n = 25$) and a spore body length of 7.0 (6.8–7.3) µm (Fig. 4–8). The spore wall was thin and smooth comprising two equal valves (Fig. 4, 5, 8), each one with a caudal projection forming the tail with a total length 10.7 (10.3–11.0) µm ($n = 25$) (Fig. 7–9). Each tail was composed of an internal dense material surrounded by a homogeneous hyaline layer 0.15 µm thick (Fig. 7, 8).

The two equal polar capsules were 2.5 (2.2–2.7) µm long and 0.85 (0.79–0.88) µm thick ($n = 25$ polar capsules) wide and the polar capsule wall was 0.23 µm in thickness (Fig. 7, 8). The number of polar filament coils ranged from six to seven (Fig. 7). The binucleated sporoplasm was located in the posterior pole of the spore with several sporoplasmosomes (Fig. 8, inset) and two nuclei located at different levels randomly distributed among a cytoplasm containing several small light areas (Fig. 3, 4). Sporoplasmosomes were globular, 0.38 (0.33–0.40) µm ($n = 15$) in diameter, and contained an eccentric dense structure with a half-crescent section (Fig. 8, inset).

DISCUSSION

The light and ultrastructural morphology of the spores described in the present work correspond to those of the genus *Henneguya* (Family Myxobolidae) (Lom and Dyková 1992, 2006). Spores of this genus are described as having an ellipsoidal spore body (biconvex in sutural view) formed by two shell valves each with one caudal projection, shell valves smooth, and two polar capsules. All characters of this genus were present in this isolate and confirmed morphological similarities to the spores of different species of the genus *Henneguya* described previously (Azevedo and Matos 1995, 1996; Kent et al. 2001; Lom and Dyková 1992, 2006), particularly to *Henneguya* spp. that were reported in teleosts from Brazil and that have spores with tails surrounded by a homogeneous hyaline sheath and containing equal polar capsules (see Table 1).

Among the species in which the spore tails are surrounded by hyaline homogeneous sheaths, *H. rondoni* shows several morphological differences when compared with other *Henneguya* spp. described from South America, in particular in the dimension of the spores, polar capsules, the polar filament coil arrangements, and site of infection (Table 1). Total size, spore body and tail size are all smaller in *H. rondoni* than in *H. theca* (Kent and Hoffmann 1984), *H. adherens* (Azevedo and Matos 1995), *H. malabarica* (Azevedo and Matos 1996), *H. striolata* (Casal et al. 1997), and *H. rhamdia* (Matos et al. 2005) (Table 1). *Henneguya rondoni* also differs from these species in the dimensions of its polar capsules, and the number of polar filament coils (6–7), is markedly smaller than in *H. rhamdia* (10–11) and *H. striolata* (13–14). Furthermore, information on the site of infection (e. g. tissue tropism and cyst location) assists in the identification of myxosporean parasites (Eszterbauer 2004). While in most *Henneguya* spp. the site of infection is the gills (Lom and Dyková 2006; Molnár 2002), in the proposed new species the cysts appeared in close contact with myelin sheaths of the nervous fibres of the lateral lines of the fish body. To our knowledge, infections of *Henneguya* spp. in close contact with the peripheral nervous fibres of fish were never been reported previously.

In a recent detailed review of myxozoan genera it was reported that only *Myxobolus* spp., *Kudoa* spp., and *Henneguya* spp. were found parasitizing the nervous system, mainly the brain of the fishes (Lom and Dyková 2006). Effectively, until recently only a few works report the presence of these genera (Cho and Kim 2003; Kent and Hoffman 1984; Longshaw, Frear, and Feist 2003; Yokoyama et al. 2004).

Table 1. Comparative measurements (in μm) of the spore from *Henneguya* spp. with tails surrounded by homogenous hyaline sheaths and with equal polar capsules.

<i>Henneguya</i> spp.	Hosts/tissues	TL	SBL	SBW	SBT	TaL	PCL	PCW	FC	References
<i>H. theca</i>	<i>Eigemannia virescens</i> Brain	48.0 40.6–52.6	24.8	3.5 3.0–4.1	—	23.2 20.3–24.2	11.1 9.8–12.5	1.4 1.0–1.6	—	Kent and Hoffmann (1984)
<i>H. adherens</i>	<i>Acestrorhynchus falcatus</i> Gill filaments	32.3 30.7–35.1	12.4 10.5–13.8	5.8 5.1–6.5	—	20.5 18.5–21.7	3.1 2.8–3.5	1.2 1.0–1.6	3–4	Azevedo and Matos (1995)
<i>H. malabarica</i>	<i>Hoplias malabaricus</i> Gill filaments	28.3 26.6–29.8	12.6 11.8–13.1	4.8	—	17.1 16.2–18.9	3.7 3.0–4.3	1.8 1.6–2.2	6–7	Azevedo and Matos (1996)
<i>H. striolata</i>	<i>Serrasalmus striolatus</i> Gill filaments	42.2 39.3–45.6	15.8 14.4–17.0	5.3 4.9–5.9	—	25.9 23.6–29.8	6.8 5.1–7.0	1.2 1.1–1.3	13–14	Casal, Matos and Azevedo (1997)
<i>H. rhamdia</i>	<i>Rhamdia quelen</i> Gill filaments	50.0 $\pm$ 1.8	13.1 $\pm$ 1.1	5.2 $\pm$ 0.5	—	36.9 $\pm$ 1.6	4.7 $\pm$ 0.4	1.1 $\pm$ 0.2	10–11	Matos, Tajdari and Azevedo (2005)
<i>H. rondoni</i> n. sp.	<i>Gymnorhamphichthys rondoni</i> Nervous fibres	17.7 16.9–18.1	7.0 6.8–7.3	3.6 3.0–3.9	2.5 2.2–2.8	10.7 10.3–11.0	2.5 2.2–2.7	0.85 0.79–0.88	6–7	Present study

TL, total length of the spore; SBL, spore body length; SBW, spore body width; SBT, spore body thickness; TaL, tail length; PCL, polar capsule length; PCW, polar capsule width; FC, number of the polar filament coils.

Even though Molnár (2002) referred to the gills as the preferential site of fish myxosporean species, in some species these parasite occur forming xenoma and cysts, containing in their walls several fibroblasts and collagen fibrils (Matos et al. 2005). The same structures were observed in the present work, suggesting that may represent a host reaction to the presence of the parasite.

Curiously, the only *Henneguya* spp. (*H. theca*) previously reported as parasitizing the nervous system of a fish was obtained from a fish from the Brazilian fauna. It was described parasitizing the nervous system (brain) of the green knife fish *Eigemannia virescens* (V.), imported from Brazil to Scripps Institution of Oceanography (San Diego) (Kent and Hoffman 1984). No other *Henneguya* spp. was described parasitizing the nervous system, so *H. rondoni* seem to be only the second species of this genus to be described parasitizing the fish nervous system.

In the present study it was not possible to determine either the origin of the plasmodia or the mechanism of invasion of the parasite. It is becoming apparent that some myxosporeans have an alternate phase of development in oligochaete hosts (Kent, Whitaker, and Margolis 1993), producing actinospores that serve to infect fish (Lom and Dyková 2006). This process of infection may occur in this parasite, but we do not have any results to confirm this hypothesis.

The presence of a high number of plasmodia in contact with the myelin sheaths of the lateral nervous fibres (which are responsible for the caudal fin movements) and the consequent alteration of the behaviour of the infected fish seem to suggest that the described parasites are pathogenic for their hosts.

Thus, our results provide a description of the second species of this genus to parasitize the fish nervous system, which we name as *H. rondoni* n. sp. and classify according to Lom and Dyková (2006):

Phylum Myxozoa Grassé, 1970
Class Myxosporea Bütschli, 1881
Order Bivalvulida Shulman, 1959
Family Myxobolidae Thélohan, 1892
Genus *Henneguya* Thélohan, 1892
Henneguya rondoni n. sp.

Diagnosis. Spherical to ellipsoidal cysts, measuring up to 110- μm across located among the nervous fibres of the lateral line of the fish body. Ellipsoidal spore 17.7 (16.9–18.1) μm long, 3.6 (3.0–3.9) μm wide, 2.5 (2.2–2.8) μm thick and tail 10.7 (10.3–11.0) μm long ($n = 25$). Two equal-sized pyriform polar capsules

measuring 2.5 (2.2–2.7) $\times$ 0.85 (0.79–0.88) μm . Polar filament coiled 6 to 7 times.

Type host. Cysts containing spores were observed only in the two lateral lines of the fish body in close contact with the myelin sheaths of nervous fibres of the fish *Gymnorhamphichthys rondoni* (Teleostei, Rhamphichthyidae).

Type locality. Amazon river near the Irituia Beach, State of Pará, Brazil (01°46'S/47°26'W).

Prevalence. 33.3% (10/30).

Hapantotype specimens. Resin-embedded block of tissue from infected fish and toluidine blue-stained semithin sections of the cyst containing spores, deposited in the International Protozoan Type Slide Collection at the Smithsonian Institution, Washington, DC 20560, USA (USNM No. 1110541), and isolated spores and cysts containing spores fixed in 80% ethanol deposited at the same Institution (USNM No. 1110542). Together these materials constitute the hapantotype of the species.

Etymology. The specific epithet derives from the generic name of the type host.

ACKNOWLEDGMENTS

This work was partially supported by Eng. A. Almeida Foundation (Porto, Portugal), CESPU (Gandra), CNPq, CAPES (Brazil), and Federal Rural University of Amazonia (Belém, Brazil). The helpful comments and suggestions of the Associate Editor and two anonymous reviewers in reviewing this manuscript are greatly appreciated.

LITERATURE CITED

- Azevedo, C. & Matos, E. 1995. *Henneguya adherens* n. sp. (Myxozoa, Myxosporea), parasite of the Amazonian fish, *Acestrorhynchus falcatus*. *J. Eukaryot. Microbiol.*, **42**:515–518.
- Azevedo, C. & Matos, E. 1996. *Henneguya malabarica* sp. nov. (Myxozoa, Myxobolidae) in the Amazonian fish *Hoplias malabaricus*. *Parasitol. Res.*, **82**:222–224.
- Azevedo, C., Corral, L. & Matos, E. 1997. Light and ultrastructural data on *Henneguya testicularis* n. sp. (Myxozoa, Myxobolidae), a parasite from the testis of the Amazonian fish *Moenkhausia oligolepis*. *Syst. Parasitol.*, **37**:111–114.
- Barassa, B., Cordeiro, N. S. & Arana, S. 2003. A new species of *Henneguya*, a gill parasite of *Astyanax altiparanae* (Pisces: Characidae) from

- Brazil, with comments on histopathology and seasonality. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro*, **98**:761–765.
- Casal, G., Matos, E. & Azevedo, C. 1997. Some ultrastructural aspects of *Henneguya striolata* sp. nov. (Myxozoa, Myxosporea), a parasite of the Amazonian fish *Serrasalmus striolatus*. *Parasitol. Res.*, **83**: 93–95.
- Cellere, E. F., Cordeiro, N. S. & Adriano, E. A. 2002. *Myxobolus absonus* sp. n. (Myxozoa: Myxosporea) parasitizing *Pimelodus maculatus* (Siluriformes: Pimelodidae), a South American freshwater fish. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro*, **97**:79–80.
- Cho, J. B. & Kim, K. H. 2003. Light- and electron-microscope description of *Kudoa paralichthys* n. sp. (Myxozoa, Myxosporea) from the brain of cultured olive flounder *Paralichthys olivaceus* in Korea. *Dis. Aquat. Org.*, **55**:59–63.
- Cordeiro, N. S., Artigas, P. T., Gióia, I. & Lima, R. S. 1984. *Henneguya pisciforme* n. sp., mixosporídeo parasito de brânquias do Lambari *Hyphessobrycon anisitsi* (Pisces, Characidae). *Mem. Inst. Butantan*, **48**:61–69.
- Cunha, A. M. & Fonseca, O. 1918. Sobre os myxosporídeos dos peixes brasileiros. *Bras. Med.*, **32**:414.
- Eiras, J. C. 2002. Synopsis of the species of the genus *Henneguya* Thélohan, 1892 (Myxozoa: Myxosporea: Myxobolidae). *Syst. Parasitol.*, **52**:43–54.
- Eiras, J. C., Pavanelli, G. C. & Takemoto, R. M. 2004. *Henneguya paranaensis* sp. n. (Myxozoa, Myxobolidae), a parasite of the teleost fish *Prochilodus lineatus* (Characiforme, Prochilodontidae) from the Paraná river, Brazil. *Bull. Eur. Ass. Fish Pathol.*, **24**:308–311.
- Eiras, J. C., Malta, J. C., Varela, A. & Pavanelli, G. C. 2004. *Henneguya schizodon* n. sp. (Myxozoa, Myxobolidae), a parasite of the Amazonian teleost fish *Schizodon fasciatus* (Characiformes, Anostomidae). *Parasite*, **11**:169–173.
- Eszterbauer, E. 2004. Genetic relationship among gill-infecting *Myxobolus* species (Myxosporea) of cyprinids: molecular evidence of importance of tissue-specificity. *Dis. Aquat. Org.*, **58**:35–40.
- Gioia, I. & Cordeiro, N. S. 1996. Brazilian myxosporidians' check-list (Myxozoa). *Acta Protozool.*, **35**:137–149.
- Gioia, I., Cordeiro, N. S. & Artigas, P. T. 1986. *Henneguya intracornea* n. sp. (Myxozoa: Myxosporea) parasita do olho do lambari, *Astyanax scabripinnis* (Jenyns, 1842) (Osteichthyes, Characidae). *Mem. Inst. Oswaldo Cruz, Rio de Janeiro*, **81**:401–407.
- Kent, M. L. & Hoffman, G. L. 1984. Two new species of Myxozoa, *Myxobolus inaequus* sp. n. and *Henneguya theca* sp. n. from the brain of a South American knife fish, *Eigmannia virescens* (V.). *J. Protozool.*, **31**:91–94.
- Kent, M. L., Whitaker, D. J. & Margolis, L. 1993. Transmission of *Myxobolus arcticus* Pugachev and Khokhlov, 1979, via a triactinomyxon from the aquatic oligochaete *Stylodrilus heringianus* (Lumbriculidae). *Can. J. Zool.*, **71**:1207–1211.
- Kent, M. L., Andree, K. B., Bartholomew, J. L., El-Matbouli, M., Desser, S. S., Devlin, R. H., Feist, S. W., Hedrick, R. P., Hoffmann, R. W., Khattra, J., Hallet, S. L., Lester, R. J. G., Longshaw, M., Palenzuela, O., Siddall, M. E. & Xiao, C. 2001. Recent advances in our knowledge of the Myxozoa. *J. Eukaryot. Microbiol.*, **48**:395–413.
- Lom, J. & Dyková, I. 1992. Myxosporidia (Phylum Myxozoa). Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science. Vol. 26. Elsevier, Amsterdam. p. 159–235.
- Lom, J. & Dyková, I. 2006. Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol.*, **53**:1–36.
- Longshaw, M., Frear, P. A. & Feist, S. W. 2003. *Myxobolus buckei* sp. n. (Myxozoa), a new pathogenic parasite from the spinal column of three cyprinid fishes from the United Kingdom. *Folia Parasitol.*, **50**:251–262.
- Martins, M. L. & de Souza, V. N. 1997. *Henneguya piaractus* n. sp. (Myxozoa: Myxobolidae), a gill parasite of *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae), in Brazil. *Rev. Brasil. Biol.*, **57**:239–245.
- Martins, M. L., de Souza, V. N., de Moraes, J. R. E. & de Moraes, F. R. 1999. Gill infection of *Leporinus macrocephalus* Garavell & Britski, 1988 (Osteichthyes: Anostomidae) by *Henneguya leporinicola* n. sp. (Myxozoa: Myxobolidae). Description, histopathology and treatment. *Rev. Brasil. Biol.*, **59**:527–534.
- Matos, E., Tajdari, J. & Azevedo, C. 2005. Ultrastructural studies of *Henneguya rhamdia* n. sp. (Myxozoa) a parasite from the Amazon teleost fish, *Rhamdia quelen* (Pimelodidae). *J. Eukaryot. Microbiol.*, **52**:532–537.
- Molnár, K. 2002. Site preference of fish myxosporeans in the gill. *Dis. Aquat. Org.*, **48**:197–207.
- Nemeczek, A. 1926. Beiträge zur Kenntnis der Myxosporidienfauna Brasiliens. *Arch. Protistenkd.*, **54**:137–150.
- Vita, P., Corral, L., Matos, E. & Azevedo, C. 2003. Ultrastructural aspects of the myxosporean *Henneguya astyanax* n. sp. (Myxozoa: Myxobolidae) parasite of the Amazonian teleost *Astyanax keithi* (Characidae). *Dis. Aquat. Org.*, **53**:55–60.
- Walliker, D. 1969. Myxosporidea of some Brazilian freshwater fishes. *J. Parasitol.*, **55**:942–948.
- Yokoyama, H., Freeman, M. A., Yoshinaga, T. & Ogawa, K. 2004. *Myxobolus buri*, the myxosporean parasite causing scoliosis of yellowtail, is synonymous with *Myxobolus acanthogobii* infecting the brain of the yellowfin goby. *Fish. Sci.*, **70**:1036–1042.

Received: 10/17/07, 12/18/07, 01/30/08; accepted: 02/05/08

Capítulo 11

**ULTRASTRUCTURAL DESCRIPTION OF A NEW MYXOSPOREAN PARASITE
KUDOA AEQUIDENS SP. N. (MYXOZOA, MYXOSPOREA), FOUND IN THE
SUB-OPERCULAR MUSCULATURE OF *AEQUIDENS PLAGIOZONATUS*
(TELEOSTEI) FROM THE AMAZON RIVER**

Acta Protozoologica (2008) **47**: 135-141

Graça Casal, Edilson Matos, Patricia Matos & Carlos Azevedo

Ultrastructural Description of a New Myxosporean Parasite *Kudoa aequidens* sp. n. (Myxozoa, Myxosporea), found in the Sub-Opercular Musculature of *Aequidens plagiozonatus* (Teleostei) from the Amazon River

Graça CASAL^{1, 2, 3}, Edilson MATOS⁴, Patricia MATOS⁵ and Carlos AZEVEDO^{1, 2, *}

¹Department of Cell Biology, Institute of Biomedical Sciences, University of Porto, Porto, Portugal; ²Laboratory of Pathology, Centre for Marine and Environmental Research (CIIMAR/UP), Porto, Portugal; ³Department of Sciences, High Institute of Health Sciences, Gandra, Portugal; ⁴Carlos Azevedo Research Laboratory (LPCA), Federal Rural University of Amazonia, Belém (Pará), Brazil; ⁵Laboratory of Aquatic Animals, Federal University of Pará, Belém (Pará), Brazil

Summary. *Kudoa aequidens* sp. n. (Phylum Myxozoa) was ultrastructurally described in the sub-opercular musculature of the fish *Aequidens plagiozonatus* (Fam. Cichlidae) from the Amazonian estuarine region of Pará State, Brazil. Out of 28 fishes examined, 10 were found to be parasitized. Some light aspects of soft flesh phenomenon were observed. Spore with a quadrate or pseudoquadrate shape in apical view with four equal valves were observed in the pseudocysts. Each valve had 4 lateral opposite cytoplasmic projections up to 2 µm long. Spore length and width ranged between 3.2 (2.9–3.5) µm and 6.8 (6.2–7.1) µm (n=25), respectively, while the equal polar capsules averaged 2.2 (2.0–2.6) x 1.2 (1.1–1.5) µm (n=20). The polar capsules were located side by side in quadrate position with the apex converging to the apical pole of the spore. Each of the polar capsules was pyriform in shape and contained a coiled filament with 3–4 coils with irregular transverse sections. There was no evidence of any immunological reaction in the parasitized muscle or encapsulated cysts. Spores seemed free among disintegrated myofibrils, showing some aspects of liquefaction of the muscle. Morphological and ultrastructural comparisons with other *Kudoa* spp. enabled us to determine this parasite to be a new species that we name *Kudoa aequidens*. These ultrastructural data are the first record obtained of a *Kudoa* sp. from Brazil.

Key words: Amazonian fish, *Kudoa aequidens* n. sp., Myxozoa, parasite, Ultrastructure.

INTRODUCTION

Myxosporeans of the genus *Kudoa* Meglitsch, 1947 (Multivalvulida), which parasitize estuarine and marine fish (Lom and Dyková 1992, 2006) are most commonly found in the somatic musculature (Moran *et al.* 1999;

Lom *et al.* 1983, 1992; Whitaker *et al.* 1996; Kalavati *et al.* 2000; Whipps *et al.* 2003a, b; Adlard *et al.* 2005; Yokoyama and Itoh 2005), intestinal musculature (Maeno *et al.* 1993), brain (Cho and Kim 2003; Wang *et al.* 2005), gill (Kpatcha *et al.* 1999; Cho and Kim 2003), cardiac muscle (Blaylock *et al.* 2004) and other organs (Dyková *et al.* 2002; Lom and Dyková, 2006). Post-mortem myoliquefaction of the muscles associated with *Kudoa* sp. causing the soft or milky flesh was found in different fish species (Langdon 1991; Langdon *et al.* 1992; Stehr 1986; Moran *et al.* 1999; Yokoyama *et al.* 2004; Yokoyama and Itoh 2005).

*Address for correspondence: Carlos Azevedo, Department of Cell Biology, Institute of Biomedical Sciences, University of Porto, Lg. Abel Salazar no 2, P-4099 – 003 Porto, Portugal; Phone: +351.22.206.22.00; Fax: + 351.22.206.22.32/33; E-mail: azevedo@icbas.up.pt; gcasal@icbas.up.pt

The genus *Kudoa* contained about 45 identified species (Moran *et al.* 1999; Swearer and Robertson 1999) and successively this number was increased by the erection of some other new species (Pampoulie *et al.* 1999; Swearer and Robertson 1999; Dyková *et al.* 2002; Yokoyama *et al.* 2004; Whipps *et al.* 2003a, b; Blaylock *et al.* 2004; Wang *et al.* 2005; Yokoyama and Itoh 2005). In a recent revision paper 63 species is the appointed number (Lom and Dyková 2006). These species are distributed in almost all geographic areas (Lom and Dyková 1992, 2006; Moran *et al.* 1999; Swearer and Robertson 1999). Although there is considerable information on the species of the genus *Kudoa* (Lom and Dyková 1992, 2006; Lom *et al.* 1992; Sarkar and Chaudhury 1996; Moran *et al.* 1999; Swearer and Robertson 1999), nothing is known about those from aquatic fauna of Brazil, and particularly those from the Amazon River (Gioia and Cordeiro 1996; Moran *et al.* 1999; Békési *et al.* 2002; Lom and Dyková 2006), where a diverse assemblage of several hundred species of fish lives. The present ultrastructural study is the first report of a *Kudoa* sp. from the Brazilian aquatic fauna. Light and transmission electron microscopic observations suggested that this species of *Kudoa* differs from previously described species.

MATERIALS AND METHODS

Twenty eight specimens of the freshwater fish *Aequidens plaggiozonatus* Kullander, 1984 (Teleostei, Cichlidae) (Brazilian common name "Cará pixuna") were collected from the Amazonian estuarine region of the Peixe Boi River (01°11'S/47°18'W) near the city of Peixe Boi, State of the Pará, Brazil. The fishes ranged from 15 to 22 cm in total length, were lightly anaesthetized with MS 222 (Sandoz Laboratories) diluted in freshwater and samples of infected muscle from sub-opercular region, were taken for light and electron microscopic studies.

For light microscopy (LM) studies, free mature spores were fixed in 3% buffered glutaraldehyde and observed by a light microscope

equipped with Nomarsky interference-contrast (DIC) optics. For transmission electron microscopic (TEM) studies small fragments of infected muscle containing pseudocysts were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4°C for 10 h. After washing overnight with the same buffer at 4°C and post-fixation in 2% OsO₄ buffered with the same buffer for 2 h at same temperature, the fragments were dehydrated through an ascending ethanol series, followed by propylene oxide and embedded in Epon. Semi-thin sections were stained with methylene blue-Azur II and observed by light microscopy and the ultrathin sections, cut with a diamond knife, contrasted with both aqueous uranyl acetate and lead citrate and were observed in a JEOL 100CXII TEM operated at 60 kV.

RESULTS

The parasitized tissue had some small spherical to ellipsoidal pseudocysts up to 125 µm containing mature spores (Fig. 1). No other life cycle stages were observed. Some fibroblasts were observed surrounding the pseudocysts. Plasmodia were observed only within the parasitized muscle tissue from sub-opercular region of the fish. The parasite was easily identified as *Kudoa* sp. when observed in semithin sections (Fig. 1) and in free spores observed by DIC optics (Fig. 1, inset).

Histological observations revealed that the parasites developed intracellularly among the muscle fibres (Fig. 2). No inflammatory response was observed to be directly related to fibres. Spores seemed liberated among disintegrated myofibrils, suggesting that liquefaction of the muscles was associated with the presence of the spores. The parasitized specimens seemed to have slower opercular movements, as they showed an evident disintegration of the myofibrils and a larger quantity of spores in the sub-opercular musculature (Fig. 2).

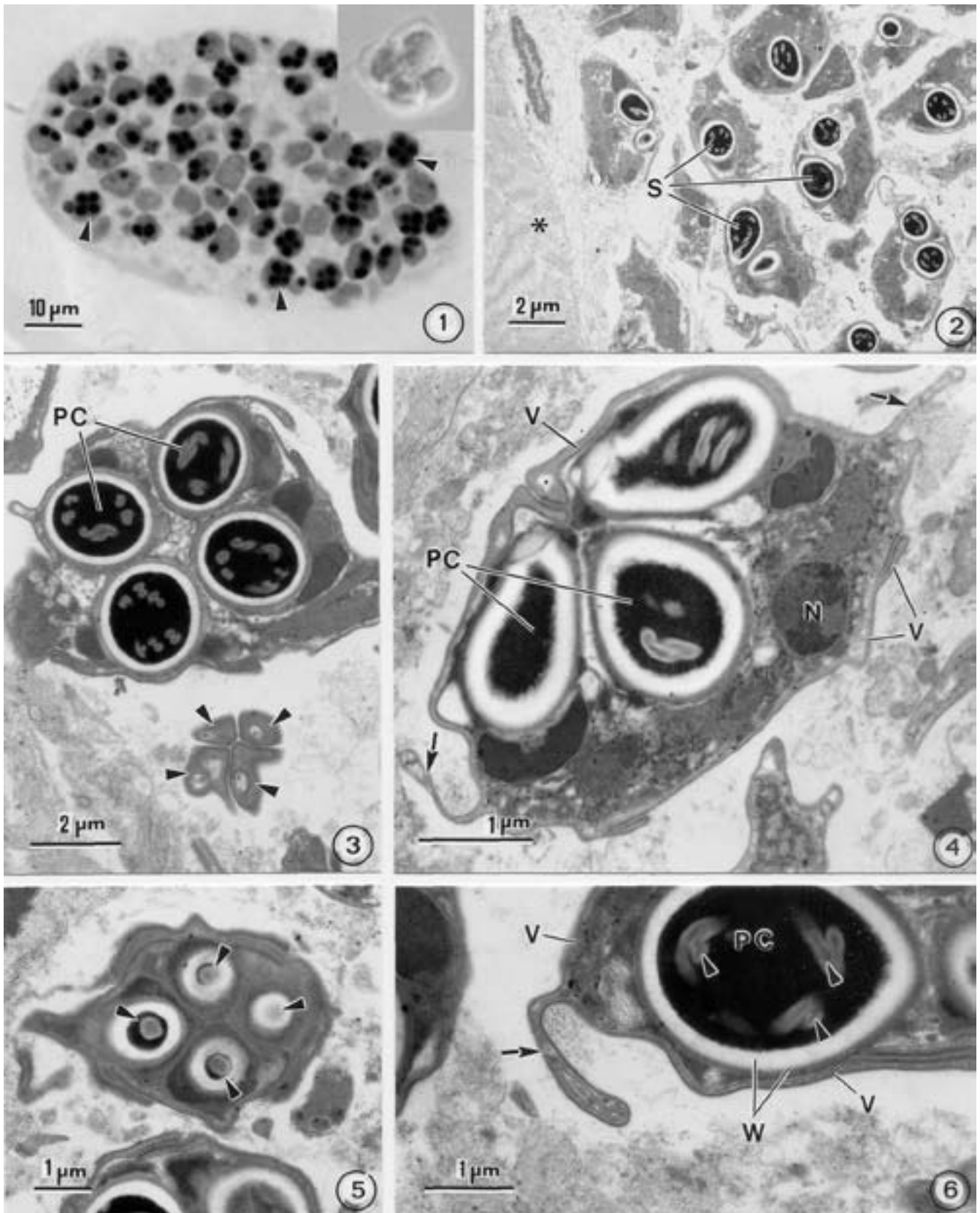
Kudoa aequidens sp. n. (Figs 1–11)

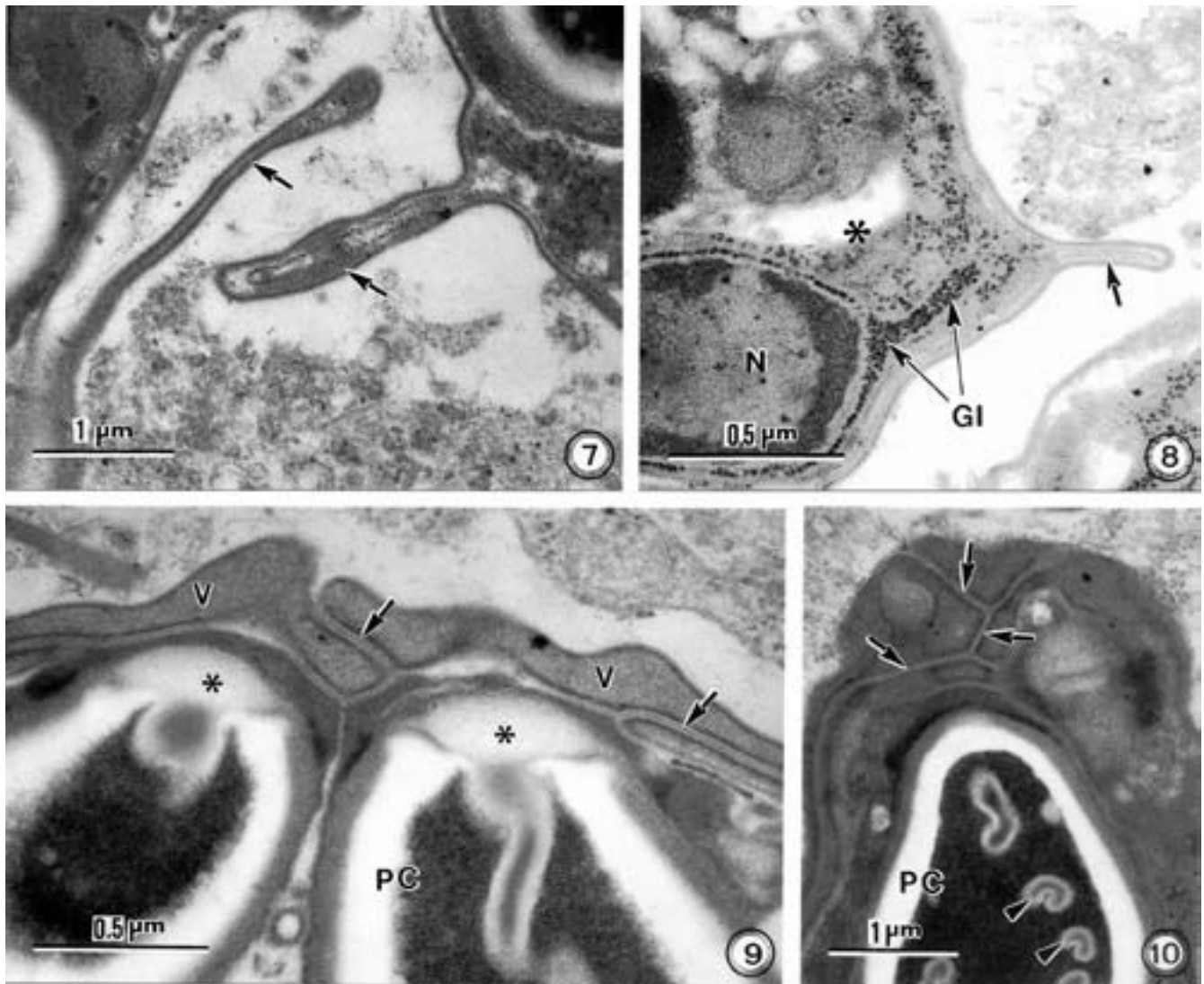
Type host: *Aequidens plaggiozonatus* Kullander, 1984 (Teleostei, Perciformes, Cichlidae)

Host size: 15 to 22 cm of the total length in average.

»

Figs 1–6. *Kudoa aequidens* sp. n. light and electron micrographs. **1** – semithin section of a pseudocyst containing several spores sectioned at different levels, some of which showing the four polar capsules (arrowheads). Inset: Wet mount preparation showing a spore observed by DIC optics; **2** – ultrathin section observed at low magnification showing several spores (S) sectioned at different levels located near the muscle tissue (*); **3** – transverse section of the spore showing the four equal polar capsules (PC) located at the same level, and a transverse section of the apical region of the spore showing the polar filament sections (arrowheads); **4** – longitudinal (lightly oblique) section through a spore showing the polar capsules (PC) and one of the two nuclei (N) of the sporoplasmic cell. Laterally two cytoplasmic projections (arrows) of the valves (V) are present; **5** – transversal ultrathin section of a spore sectioned at the tips of the polar capsules (arrowheads); **6** – detail of a cytoplasmic projection (arrow) of the valve (V) and the polar capsule section (PC) showing the polar capsules wall (W) and polar filament sections (arrowheads).





Figs 7–10. *Kudoa aequidens* sp. n. electron micrographs. **7** – two different aspects of the cytoplasmic projections (arrows) showing the internal organization; **8** – detail of the sporoplasmic cell (*) showing one of the two nuclei (N) and the surrounding glycogen particles (Gl). A cytoplasmic projection of the valve (arrow) is shown; **9** – the apical zone of the shell valves cut obliquely showing the suture-like connections between adjacent edges of the valves (V) (arrows) and the apical regions of the polar capsules (PC) with the apical plug-like structures (*) in continuity of the polar filaments; **10** – the four apical zones of the shell valves cut obliquely showing the suture-like connections (arrows) between the adjacent edges of the valves and a longitudinal section of a polar capsule (PC) with their polar filament sections (arrowheads).

Type locality: Amazonian estuarine region of the Peixe Boi river (01°11'S/47°18'W), near the city of Peixe Boi (State of the Pará), Brazil.

Site of infection: Spherical to ellipsoidal pseudocysts (up to 125 µm long) with numerous spores were found intermingled with the sub-opercular skeletal musculature. Some fibroblasts were observed surrounding the pseudocysts.

Prevalence and intensity: 10 out of 28 fishes (35.7%) were parasitized with no observed difference in prevalence between sexes.

Type specimens: One slide of semithin sections containing mature spores of the syntypes was deposited in the International Protozoan Type Slide Collection at Smithsonian Institute Washington, DC. 20560 (USNM no 1112643)

Etymology: The specific name is derived from the name of the host species.

Description of the spores: For description of the spores, light microscopy (DIC) (Figs 1 and inset), TEM (Figs 3–10) and a schematic drawing (Fig. 11) were used. Pseudocysts were observed within the sub-opercular musculature and did not elicit an inflammatory response. The parasite was identified as the genus *Kudoa* Meglisch, 1947 by spore shape, e. g. quadrate or pseudoquadrate in polar view with four equal pyriform polar capsules located side by side (Figs 1 and inset, 3) with the apex converging at the apical pole of the spore (Figs 4, 5).

The spore contains rounded edges with a total length of 3.2 (2.9–3.5) μm and width of 6.8 (6.2–7.1) μm ($n=25$). Each valve possessed an opposite cytoplasmic projection with total length up to 2 μm (rarely more) (Figs 6–8). These structures contained cytoplasmic structures in continuity with the valves (Figs 4, 6–8). The 4 polar capsules (PC) of equal size, averaging 2.2 (2.0–2.6) $\times$ 1.2 (1.1–1.5) μm ($n=20$), were ellipsoidal and located at same level, as they converge at the apical pole (Figs 3–5, 9, 10). The wall of the PC has a thin dense outer layer (100–120 nm) and an internal lucent layer (160–190 nm) (Figs 4, 6, 9, 10). Each PC contained a coiled filament with 3–4 coils with irregular transverse section (Figs 3, 4, 10) within an electron-dense matrix (Figs 4, 6, 10). The tips of the PC form small prominences at apical meeting region (Figs 3, 5, 8). The sporoplasm contained two prominent nuclei with evident dense chromatin surrounded by numerous glycogen particles, randomly distributed throughout the cytoplasm (Figs 4, 8).

DISCUSSION

Our results demonstrate that morphological aspect observed in DIC and the ultrastructural morphology of the spores correspond to the phylum Myxozoa and among them they are similar to those defined in different species of the genus *Kudoa* Meglitsch, 1947 (Lom and Dyková 1992, 2006; Kpatcha *et al.* 1999; Swearer and Robertson 1999; Dyková *et al.* 2002).

Based on ultrastructural morphology of the spore (shape, dimensions and internal organization), host species, site of infection and geographic localization, the myxosporean identified here is a new species of the genus *Kudoa* that is named *Kudoa aequidens*.

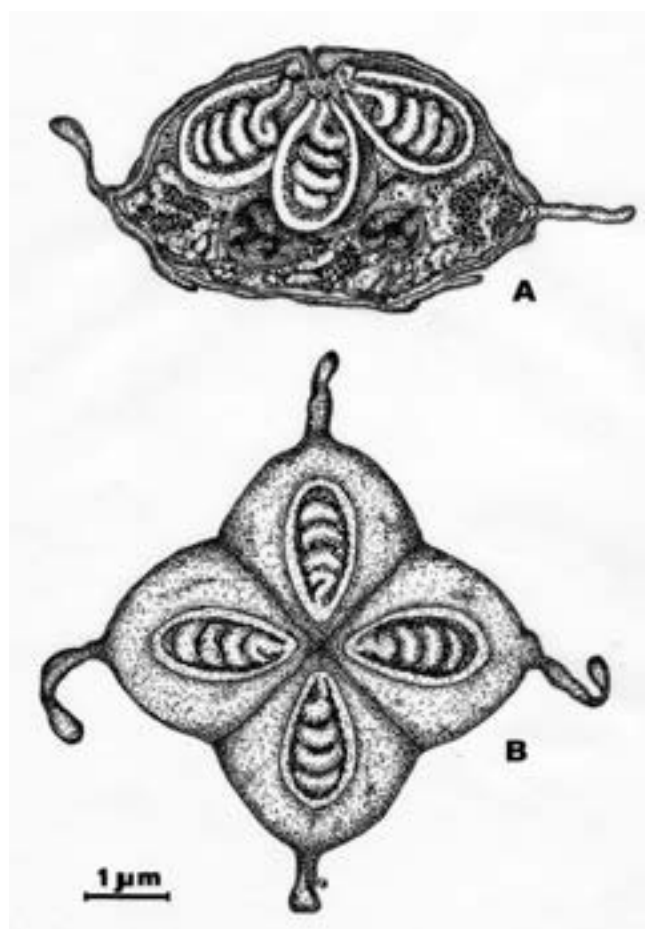


Fig. 11. Semischematic drawing of the spore of *Kudoa aequidens* sp. n. observed in lateral view (A) and in frontal view (B).

Comparing the spores described in the present study with another one with comparable morphology, a similarity with *K. lunata* (Lom *et al.* 1983) was observed, the only described parasite that has 4 lateral cytoplasmic projections from the shell valves (Lom and Dyková, 1988), similar to that in the spore of the present species (Table 1).

On the other hand, while in *K. lunata* these structures had an oblique insertion on the latero-apical region of each valves, in *K. aequidens* they are laterally projected from the valve. The *K. lunata* valves had 4 beak-like projections in the apical zone of the contact of four shell valves, never observed in *K. aequidens* in which the apical region of the shell valves does not contain any valvar projection. The microtubular reinforcement of their apical projections by microtubular bundles in *K. lunata* (Lom and Dyková, 1988) is a well-developed

Table 1. Comparison of shape and measurements of spore from closely related species of *Kudoa* sp.

	<i>Kudoa lunata</i>	Present study
Spore: Length	5.3 (4.5–6.2)	3.2 (2.9–3.5)
Width	10 (9.0–11.4)	6.8 (6.2–7.1)
Spore shape	Stellate	Quadrangle or pseudoquadrangle
Apical projections	4 sharps with microtubules	Without
Lateral projections of shell valves	4 Lateral opposite projections (~ 2 µm long)	4 lateral opposite projections (up to 2 µm long)
Polar capsule	1.5 x 2.5n	1.25 x 2.15
outer wall layer	60–80 nm	100–120 nm
inner wall layer	100–130 nm	160–190 nm
Coils	3	3–4
Authors	Lom and Dyková, 1988	Casal <i>et al.</i> , 2007

structure not observed in the shell valves of the species presently studied. The canals for polar filament discharge extend through the apical spore projections in *K. lunata*, which are of a length never observed in other *Kudoa* sp., additionally, the polar capsule wall in *K. aequidens* is thinner than that of *K. lunata*.

Furthermore, the organization of the suture lines of these two species is different. In *K. aequidens* the suture lines are almost parallel to the spore surface, most occupying a long extension, while in *K. lunata* they are oblique in relation the surface of the spore valves (Lom and Dyková, 1988).

The spore shapes (stellate in *K. lunata* and quadrangle or pseudoquadrangle in the *K. aequidens*) are additional evidence that reinforces our conclusion that two species are different. The presence of this parasite has never been reported previously from this country (Békési *et al.* 2002; Lom and Dyková, 2006).

Acknowledgments. This work was partially supported by the Engo. António Almeida Foundation (Porto, Portugal), a CESPU PhD grant (Portugal), CNPq and CAPES (Brazil).

REFERENCES

Adlard R. D., Bryant M. S., Whipps C. M., Kent M. L. (2005) Multivalvulid myxozoans from eastern Australia: Three new species of *Kudoa* from scombrid and labrid fishes of the Great Barrier Reef, Queensland, Australia. *J. Parasitol.* **91**: 1138–1142

Békési L., Székely C., Molnár K. (2002) Recent information on the Myxosporean (Myxozoa) fish parasites. An alternate stage of the parasites in Brazil. *Braz. J. Vet. Res. Anim. Sci.* **39**: 1–11 (in Portuguese)

Blaylock R. B., Bullard S. A., Whipps C. M. (2004) *Kudoa hypoepicardialis* n. sp. (Myxozoa: Kudoidae) and associated lesions from the heart of seven perciform fishes in the northern Gulf of Mexico. *J. Parasitol.* **90**: 584–593

Cho J. B., Kim K. H. (2003) Light- and electron microscope description of *Kudoa paralichthys* n. sp. (Myxozoa, Myxosporea) from the brain of cultured olive flounder *Paralichthys olivaceus* in Korea. *Dis. Aquat. Org.* **55**: 59–63

Dyková I., Avila E. J. F., Fiala I. (2002) *Kudoa diana* sp. n. (Myxosporea: Multivalvulida), a new parasite of bullseye puffer, *Sphoeroides annulatus* (Tetraodontiformes: Tetraodontidae). *Folia Parasitol.* **49**: 17–23

Gioia I., Cordeiro N. S. (1996) Brazilian myxosporidians' check-list (Myxozoa). *Acta Protozool.* **35**: 137–149

Kalavati C., Brickle P., MacKenzie K. (2000) Two new species of myxozoan parasites (Myxosporea, Multivalvulida, Bivalvulida) from fishes of the Falkland Islands. *Acta Parasitol.* **45**: 285–288

- Kpatcha T. K., Diebakate C., Faye N., Toguebaye B. S. (1999) Light and electron microscopic observations on *Kudoa boopsi* sp. n. (Myxosporea: Kudoidae), a gill parasite of *Boops boops* (Pisces: Teleostei: Sparidae) from coasts of Senegal (West Africa). *Acta Protozool.* **38**: 317–321
- Langdon J. S. (1991) Myoliquefaction post-mortem ('milky flesh') due to *Kudoa thyrsites* (Gilchrist) (Myxosporea: Multivalvulida) in mahi mahi, *Coryphaena hippurus* L. *J. Fish Dis.* **14**: 45–54
- Langdon J. S., Thorne T., Fletcher W. J. (1992) Reservoir hosts and new clupeoid host records for the myoliquefactive myxosporean parasite *Kudoa thyrsites* (Gilchrist). *J. Fish Dis.* **15**: 459–471
- Lom J., Dyková I. (1988) Sporogenesis and spore structure in *Kudoa lunata* (Myxosporea, Multivalvulida). *Parasitol. Res.* **74**: 521–530
- Lom J., Dyková I. (1992) Myxosporidia (Phylum Myxozoa). In: Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science, Elsevier, Amsterdam, Netherlands, **26**: 159–235
- Lom J., Dyková I. (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol.* **53**: 1–36
- Lom J., Dyková I., Lhotáková S. (1983) *Kudoa lunata* n. sp. (Myxozoa, Myxosporea) and notes on the nature of muscular "cysts" of the genus *Kudoa*. *Arch. Protistenk.* **127**: 387–397
- Lom J., Rohde K., Dyková I. (1992) Studies on protozoan parasites of Australia fishes I. New species of the genera *Coccomyxa* Léger et Hesse, 1907, *Ortholinea* Shulman, 1962 and *Kudoa* Meglitsch, 1947 (Myxozoa, Myxosporea). *Folia Parasitol.* **39**: 289–306
- Maeno Y., Nagasawa K., Sorimachi M. (1993) *Kudoa intestinalis* n. sp. (Myxosporea: Multivalvulida) from the intestinal musculature of the striped mullet, *Mulgil cephalus*, from Japan. *J. Parasitol.* **79**: 190–192
- Moran J. D. W., Whitaker D. J., Kent M. L. (1999) A review of the myxosporean genus *Kudoa* Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture* **172**: 163–196
- Pampoulie C., Marques A., Rosecchi E., Crivelli A. J., Bouchereau J. L. (1999) A new myxosporean parasite, *Kudoa camarguensis* n. sp., recorded on two goby species (Teleostei: Pisces) in the Rhône delta (Mediterranean Sea, France). *J. Eukaryot. Microbiol.* **46**: 304–310
- Sarkar N. K., Chaudhury S. R. (1996) *Kudoa cascasi* sp. n. (Myxosporea: Kudoidae) parasitic in the mesentery of *Sicamugil cascasi* (Ham.) from Hooghly estuary of West Bengal, India. *Acta Protozool.* **35**: 335–338
- Stehr C. (1986) Sporogenesis of the myxosporean *Kudoa paniformis* Kabata & Whitaker, 1981 infecting the muscle of the Pacific whiting, *Merluccius productus* (Ayres). *J. Fish Dis.* **9**: 493–504
- Swearer S. E., Robertson D. R. (1999) Life history, pathology, and description of *Kudoa ovivora* n. sp. (Myxozoa, Myxosporea): an ovarian parasite of Caribbean labroid fishes. *J. Parasitol.* **85**: 337–353
- Wang P.-C., Huang J.-P., Tsai M.-A., Cheng S.-Y., Tsai S.-S., Chen S.-D., Chen S.-P., Chui S.-H., Liaw L.-L., Chang L.-T., Chen S.-C. (2005) Systemic infection of *Kudoa lutjanus* n. sp. (Myxozoa: Myxosporea) in red snapper *Lutjanus erythropterus* from Taiwan. *Dis. Aquat. Org.* **67**: 115–124
- Whipps C. M., Adlard R. D., Bryant M. S., Kent M. L. (2003a) Two unusual myxozoans, *Kudoa quadricornis* n. sp. (Multivalvulida) from the muscle of goldspotted trevally (*Carangoides fulvoguttatus*) and *Kudoa permulticapsula* n. sp. (Multivalvulida) from the muscle of Spanish mackerel (*Scomberomorus commerson*) from the Great Barrier Reef, Australia. *J. Parasitol.* **89**: 168–173
- Whipps C. M., Adlard R. D., Bryant M. S., Lester R. J. G., Findlay V., Kent M. L. (2003b) First report of three *Kudoa* species from Eastern Australia: *Kudoa thyrsites* from Mahi mahi (*Coryphaena hippurus*), *Kudoa amamiensis* and *Kudoa minithyrsites* n. sp. from sweeper (*Pempheris ypsilychnus*). *J. Eukaryot. Microbiol.* **50**: 215–219
- Whitaker D. J., Kent M. L., Sakanari J. A. (1996) *Kudoa miniauriculata* n. sp. (Myxozoa, Myxosporea) from the musculature of bocaccio (*Sebastes paucispinis*) from California. *J. Parasitol.* **82**: 312–315
- Yokoyama H., Itoh N. (2005) Two multivalvulid myxozoans causing postmortem myoliquefaction: *Kudoa megacapsula* n. sp. from red barracuda (*Sphyraena pinguis*) and *Kudoa thyrsites* from splendid alfonso (*Beryx splendens*). *J. Parasitol.* **91**: 1132–1137
- Yokoyama H., Whipps C. M., Kent M. L., Mizuno K., Kawakami H. (2004) *Kudoa thyrsites* from Japanese flounder and *Kudoa lateolabracis* n. sp. from Chinese sea bass: Causative myxozoans of post-mortem myoliquefaction. *Fish Pathol.* **39**: 79–85

Received on 24th September, 2007; revised version on 19th February, 2008; accepted on 22nd February, 2008

Capítulo 12

**FINE STRUCTURE OF *CHLOROMYXUM MENTICIRRHUS* N. SP. (MYXOZOA)
INFECTING URINARY BLADDER OF THE MARINE TELEOST
MENTICIRRHUS AMERICANUS (SCIAENIDAE) IN SOUTHERN BRAZIL**

European Journal of Protistology (2009) **45**: 139-146

Graça Casal, Patrícia Garcia, Patrícia Matos, Emanuel Monteiro,
Edilson Matos & Carlos Azevedo

Fine structure of *Chloromyxum menticirrhi* n. sp. (Myxozoa) infecting the urinary bladder of the marine teleost *Menticirrhus americanus* (Sciaenidae) in Southern Brazil

Graça Casal^{a,b,c}, Patrícia Garcia^d, Patrícia Matos^e, Emanuel Monteiro^f,
Edilson Matos^g, Carlos Azevedo^{a,b,*}

^aDepartment of Cell Biology, Institute of Biomedical Sciences (ICBAS/UP), University of Porto, Largo A. Salazar, No. 2, P-4099-003 Porto, Portugal

^bLaboratory of Pathology, Centre for Marine and Environmental Research (CIIMAR/UP), University of Porto, P-4050-123, Porto, Portugal

^cLaboratory of Sciences, High Institute of Health Sciences (CESPU), P-4585-116 Gandra, Portugal

^dLaboratory of Diagnostic and Pathology in Aquaculture, Federal University of Santa Catarina, 88040-970 Florianópolis, SC, Brazil

^eLaboratory of Aquatic Animals, Federal University of Pará, 66075-110 Belém, PA, Brazil

^fDepartment of Anatomy, Institute of Biomedical Sciences (ICBAS/UP), University of Porto, P-4099-003 Porto, Portugal

^gCarlos Azevedo Research Laboratory, Federal Rural University of Amazonia, 66077-530 Belém, PA, Brazil

Received 29 January 2008; received in revised form 27 August 2008; accepted 28 August 2008

Abstract

A myxosporidian was found in the urinary bladder of the teleost *Menticirrhus americanus* Linnaeus, 1758 (Sciaenidae) collected from the South Atlantic coast of Brazil. Polysporic amoeboid plasmodia containing sporoblasts, developing pansporoblasts and spores were free in the bladder lumen. The prevalence of infection was 17.64% (15/85). Unfixed spores were spherical to subspherical, on average 10.5 µm long, 9.8 µm wide and 10.1 µm thick ($n = 25$), and fixed spores measured 10.1 × 9.5 × 9.7 µm. The two spore valves were of equal size and each possessed prominent sutural lines and about 41 (37–45) surface ridges aligned parallel with the suture line. These ridges gave transverse sections a cog-wheel-like outline. The spores contained four pyriform polar capsules of equal size (3.20 × 2.0 µm) ($n = 25$) (fixed), each with a polar filament having 3–4 (rarely 5) coils. The binucleate sporoplasm was irregular in shape, with granular matrix and randomly distributed dense bodies. The shape and dimensions of the spore, as well as the number, position and arrangement of the surface ridges, polar capsules and polar filament indicate that this is a new species, herein designated *Chloromyxum menticirrhi*. The gill, liver, gall bladder and intestine of the host showed no abnormalities.

© 2008 Elsevier GmbH. All rights reserved.

Keywords: Teleost; *Chloromyxum menticirrhi* n. sp.; Myxosporea; Ultrastructure; Sporogenesis

*Corresponding author at: Department of Cell Biology, Institute of Biomedical Sciences (ICBAS/UP), University of Porto, Largo A. Salazar, No. 2, P-4099-003 Porto, Portugal. Tel.: +351 22 206 2200; fax: +351 22 206 2232/33.

E-mail address: azevedoc@icbas.up.pt (C. Azevedo).

Introduction

The Myxosporea of the phylum Myxozoa is an assemblage of more than 2180 species distributed among some 60 genera (Lom and Dyková 2006). They have been reported from different geographic areas, mainly as parasites and pathogens of fish, and are of importance in fisheries and aquaculture (Lom and Dyková 2006). Among them, members of the genus *Chloromyxum* Mingazzini, 1890, the fourth largest genus of Myxozoa, with 115 nominal species, are commonly coelozoic in the urinary tract and gall bladder of freshwater and marine fishes (Lom and Dyková 2006), as well as some non-fish hosts such as batrachian amphibians (Duncan et al. 2004; Joseph 1905; Lom and Dyková 2006; Mutschmann 1999; Upton et al. 1995). The taxonomy of the group is difficult when relying solely on light microscopy of the spore because of the limited number of distinct characters for separation of species. Recent revision of the genus makes use of the pattern of ridges on the spore surface revealed by electron microscopy, particularly scanning electron microscope (SEM) (Lom and Dyková 1993).

Considering the high number of Brazilian fish species (about 8000) (Celere et al. 2002), the number of described parasite species is low (Gioia and Cordeiro 1996). Only two myxosporean species of the genus *Chloromyxum*, (*C. leydigi* Mingazzini, 1890 and *C. sphyrynae* Cunha and Fonseca, 1918) have been observed by light microscopy from the Brazilian fauna and illustrated by diagrammatic drawings (Cunha and Fonseca 1918; Guimarães 1931, Pinto 1928). One other described member of this genus from South America was found in the flatfish *Paralichthys adspersus* from the Pacific coast of the Chile (Oliva et al. 1996).

Previously, only metazoan parasites have been reported from the host *Menticirrhus americanus* (Sciaenidae) (Luque and Oliva 1999). Myxosporeans belonging to the genera *Henneguya* and *Parvicapsula* (Landsberg 1993), *Ceratomyxa* (Sarkar and Pramanik 1994), *Myxoproteus* and *Zschokkella* (Sarkar 1996), *Sinuolinea* (Sarkar 1997), *Myxidium* (Diamant 1998) and *Kudoa* (Blaylock et al. 2004; Oliva et al. 1992) have been described as parasites of members of the family Sciaenidae.

We here describe *Chloromyxum menticirrhii* n. sp. from the urinary bladder of the marine teleost *Menticirrhus americanus* from coastal waters of Southern Brazil from studies using light (LM), SEM and transmission electron microscopes (TEM).

Materials and methods

The marine teleost *Menticirrhus americanus* Linnaeus, 1758 (Sciaenidae) (Southern kingfish) (Brazilian

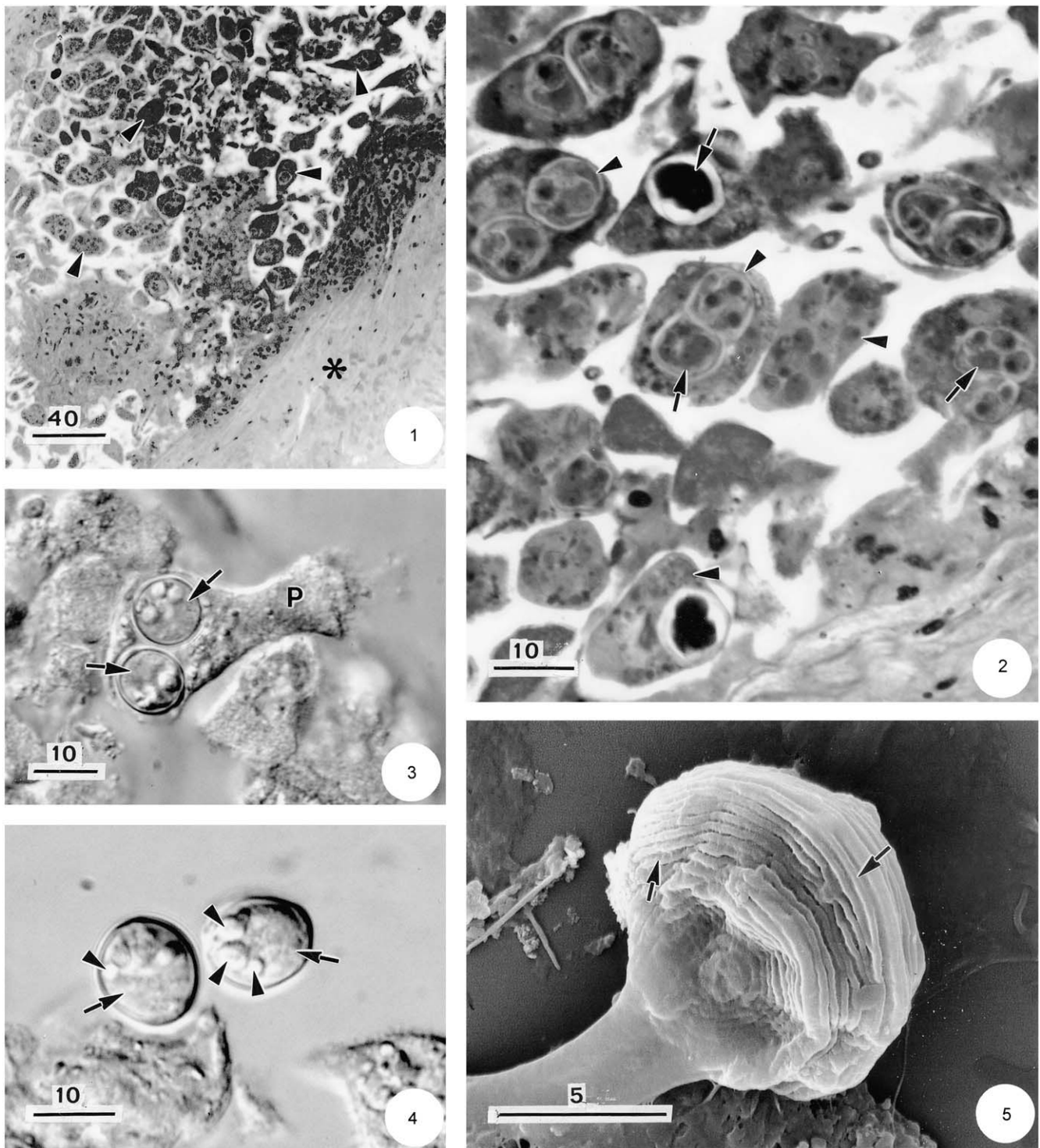
common name “Papa-Terra”) was collected in the surf zone of the “Barra da Lagoa” Beach (27° 34'S, 48° 25'W) near Florianópolis (Santa Catarina State), on the southern Atlantic coast of Brazil. Specimens were collected once a month between October 2006 and September 2007. Altogether, 85 fishes (10–22 cm long) were taken alive to the laboratory, where they were anaesthetized with benzocain, killed and necropsied. Each fish was measured (total length) and its sex determined. No specimens showed macroscopical signs of disease.

Smears of fresh gill, liver, gall bladder, urinary bladder and intestine were microscopically examined. Smears of fresh urinary bladders, the only parasitized organ, containing plasmodia and free spores were prepared for observation by LM using differential interference contrast (DIC) microscopy and unfixed and fixed plasmodia and free spores were measured with an ocular micrometer.

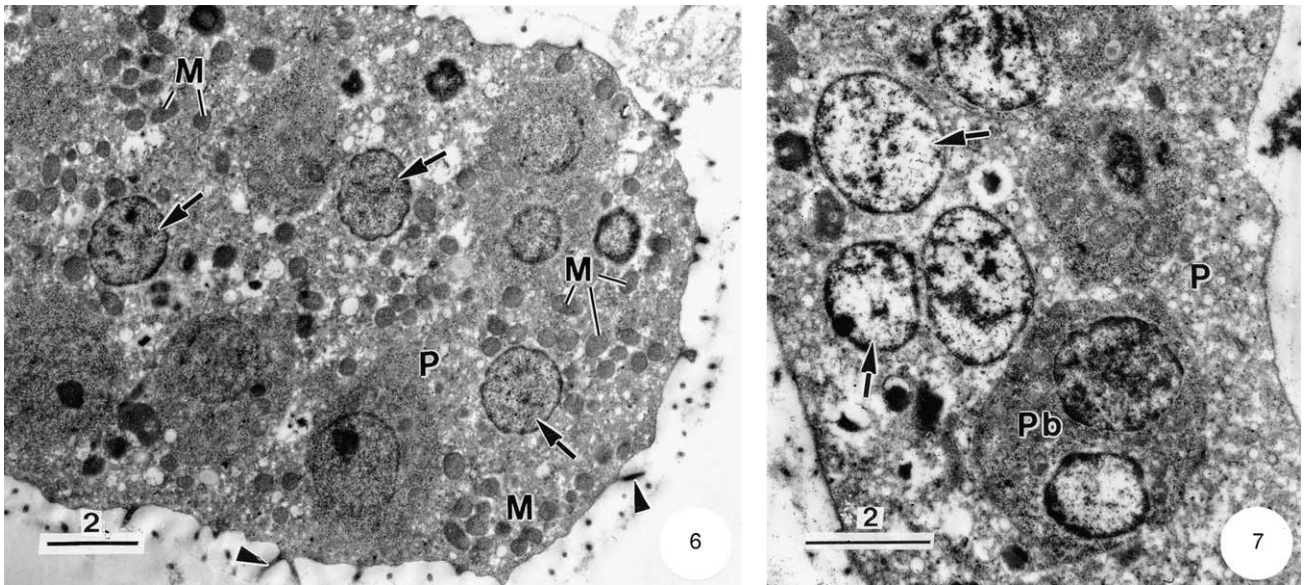
For SEM, the plasmodia were teased apart to release spores, which were fixed at 4 °C for 24 h in 5% glutaraldehyde buffered with 0.2 M sodium cacodylate (pH 7.4), washed in three changes of the same buffer, dehydrated in an ascending ethanol series, critical point dried, coated with gold and examined in a JSM-630 SEM operated at 15 kV. For TEM, small fragments of the parasitized tissues were fixed as for SEM, washed overnight in buffer at 4 °C, post-fixed in 2% osmium tetroxide with the same buffer and temperature for 3 h, dehydrated in an ascending ethanol series followed by propylene oxide, and embedded in Epon. Semithin sections were stained with methylene blue-Azure II for LM and ultrathin sections were double stained with uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM operated at 60 kV.

Results

During a parasitological survey it was observed that some specimens presented hypertrophy of the urinary bladder, which contained several masses of plasmodia. The prevalence of infection was 17.64% (15/85) in a host population where the numbers of females and males were about equal. Histological sections revealed that the plasmodia were on or near the surface of the inner bladder epithelium, sometimes forming several layers (Fig. 1). The largest polysporic plasmodia reached nearly 52 µm long and contained all sporogonic stages as well as developed spores (up to 8) (Figs 2, 3, 6–8). Some free spores were observed among the plasmodia (Figs 4, 5). Plasmodia had irregular contours and the surfaces were irregularly covered by slender cytoplasmic extensions (up to 1.3 µm long) (Figs 6–8). Numerous mitochondria (M) with dense contents were located in plasmodial cytoplasm among the developmental stages,



Figs 1–5. Light and scanning electron micrographs of developmental stages of *Chloromyxum menticirrhoi* sp. n. infecting the urinary bladder of the teleost *Menticirrhus americanus* (scale bars in µm). **1.** Semithin section showing several plasmodia (arrowheads) located free in the lumen and attaching to the urinary bladder wall (*). **2.** Semithin section showing several plasmodia (arrowheads), most of them containing spores in different phases of development (arrows). **3.** Two unfixed spores (arrows) in the same plasmodium (P) observed in DIC. **4.** Two free-floating fixed spores (arrows) observed in DIC, showing polar capsules (arrowheads). **5.** SEM of a spore showing the valve ridge arrangement (arrows).



Figs 6–7. Transmission electron micrographs of developmental stages of *Chloromyxum menticirrhii* sp. n. (scale bars in μm). **6.** Plasmodium (P) showing nuclei of several developmental stages (arrows) located among numerous mitochondria (M). The plasmodium shows several peripheral cytoplasmic extensions (arrowheads). **7.** Plasmodium (P) showing some vegetative nuclei (arrows) and developing pansporoblasts (Pb).

more frequently in the peripheral areas (Fig. 6). Various stages of developing pansporoblasts (Pb) were randomly distributed throughout the plasmodial cytoplasm (Figs 7, 8).

Diagnosis

Phylum Myxozoa Grassé, 1970
 Class Myxosporea Bütschli, 1881
 Order Bivalvulida Schulman, 1959
 Family Chloromyxidae Thélohan, 1892
 Genus *Chloromyxum* Mingazzini, 1890

Chloromyxum menticirrhii n. sp. (Figs 1–14)

Specific characters: spores spherical to subspherical, $10.5 \pm 0.4 \mu\text{m}$ long in lateral view; $9.8 \pm 0.6 \mu\text{m}$ wide and $10.1 \pm 0.6 \mu\text{m}$ thick ($n = 25$) in apical view when unfixed

(Figs 3, 4); and $10.1 \pm 0.5 \times 9.5 \pm 0.5 \times 9.7 \pm 0.7 \mu\text{m}$ when fixed (Fig. 5) (Table 1). Valves adhering together along the prominent longitudinal sutural lines (Figs 9–13). Two equal-sized valves without caudal projections but with longitudinal surface ridges which appear like cog-wheel teeth around transverse sections (Figs 9–12). Each valve with ~ 41 (37–45) ridges aligned parallel with the suture line (74–90 ridges per spore) and of equal thickness 0.25 (0.21 – 0.30) μm ($n = 25$) (Figs 9–13). Some ridges coalesce towards the poles. Suture ridges form the outer edges of the valves (Figs 9–13). Four equal-sized pyriform polar capsules (PC) (Figs 9–11); $3.2 \pm 0.4 \mu\text{m}$ long and $2.0 \pm 0.3 \mu\text{m}$ wide ($n = 25$); polar filament with 3–4 (rarely 5) coils (Figs 9, 11, 14; Table 1). Sporoplasm irregular in shape with two nuclei in a granular matrix of variable density (Fig. 10). A longitudinal section of the spore structure is shown in the schematic drawing (Fig. 14).

Figs 8–13. Transmission electron micrographs of developmental stages of *Chloromyxum menticirrhii* sp. n. (scale bars in μm). **8.** Plasmodium (P) showing several developmental stages: vegetative nuclei (arrows), pansporoblasts (Pb) and immature spores (*), one of which shows the surface ridges having a cog-wheel-like appearance (**). The plasmodial periphery shows several cytoplasmic extensions (arrowheads). Inset: Detail of a similar cytoplasmic extensions (arrowheads) boxed in this figure. **9.** Transverse oblique section of the apical region of a spore showing four transverse sections of the polar capsules (PC) located side-by-side. The valves show the suture lines (arrows) and the ridge sections (arrowheads). In this picture it is possible to count more than 30 ridges on the right valve. The surrounding space in contact with the valves is occupied by anastomosing filaments (*). **10.** Longitudinal section of an immature spore showing the valvogenic cells (VC), the sporoplasm (S) and longitudinal sections of two polar capsules (PC) with the wall composed of two layers. **11.** Detail of a portion of the spore valves showing the suture line (arrow) and the position of the ridges (arrowheads) giving a cog-wheel outline, and transverse sections of two polar capsules (PC). **12.** Transverse section of a portion of the valves showing the suture line (arrow) and adjacent valve ridges (arrowheads). The space between the spore and plasmodium is occupied by numerous anastomosing filaments (*). **13.** Tangential section of the spore valve periphery showing the position of the suture line (arrows) and neighbouring valve ridges (arrowheads). The convergence of two ridges is clearly visible (double arrow). The surrounding space in contact with the ridges is occupied by numerous anastomosing filaments (*).

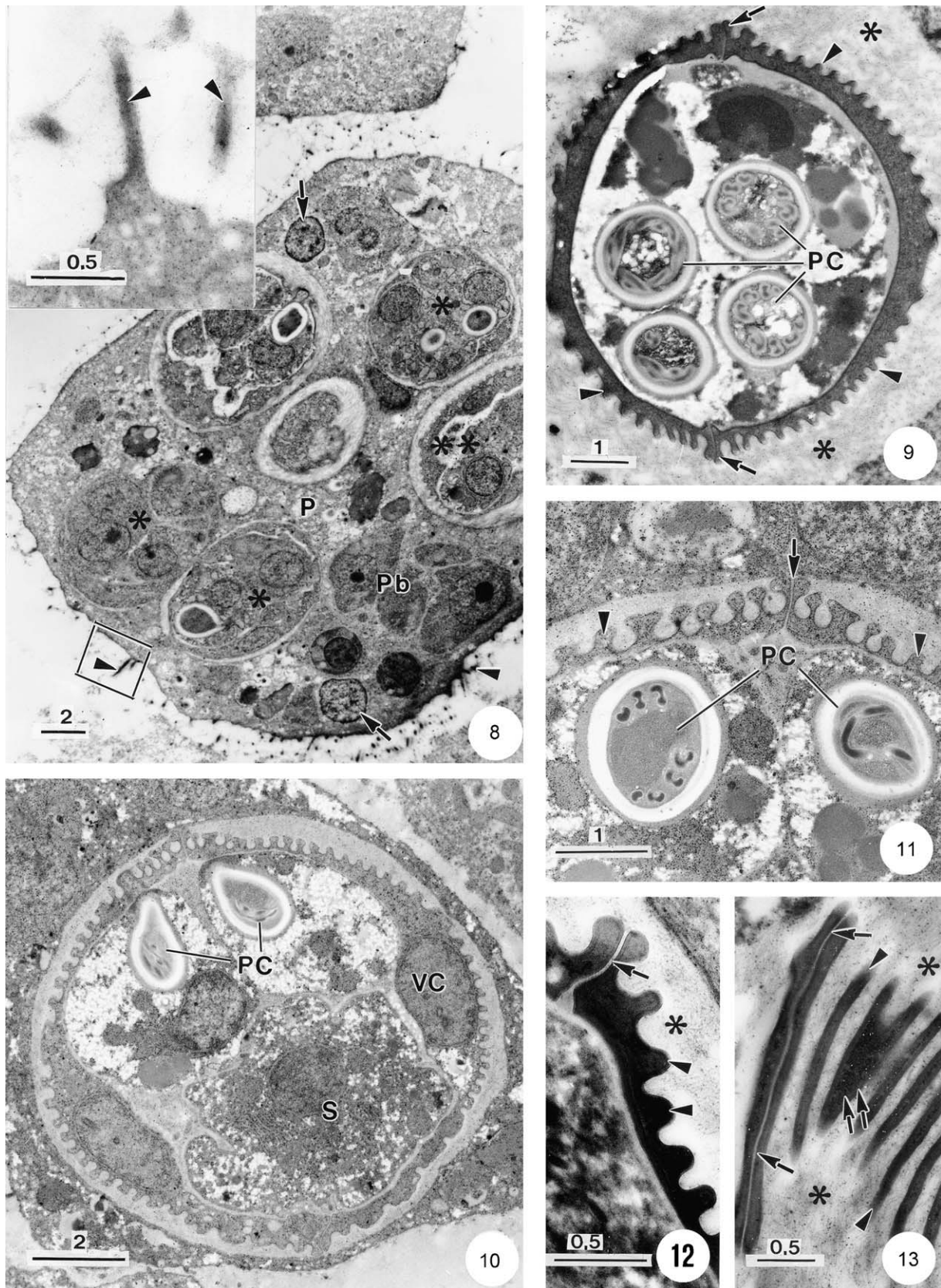


Table 1. Reports of *Chloromyxum* spp. having spherical or subspherical spores and with surface valve ridges or protuberances

<i>Chloromyxum</i> spp.	Spore diameter (µm)	Valvar ridges	Four equal PC		References
			L × W	Coils	
<i>C. legeri</i>	~7.5	Shallow and indistinct 6–7 r.p.v. parallel to the SL	2.5 × 3.4	–	Tourraine (1931)
<i>C. majori</i>	~7.9	18–23 r.p.v. running obliquely to the SL	3.5 × 4.0;	–	Yasutake and Wood (1957)
	(F) 5.6 × 7.4		(F) 2 × 3.6 La 3.7 × 2.8 Sm 3.0 × 2.4	4	Lom and Dyková (1993)
<i>C. pseudomucronatum</i>	9.8	Two r.p.v. running parallel; others obliquely to the SL	La 4.3 × 2.7 Sm 3.5 × 2.2	La 5-6-Sm 4	Lom et al. (1988)
<i>C. reticulatum</i>	(uF) 8.1 (F) 5.5–6.1	Mushroom-like buttons protruding all over the shell valves	3.4 × 2.5	–	Lom et al. (1988)
<i>C. cristatum</i>	9.0–15.9	High surface ridges, 1 parallel and the others not	Uneven size		Lom and Dyková (1993)
<i>C. paulini</i>	11.9	17–21 r.p.v. not concentric to the SL	La 4.9 × 3.9 Sm 4.0 × 3.2	–	Lom and Dyková (1993)
<i>C. truttae</i>	7.4–10.9	13–19 r.p.v. not running parallel to the SL	Uneven size	–	Lom and Dyková (1993)
<i>C. schurovi</i>	(F) 5.5–6.6	–	(F) 3.3 × (1.7–2.2)	–	Shul'man and Ieshko (2003)
	7.7–8.5	~20 r.p.v. straight suture line	La 3.6 × 2.9 Sm 3.0 × 2.2	La 5-Sm 4	Holzer et al. (2006)
<i>C. auratum</i>	~12.6	6–9 r.p.v. aligned along the longitudinal axis	4.4 × 3.5	4	Hallett et al. (2006)
<i>C. menticirrhi</i>	(F)~9.5 (uF)~9.8	Up 45 r.p.v. convergent at both poles	(F) 3.1 × 2.0	3–4 (rarely 5)	Present study

D – diameter; F – fixed; L – length; La – largest; r.p.v. – ridges per valve; SL – sutural line; Sm – smallest; uF – unfixed; W – width.

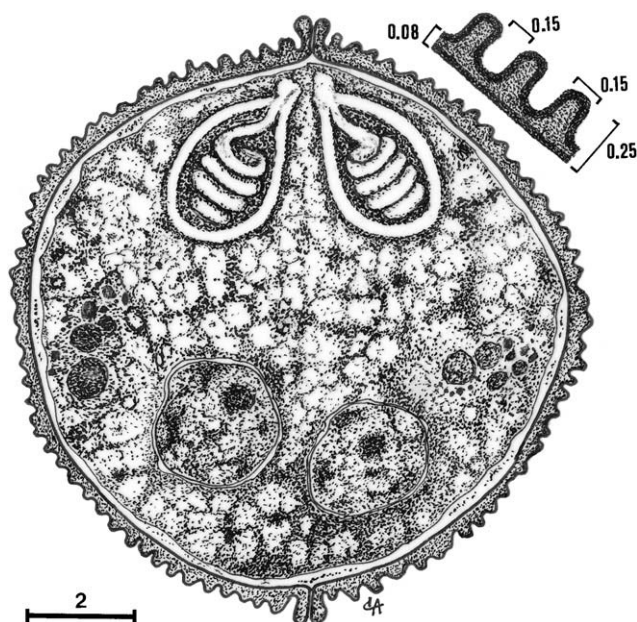


Fig. 14. Schematic drawing of a longitudinal section of the spore of *Chloromyxum menticirrhi* sp. n. in frontal view showing the external organization of the valve ridges and the internal organization including the equal-sized polar capsules. Detail of a small part of a valve, indicating some measurements in µm is shown at the right side. Scale bar in µm.

Type host

Menticirrhus americanus Linnaeus, 1758 (Teleostei, Sciaenidae).

Site of infection

Urinary bladder.

Prevalence of infection

17.64% (15/85).

Type locality

“Barra da Lagoa” Beach (27° 34’S, 48° 25’W) near Florianópolis, Santa Catarina State, Brazil.

Type specimens

Hapantotypes (one glass slide with semithin sections from spores and plasmodia) (No.: USNM 1100738) and fragments of the parasitized tissue fixed in 80% ethanol were deposited in the International Protozoan Type Slide Collection at the Smithsonian Institution, Washington, DC 20506, USA.

Etymology

The specific name “*menticirrhi*” is derived from the generic name of the host species.

Discussion

The morphology of the spores described in the present study places this parasite in the genus *Chloromyxum* Mingazzini, 1890 according to the key for determination of myxosporean genera published by Lom and Dyková (2006). The morphology and ultrastructural organization of our species is similar to that of the previously described species of this genus (Ali 1998; Baska 1990, 1993; Hallett et al. 2006; Lom and Dyková 1992, 2006; Lom et al. 1988; Molnár 1992; Mutschmann 1999; Shul'man and Ieshko 2003; Upton et al. 1995), but the genus is a large one with about 115 recognized species (Lom and Dyková 2006). The genus *Chloromyxum*, which includes coelozoic myxosporeans that develop in polysporic plasmodia, has previously been reported in the South American aquatic fauna, with two species from Brazil and one from Chile, but no species of *Chloromyxum* has been described from fish of the family Sciaenidae (see Introduction). This study provides the first ultrastructural description of this genus from the Brazilian fauna as well as the first from a sciaenid fish.

Such morphological features as the surface structure of the spore wall, and particularly the ridges, which occur only in some species, are important characteristics used to distinguish species of *Chloromyxum* (Lom and Dyková 1993). We therefore compared the morphology, size and ultrastructural organization of the spore valves, especially the number, organization and distribution of the valvular ridges observed in our specimens, with similar features of other *Chloromyxum* spp. with spherical or subspherical spores (or similar form) possessing surface ridges or protuberances, namely, *C. legeri* (Tourraine 1931), *C. majori* (Lom and Dyková 1993; Yasutake and Wood 1957), *C. pseudomucronatum* (Lom et al. 1988), *C. reticulatum* (Lom et al. 1988), *C. cristatum* (Lom and Dyková 1993), *C. paulini* (Lom and Dyková 1993), *C. truttae* (Lom and Dyková 1993), *C. schurovi* (Holzer et al. 2006; Shul'man and Ieshko 2003) and *C. auratum* (Hallett et al. 2006) (Table 1), and found that only four species show some similarities with our results. *C. majori* and *C. truttae* have approximately 20 ridges per valve but the PC are of different size. Although the species *C. auratum* and *C. legeri* possess equal PC, the spores of *C. legeri* have 6–7 shallow and indistinct ridges per valve parallel with the suture line and *C. auratum* have 6–9 ridges per valve aligned along the longitudinal axis. Spores observed in the present study have up to 90 ridges per spore, with an external organization that is clearly very different from the ridge ornamentalions reliably described and figured for any *Chloromyxum* spp. previously described. These ultrastructural differences among the spores of different *Chloromyxum* spp., coupled with the host identity,

support our conclusion that this parasite is a new myxosporean species, which we have named *Chloromyxum menticirrho*.

The developmental stages observed in the plasmodia of *C. menticirrho* are similar to those previously described in other myxozoans (Lom and Dyková 2006). Recently SSU rDNA sequence data have been used to prove that a *Chloromyxum* sp. has a two-host life cycle which involves an actinospore discharged from an oligochaete into water (Atkinson et al. 2007). Sadly, although attempts have been made to obtain further specimens of *C. menticirrho*, in order to determine its SSU rDNA sequence, these have not so far been successful.

Acknowledgments

This work was partially supported by the Eng. A. Almeida Foundation (Porto, Portugal), a Ph.D. grant from “CESPU” (G. Casal), “CNPq” and “CAPES” – Brazil. We would like to thank Prof. M. Martins (UFSC-Florianópolis) for use of the facilities in his Laboratory.

References

- Ali, M.A., 1998. Light and scanning electron microscopy of *Chloromyxum vanasi* sp. n. (Myxozoa: Myxosporea) infecting gall bladder of the Nile catfish *Bagrus bayad* (Forsk., 1775) (Teleostei: Bagridae). *Acta Protozool.* 37, 57–61.
- Atkinson, S.D., Hallett, S.L., Bartholomew, J.L., 2007. The life cycle of *Chloromyxum auratum* (Myxozoa) from goldfish, *Carassius auratus* (L.), involves an antonactinomyxon actinospore. *J. Fish Dis.* 30, 149–156.
- Baska, F., 1990. *Chloromyxum inexpectatum* n. sp. and *Sphaerospora colomani* n. sp. (Myxozoa, Myxosporea), parasites of the urinary system of the starlet, *Acipenser ruthenus*. *Syst. Parasitol.* 16, 185–193.
- Baska, F., 1993. Light and electron microscopic studies on the development of *Sphaerospora colomani* Baska, 1990 and *Chloromyxum inexpectatum* Baska, 1990. *Acta Vet. Hung.* 41, 59–72.
- Blaylock, R.B., Bullard, S.A., Whipps, C.M., 2004. *Kudoa hypoepicardialis* n. sp. (Myxozoa: Kudoidae) and associated lesions from the heart of seven perciform fishes in the northern Gulf of Mexico. *J. Parasitol.* 90, 584–593.
- Celere, E.F., Cordeiro, N., Adriano, E.S., 2002. *Myxobolus absonus* sp. n. (Myxozoa: Myxosporea) parasitizing *Pimelodus maculatus* (Siluriformes: Pimelodidae), a South American freshwater fish. *Mem. Inst. Oswaldo Cruz* 97, 79–80.
- Cunha, A.M., Fonseca, O., 1918. About the myxosporidians from Brazilian fishes. *Braz.-Méd.* 32, 393 (in Portuguese).
- Diamant, A., 1998. Red drum *Sciaenops ocellatus* (Sciaenidae), a recent introduction to Mediterranean mariculture, is susceptible to *Myxidium leei*. *Aquaculture* 162, 33–39.

- Duncan, A.E., Garner, M.M., Bartholomew, J.L., Reichard, T.A., Nordhausen, R.W., 2004. Renal myxosporidiasis in Asian horned frog (*Megophrys nasuta*). *J. Zoo Wildl. Med.* 35, 381–386.
- Gioia, I., Cordeiro, N.S., 1996. Brazilian Myxosporidians' check-list (Myxozoa). *Acta Parasitol.* 35, 137–149.
- Guimarães, J.R.A., 1931. Myxozoans of the Brazilian ictiofauna. Thesis. Faculty of Medicine, S. Paulo, Brazil, 1–50 (in Portuguese).
- Hallett, S.L., Atkinson, S.D., Holt, R.A., Banner, C.R., Bartholomew, J.L., 2006. A new myxozoan from feral goldfish (*Carassius auratus*). *J. Parasitol.* 92, 357–363.
- Holzer, A.S., Sommerville, C., Wootten, R., 2006. Molecular identity, phylogeny and life cycle of *Chloromyxum schurovi* Shul'man & Ieshko 2003. *Parasitol. Res.* 99, 90–96.
- Joseph, H., 1905. *Chloromyxum protei* n. sp. *Zool. Anz.* 29, 445–451.
- Landsberg, J.H., 1993. Kidney myxosporean parasites in red drum *Sciaenops ocellatus* (Sciaenidae) from Florida, USA, with a description of *Parvicapsula renalis* n. sp. *Dis. Aquat. Org.* 17, 9–16.
- Lom, J., Dyková, I., 1992. Myxosporidia (phylum Myxozoa). In: Lom, J., Dyková, I. (Eds.), *Protozoan Parasites of Fishes*. Elsevier, Amsterdam, pp. 159–235.
- Lom, J., Dyková, I., 1993. Scanning electron microscopic revision of common species of the genus *Chloromyxum* (Myxozoa: Myxosporea) infecting European freshwater fishes. *Folia Parasitol.* 40, 161–174.
- Lom, J., Dyková, I., 2006. Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol.* 43, 1–36.
- Lom, J., Dyková, I., Kepr, T., 1988. Species of the genus *Chloromyxum* Mingazzini (Myxozoa: Myxosporea) infecting burbot (*Lota lota* L.). *Syst. Parasitol.* 11, 231–237.
- Luque, J.L., Oliva, M.E., 1999. Metazoan parasite infracommunities of *Menticirrhus* (Teleostei: Sciaenidae): an amphioceanic approximation. *J. Parasitol.* 85, 379–381.
- Molnár, K., 1992. *Ceratomyxa hungarica* n. sp. and *Chloromyxum proterorhini* n. sp. (Myxozoa, Myxosporea) from the freshwater gobi *Proterorhinus marmoratus* (Pallas). *Syst. Parasitol.* 22, 25–31.
- Mutschmann, F., 1999. A new myxozoan, *Chloromyxum careni* sp. n. (Myxosporea: Chloromyxidae) from kidney of *Megophrys nasuta* Schlegel, 1858 (Anura: Pelobatidae) from Indonesia. *Acta Protozool.* 38, 83–86.
- Oliva, M., Luque, J., Teran, L., Llican, L., 1992. *Kudoa sciaenae* (Myxozoa, Multivalvulidae) cysts distribution in the somatic muscles of *Stellifer minor* (Tschudi, 1884) (Pisces, Sciaenidae). *Mem. Inst. Oswaldo Cruz* 87, 33–35.
- Oliva, M.E., Castro, R.E., Burgos, R., 1996. Parasites of the Flatfish *Paralichthys adspersus* (Steindachner, 1867) (Pleuronectiformes) from Northern Chile. *Mem. Inst. Oswaldo Cruz* 91, 301–306.
- Pinto, C., 1928. Myxosporidians and other intestinal protozoans observed in South America. *Arch. Inst. Biol.* 1, 101–126 (in Portuguese).
- Sarkar, N.K., 1996. *Zschokkella pseudasciaena* sp. n. and *Myoproteus cujaeus* sp. n. (Myxozoa: Myxosporea) from sciaenid fish of Hooghly Estuary, West Bengal, India. *Acta Protozool.* 35, 331–334.
- Sarkar, N.K., 1997. *Sinuolinea indica* sp.n. (Myxosporea: Sinuolineidae) parasitic in the urinary bladder of a sciaenid fish from the Hooghly Estuary, West Bengal, India. *Acta Protozool.* 36, 305–309.
- Sarkar, N.K., Pramanik, A.K., 1994. *Ceratomyxa daysciaenae* sp. n. (Myxozoa, Ceratomyxidae) a myxosporean parasite in the gall bladder of a teleost from the Hooghly Estuary, West Bengal, India. *Acta Protozool.* 33, 121–124.
- Shul'man, B.S., Ieshko, E.P., 2003. *Chloromyxum schurovi* sp. n. – a new myxosporidian species (Myxosporea: Sphaerosporidae) from salmonid fishes (Salmonidae). *Parazitologiya* 37, 246–247.
- Tourraine, F., 1931. Sur une nouvelle myxosporidie du genre *Chloromyxum* observée chez le carpe. *C. R. Acad. Sci.* 192, 1125–1127.
- Upton, S.J., McAllister, C.T., Trauth, S.E., 1995. A new species of *Chloromyxum* (Myxozoa, Chloromyxidae) from the gall bladder of *Eurycea* spp. (Caudata, Plethodontidae) in North America. *J. Wildl. Dis.* 31, 394–396.
- Yasutake, W.T., Wood, E.M., 1957. Some myxosporidia found in Pacific Northwest salmonids. *J. Parasitol.* 43, 633–642.

Capítulo 13

ULTRASTRUCTURAL AND PHYLOGENTIC DATA OF *CHLOROMYXUM RIORAJUM* SP. NOV. (MYXOZOA), A PARASITE OF THE STINGRAY *RIORAJA AGASSIZII* IN SOUTHERN BRAZIL

Diseases of Aquatic Organisms (2009) **85**: 41-51

Carlos Azevedo, Graça Casal, Patrícia Garcia, Patrícia Matos, Leonor
Teles Grilo & Edilson Matos

Ultrastructural and phylogenetic data of *Chloromyxum riorajum* sp. nov. (Myxozoa), a parasite of the stingray *Rioraja agassizii* in Southern Brazil

Carlos Azevedo^{1,2,*}, Graça Casal^{1,2,3}, Patrícia Garcia⁴, Patrícia Matos⁵,
Leonor Teles-Grilo⁶, Edilson Matos⁷

¹Department of Cell Biology, Institute of Biomedical Sciences (ICBAS/UP), University of Porto, 4099-003 Porto, Portugal

²Laboratory of Pathology, Centre for Marine and Environmental Research (CIIMAR/UP), University of Porto, 4050-123 Porto, Portugal

³Departamento de Ciências, Instituto Superior de Ciências da Saúde-Norte, 4585-116 Gandra, Portugal

⁴Laboratory of Diagnostic and Pathology in Aquaculture, Federal University of Santa Catarina, 88040-970 Florianópolis, SC, Brazil

⁵Laboratory of Histology of Aquatic Animals, Federal University of Pará, 66075-110 Belém, PA, Brazil

⁶Laboratory of Molecular Genetics, Institute of Biomedical Sciences (ICBAS/UP), University of Porto, 4099-003 Porto, Portugal

⁷Carlos Azevedo Research Laboratory, Federal Rural University of Amazonia, 66077-530 Belém, PA, Brazil

ABSTRACT: We describe a new myxozoan parasite found infecting the gall bladder of the cartilaginous fish *Rioraja agassizii* (Rajidae) from the South Atlantic coast of Brazil. Light microscopy, scanning and transmission electron microscopy and phylogenetic data were used. Numerous irregular polysporic plasmodia externally covered by numerous microvilli containing different stages of sporogony, including free spores, were observed in bile. Ellipsoidal spores, on average 11.41 µm long, 8.48 µm wide and 7.32 µm thick, were formed by 2 equal-sized valves, each possessing 3 to 4 (rarely 5) elevated ridges which projected from the basal portion of the spore, and joined along a sinuous S-shaped sutural line. The basal portion of the valves bore a bundle of 33 to 37 extended tapering caudal filaments attached to the basal portion of the last ridge and basal portion of the sutural edge of the 2 valves. The caudal filaments, formed of material similar to the valves, were attached to the shell wall by a conical basis. The spores contained 4 equal-sized pyriform polar capsules (4.5 × 2.4 µm), located at the same level, each with a polar filament with 6 (rarely 7) coils. Binucleate sporoplasm was irregular in shape, with a granular matrix and dense bodies randomly distributed in a light area. Based on the shape and dimensions of the spore, on the number, position and arrangements of the surface ridges, caudal bundle of filaments, polar capsules and polar filament arrangements, as well as phylogenetic analyses using the small subunit ribosomal DNA (SSU rDNA) sequences, we propose the name *Chloromyxum riorajum* for this new myxozoan.

KEY WORDS: Cartilaginous fish · *Chloromyxum riorajum* sp. nov. · Parasite · Phylogeny · Ultrastructure

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The Myxosporea of the phylum Myxozoa is an assemblage of more than 2180 species distributed among some 60 genera (Lom & Dyková 2006) with

more species being added regularly. They have been reported from different geographic areas, mainly as parasitic and pathogenic of fish, where they are of importance in fisheries and aquaculture (Lom & Dyková 2006). Among them, the genus *Chloromyxum*

*Email: azevedoc@icbas.up.pt

Mingazzini, 1890, the fourth largest genus of Myxozoa with 115 nominal species, is commonly coelozoic in the urinary tract and gall bladder of freshwater and marine fishes (Lom & Dyková 2006) and in some non-fish host such as batrachian amphibians (Duncan et al. 2004, Lom & Dyková 2006). Determining the taxonomy of the group is difficult when relying solely on light microscopy of the spore because there is only a limited number of distinct characters which can be used to separate the species. Revision of the genus has shown that ultrastructural microscopy, particularly scanning electron microscopy (SEM), provides taxonomic data that considers the pattern of the ridges on the spore surface (Lom & Dyková 1993).

Considering the high number of Brazilian fish species (about 8000 species) (Cellere et al. 2002), the number of described parasite species is low. Little has been published on the myxosporeans, and that which has been published mainly concerns the genus *Chloromyxum*. From the Brazilian fauna, only 2 species (*C. leydigii* Mingazzini, 1890 and *C. sphyrnae* Cunha and Fonseca, 1918) have been observed by light microscopy and represented by diagrammatic drawings (Cunha & Fonseca 1918, Pinto 1928, Gioia & Cordeiro 1996). In South American fauna, there is another description of this genus found in the flatfish *Paralichthys adspersus* from the Pacific coast of the Chile (Oliva et al. 1996). Recently, on the bases of the morphological and ultrastructural data, a new species *C. menticirrhii* was described parasitizing a Brazilian marine teleost fish (Casal et al. 2009).

With respect to molecular data, there is information on the 18S rDNA gene for only 7 *Chloromyxum* species (Fiala & Dyková 2004, Holzer et al. 2004, 2006, Hallett et al. 2006). An analysis of the small subunit ribosomal DNA (SSU rDNA) in phylogenetic studies shows that the majority of myxosporea can be divided into 2 main clades: marine and freshwater clades. However, there are exceptions to this division, notably regarding some species infecting anadromous hosts and species of the genus *Chloromyxum* (Fiala 2006). The present study describes *C. riorajum* sp. nov. from the gall bladder of the marine stingray *Rioraja agassizii* from coastal waters off Southern Brazil, making use of light microscopy (LM), SEM, transmission electron microscopy (TEM) and phylogenetic data pertaining to the 18S rDNA sequence.

MATERIALS AND METHODS

The marine stingray *Rioraja agassizii* (Müller & Henle, 1841) (Chondrichthyes, Rajidae) (Brazilian common name 'Raia-santa') was collected during February and March 2008 in the surf zone of Joaquina

Beach (27° 37' S, 48° 26' W) located on the South Atlantic near the city of Florianópolis, Santa Catarina State, Brazil. After collection, 4 stingrays (30 to 52 cm total length) were transported alive to the laboratory, where they were anaesthetized with MS-222 (Sandoz Laboratories), and necropsied. Smears of fresh gall bladder and bile, urinary bladder, gill, liver and intestine were examined microscopically. Smears of fresh gall bladder contents, the only organ observed to be parasitized, containing free spores and plasmodia were prepared for observation by LM using Nomarski differential interference contrast (DIC) optics, and free spores were measured with an ocular micrometer adapted to the photomicroscope.

Electron microscopy. For SEM, gall bladders were teased apart to release spores. These were fixed in 5% glutaraldehyde buffered in 0.2 M sodium cacodylate (pH 7.4) at 4°C for 20 h, washed in 3 changes of the same buffer, dehydrated in an ascending ethanol series, critical point dried, coated with gold and examined in a JSM-630 SEM operated at 15 kV. After dehydration some spores were observed in a DIC microscope. For TEM, small fragments of the parasitized gall bladder and fluid bile containing plasmodia and free spores were fixed as for the SEM procedure, washed overnight in buffer at 4°C, and post-fixed in 2% osmium tetroxide with the same buffer and at the same temperature for 3 h, dehydrated in an ascending ethanol series followed by propylene oxide, and embedded in Epon. Semithin sections were stained with methylene blue-Azure II for LM, and ultrathin sections were double-stained with uranyl acetate and lead citrate and observed and photographed using a JEOL 100CXII TEM operated at 60 kV.

DNA isolation and PCR amplification. Several plasmodia and spores were preserved in 80% ethanol at 4°C before genomic DNA extraction which was performed using a GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma) following the manufacturer's instructions for animal tissue, except for incubation time. The DNA was stored in 50 µl of TE buffer at –20°C until used. Initial amplification of the SSU rDNA gene was achieved using the universal eukaryotic primers 18e (Hillis & Dixon 1991) and 18r (Whipps et al. 2003). PCR was carried out in 50 µl reactions using 10 pmol of each primer, 10 nmol of each dNTP, 2.5 mM MgCl₂, 5 µl 10× *Taq* polymerase buffer, 1.5 units *Taq* DNA polymerase (Invitrogen), and 5 µl of the genomic DNA. The reactions were run on a Hybaid PxE Thermocycler (Thermo Electron Corporation). The amplification program consisted of 95°C denaturation for 3 min, followed by 35 cycles of 94°C for 45 s, 53°C for 45 s and 72°C for 90 s. A final elongation step was performed at 72°C for 7 min. Nested PCR was done using as template 2 µl of initial PCR: 5'-end with the primers

18e/MyxospecR, the central region of the gene with primers MyxospecF/ChloromyxR1 and finally the 3'-end with the primers ChloromyxF1/18r (Table 1). The amplification program consisted of 95°C denaturation for 5 min, followed by 30 cycles of 95°C for 1 min, 52°C for 1 min and 72°C for 2 min. A final elongation step was performed at 72°C for 10 min. Then 5 µl aliquots of PCR products were electrophoresed through a 1% agarose 1× Tris-acetate-EDTA buffer gel stained with ethidium bromide.

DNA cloning and sequencing. PCR products for the SSU rDNA gene with an approximate size of 300 bp (18e/MyxospecR), 900 bp (MyxospecF/ChloromyxR1) and 600 (ChloromyxF1/18r) were obtained from the excised band. Before cloning, the bands were purified with NucleoSpin Extract II (Macherey-Nagel). DNA was cloned into a pGEM-T Easy Vector System II (Promega) following the manufacturer's instructions. JM109 Competent Cells with high efficiency (Promega) were transformed and then 2 positive clones were selected using the blue-white colour screening method. The minipreps were carried out with a NucleoSpin Plasmid (Macherey-Nagel) according the manufacturer's instructions. The cloned inserts were confirmed by digestion with restriction enzyme *EcoRI* (Promega), and then they were sequenced in both directions with the universal sequencing primers T7 forward/SP6. Sequencing was done using BigDye Terminator v1.1 from the Applied Biosystems Kit, and the sequence reactions were run on an ABI3700 DNA analyzer (Perkin-Elmer Applied Biosystems).

Distance and phylogenetic analysis. To evaluate the relationship of *Chloromyxum riorajum* to other myxosporean species, we used 29 18SSU rDNA sequences, obtained from GenBank data: *Ceratomyxa labracis* (AF411472), *Ceratomyxa sparusaurati* (AF411471), *Ceratomyxa shasta* (AF001579), *Chloromyxum auratum* (AY971521), *Chloromyxum legeri* (AY604197), *Chloromyxum leydigi* (AY604199), *Chloromyxum leydigi* (DQ377710), *Chloromyxum cyprini* (AY604198), *Chloromyxum trijugum* (AY954689), *Chloromyxum truttae* (AJ581916), *Chloromyxum* sp. (AJ581917),

Enteromyxum leei (AF411334), *Enteromyxum scopthalmi* (AF411335), *Henneguya ictaluri* (AF195510), *Henneguya salminicola* (AF031411), *Hoferellus gilsoni* (AJ582062), *Kudoa amamiensis* (AF034638), *Kudoa crumena* (AF378347), *Kudoa diana* (AF414692), *Myxidium lieberkuehni* (X76639), *Myxidium truttae* (AF201374), *Myxidium* sp. (U13829), *Myxobolus bibulatus* (AF378336), *Myxobolus cerebralis* (U96492), *Myxobolus osburni* (AF378338), *Parvicapsula minibicornis* (AF201375), *Raabeia* sp. (AF378352), *Sphaerospora oncorhynchi* (AF201373), and *Zschokkella mugilis* (AF411336). The corresponding sequences and GenBank/NCBI accession number of *Tetracapsuloides bryosalmonae* (U70623) and *Buddenbrockia plumatellae* (AY074915) were used as the outgroup.

Sequences were aligned as described by Azevedo et al. (2006). Alignment was made using Clustal W (Thompson et al. 1994) with MEGA 4 software (Tamura et al. 2007), with an opening gap penalty of 10 and a gap extension penalty of 4 for both pairwise and multiple alignments. Subsequent phylogenetic and molecular evolutionary analyses were conducted using MEGA 4, with the 29 rDNA sequences for myxosporidian species and the outgroup species selected. Distance estimation was carried out using the Kimura 2-parameter model distance matrix for transitions and transversions. For the phylogenetic tree reconstructions, maximum parsimony analysis was conducted using the close neighbour interchange heuristic option with a search factor of 2 and random initial tree additions of 2000 replicates. Bootstrap values were calculated over 100 replicates.

RESULTS

Morphology of the parasite

During a parasitological survey conducted to detect parasites it was observed that some specimens of the stingray *Rioraja agassizii* presented hypertrophy of the gall bladder, the bile of which contained several masses of plasmodia and numerous free spores.

Table 1. Primer sequences and location used to amplify small subunit ribosomal DNA of *Chloromyxum riorajum* sp. nov.

Primers	Sequence (5'-3')	Position	Used with	Source
18e	CTG GTT GAT CCT GCC AGT	1	MyxospecR, 18r	Hillis & Dixon (1991)
MyxospecF	TTC TGC CCT ATC AAC TTG TTG	312	ChloromyxR1	Fiala (2006)
ChloromyxF1	CTT AAA GGA ATT GAC GGA AGG	1209	18r	Present study
MyxospecR	CAA CAA GTT GAT AGG GCA GAA	332	18e	Present study
ChloromyxR1	CCT TCC GTC AAT TCC TTT AAG	1229	MyxospecF	Present study
18r	CTA CGG AAA CCT TGT TAC G	1832	18e, ChloromyxF1	Whipps et al. (2003)

Light microscopy

Numerous plasmodia and free spores were observed immersed in the bile (Figs. 1 to 3). Based on the morphological aspects of the spores and particularities of the spore valves and attached caudal bundle of the tapering filamentous projections forming tails, the parasite was identified as belonging to the genus *Chloromyxum* as classified according to Lom & Dyková (2006). Our results provide a description of a new species:

Phylum Myxozoa Grassé, 1970

Class Myxosporea Bütschli, 1881

Order Bivalvulida Schulman, 1959

Family Chloromyxidae Thélohan, 1892

Genus *Chloromyxum* Mingazzini, 1890

Chloromyxum riorajum sp. nov.

Life history stages observed: All developmental stages in the polysporic plasmodia (up to 150 µm) and free spores immersed in the bile (Figs. 1 to 3).

Description: Cell membrane of the plasmodia and its pseudopodia is covered by numerous microvilli (Figs. 4 & 5). Spores are ellipsoidal to pyriform, 11.41 ± 0.31 µm long in lateral view; 8.48 ± 0.45 µm wide and 5.92 ± 0.54 µm thick ($n = 25$) in apical view (Figs. 1 & 2). Two equal-sized valves with 3 to 4 (rarely 5) surface ridges in the posterior half of the spore. Valves adhering together along a sinuous S-like structure of the sutural line (Figs. 6 & 7). Ridges are located on the last half of the spore and run parallel to the basal portion of the sutural ridges (Figs. 9 to 13). The ridges coalesce towards the apical pole of the spore (Figs. 9 to 11). Each valve consists of a continuous layer of external and internal dense material surrounding a middle lighter area (Figs. 6 to 8). A bundle of 33 to 37 tapering caudal filamentous projections or tails (12.10 ± 0.87 µm long) is attached to the basal part of the last ridge and sutural ridge of the 2 valves (Figs. 1, 2, 10, 12 & 13). There were no visible junctions between the tails and the wall. The tails were formed of the same material as the valves, and had a circular cross-section measuring 0.2 to 0.3 µm in diameter near the valvar insertion (Fig. 18), reducing gradually in diameter towards the end of the tail (Figs. 1, 17 & 19). Four anteriorly pointed equal-sized pyriform polar capsules (3.2 ± 0.4 µm long, 2.0 ± 0.3 µm wide; $n = 25$) were located all on the same level within the spore (Figs. 1 & 2); each contained 6 (rarely 7) obliquely coiled polar filament (Figs. 15 & 20). Sporoplasm was irregular in shape with 2 nuclei randomly distributed within a granular matrix with numerous light areas where the sporoplasmosomes were hardly visible. The spore morphology is presented in schematic drawings (Fig. 20) showing the arrangement of the valvar ridges and caudal bundle of

filaments and the ultrastructural details of the spore longitudinal sections.

Type host: *Rioraja agassizii* (Müller & Henle, 1841) (Chondrichthyes, Rajidae).

Type locality: Joaquina Beach (27° 37' S, 48° 26' W) on the South Atlantic coast situated near the city of Florianópolis, Santa Catarina State, Brazil.

Site of infection: Gall bladder in bile.

Prevalence: 75 % (3/4).

Type specimens: One slide with semi-thin sections of tissues containing spores and developmental stages of hapantotype was deposited in the International Protozoan Type Slide Collection at the Smithsonian Institution Washington, DC, USA, with the acquisition number USNM 1122327. Another slide with semi-thin sections was deposited at the Laboratory of Pathology, Centre for Marine and Environmental Research, University of Porto, Porto, Portugal.

Molecular analysis

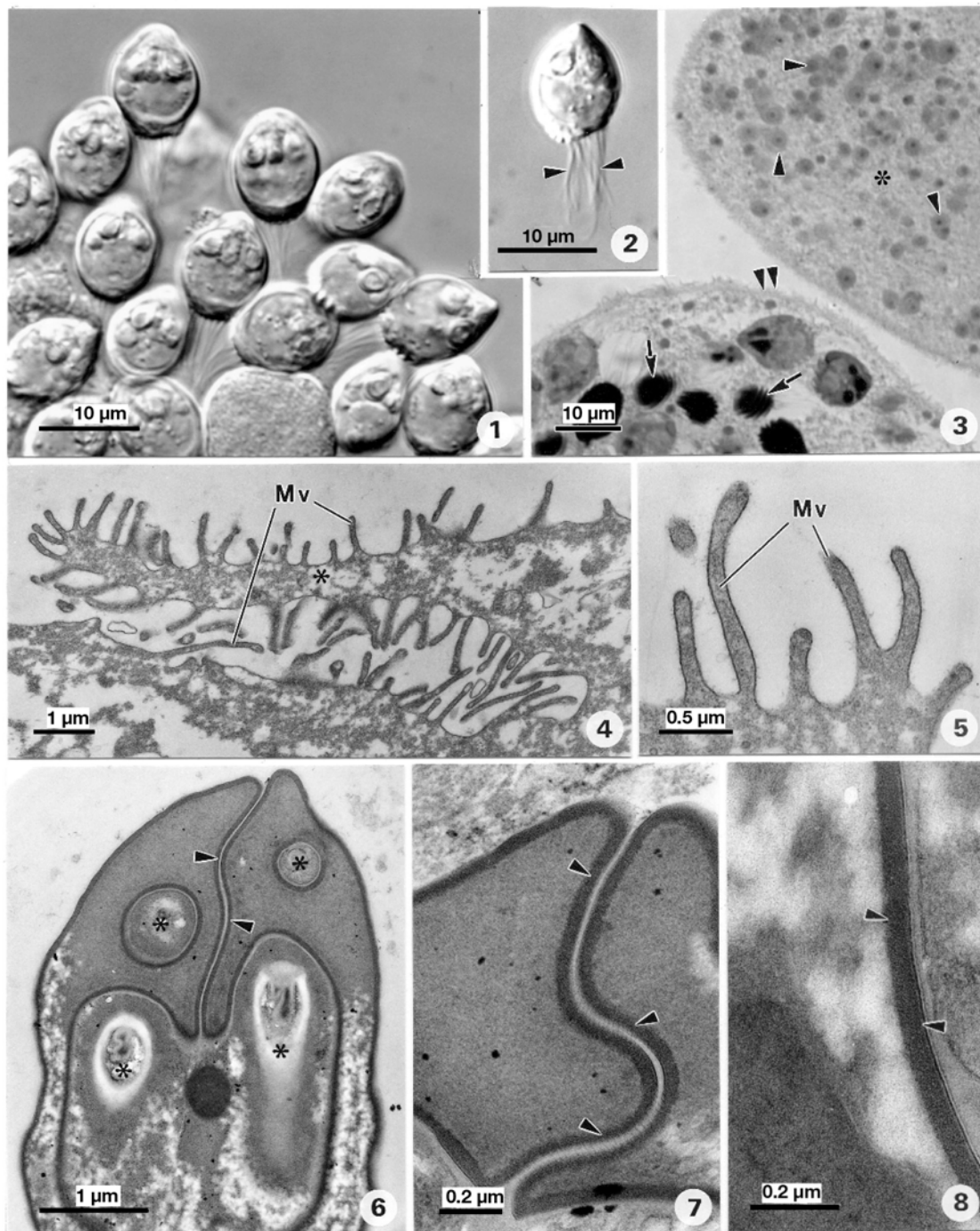
The amplified sequences were assembled, and the resulting consensus DNA sequence of the partial SSU rRNA gene, which was 1807 bp in length, was deposited in GenBank (Accession number FJ624481). In total, 29 SSU rDNA sequences, including those with the highest BLAST scores, were aligned with the *Chloromyxum riorajum* sp. nov. SSU rDNA sequence. The resulting alignment consisted of 1629 positions after trimming the 3'-end (642 ambiguously aligned positions were excluded).

Based on pairwise comparisons among the SSU rDNA sequences, the maximal similarity was observed with all *Chloromyxum* species: *C. leydigi* (97.7 and 97.9%), *C. auratum* (85.4%), *C. cyprini* (85.2%), *C. truttae* (84.6%), *C. trijugum* (83.8%), *C. schurovi* (82.3%) and *C. legeri* (80.9%) (Table 2).

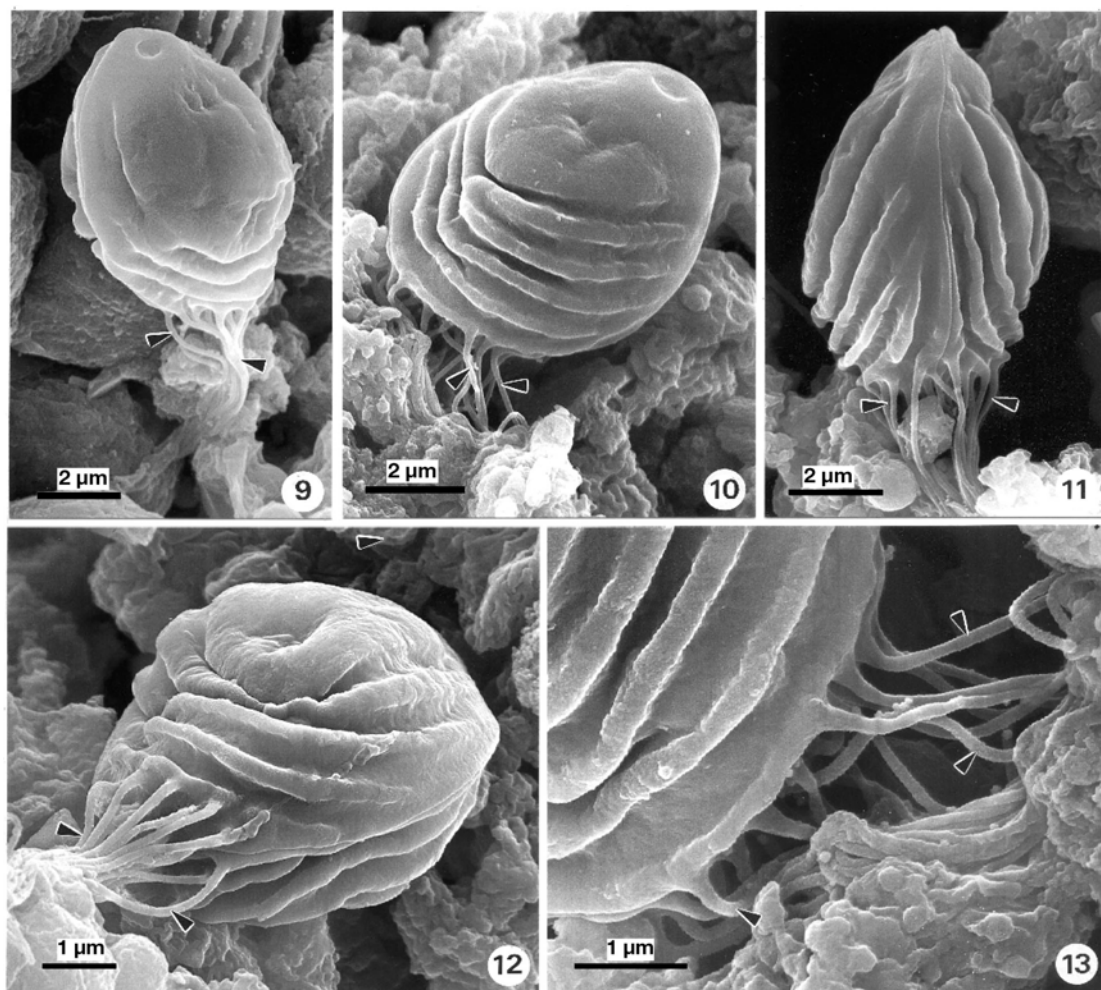
Maximum parsimony analysis of SSU rDNA gene sequence places *Chloromyxum riorajum* sp. nov. within a clade comprising almost all *Chloromyxum* species, with 2 exceptions: *C. legeri* (AY604197) and *C. schurovi* (AJ581917). The most closely related species is *C. leydigi* (AY604199, DQ377710) with 100 % bootstrap support.

DISCUSSION

The morphology of the spores described in the present study, i.e. ellipsoidal spores with 2 shell valves, 4 equal-sized polar capsules and a bundle of caudal filamentous projections, shows the characters of a parasite belonging to the genus *Chloromyxum* (Lom & Noble 1984, Lom & Dyková 2006). A comparison of our results with the morphology and ultrastructural organization



Figs. 1 to 8. Light and transmission electron micrographs of the myxosporean *Chloromyxum riorajum* sp. nov. from gall bladder of *Rioraja agassizii*. Fig. 1. Several unfixed mature spores observed using differential interference contrast (DIC). Fig. 2. A free unfixed mature spore observed under DIC showing a bundle of filamentous tails (arrowheads) attached to the base of one of the spores. Fig. 3. Semithin section showing 2 plasmodia, one of which (*) contains several of the developmental stages (arrowheads); the other one contains spores (arrows). The periphery of the plasmodia shows numerous microvilli (double arrowheads). Fig. 4. Periphery of a plasmodium (*) showing pseudopodia and several microvilli (Mv). Fig. 5. Details of the microvilli (Mv). Fig. 6. Oblique section of the apical end of a spore showing the apical end of the polar capsules (*) and the sutural line (arrowheads). Fig. 7. Detail of the longitudinal section of the apical region of the S-like sutural line (arrowheads). Fig. 8. Detail of the dense internal layer of the valve (arrowheads).



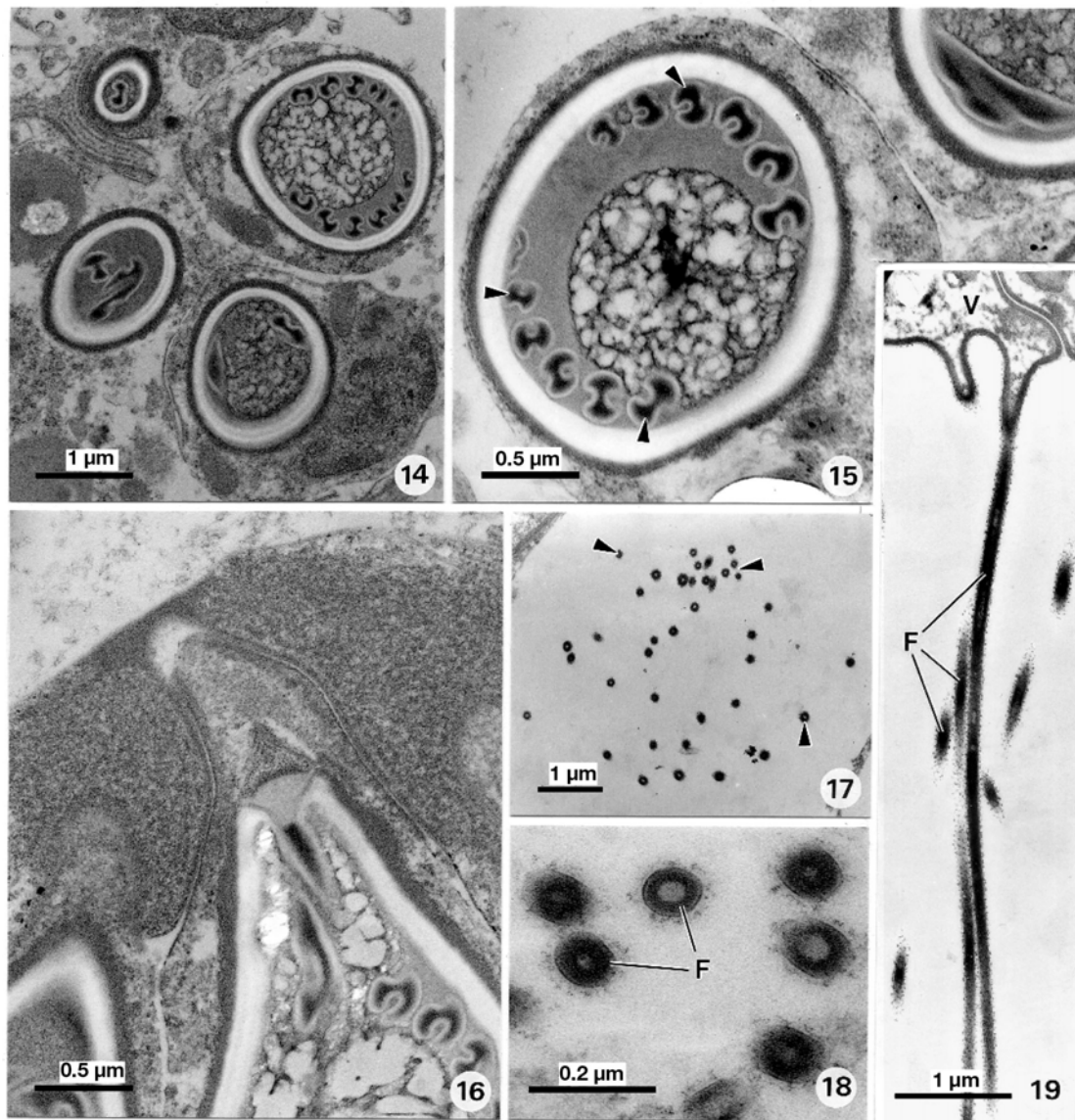
Figs. 9 to 13. Scanning electron micrographs of *Chloromyxum riorajum* sp. nov. spore morphology. Note particularly the bundle of filaments (arrowheads), as well the organization of the ridges

of previously described species of this genus, shows that the morphology is similar, which consequently confirms that this parasite belongs to this genus (Lom & Noble 1984, Lom et al. 1988, Baska 1990, 1993, Molnár 1992, Shul'man & Ieshko 2003, Hallett et al. 2006, Lom & Dyková 1992, 2006), although few of the previously described species have attached caudal filaments.

Amongst the 115 recognized species of this genus, only *Chloromyxum leydigi* Mingazzini, 1890 (Pinto 1928, Gioia & Cordeiro 1996), *C. ovatum* and *C. transversocostatum* (Kuznetsova 1977) have attached filaments (Lom & Dyková 2006). Our species differed from these 3 species, i.e. the *C. leydigi* spore has sutural edge projections at the apex shell valves bearing 7 elevated ridges each; *C. ovatum* has large spores with different patterns of surface ridges, while *C. transversocostatum* differs from these 2 species in that it has a spore with transversal concentric surface ridges.

Recently, Kovaljova (1988) described 4 new species of the genus *Chloromyxum* (*C. dogieli*, *C. lissosporum*, *C. schulmani* and *C. striatellus*) from several cartilaginous fishes captured off the Atlantic coast of Africa. This description was based on diagrammatic drawings. However, it is particularly difficult to compare the present species with the species described in the Kovaljova (1988) study, because their data consisted solely of spore dimensions and drawings showing the distribution and position of the surface ridges. No references were made to the bundle of filaments shown in the drawings in the figures. However, these structures appear to be very different to those of the parasite described here.

The genus *Chloromyxum* has also been previously reported in South American aquatic fauna. Two species described in Brazilian fauna (*C. leydigi* and *C. sphyrynae*) (Gioia & Cordeiro 1996) and another one in the urinary bladder of flatfish *Paralichthys adspersus*



Figs. 14 to 19. Transmission electron micrographs of the myxosporean *Chloromyxum riorajum* sp. nov. from the gall bladder of *Rioraja agassizii*. Fig. 14. Transverse section at the apical region of the 4 polar capsules. Fig. 15. Detail of a transverse section of a polar capsule showing different sections of a polar filament (arrowheads). Fig. 16. Longitudinal section of the apical zone of a polar capsule. Fig. 17. Transverse section of a bundle of 37 filaments (arrowheads). Fig. 18. Transverse sections showing details of several filaments (F). Fig. 19. Longitudinal and oblique sections of several filaments (F), one of which is attached to the valve (V)

from the Pacific coast of the Chile (Oliva et al. 1996) were described by use of LM only and represented by diagrammatic drawings. Recently, *C. menticirrhii* spores, which do not have external filaments attached to the wall, were described on the basis of SEM and TEM studies (Casal et al. 2009).

Morphological aspects, such as the surface structure of the spore, the different patterns of spore ridges and the caudal bundle of 33 to 37 filaments (number never

referred to in other species) which is attached to the more basal ridge and suture line of the *Chloromyxum* spore wall, which differentiate only in some species, are important characteristics which can be used to distinguish *Chloromyxum* species (Lom & Dyková 1993, 2006).

The external organization of the present species is clearly very different from that of all other *Chloromyxum* spp. previously discussed, including those for

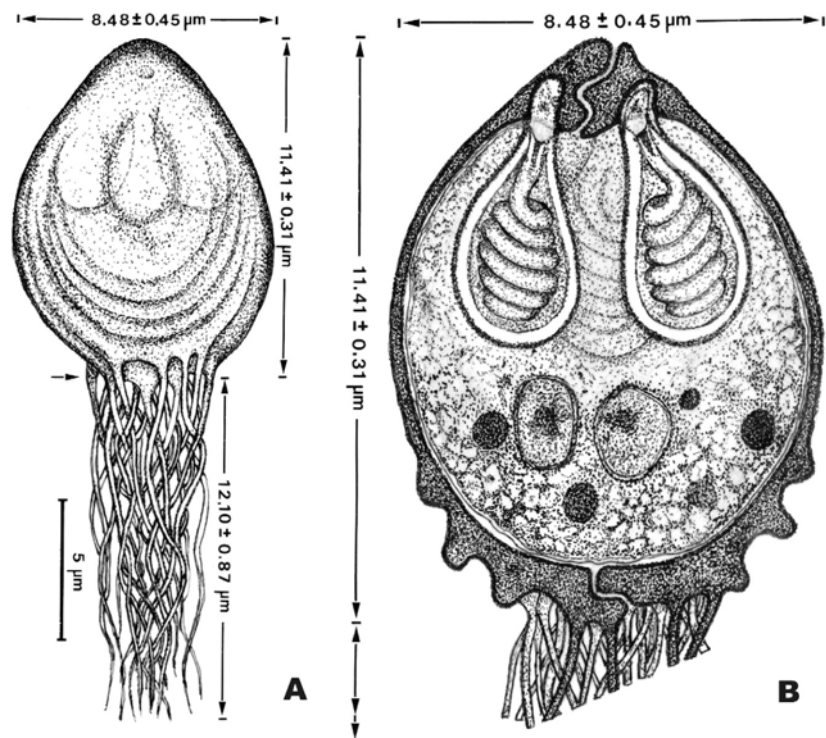


Fig. 20. Spore of *Chloromyxum riorajum* sp. nov. from the gall bladder of *Rioraja agassizii*. (A) Morphological aspect of the spore as observed under differential interference contrast (DIC), showing the basal bundle of filamentous tails attached to the valves. (B) Longitudinal section (frontal view) with special emphasis on the polar capsules and basal bundle of the filamentous tails

which a bundle of filamentous projections and ridge ornamentations on the spore valves have been reported. A comparison of the spore morphology of all *Chloromyxum* spp. which have tailed spores showed that there are similarities between *C. riorajum* and *C. leydigi*. However, *C. riorajum* differs from *C. leydigi* in that the former have larger spores and polar capsules,

as well as different patterns of surface ridges. Moreover, the bundle of filamentous tails of *C. riorajum* differs from that of *C. leydigi*, because it has a large number of filaments and is longer.

All morphological and ultrastructural aspects are useful for the description of a new species despite the fact that of some them such as caudal appendages are

Table 2. Comparison of some small subunit ribosomal (SSU) rDNA sequences: percentage of identity (above diagonal) and pairwise distance (below diagonal) obtained by Kimura 2-parameter analysis. *C.*: *Chloromyxum*; *M.*: *Myxidium*; *S.*: *Sphaerospora*

	<i>C.</i> <i>riorajum</i>	<i>C.</i> <i>leydigi</i> 1	<i>C.</i> <i>leydigi</i> 2	<i>C.</i> <i>auratum</i>	<i>C.</i> <i>cyprini</i>	<i>C.</i> <i>truttae</i>	<i>C.</i> <i>trijugum</i>	<i>C.</i> <i>schurovi</i>	<i>C.</i> <i>legeri</i>	<i>M.</i> <i>truttae</i>	<i>Myxidium</i> sp.	<i>S.</i> <i>oncorhynchi</i>
<i>C. riorajum</i> (FJ624481)		97.9	97.7	85.4	85.2	84.6	83.8	82.3	80.9	82.8	81.9	81.7
<i>C. leydigi</i> 1 (DQ377710)	0.021		99.4	86.0	85.7	85.2	84.3	82.6	81.2	83.3	82.5	81.9
<i>C. leydigi</i> 2 (AY604199)	0.023	0.004		85.9	85.6	85.2	84.3	82.6	81.1	83.3	82.5	81.9
<i>C. auratum</i> (AY971521)	0.146	0.140	0.141		99.8	97.7	91.8	91.8	88.4	91.3	91.3	92.3
<i>C. cyprini</i> (AY604198)	0.148	0.143	0.143	0.002		97.4	91.6	91.6	88.1	90.9	90.9	92.0
<i>C. truttae</i> (AJ581916)	0.154	0.148	0.148	0.023	0.026		92.1	91.6	88.6	90.9	90.9	92.0
<i>C. trijugum</i> (AY954689)	0.162	0.157	0.157	0.082	0.084	0.079		90.5	86.2	95.5	95.9	89.9
<i>C. schurovi</i> (AJ581917)	0.177	0.174	0.174	0.082	0.084	0.084	0.105		87.9	88.5	88.5	92.3
<i>C. legeri</i> (AY604197)	0.191	0.188	0.189	0.116	0.119	0.114	0.138	0.121		85.8	84.8	89.9
<i>M. truttae</i> (AF201374)	0.172	0.167	0.167	0.087	0.089	0.089	0.045	0.115	0.142		97.7	87.6
<i>Myxidium</i> sp. (U13829)	0.181	0.175	0.175	0.087	0.089	0.089	0.041	0.115	0.152	0.023		88.1
<i>S. oncorhynchi</i> (AF201373)	0.183	0.181	0.181	0.077	0.080	0.080	0.101	0.077	0.101	0.124	0.119	

not a phylogenetic character. Unfortunately, with the exception of *Chloromyxum leydigi*, there is no SSU rDNA information for the other *Chloromyxum* species with caudal appendages. Apparently, the habitat, the host and the location of infection are the characteristics that have been used for species classification.

A BLAST search using SSU rDNA sequence data found only 7 *Chloromyxum* spp. available in GenBank: All species, except *C. leydigi* (AY604199) isolated from the gall bladder of *Torpedo marmorata* caught in the Mediterranean Sea (Fiala & Dyková 2004) and *C. leydigi* (DQ377710) from the gall bladder of *Centroscymnus coelolepis* caught in the North Atlantic (Fiala 2006), infect freshwater fishes. *C. cyprini* (AY604198) and *C.*

legeri (AY604197) were found in the gall bladder of *Hypophthalmichthys molitrix* and *Cyprinus carpio*, respectively, from the Czech Republic (Fiala & Dyková 2004). *C. auratum* (AY971521) and *C. trijugum* (AY954689) were found in the gall bladder of *Carassius auratus* and *Pomoxis nigromaculatus*, respectively, from Oregon, USA (Hallett et al. 2006). *C. truttae* (AJ581916) was found in the gall bladder epithelium and *C. schurovi* (AJ581917) in kidney tubules of *Salmo salar* in Scotland (Holzer et al. 2004). We obtained an almost complete SSU rRNA gene sequence with 1807 bp.

Our analysis of the phylogenetic relationship for maximum parsimony is in concordance with previous cladograms (Fiala & Dyková 2004, Fiala 2006, Holzer et

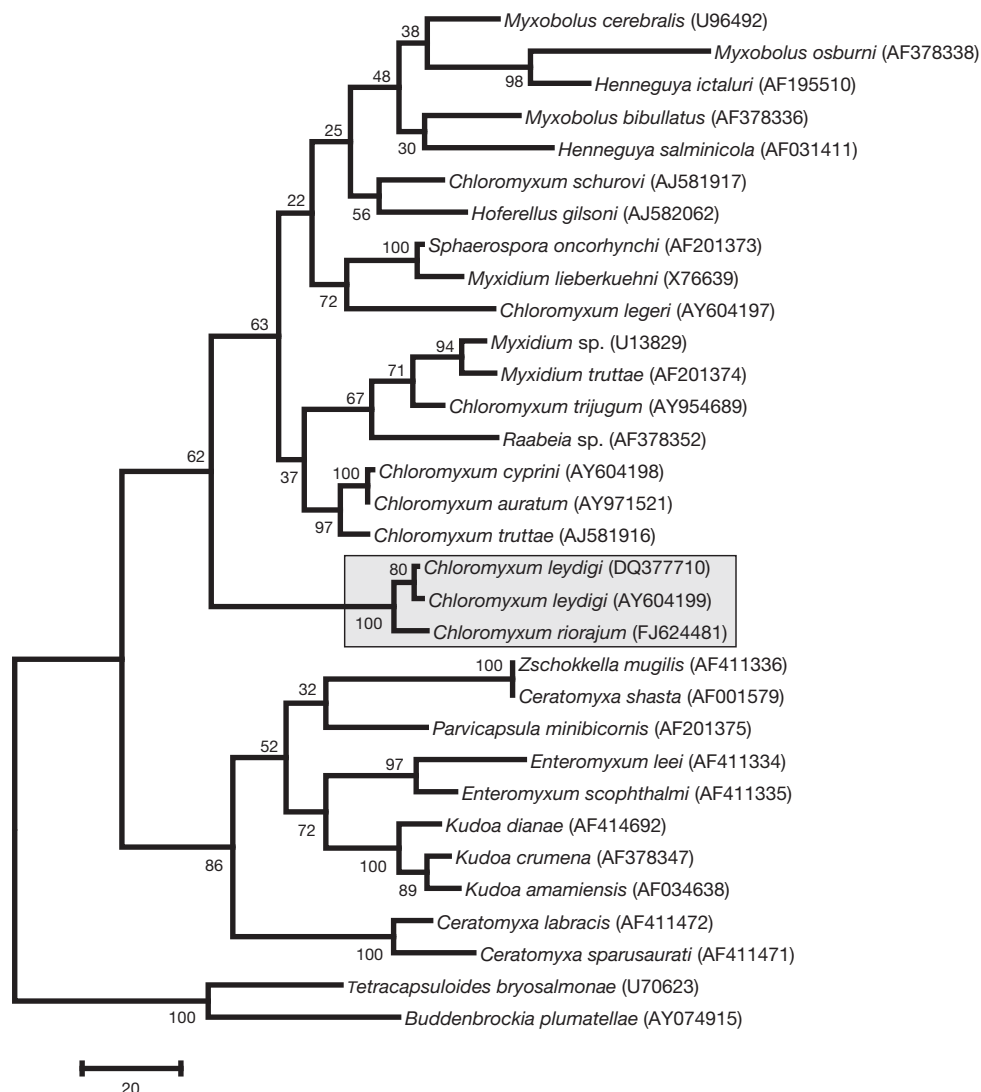


Fig. 21. Maximum parsimony tree of small subunit ribosomal (SSU) rDNA sequences of *Chloromyxum riorajum* sp. nov. and other selected myxosporean species. Numbers on the branches are bootstrap confidence levels on 100 replicates. GenBank accession numbers in parentheses after the species names; scale is given under the tree. *C. riorajum* and *C. leydigi* are placed in the basal clade with several freshwater myxosporean species (shaded box)

al. 2006). The bootstrap for the 2 *Chloromyxum* species both found in the gall gladder of cartilaginous fishes, *C. leydigi* and *Chloromyxum* sp. described here, is 100% supported, and the pairwise sequence analyses presented 97.7 to 97.9% similarity (Table 2).

Most genera present in the myxosporean SSU rDNA tree are poly/paraphyletic (Kent et al. 2001). Presently, 8 sequences of *Chloromyxum* species are known, and the data suggest that they are paraphyletic groups. Monophyly was only observed in some freshwater *Chloromyxum* species, *C. auratum*, *C. cyprini*, and *C. truttae*, and were supported by a bootstrap value of 94%. Previously phylogenetic analysis clearly shows a division into 2 clades, namely freshwater and marine species (Kent et al. 2001). One possible justification for this is the presence of numerous insertions in the V7 region of the SSU rDNA of all freshwater myxosporeans which have longer sequences than marine species. The exception are *C. leydigi* (Fiala & Dyková 2004, Holzer et al. 2006) and the species we describe, *C. riorajum* sp. nov. These 2 species have marine cartilaginous fishes as hosts and do not have insertions near the 3'-end of the SSU rDNA gene, but they are clustered with freshwater myxosporeans. For this reason, they are basal species in the freshwater myxosporean clade (Fiala & Dyková 2004).

Additional parsimony analyses of SSU rDNA gene sequences support a close relationship between the different *Chloromyxum* species, as well as a very good bootstrap (100%) for the clade to which *C. leydigi* and *C. riorajum* belong (Fig. 21). In conclusion, molecular phylogenetic analysis reinforced by morphological and ultrastructural data and specificity of the host suggest that the parasite from *Rioraja agassizii* is a new species, named *Chloromyxum riorajum* sp. nov.

Acknowledgements. This work was partially supported by the Engº. A. Almeida Foundation (Porto, Portugal), PhD grant from 'CESPU' (to G.C.), 'CNPq' and 'CAPES'-Brazil. We thank Prof. M. Martins (UFSC-Florianópolis) for use of the facilities in his laboratory and the technical assistance of G. Ribeiro MSc (NEMAR—Florianópolis-Brazil) and J. Carneiro (ICBAS/UP). This work complies with the current laws of the countries in which it was performed. The helpful comments and suggestions of the anonymous reviewers in reviewing this manuscript are greatly appreciated.

LITERATURE CITED

- Azevedo C, Balseiro P, Casal G, Gestal C and others (2006) Ultrastructural and molecular characterization of *Haplosporidium montforti* sp. nov., parasite of the European abalone *Haliotis tuberculata*. J Invertebr Pathol 92:23–32
- Baska F (1990) *Chloromyxum inexpectatum* sp. nov. and *Sphaerospora colomani* sp. nov. (Myxozoa, Myxosporea), parasites of the urinary system of the starlet, *Acipenser ruthenus*. Syst Parasitol 16:185–193
- Baska F (1993) Light and electron microscopic studies on the development of *Sphaerospora colomani* Baska, 1990 and *Chloromyxum inexpectatum* Baska, 1990. Acta Vet Hung 41:59–72
- Casal G, Garcia P, Matos P, Monteiro E, Matos P, Azevedo C (2009) Fine structure of *Chloromyxum menticirrhii* sp. nov. (Myxozoa) infecting the urinary bladder of the marine teleost *Menticirrhus americanus* (Sciaenidae) in southern Brazil. Eur J Protistol 45:139–146
- Cellere EF, Cordeiro N, Adriano ES (2002) *Myxobolus absonus* sp. n. (Myxozoa: Myxosporea) parasitizing *Pimelodus maculatus* (Siluriformes: Pimelodidae), a South American freshwater fish. Mem Inst Oswaldo Cruz 97: 79–80
- Cunha AM, Fonseca O (1918) Sobre os mixosporídios dos peixes brasileiros. Bras Med 32:393
- Duncan AE, Garner MM, Bartholomew JL, Reichard TA, Nordhausen RW (2004) Renal myxosporidiasis in Asian horned frog (*Megophrys nasuta*). J Zoo Wildl Med 35:381–386
- Fiala I (2006) The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. Int J Parasitol 36:1521–1534
- Fiala I, Dyková I (2004) The phylogeny of marine and freshwater species of the genus *Chloromyxum* Mingazzini, 1890 (Myxosporea: Bivalvulida) based on small subunit ribosomal RNA gene sequences. Folia Parasitol 51: 211–214
- Gioia I, Cordeiro NS (1996) Brazilian Myxosporidians checklist (Myxozoa). Acta Parasitol 35:137–149
- Hallett SL, Atkinson SD, Holt RA, Banner CR, Bartholomew JL (2006) A new myxozoan from feral goldfish (*Carassius auratus*). J Parasitol 92:357–363
- Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66: 411–453
- Holzer AS, Sommerville C, Wooden R (2004) Molecular relationships and phylogeny in a community of myxosporeans and actinosporians based on their 18S rDNA sequences. Int J Parasitol 34:1099–1111
- Holzer AS, Sommerville C, Wooden R (2006) Molecular identity, phylogeny and life cycle of *Chloromyxum schurovi* Shul'man and Ieshko, 2003. Parasitol Res 99:90–96
- Kent ML, Andree KB, Bartholomew JL, El-Matbouli M and others (2001) Recent advances in our knowledge of the Myxozoa. J Eukaryot Microbiol 48:395–413
- Kovaljova AA (1988) Myxosporidia of the genus *Chloromyxum* (Cnidospora, Myxosporea) of cartilaginous fishes from the Atlantic coast of Africa. Parazitologiya 22:384–388 (in Russian with English summary)
- Kuznetsova IG (1977) Myxosporidians of Chondrostei from the Patagonian shelf. Parazitologiya 11:74–77
- Lom J, Dyková I (1992) Myxosporidia (Phylum Myxozoa). In: Lom J, Dyková I (eds) Protozoan parasites of fishes. Elsevier, Amsterdam
- Lom J, Dyková I (1993) Scanning electron microscopic revision of common species of the genus *Chloromyxum* (Myxozoa: Myxosporea) infecting European freshwater fishes. Folia Parasitol 40:161–174
- Lom J, Dyková I (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol 43:1–36
- Lom J, Noble ER (1984) Revised classification of the class Myxosporea Bütschli, 1881. Folia Parasitol 31:193–205
- Lom J, Dyková I, Kepr T (1988) Species of the genus *Chloromyxum* Mingazzini (Myxozoa: Myxosporea) infecting burbot (*Lota lota* L.). Syst Parasitol 11:231–237

- Molnár K (1992) *Ceratomyxa hungarica* sp. nov. and *Chloromyxum proterorhini* sp. nov. (Myxozoa, Myxosporea) from the freshwater gobi *Proterorhinus marmoratus* (Pallas). Syst Parasitol 22:25–31
- Oliva ME, Castro RE, Burgos R (1996) Parasites of the flatfish *Paralichthys adspersus* (Steindachner, 1867) (Pleuronectiformes) from Northern Chile. Mem Inst Oswaldo Cruz 91: 301–306
- Pinto C (1928) Mixosporídeos e outros protozoários intestinais observados na América do Sul. Arch Inst Biol 1: 101–126
- Shul'man BS, Ieshko EP (2003) *Chloromyxum schurovi* sp. n. — a new myxosporidian species (Myxosporea: Sphaerosporidae) from salmonid fishes (Salmonidae). Parazitologiya 37:246–247
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
- Thompson JD, Higgins DG, Gilson TJ (1994) Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
- Whipps CM, Adlard RD, Bryant MS, Lester RJG, Findlay V, Kent ML (2003) First report of three *Kudoa* species from Eastern Australia: *Kudoa thyrsites* from Mahi mahi (*Coryphaena hippurus*), *Kudoa amamiensis* and *Kudoa minithyrsites* sp. nov. from sweeper (*Pempheris ypsilychnus*). J Eukaryot Microbiol 50:215–219

Editorial responsibility: Sven Klimpel,
Düsseldorf, Germany

Submitted: January 15, 2009; Accepted: April 7, 2009
Proofs received from author(s): May 20, 2009

PARTE IV

CONSIDERAÇÕES GERAIS E CONCLUSÕES FINAIS

Capítulo 14

14.1. Considerações Gerais

A classificação dos microparasitas tem sido, muitas vezes, baseada unicamente na descrição da morfologia do esporo, principalmente no grupo dos mixosporídios. Vários estudos indicam não ser este o tipo de abordagem mais correcta, dado que dentro da mesma espécie podem existir variações morfológicas para as diferentes idades dos parasitas. Também a variabilidade do hospedeiro deve ser tida em conta. Por outro lado, é igualmente notório, que espécies biologicamente diferentes podem ser muito semelhantes em morfologia. Na grande maioria das espécies de mixosporídios, para além do esporo, foram caracterizados poucos aspectos inerentes ao ciclo de vida. Já os microsporídios, dificilmente, são classificados apenas com base na morfologia e ultraestrutura do esporo.

A especificidade do hospedeiro tem sido referida como um factor importante, por vezes subestimado em taxonomia, havendo mesmo quem refira que a determinação do hospedeiro definitivo (invertebrado) dos mixosporídios é importante para a correcta classificação do taxon. Pensa-se que a análise de modelos de evolução, baseada no hospedeiro definitivo, pode reflectir modelos de evolução mais correctos, comparativamente aos utilizados em hospedeiros intermediários (peixes). Infelizmente, para que se possa comprovar esta teoria, necessitam de ser determinados muitos dos ciclos de vida dos Myxozoa.

Na última década, paralelamente às observações microscópicas, têm sido feitos esforços para caracterizar o grupo dos microsporídios e mixosporídios através de dados fornecidos pela sequenciação de genes conservados, tais como o SSU e LSU rDNA. Está provado que as análises moleculares em muito têm contribuído para o conhecimento actual destes grupos de parasitas. Muitas das análises filogenéticas, baseadas nos caracteres moleculares diferem substancialmente das classificações morfológicas ao nível dos géneros, tendo estes sido sofrivelmente descritos, em muitos casos, através de desenhos esquemáticos.

Em 2003, Tauz e colaboradores chegaram mesmo a propor o abandono das classificações morfológicas e esquemáticas, em favor de uma taxonomia baseada exclusivamente em sequências de DNA. Efectivamente, a aposta no crescente aumento de informação genética muito tem contribuído para a identificação do taxon ao nível do género e espécie e, consequentemente, para propor pistas, de modo a que possamos

compreender a biologia destes parasitas e quais os seus caracteres, que estão na base da divergência filogenética.

Na nossa opinião, a classificação de qualquer grupo de organismos não deve ser baseada numa única característica, mas antes tendo em consideração a combinação de vários factores, tais como o habitat, especificidade do hospedeiro, local de infecção, interacção com as células hospedeiras, características morfológicas e detalhes ultrastruturais do ciclo de vida do parasita, bem como a análise de sequências moleculares e, consequentemente, as inferências filogenéticas que se possam obter.

Durante a execução deste trabalho, foram diagnosticados, em ambas as faunas e habitats, vários parasitas em diversos tecidos e órgãos, totalizando 13 microsporídios (anexo 1) e 21 mixosporídios (anexo 2). Até à presente data, a análise dos resultados e discussão dos mesmos permitiu redigir 12 artigos científicos (10 publicados, 1 em revisão, 1 submetido) em revistas indexadas de divulgação internacional, que são apresentados, nesta tese, sob a forma de capítulos.

Assim, na **Parte II** descrevemos, com base na ultraestrutura dos diferentes estádios do ciclo de vida do parasita, 1 novo género, *Potasporea*, e 4 novas espécies de microsporídios, todas ocorrendo na fauna brasileira: *Potasporea morhaphis*, *Loma psittaca*, *Microsporidium rondoni* e *Spraguea gastrophysus*. Geralmente, neste grupo de parasitas, dados baseados unicamente em aspectos morfológicos ou ultrastruturais da esporogénese tardia não facilitam a sua classificação. Nesse sentido, procurámos fazer, paralelamente, uma análise filogenética com os genes para os SSU e LSU rRNA.

Em relação às mixosporidioses diagnosticadas (**Parte III**), 7 novas espécies, *Chloromyxum menticirrho*, *Chloromyxum riorajum*, *Myxobolus maculatus*, *Myxobolus metynnis*, *Henneguya friderici*, *Henneguya rondoni* e *Kudoa aequidens*, todas ocorrendo na fauna aquática brasileira, foram descritas morfológica e ultrastruturalmente. Fez-se igualmente uma caracterização ultrastrutural do mixosporídio *Ceratomyxa tenuispora*, parasita do peixe-espada capturado na costa da Ilha da Madeira. Apenas para a espécie *C. riorajum* foram realizadas análises moleculares e filogenéticas.

Alguns dos resultados obtidos no decurso da realização desta tese (anexos 1 & 2), não foram apresentados e discutidos em nenhum dos capítulos, por motivos variados. Entre eles, incluem-se a existência de dados parciais relativos aos aspectos ultrastruturais do ciclo de vida e/ou a ausência de informação molecular, inviabilizando a classificação ao nível da espécie, no caso de algumas microsporidioses. Não obstante, entendeu-se que seria pertinente incluir neste capítulo, dedicado às considerações gerais e conclusões,

todos os dados moleculares e filogenéticos obtidos (anexos 3, 4 & 5), de modo a permitir que a discussão da mesma seja feita de uma forma mais ampla e conclusiva.

14.2. Conclusões finais

- De acordo com referências prévias na literatura, os peixes são organismos susceptíveis de serem parasitados por vários organismos. Nesta tese, em algumas das espécies analisadas - *Gymnorhamphichthis rondomi* (Ituí transparente), *Colomesus psittacus* (Baiacú), *Trachinotus coralinus* (Pampo), *Aequidens plagiozonatus* (Cará pixuna), *Trisopterus luscus* (Faneca) e *Gaidropsarus vulgaris* (Lulão) - foram observados, simultaneamente, microsporídios e mixosporídios, muitas vezes no mesmo tecido/órgão.
- Nesta tese foram, pela primeira vez, obtidos dados referentes à sequenciação de genes conservados para os SSU e LSU rRNAs, nomeadamente de vários microsporídios provenientes da ictiofauna capturada em águas continentais portuguesas e brasileiras. No futuro, a sequenciação do rDNA para mais microsporídios, bem como os genes de proteínas, irá permitir investigar se a área geográfica é ou não um factor determinante nas relações filogenéticas entre os organismos pertencentes ao mesmo taxon (p. e. género, família), à semelhança do que acontece com os mixosporídios (Fiala 2006). Por outro lado, a sequenciação de genes conservados poderá ajudar a determinar se existem hospedeiros definitivos em peixes, tal como sucede com os mixosporídios.
- Para muitas das descrições de novos taxa (espécies, géneros) ou reclassificações de espécies já existentes, aparentemente parece bastar uma análise dos dados obtidos através da microscopia de luz e da sequenciação de genes ribossomais. Pelo que se pode constatar, durante a realização desta tese, este tipo de abordagem nem sempre foi possível, nomeadamente para o grupo dos microsporídios. Regra geral, este grupo de parasitas carece de uma conveniente caracterização ultrastrutural dos aspectos inerentes às fases merogónicas, esporogónicas, organização estrutural do xenoma, para que possam ser devidamente classificados ao nível da família, género e espécie.
- Em todos os cladogramas elaborados para os microsporídios de peixes verificou-se um baixo “bootstrap” da espécie *L. acerinae*, juntamente com a espécie recém criada *L. psittaca* (FJ843104), com as restantes espécies do grupo 1. A percentagem de identidade destas 2 espécies é maior quando comparada com a das espécies do género *Glugea*. Infelizmente, as observações ultrastruturais efectuadas em *L. psittaca* não evidenciaram caracteres morfológicos significativos que permitissem diferenciar estas duas espécies dos géneros *Loma* e *Glugea* e, assim, caracterizar um novo género, de acordo com a sugestão proposta por Lom e Nilsen (2003).

- Sete das sequências referentes ao gene para o SSU rRNA, obtidas no decurso da execução dos trabalhos desta tese, dizem respeito a microsporídios posicionados no **grupo 4** das árvores filogenéticas: *Potasporea morhaphis* (EU534408), *Potasporea* 1 sp. (hospedeiro: Cará pixuna), *Spraguea gastrophysus* (GQ868443), *Microsporidium rondoni* (FJ843105), *Microgemma* 1 sp. (hospedeiro: Pampo), *Tetramicra* 1 sp. (Faneca) e *Tetramicra* 2 sp. (Lulão). Os dados morfológicos e ultrastruturais para este grupo de parasitas sequenciados demonstram haver várias características genéricas comuns, nomeadamente nenhum possuir o núcleo em diplocário e não diferenciar vacúolos parasitóforos. As espécies deste grupo demonstram também algumas afinidades filogenéticas em função do habitat. Sendo um grupo maioritariamente marinho, não é de estranhar, nos cladogramas, o posicionamento basal do grupo formado por duas espécies de água doce pertencentes ao género *Potasporea*. E por último, o microsporídio *Microsporidium rondoni*, classificado provisoriamente no grupo colectivo, apresenta muitas semelhanças ultrastruturais com as espécies do género *Kabatana*, apesar das análises filogenéticas não definirem, com clareza, com qual espécie ou espécies tem afinidade. No entanto, vários indícios apontam para que seja uma nova espécie *Kabatana*, dado que o grupo é parafilético, composto por espécies marinhas e de água doce, com afinidade para se diferenciarem no tecido muscular esquelético.
- Em relação à sequenciação dos rDNAs de duas espécies que diferenciam simultaneamente macrosporos e microsporos, estas foram classificadas como pertencentes aos géneros *Pleistophora* (*Microsporidium brevirostris*) e *Pleistophora* (hospedeiro: Ituí tuanga). As análises filogenéticas, uma vez mais, comprovam as evidências ultrastruturais, visto que o “bootstrap” do **grupo 3**, grupo composto por espécies dos géneros *Pleistophora*, *Heterosporis* e *Ovipleistophora*, é elevado.
- Relativamente às mixosporidioses descritas nesta tese (**Parte III**), somente a espécie *Chloromyxum riorajum* foi, simultaneamente, caracterizada por análises ultrastruturais e filogenéticas. A sequenciação do gene para o SSU rRNA permitiu corroborar as análises filogenéticas previamente efectuadas por Kent e colaboradores (2001), Fiala e Dyková (2004) e Fiala (2006) onde o seu posicionamento basal dentro do clado dos peixes de água doce é facilmente explicado, por ter como hospedeiro um peixe cartilágíneo marinho. A sequência para o gene SSU rDNA da espécie *Kudoa* sp., que ocorre em *Trisopterus luscus* (faneca), foi igualmente obtida. Esta espécie forma um clado com a espécie *Kudoa neurophila*.
- Ao caracterizar as mixosporidioses identificadas, descrevemos vários pormenores ultrastruturais: a estrutura do revestimento em torno das valvas e projecções; a estrutura dos esporoplasmosomas; a diferenciação da cápsula polar, bem como as interacções do

parasita-hospedeiro. Assim, verificou-se, na superfície externa das valvas e das projecções caudais dos esporos de alguns mixosporídios, a presença de material aderente de diferente natureza. Em *H. rondoni* um revestimento homogéneo hialino cobre ambas as superfícies, enquanto que na espécie *M. metynnis* foi descrita a presença de microfibrilas anastomosadas aderentes à superfície. Possivelmente, a presença de revestimentos confere alguma protecção aos efeitos fagocíticos, que as células hospedeiras possam infringir.

- Em algumas espécies, os esporoplasmosomas, foram observados e caracterizados: *H. friderici* possui vesículas em forma de gota sendo externamente revestidas por material electrodense, em *M. metynnis* foi descrita uma estrutura densa e excêntrica em forma de meia-lua aderente à vesícula, enquanto as da espécie *Ceratomyxa tenuispora* são arredondadas, com uma matriz de conteúdo moderadamente electrodense e homogéneo. Presentemente, nos mixosporídios, desconhece-se a função destas vesículas electrodensas.

- Na diferenciação do primórdio capsular da espécie *M. maculatus* observaram-se diferentes graus de condensação da matriz, disposta em várias camadas. No final da esporogénese, foram descritos, igualmente, feixes de tubulina, agregados ou dispersos na matriz capsular. Curiosamente, na espécie *C. tenuispora*, foram descritas centenas de microtúbulos agregados em vários feixes com diferentes organizações. A presença de tubulina e de microtúbulos nas células capsulogénicas sugere que estão envolvidas no mecanismo que força a inversão do tubo externo para dentro do primórdio capsular e, possivelmente, que assumem também um papel importante durante a extrusão do filamento polar, permitindo a fixação do espora às células hospedeiras.

- Nesta tese, ao descrever 5 parasitas histozóicos (*M. maculatus*, *M. metynnis*, *H. friderici*, *H. rondoni* e *Kudoa aequidens*) e 3 coelozóicos (*C. menticirrho*, *C. riorajum*, *C. tenuispora*), foram caracterizados alguns aspectos ultrastruturais relativos à interface parasita-hospedeiro, que correlacionam o tipo de nutrição do parasita. Expansões citoplasmáticas no lado externo da membrana plasmodial foram descritas nas 3 espécies coelozóicas, enquanto que na espécie histozóica, *M. maculatus*, se observaram plasmódios polispóricos delimitados por dupla membrana diferenciando a membrana interna e vários canais pinocíticos. Em *M. metynnis* verificou-se a formação de pequenas microvilosidades à superfície da membrana plasmodial.

14.3. Perspectivas para futuras investigações

O estudo e descrição de novas espécies de microsporídios e mixosporídios, através da caracterização nas vertentes morfológica, ultrastrutural, molecular e filogenética em ictiofaunas, para as quais são escassas as referências na literatura, permitirão incrementar o conhecimento actual destes grupos de parasitas. Futuramente, estes resultados irão contribuir para um conhecimento mais amplo das relações filogenéticas, bem como na definição de caracteres mais específicos para cada taxon, facilitando a classificação taxonómica dos grupos.

Após a conclusão desta tese, gostaríamos de dar continuidade a este estudo, descrevendo e publicando algumas das restantes parasitoses, cujos estudos já foram iniciados, dando prioridade às espécies diagnosticadas na ictiofauna portuguesa.

Referências

- Fiala, I. (2006) The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. *Intern. J. Parasitol.* 36:1521-1534.
- Fiala, I. & Dyková, I. (2004) The phylogeny of marine and freshwater species of the genus *Chloromyxum* Mingazzini, 1890 (Myxosporea: Bivalvulida) based on small subunit ribosomal RNA gene sequences. *Folia Parasitol.* 51: 211-214.
- Kent, M.L., Andree, K.B., Bartholomew, J.L., El-Matbouli, M., Dessler, S.S., Devlin, R.H., Feist, S.W., Hedrick, R.P., Hoffmann, R.W., Khattra, J., Hallett, S.L., Lester, R.J.G., Longshaw, M., Palenzeula, O., Siddall, M.E. & Xiao, C. (2001) Recent advances in our understanding of the Myxozoa. *J. Euk Microbiol.* 48: 395-413.
- Lom, J. & Nilsen, F. (2003) Fish microsporidia: fine structural diversity and phylogeny. *Int. J. Parasitol.* 33: 107-127.
- Tautz, D., Arctander, P., Minelli, A., Thomas, R.H. & Vogler, A.P. (2003) A plea for DNA taxonomy. *Trends Ecol. Evol.* 18: 70-74.

Anexo 1 - Listagem das microsporidioses diagnosticadas em hospedeiros da ictiofauna portuguesa e brasileira

Hospedeiro (nome vulgar)	Género / Espécie	Local de infecção	Proveniência (Estado e País)	Habitat	Morfologia e ultraestrutura	Biologia Molecular (genes do rDNA sequenciados)	Capítulo Referência bibliográfica
<i>Gaidropsarus vulgaris</i> (Lulão)	<i>Microsporidium</i> sp.	Músculo esquelético	Região Norte, Portugal	M	Estádios tardios	SSU + ITS + LSU	*
<i>Diplodus annularis</i> (Sargo)	<i>Loma</i> sp.	Filamentos brânquias	Região Norte, Portugal	M	Estádios tardios	—	**
<i>Boops boops</i> (Boga)	<i>Microsporidium</i> sp.	Fígado	Região Norte, Portugal	M	Estádios tardios	—	**
<i>Trisopterus luscus</i> (Faneca)	<i>Microsporidium</i> sp.	Músculo esquelético	Região Norte, Portugal	M	Estádios tardios	SSU + ITS + LSU	*
<i>Potamorhaphis guianensis</i> (Agulha)	<i>Potaspora morhaphis</i> n. gen. n. sp.	Cavidade celômica	Pará, Brasil	D	Todos os estádios	SSU + ITS + LSU	Cap. 2 - Casal <i>et al.</i> (2008) <i>Parasitology</i> 135: 1053-1064.
<i>Colomesus psittacus</i> (Baiaçú)	<i>Loma psittaca</i> n. sp.	Mucosa intestinal	Pará, Brasil	D	Estádios tardios	SSU	Cap. 3 - Casal <i>et al.</i> (2009) <i>Res. Parasitol.</i> (in press)
<i>Gymnorhamphichthys rondoni</i> (Ituí transparente)	<i>Microsporidium rondoni</i> n. sp.	Músculo esquelético	Pará, Brasil	D	Todos os estádios	SSU + ITS + LSU	Capítulo 4
<i>Lophius gastrophysus</i> (Tamboril)	<i>Spraguea gastrophysus</i> n. sp.	Tecido muscular da cavidade abdominal	Rio de Janeiro, Brasil	M	Estádios tardios	SSU + ITS + LSU	Capítulo 5
<i>Trachinotus coralinus</i> (Pampo)	<i>Microgemma</i> sp.	Fígado	Santa Catarina, Brasil	M	Todos os estádios	SSU + ITS + LSU	*
<i>Brachyhypopomus brevirostris</i> (Ituí rajado)	<i>Pleistophora brevirostris</i>	Tecido muscular da cavidade abdominal	Pará, Brasil	D	Estádios tardios	SSU	* Reclassificação da espécie <i>Microsporidium brevirostris</i>
<i>Brachyhypopomus</i> sp. (Ituí tuanga)	<i>Pleistophora</i> sp.	Tecido muscular da cavidade abdominal	Pará, Brasil	D	Estádios tardios	SSU + ITS + LSU	*
<i>Aequidens plagiozonatus</i> (Cará pixuna)	<i>Potaspora</i> sp.	Musculatura da cavidade orofaríngea	Pará, Brasil	D	Estádios tardios	SSU + ITS + LSU	*
<i>Parauchenipterus galeatus</i> (Anujá)	<i>Amazonspora</i> sp.	Papila genital	Pará, Brasil	D	Todos os estádios	—	**

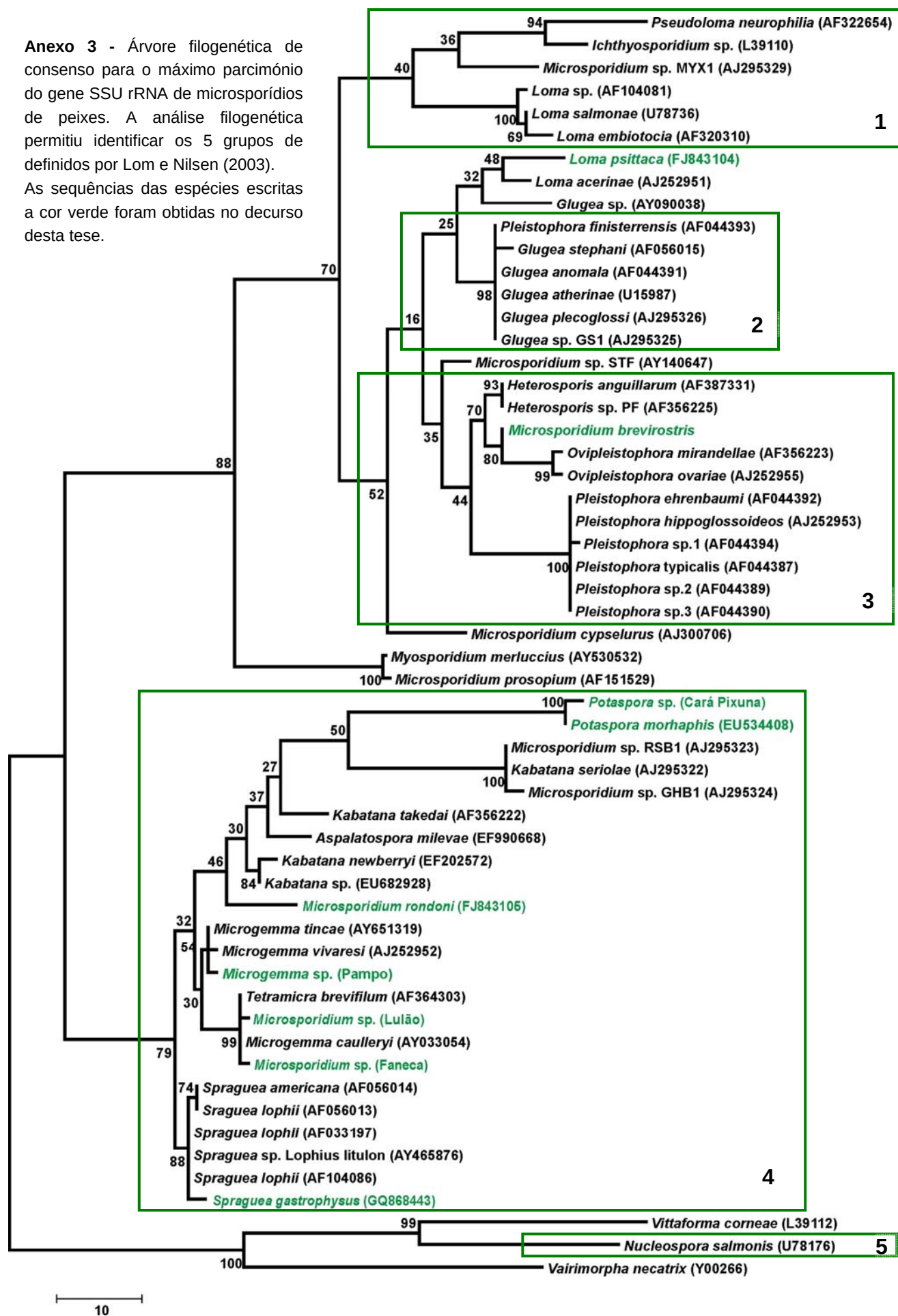
***Estudo em fase de conclusão; ** Estudo parcial**

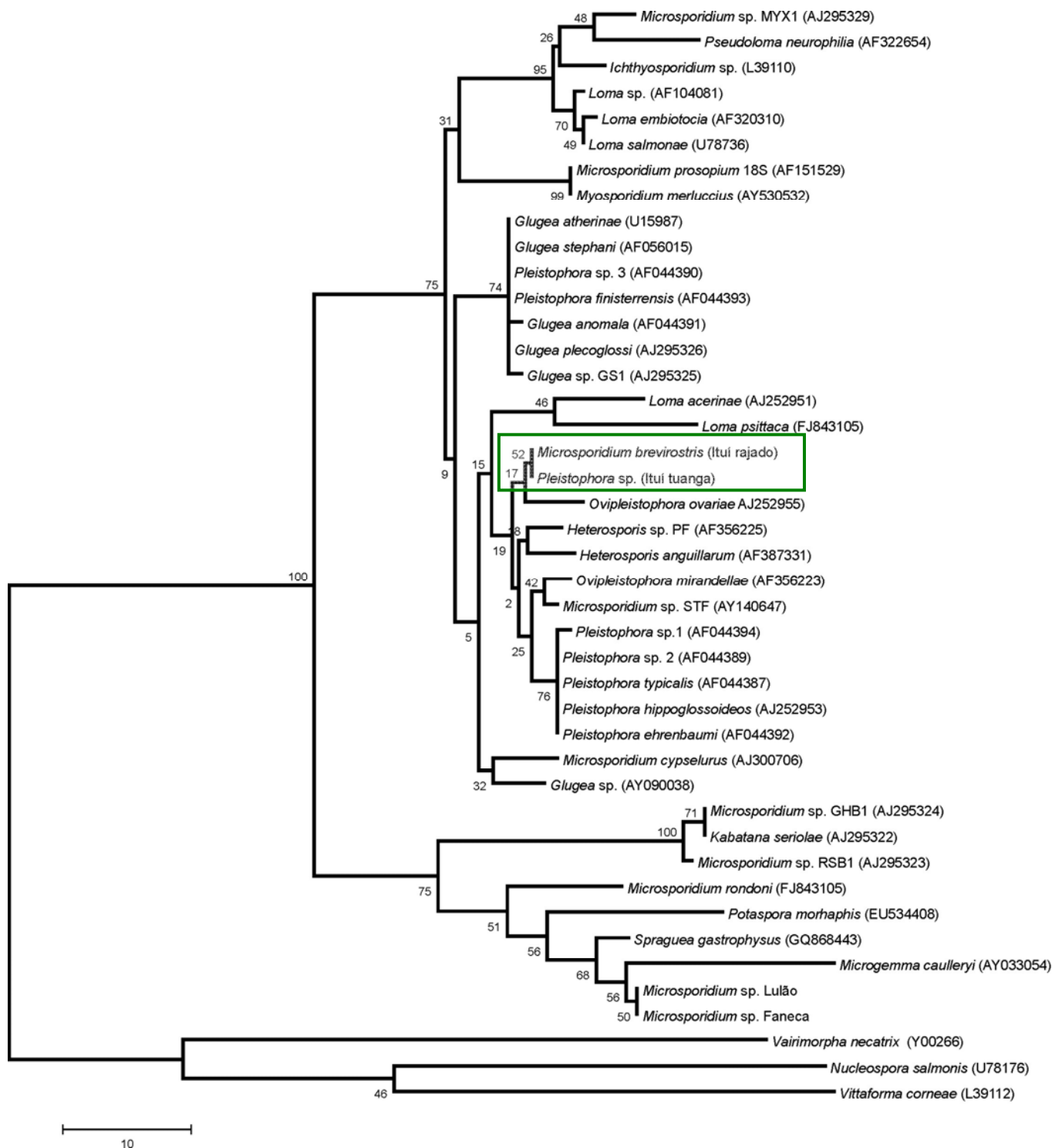
Anexo 2 - Listagem das mixosporidioses diagnosticadas em hospedeiros da ictiofauna portuguesa e brasileira

Hospedeiro (nome vulgar)	Gênero / Espécie	Local de infecção	Proveniência (Estado e País)	Habitat	Morfologia e ultraestrutura	Biologia Molecular (genes do rDNA sequenciados)	Capítulo Referência bibliográfica
<i>Trisopterus luscus</i> (Faneca)	<i>Kudoa</i> sp.	Tecido muscular esquelético	Portugal	M	Estádios tardios	SSU	*
<i>Scomber japonicus</i> (Cavala)	<i>Kudoa</i> sp.	Tecido muscular esquelético	Portugal	M	Todos os estádios	—	**
<i>Trachurus trachurus</i> (Carapau)	<i>Kudoa</i> sp.	Filamentos branquiais	Portugal	M	Estádios tardios	—	**
<i>Raja clavata</i> (Raia-lenga)	<i>Chloromyxum</i> sp.	Vesícula biliar	Portugal	M	Estádios tardios	—	**
<i>Gaidropsarus vulgaris</i> (Lulão)	<i>Ceratomyxa</i> sp.	Vesícula biliar	Portugal	M	Estádios tardios	—	**
<i>Merluccius merluccius</i> (Pescada)	<i>Ceratomyxa</i> sp.	Vesícula biliar	Portugal	M	Estádios tardios	—	**
<i>Aphanopus carbo</i> (Peixe-espada)	<i>Ceratomyxa tenuispora</i>	Vesícula biliar	Ilha da Madeira	M	Todos os estádios	—	Cap. 9 Casal et al. (2007) <i>Folia Parasitol.</i> 54:165-171
<i>Metynnis maculatus</i> (Pacú)	<i>Myxobolus maculatus</i> n. sp.	Rim	Pará, Brasil	D	Todos os estádios	—	Cap. 6 Casal et al. (2002) <i>Dis. Aquat. Org.</i> 51: 107-112
<i>Leporinus friderici</i> (Aracú)	<i>Henneguya friderici</i> n. sp.	Brânquia, intestino, rim e fígado	Pará, Brasil	D	Todos os estádios	—	Cap. 7 Casal et al. (2003) <i>Parasitology</i> 126: 313-319
<i>Metynnis argenteus</i> (Piaba chata)	<i>Mxyobolus metynnis</i> n. sp.	Tecido conjuntivo	Pará, Brasil	D	Todos os estádios	—	Cap. 8 Casal et al. (2006) <i>J. Parasitol.</i> 92: 817-821
<i>Gymnorhamphichthys rondoni</i> (Ituí transparente)	<i>Henneguya rondoni</i> sp.	Tecido nervoso e muscular	Pará, Brasil	D	Todos os estádios	—	Cap. 10 Azevedo et al. (2008) <i>J. Euk. Microbiol.</i> 55: 229-234
<i>Aequidens plagiozonatus</i> (Cará pixuna)	<i>Kudoa aequidens</i> n. sp.	Músculo esquelético	Pará, Brasil	D	Todos os estádios	—	Cap. 11 Casal et al. (2008) <i>Acta Protozool.</i> 47:135-141
<i>Menticirrhus americanus</i> (Papa-terra)	<i>Chloromyxum menticirrhii</i> n. sp.	Bexiga urinária	Santa Catarina, Brasil	M	Todos os estádios	—	Cap. 12 Casal et al. (2009) <i>Europ. J. Protistol.</i> 45:139 146
<i>Rioraja agassizii</i> (Raia-santa)	<i>Chloromyxum riorajum</i> n. sp.	Vesícula biliar	Santa Catarina, Brasil	M	Todos os estádios	SSU	Cap. 13 Azevedo et al. (2009) <i>Dis. Aquat. Org.</i> 85 : 41-51
<i>Centromochlus</i> sp. (Carataí)	<i>Myxobolus heckelii</i> n. sp.	Filamentos branquiais	Pará, Brasil	D	Todos os estádios	—	Azevedo et al. (2009) (in press)
<i>Hemiodopsis microlepes</i> (Flexeiro)	<i>Henneguya hemiodopsis</i>	Filamentos branquiais	Piauí, Brasil	D	Todos os estádios	—	Azevedo et al. (2009) (in press)
<i>Colomesus psittacus</i> (Baiaçú)	<i>Triangulamyxa</i>	Bexiga urinária	Pará, Brasil	D	Todos os estádios	—	**
<i>Trachinotus coralinus</i> (Pampo)	<i>Henneguya</i>	Intestino e cecos pilóricos	Santa Catarina, Brasil	M	Todos os estádios	—	**
<i>Astyanax bimaculatus</i> (Piaba de rabo vermelho)	<i>Henneguya</i>	Brânquia	Pará, Brasil	D	Todos os estádios	—	**
<i>Brachyhypopomus</i> sp. (Ituí tuanga)	<i>Henneguya</i>	Tecido nervoso na região dorsal	Pará, Brasil	D	Estádios tardios	—	**
<i>Brycon hilarii</i> (Piraputanga)	<i>Myxobolus</i>	Filamentos branquiais	Mato Grosso do Sul, Brasil	D	Estádios tardios	—	**

* Estudo em fase de conclusão; ** Estudo parcial

Anexo 3 - Árvore filogenética de consenso para o máximo parcimônio do gene SSU rRNA de microsporídios de peixes. A análise filogenética permitiu identificar os 5 grupos de definidos por Lom e Nilsen (2003). As sequências das espécies escritas a cor verde foram obtidas no decurso desta tese.





Anexo 4 – Árvore filogenética da região SSU, ITS e LSU do gene rRNA (sequência parcial) de microsporídios de peixes. Árvore de consenso para o máximo parcimónio. Em destaque duas sequências de *Pleistophora* spp. obtidas no decurso da tese.

Anexo 5 - Árvore filogenética de consenso para o máximo parcimónio do gene SSU rRNA de espécies de mixosporídios. As setas indicam as espécies sequenciadas no decurso desta tese.

