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ROGER LEOMAR DA SILVA FERREIRA

CARACTERIZAÇÃO MOLECULAR E MORFOLÓGICA DE MYXOZOA EM *Satanoperca jurupari* Heckel, 1840 NA AMAZÔNIA ORIENTAL

MACAPÁ - AP
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(Albert Einstein – Adaptado pela quantidade de lâminas histológicas que fiz nessa dissertação)

FERREIRA, Roger Leomar da Silva. FERREIRA, R.L.S. CARACTERIZAÇÃO MOLECULAR E MORFOLÓGICA DE MYXOZOA EM *Satanoperca jurupari* Heckel, 1840 NA AMAZÔNIA ORIENTAL. Dissertação – Departamento de Meio Ambiente e Desenvolvimento, Universidade Federal do Amapá, Macapá, 2020.

RESUMO

O *Satanoperca jurupari* é um peixe comum nos rios da bacia Amazônica e pode chegar a 25cm de comprimento, possui comportamento bentopelágico, alimenta-se de vegetais aquáticos, insetos e peixes. Apesar de pouco estudado, este ciclídeo é uma das espécies mais encontradas na ictiofauna da Área de Proteção Ambiental do Rio Curiaú em Macapá-AP. Em 8 coletas, foram capturados 72 espécimes de *S. jurupari* com auxílio de tarrafas de 30mm entre nós, no período de Março/2018 à outubro/2019 e transportados vivos para o Laboratório de Morfofisiologia e Sanidade Animal -UEAP, onde foi realizada eutanásia e analisado sua parte externa, seguida necropsia, para observação da presença de parasitos do subfilo Myxozoa. Na fauna microparasitária foram encontrados dois gêneros de Myxozoa infectando dois órgãos, a brânquia e a vesícula biliar. Nas vesículas biliares, foram observados esporos em forma elipsóide com duas cápsulas polares em posições opostas, característica do gênero *Ellipsomyxa* (Ceratomyxidae) com prevalência de 80,95% . Nas brânquias foi constatado a presença de cistos com esporos de microparasitas pertencentes ao gênero *Henneguya* com prevalência de 68,25%, pertencente à família Myxobolidae. Em todas as espécies encontradas no *S. jurupari* foram feitas análises morfológicas, morfométricas e filogenéticas, usando 18s rDNA e verificou-se que não correspondiam a nenhuma espécie já descrita na literatura, sendo assim, foram descritas nesse trabalho duas espécies novas, dispostas em dois artigos submetidos a periódicos para avaliação. Assim como esse, vários estudos demonstram cada vez mais a necessidade de descrever espécies de Myxozoa, pois são agentes que vem comprometendo a sanidade de peixes de interesses comerciais para consumo, forragem ou ornamentação e são muitas vezes subdiagnosticados.

Palavras-chave: Amapá, Ceratomyxidae, Myxobolidae, 18s rDNA

ABSTRACT

FERREIRA, Roger Leomar da Silva. FERREIRA, R.L.S. MORPHOLOGICAL AND MORPHOLOGICAL DESCRIPTION IN Satanoperca jurupari Heckel, 1840 IN THE EASTERN AMAZON. Dissertation - Department of Environment Environment and Development, Federal University of Amapá, Macapá, 2020.

Satanoperca jurupari is a common fish in the rivers of the Amazon basin and can reach at 25cm long, has bentopelagic behavior, feeds on vegetables aquatic, insects and fish. Although little studied, this cichlid is one of the species found in the ichthyofauna of the Curiaú Environmental Protection Area in Macapá-AP. In 8 collects, 72 specimens of *S. jurupari* were captured with the aid of 30mm between knot, from March/2018 to October/2019 and transported alive to the Morphophysiology and Animal Health Laboratory - EUAP, where euthanasia was performed and its external part was analyzed, followed by necropsy to observation of the presence of parasites of the Subphylum Myxozoa. In microparasitic fauna two genera of Myxozoa were found infecting two organs, the gill and the gallbladder. In the gallbladders, ellipsoid spores were observed with two polar capsules in opposite positions, characteristic of the genus *Ellipsomyxa* (Ceratomyxidae) with a prevalence of 80.95%. In the gills it was found the presence of of cysts with microparasite spores belonging to the genus *Henneguya* with prevalence of 68.25%, belonging to the Myxobolidae family. In all species found in *S. jurupari*, was made morphological, morphometric and phylogenetics analyzes using 18s rDNA and were found not correspond to any species already described in the literature, thus, this work described two new species, arranged in two articles submitted to journals for evaluation. Like this, several studies increasingly demonstrate the need to describe Myxozoa species, as they are agents that have been compromising the health of commercial fish interests for consumption, fodder or ornamentation and are often underdiagnosed.

Keywords: Amapá, Ceratomyxidae, Myxobolidae, 18s rDNA

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LISTA DE ABREVIATURAS E SIGLAS

AP	Amapá
APA	Área de Preservação Ambiental
Art.	Artigo
BM	Biologia Molecular
CEUA	Comitê de Ética de Uso Animal
CPAFAP	Centro de Pesquisa Agroflorestais do Amapá
DIC	Microscópio de Interferência Diferencial
DNA	Ácido Desoxirribonucléico
H&E	Hematoxilina e Eosina
LABMORSA	Laboratório de Morfosiologia e Sanidade Animal
LGA	Laboratório de Genética Aplicada
LPCA	Laboratório de Pesquisa Carlos Azevedo
MET	Microscopia Eletrônica de Transmissão
MEV	Microscopia Eletrônica de Varredura
ML	Microscopia de Luz
MP	Máxima Parcimônia
MS222	Tricaína Metano Sulfonato
MV	Máxima Verossimilhança
PA	Pará
PCR	Reação em Cadeia Polimerase
rDNA	DNA Ribossomal
SISBIO	Sistema de Informação em Biodiversidade
TP	Tubo Polar
UEAP	Universidade do Estado do Amapá
UFRA	Universidade Federal Rural da Amazônia

LISTA DE SIMBOLOS

nm	Nanômetro (10^{-9} m)
m	Metros
µm	Micrometro (10^{-6} m)
kV	Quilovolt
mm	Milimetros (10^{-3} m)
mg	Miligramas
L	Litro
g	Gramas
cm	Centímetro
M	Mol
ºC	Graus Celsius

SUMÁRIO

1 INTRODUÇÃO GERAL

O Estado do Amapá, no extremo norte do Brasil possui particularidades ambientais em função principalmente de sua localização na foz do Rio Amazonas, a qual lhe confere características ímpares no uso dos recursos naturais (TAKIYAMA et al., 2002).

A bacia hidrográfica do Rio Curiaú mede aproximadamente 584,47 km^2, das quais, cerca de 40%, encontra-se dentro dos limites da Área de Preservação Ambiental (APA) do Rio Curiaú. Ao longo de seu percurso, o Rio Curiaú atravessa as áreas de campos inundáveis percorrendo 4,5km dentro da floresta de várzea até desembocar no Rio Amazonas (FACUNDES & GIBSON, 2000). O regime das marés quanto os pluviais são os responsáveis pela influência da dinâmica no Rio Curiaú (SILVA et al., 2013).

Durante o período chuvoso, as águas da chuva alagam totalmente os campos permanecendo assim grande parte do ano (CHAGAS, 1997). A Biomassa de peixes aumenta rapidamente durante as cheias, em grande parte devido ao rápido crescimento das formas jovens do ano (LOWEMCCONNELL, 1999).

Estudos ictiológicos ainda são escassos quando comparados com a alta diversidade de peixes que o Brasil apresenta, devido ao seu extenso conjunto de bacias hidrográficas formadas por seus rios e lagos. Os peixes de água doce podem abrigar uma grande variedade de espécies de parasitos, sejam esses ectoparasitos ou endoparasitos, pertencentes a numerosos filos (EIRAS et al., 2010).

Nas últimas décadas têm aumentado consideravelmente a relevância dos estudos relacionados com parasitos e outros patógenos de organismos aquáticos, principalmente daqueles hospedeiros com potencial para o cultivo e para a comercialização, face ao aumento significativo destas atividades no Brasil e no mundo (LUQUE et al., 2004).

O peixe escolhido para esse estudo, o acará-bicudo, *Satanoperca jurupari* Heckel, 1840, apresenta porte médio de até 25 cm, com característica marcantes em seu lóbulo, que é bem desenvolvido na parte superior no início do arco branquial, dando-lhe uma aparência de um "bico" (SOARES et al., 2007). Outra característica marcante e auxiliadora para a sua identificação é uma pequena mancha em forma de ocelo na porção superior da base da nadadeira caudal (SOARES et al., 2007).

FARIA et al. (2008) afirmam que "O *S. jurupari* é um peixe forrageiro de alta adaptabilidade ao cativeiro, porém aspectos como: manejo nutricional, reprodutivo, sanitário são desconhecidos".

FERREIRA et al. (2017) identificaram uma elevada diversidade taxonômica de microparasitos em *S. jurupari* na Área de Proteção Ambiental do Rio Curiaú, distrito de Macapá-AP, com características distintas das descritas em revisões de literatura, com grandes possibilidades de descrever novas espécies. Assim, este estudo visa descrever os aspectos biomoleculares e morfológicos destes microparasitos, contribuindo para o aumento do conhecimento do estado sanitário da fauna ictiológica amazônica, em especial no estado do Amapá.

1.1 A atividade pesqueira no Estado do Amapá

O consumo de pescado per capta da população urbana na cidade de Macapá em 1996, era de 54 $kg.ano^{-1}$, com aumento significativo na população rural (ISAAC et al., 1998). No Brasil o consumo médio nacional de peixes de água doce (da pesca de captura continental e aquicultura de água doce) é bastante baixo - somente 3,95 kg per capita por ano em 2013 - mas na Amazônia, esse mesmo consumo está próximo de 150 kg per capita por ano (FAO, 2018).

Segundo FREITAS et al. (2006), a pesca artesanal é uma atividade difusa, praticada pelas populações ribeirinhas de toda a Amazônia, sem local específico para desembarque. A presença de embarcações de estados vizinhos e de outros países na costa amapaense, representa uma ameaça à sustentabilidade da atividade pesqueira na região, já que a exploração econômica desordenada e o uso sustentável desses recursos naturais entram em conflito com o lucro, o qual representa sempre o objetivo final e imediato na pesca industrial (SLVA & TAVARES-DIAS, 2010).

A pesca na Amazônia sempre foi uma atividade fundamental e está atrelada aos hábitos culturais e a história da própria região e se destaca em relação às demais regiões brasileiras, tanto costeiras quanto de águas interiores, pela riqueza de espécies exploradas, pela quantidade de pescado capturado e pela dependência da população tradicional a esta atividade (BARTHEM et al., 2004).

A profissionalização da pesca, aliada ao aumento dos incrementos tecnológicos introduzidos na Amazônia nas últimas três décadas tem influenciado pouco na produção do estado do Amapá, apesar de estarem presentes em sua costa as frotas dos estados vizinhos (PA e MA) e toda sua

produção era levada ao Mercado do Ver-o-Peso, capital paraense (SILVA & TAVARES-DIAS, 2010).

1.2 A Área de Proteção Ambiental do Rio Curiaú (APA)

Vista como sinônimo da cultura e das tradição popular negra dentro do estado do Amapá, a vila do Curiaú foi a primeira no Estado do Amapá a ter reconhecidos seus direitos sobre a propriedade das terras que ocupavam há séculos. Na região da APA do Rio Curiaú existe a comunidade quilombola do Curiaú, tombada como Vila do Curiaú pelo decreto 1417/1992, devido sua representação significativa da tradição afroamapaenses (CANTUÁRIA et al., 2011).

Após a Assembléia legislativa do estado do Amapá decretar o tombamento da Vila do Curiaú, o Governo do Estado do Amapá sancionou em 15 de novembro de 1998 a lei nº 0431, que dispõe em seu Art. 1º diz:

> "Fica criada a Área de Proteção Ambiental do Rio Curiaú (APA do Rio Curiaú), situada no Município de Macapá, Estado do Amapá, com o objetivo de proteger e conservar os recursos naturais ali existentes, visando a melhoria da qualidade de vida das comunidades tradicionais residentes no local."

AMARAL et al. (2015) enfatizaram que a pesca na área do Quilombo do Curiaú é realizada de forma artesanal por pescadores desta comunidade, proporcionando às famílias uma fonte de renda e subsistência. Localizada próximo à área urbana da cidade de Macapá, APA do Rio Curiaú encontra-se sob forte influência de ações antrópicas. Segundo CANTUÁRIA et al., (2011) em função dos riscos que a expansão urbana da cidade de Macapá-AP vinha causando aos ecossistemas da bacia do Rio Curiaú, que deságua no Amazonas e pela necessidade de garantir a territorialidade das comunidades residentes na área compostas predominantemente por afrodescendentes.

O Rio Curiaú na região de sua desembocadura no rio Amazonas, apresenta características meândricas distintas da sua região de meso-rio nascente. Essa diferença deve-se, provavelmente, à maior turbulência no rio, provocada pela maior velocidade de correnteza da água do rio Amazonas e pelo regime de maré (VASCONCELOS & AS-OLIVEIRA, 2011).

1.3 Peixes: aspectos gerais

Estima-se que existam cerca de 55.000 espécies de vertebrados em todo o mundo, das quais aproximadamente 28.000 são peixes (NELSON, 2006). Segundo REIS et al. (2016) existem atualmente cerca de 5.160 espécies de peixes de água doce para América do Sul, e a estimativa para a ictiofauna de água doce do mundo pode representar uma diversidade aproximada entre 8000 e 9000 espécies.

A partir desse conhecimento é importante a realização de estudos que caracterizem o perfil parasitológico de peixes, a fim de verificar e controlar a disseminação de parasitos que geram desequilíbrio nos ecossistemas aquáticos, uma vez que esses, segundo MALTA et al. (1984), apresentam peculiaridades que facilitam a propagação, complementação do ciclo de vida e outros fatores de relevante importância para sobrevivência de cada grupo de organismos parasitas.

Os parasitos possuem uma distribuição mundial, afetam todas as espécies e das águas tropicais às polares, de qualquer nicho ecológico e habitat (EIRAS 1994; THATCHER 2006). Num ecossistema, é possível encontrar diversas relações entre os organismos aí presentes, sendo que essas relações bióticas são categorizadas de acordo com o saldo benefício-prejuízo dos organismos envolvidos (MACHADO & CASTRO, 2019).

1.4 O hospedeiro: *Satanoperca jurupari*

O gênero *Satanoperca* pertence à subfamília Geophaginae, família Cichlidae, ordem Cichliformes e compreendendo a sete espécies distribuídas por toda América do Sul, sendo que na bacia amazônica ocorrem cinco: *S. jurupari* (Figura 1), *S. daemon, S. acuticeps, S. papaterra, S. lilith* (KULLANDER, 2003).

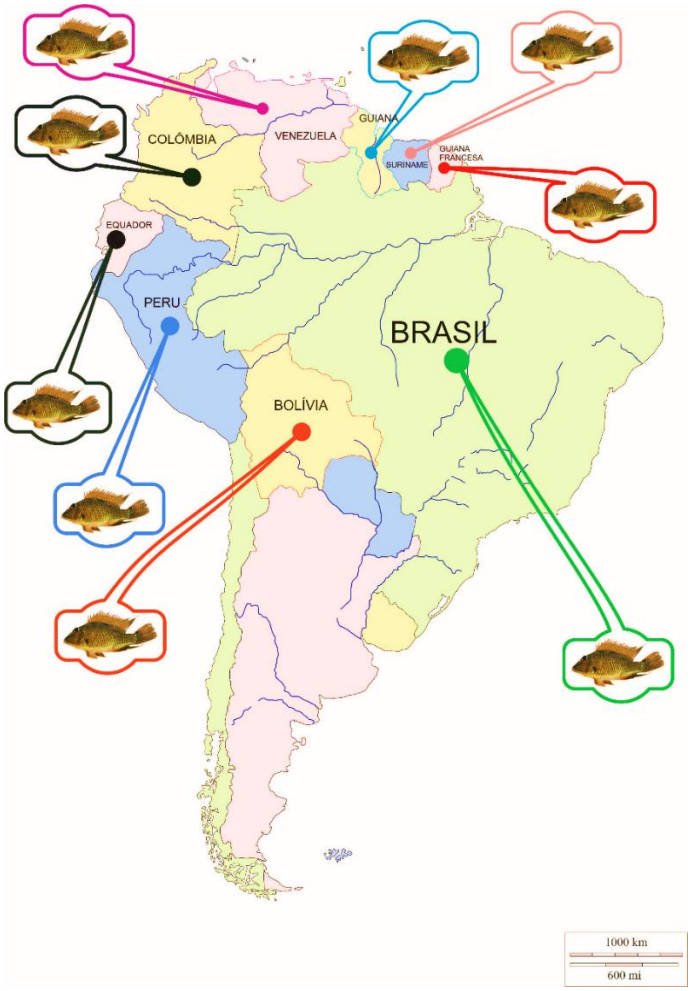

Figura 1 Países que apresentam registro de ocorrência da espécie de *Satanoperca jurupari* na América do Sul de acordo com KULLANDER (2003).

Os ciclídeos possuem hábitos diurnos e preferência por ambientes lênticos. Apresentam cuidado parental, protegendo ovos e jovens, e fazendo ninhos (BRITSKI et al., 2007). Membros dessa família são muito apreciados por aquariofilistas e pescadores esportivos. A preferência por ambientes lênticos, juntamente com o cuidado parental, proporcionam amplo sucesso reprodutivo em reservatórios. Essa família caracteriza-se por reunir espécies com linha lateral dividida em dois ramos (superior e inferior), boca protrátil, pré-maxilar móvel e dentes cônicos (REIS et al. 2003) (Figura 2).

Figura 2 Exemplar de *Satanoperca jurupari* coletado na Área de Proteção Ambiental do Rio Curiaú –AP. Fonte: (LABMORSA, 2017)

Em recente abordagem filogenética (Figura 3) essa família foi revisada por BETANCUR-R. et al. (2013) sendo que, atualmente, pertence à Ordem Cichliformes.

REINO Metazoa
FILO Chordata
CLASSE ActinopterygiI
ORDEM Cichliformes
FAMÍLIA Cichlidae
GÊNERO *Satanoperca*
ESPÉCIE *Satanoperca jurupari*

Figura 3 Classificação taxonômica do *Satanoperca jurupari*

O *Satanoperca jurupari* em geral apresenta cuidado com a prole via oral, realiza pequenos deslocamentos e reproduz-se no período de águas baixas (estiagem), apresentando grandes ovócitos característicos de peixes de desova parcelada, não apresentando valor comercial na região amazônica, porém é um dos peixes consumidos pela população ribeirinha (QUEIROZ et al., 2014). MATOS et al. (2002), em seus estudos sobre espermiogênese e descrição ultraestrutural do espermatozoide de *S. jurupari*, afirmam que esta é uma espécie hermafrodita simultânea e sofre autofertilização interna.

Este peixe ornamental é onívoro e se alimenta de microcrustáceos, sementes de frutas, gramíneas e peixes pequenos, bem como larvas de insetos aquáticos e terrestres (TAVARES-DIAS et al., 2017).

1.5 Peixes: diversidade parasitológica

Os peixes podem ser hospedeiros de uma grande diversidade de organismos que estão distribuídos dentro de diversos grupos, podendo ter o seu desenvolvimento afetado tanto em ambientes naturais como em ambientes de cultivo (EIRAS et al., 1994).
Os parasitos causadores de enfermidades em peixes são abundantes e apresentam membros dos diferentes grupos zoológicos. A patologia e o grau de parasitismo que acometem os peixes dependem de diversos fatores como a natureza do parasito, intensidade da infecção, condições ambientais, a biologia de cada espécie, comportamento alimentar, população e filogenia (FLORINDO, 2016).

Desde 2009 estudos parasitológicos vem sendo descritos para esta espécie de interesse forrageiro, com destaque para o estado do Amapá, que apresenta a maior quantidade de informações ictiosanitárias para o hospedeiro em estudo. Já foram descritos protozoa, platelmintos, Crustácea e nematoides e até o presente momento nenhum Myxozoa foi descrito, como pode ser visto na tabela 1.

PARASITO	ESTADO	AUTOR	ANO
Ichthyophthirius multifiliis	AMAPÁ	BITTENCOURT et al.	2014
	AMAPÁ	BITTENCOURT et al.	2014
	AMAPÁ	TAVARES-DIAS et al.	2017
Piscinoodinium pillulare	AMAPÁ	TAVARES-DIAS et al.	2017
Sciadicleithum juruparii	PARÁ	MELO et al.	2012
	AMAPÁ	BITTENCOURT et al.	2014
	AMAPÁ	OLIVEIRA et al.	2017
	AMAPÁ	TAVARES-DIAS et al.	2017
Sciadicleithrum edgari	AMAPÁ	PASCHOAL et al.	2016
Sciadicleithrum satanoperca	PARÁ	YAMADA et al.	2009
	PARÁ	MENDONZA-FRANCO	2010
	AMAPÁ	BITTENCOURT et al.	2014
Posthodiplostomum Dubois	AMAPÁ	TAVARES-DIAS et al.	2017
Clinostomum marginatum Rudolphi,	AMAPÁ	TAVARES-DIAS et al.	2017
Genarchella genarchella	AMAPÁ	TAVARES-DIAS et al.	2017
	AMAPÁ	OLIVEIRA et al.	2017

Icthyouris sp.	AMAPÁ	BITTENCOURT et al.	2014
chthyouris Inglis	AMAPÁ	TAVARES-DIAS et al.	2017
Gorytocephalus spectabilis	AMAPÁ	BITTENCOURT et al.	2014
	AMAPÁ	TAVARES-DIAS et al.	2017
Argulus multicolor	AMAPÁ	OLIVEIRA et al.	2016
	AMAPÁ	OLIVEIRA et al.	2017
	AMAPÁ	TAVARES-DIAS et al.	2017
Ergasilus coatiarus	AMAPÁ	TAVARES-DIAS et al.	2017
	AMAPÁ	OLIVEIRA et al.	2017
Posthodiplostomum sp.	AMAPÁ	OLIVEIRA et al.	2017
Sciadicleithrum araguariensis	PARÁ	PASCHOAL et al.	2016
	AMAPÁ	PASCHOAL et al.	2016
Calyptospora sp.	AMAPÁ	SILVA NEGRÃO et al.	2019
Protozoa	AMAPÁ	BAIA et al.	2018
Derogenidae gen. sp. (metacercariae)	AMAPÁ	BITTENCOURT et al.	2014
Procamallanus (Spirocamallanus) rarus	PARÁ	MELO et al.	2012
Procamallanus (*Spirocamallanu*s) sp.	PARÁ	MELO et al.	2011b
Raphidascaroides sp.	PARÁ	MELO et al.	2011b
Neoechinorhynchus (Neoechinorhynchus) paraguayensis	PARÁ	MELO et al.	2011b
Raphidascaris (Sprentascaris) lanfrediae	PARÁ	MELO et al.	2011a

Tabela 1 – Levantamento bibliográficos dos taxas que já foram descritos parasitando o *Satanoperca jurupari*.

1.5.1 Filo Cnidaria:Myxosporea (Myxozoa) Bütschli, 1970

Filo Cnidária: Myxozoa Bütschli, 1970 contém 62 gêneros registrados. Os dois principais gêneros, dentro da classe Myxosporea são *Myxobolus* Bütschli, 1882 e *Henneguya* Thélohan, 1892, este subfilo possui uma grande diversidade, sendo conhecidas cerca de 2.300 espécies,

as quais infectam principalmente peixes, mas também anfíbios, répteis e aves (CARRIERO et al., 2011).

Muito se discute sobre a filogenia desse grupo taxonômico (Figura 4), classificado como protistas até 1940, devido a sua ausência de centríolos, criptomitose e a presença de cristais tubulares mitocondriais em alguns táxons (MARQUÈS 1987; LOM & DYKOVÁ 1997; OKAMURA et al., 2015) e devido ao posicionamento das suas cápsulas polares e sua similaridade com nematocisos dos cnidários, levantou-se dúvidas sobre esse táxon (CARRIERO et al., 2011).

Segundo SMOTHERS et al. (1994) analisaram filogeneticamente através do 18s rDNA de mixosporídeo e confirmaram seu agrupamento dentro dos Metazoa. Em estudos HOLZER et al. (2002) fizeram as análises da evolução molecular mostram que os Myxozoa originaram quase 600 milhões de anos atrás, tendo divergido da Cnidária de vida livre 100 milhões de anos após sua origem comum.

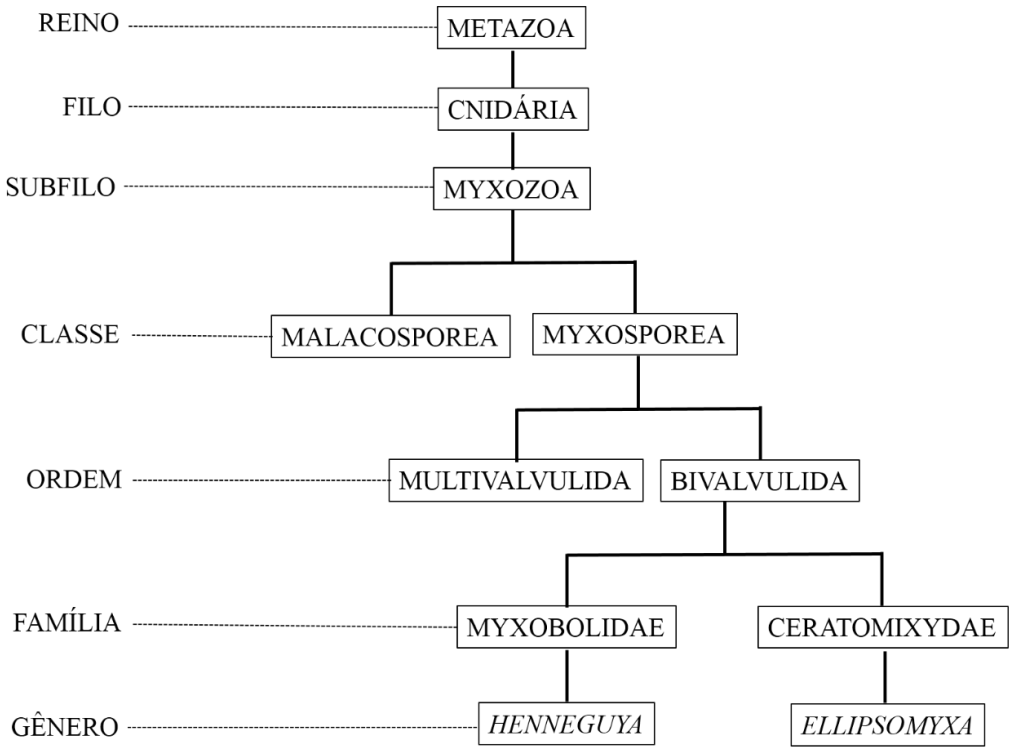

Figura 4 Descrição taxonômica dos dois gêneros que estão descritos no trabalho, *Henneguya* e *Ellipsomyxa*.

MATOS et al. (2001) afirmaram que a fase esporal do subfilo Myxozoa é a que melhor caracteriza o grupo. Os esporos são compostos por várias células que, embora com morfologia diferente, vão se organizar e formar o esporo. Segundo MATOS et al. (2004), são parasitos de

morfologia e estrutura muito diversa, encontrados, principalmente em peixes e caracterizados por um ciclo de vida complexo, alternando entre hospedeiros, de peixes e invertebrados, bem como diversidade de espécies (Figura 5).

WOLF & MARKIW (1984) demonstraram, primeiro, no caso de *Myxobolus cerebralis* (Myxobolidae) da truta (doença do rodopio), que o ciclo vital dos mixosporídeos ocorre paralelamente em dois hospedeiros: vertebrado (peixe) e em um invertebrado (oligoqueta).

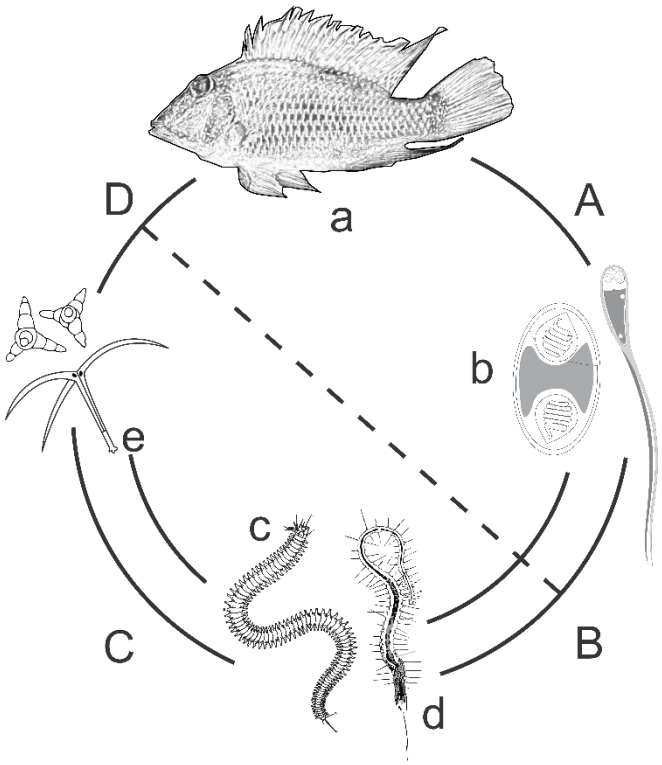

Figura 5 Ciclo de vida dos Myxozoa de acordo com WOLF e MARKIW (1984). O desenvolvimento do estágio dos myxosporos ocorrem no hospedeiro (a) e culmina com a liberação (A) dos esporos de myxosporos (b), que vão para o substrato ou ficam na coluna d´água (B) e são ingeridos pelo hospedeiro oligoquetas (c/d). O desenvolvimento da fase actinosporeana ocorre (C) e produz os esporos de actinosporos (e) que são aquáticos e infecciosos (D) para o hospedeiro (adaptado - HEDRICK et al. 1998).

Análises genômicas recentes sugeriram uma origem comum do *Polypodium hydriforme*, um parasito cnidário de peixes acipenseriformes, e os Myxozoa, e propuseram os peixes como hospedeiros originais para ambos os "irmãos" de linhagens (HOLZER et al., 2002).

Os Myxosporea são caracterizados, na sua fase de parasitos de peixes (ou répteis ou anfíbios) por possuírem esporos compostos por várias valvas (2 a 7), unidas por linhas de sutura, 1 a 2 esporoplasmas e 1 a 15 cápsulas polares, contendo cada uma 1 filamento enrolado em espiral. Os esporos podem ter formas e dimensões muito diferentes e as suas características morfológicas e morfométricas constituem a base para a identificação destes parasitos (EIRAS et al., 2010; NALDONI et al., 2018).

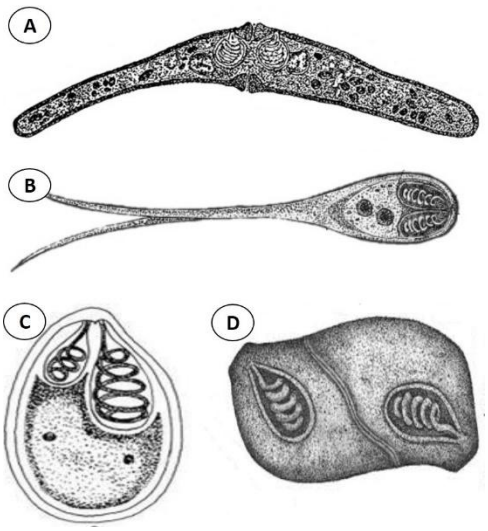

Figura 6 Esporo de Myxosporídeos em diversas formas. A. Gênero *Ceratomyxa* (AZEVEDO et al., 2013a); B. Gênero *Henneguya* (VELASCO et al., 2016); C. Gênero *Myxobolus* (CELLERE et al., 2002) e D. Gênero *Ellipsomyxa* (AZEVEDO et al., 2013b).

1.5.1.1 Myxobolidae: *Henneguya* Thélohan, 1892

O gênero *Henneguya* Thélohan, 1892 (filo Cnidaria) é um dos gêneros desta família com maior diversidade para as espécies descritas (LOM & DYKOVÁ, 1992) (Figura 7). O conhecimento sobre a biodiversidade parasitária na América do Sul é limitada, principalmente na região amazônica, devido a diversidade da ictiofauna e suas áreas remotas (REIS et al. 2016; ZATTI et al., 2018). A maioria destas espécies são principalmente parasitos de peixe de água doce e marinha e apresentam ampla distribuição geográfica (LOM E DYKOVÁ 1992; VELASCO et al., 2016, SILVA et al., 2018).

A morfologia deste gênero é caracterizada pelo número de voltas do filamento polar, forma do esporo, posição e número de cápsulas polares (redonda, piriforme ou elipsóide), a posição relativa da linha de sutura e cápsulas polares, presença de sulcos e apêndices superficiais e número voltas de filamentos polares (FEIST & LONGSHAW, 2006; LOM & DYKOVÁ 2006; KAUR & ATTRI, 2015).

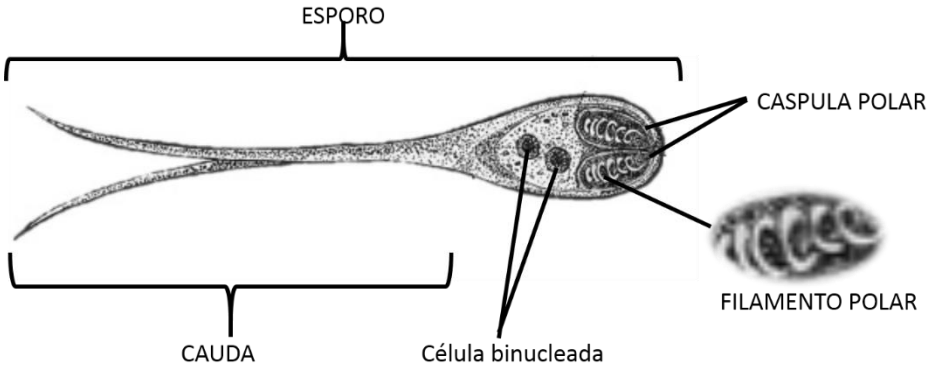

Figura 7 Desenho esquemático de *Henneguya paraensis* (VELASCO et al., 2006).

1.5.1.2 Ceratomixydae: *Ellipsomyxa* Køie, 2003

O gênero *Ellipsomyxa* Køie, 2003 representa ainda um grupo pequeno com 12 espécies descritas e se caracteriza, morfologicamente por apresentar esporo de formato elipsoide (ligeiramente oval), duas cápsulas esféricas perpendiculares a linha de sutura, ligeiramente assimétricas (KØIE, 2003) (Figura 8).

Os esporos dos parasitos desse grupo apresentam de 2 à 7 voltas no filamento polar, unidas por uma linha de sutura e de 1 à 2 esporoplasmas. Apresenta estrutura complexa com um tubo (ou filamento) eversível que facilita sua interação com o hospedeiro (EIRAS et al., 2010; CHANG et al., 2015)

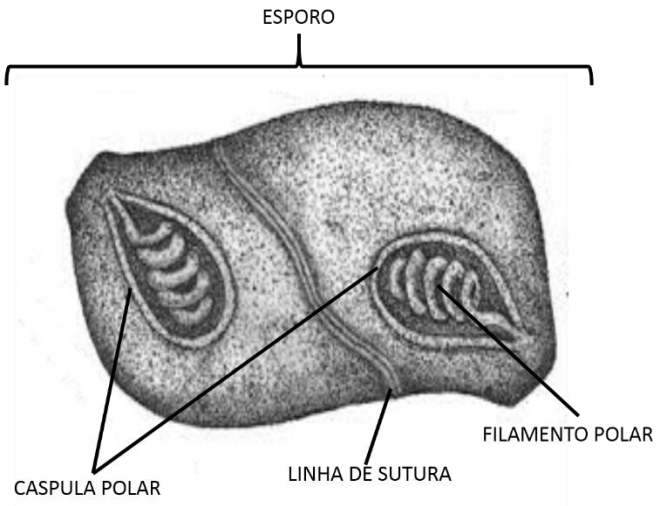

Figura 8 Desenho esquemático de *Ellipsomyxa gobioides* (AZEVEDO et al., 2006).

Ressalta-se a importância da caracterização parasitológica das espécies de peixes, pois estudos como esse oferecem conhecimentos para combates a causas de infecções em aquicultura, caso seja cultivado, melhor entendimento sobre enfermidades, ao entender a preferência por órgãos

infectados, e geram respostas ao desequilíbrio nos ecossistemas aquáticos para estudos ecológicos.

Para determinação taxonômica, algumas técnicas são necessárias para que se possa chegar a especificações plausíveis para que sua ecologia e comportamento patogênico seja relacionados a alguma enfermidade. Para isso faz-se uso de ferramentas moleculares e histológicas.

2 OBJETIVOS

2.1 Geral

Caracterizar os parasitos do subfilo Myxozoa que ocorrem em *Satanoperca jurupari* capturado na Área de Proteção Ambiental do Rio Curiaú, Macapá-A

2.2 Específicos

- Identificar as espécies de Myxozoa encontrados em *S. jurupari* na Área de Proteção Ambiental do Rio Curiaú, Macapá-AP ao menor nível taxonômico;
- Descrever as características morfológicas e moleculares desses microparasitos;
- Avaliar as relações filogenéticas dos taxas em estudo.

3 MATERIAL E MÉTODOS

3.1 Peixes: coleta e transporte

No período de Setembro/2018 à Outubro/2019 foram capturados 72 espécimes de *Satanoperca jurupari* (Artigo 1: N = 42; Artigo 2: N = 63) com peso médio de 54,13 ±14,12g e comprimento médio de 15,21 ±1,42cm, todos provenientes do Rio Curiaú no entorno correspondente aos pontos 1, 2 e 3 (0º8'N, 51º2'W) (Figura 9).

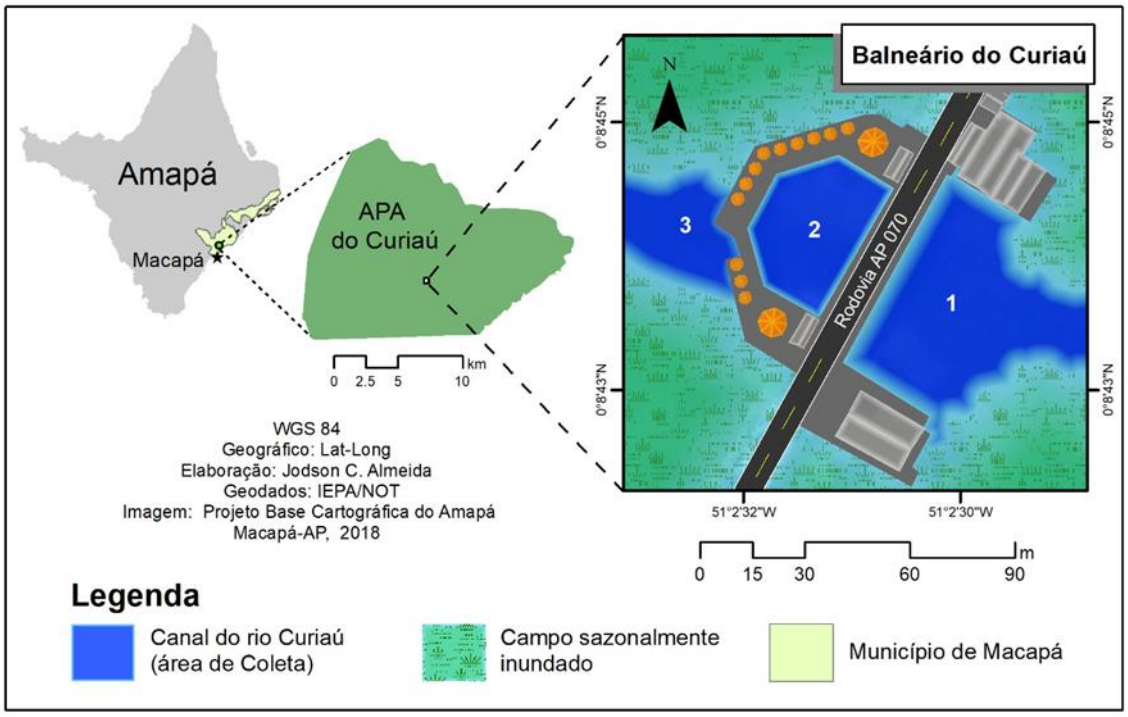

Figura 9 Mapa do estado do Amapá com destaque na Área de Proteção Ambiental do Rio Curiaú (Norte), na imagem maior vemos o local de estudo com os 3 pontos de coleta (1, 2 e 3).
Fonte: (ALMEIDA, 2018)

As coletas foram submetidas à autorizações da Secretaria de Estado do Meio Ambiente do Estado do Amapá, aprovado pelo Comitê de Uso Animal da Empresa Brasileira de Pesquisa Agropecuária - Amapá (EMBRAPA/SEI 012) (ANEXO A) e com cadastro no Sistema de Autorização e Informação em Biodiversidade (SISBIO/ICMBIO Licença 50376-1). Os espécimes foram capturados pela equipe do Laboratório de Morfofisiologia e Sanidade Animal da Universidade do Estado do Amapá (LABMORSA/UEAP) auxiliados por pescadores locais com a utilização de tarrafa de 30mm entre nós.

Os animais capturados foram acondicionados e transportados vivos em caixas térmicas com auxílio de bombas a pilha até o LABMORSA/UEAP (Figura 10 A e B), foram colocados em aquários de vidros com bombas elétricas, seguidos das análises parasitológicas.

Nos aquários, os animais foram anestesiados com Tricaína Metano Sulfonato (MS222 SIGMA) na concentração de 50mg.L^{-1}.Posteriormente, realizou-se a biometria: pesados (g) com balança digital e medidos (cm) com paquímetro. Analisou-se todo tegumento através de microscópio estereoscópico binocular (Figura 10 C), para verificar se existiam lesões (cistos) ou perda de revestimento (escamas e epiderme), sendo as observações registradas em ficha padronizada pelo LABMORSA/UEAP (APÊNDICE A). Em seguida foram realizadas incisões, na região ventral, a fim de expor suas vísceras para analisar cada órgão minuciosamente (Figura 10D).
Dos tecidos encontrados possíveis focos de desenvolvimento de parasitos, retirou-se pequenos fragmentos, coletados com pinças e colocados entre lâmina e lamínula, para serem observados através de microscopia de luz (ML) e para cálculo de prevalência a metodologia adotada foi a de BUSH (1997).

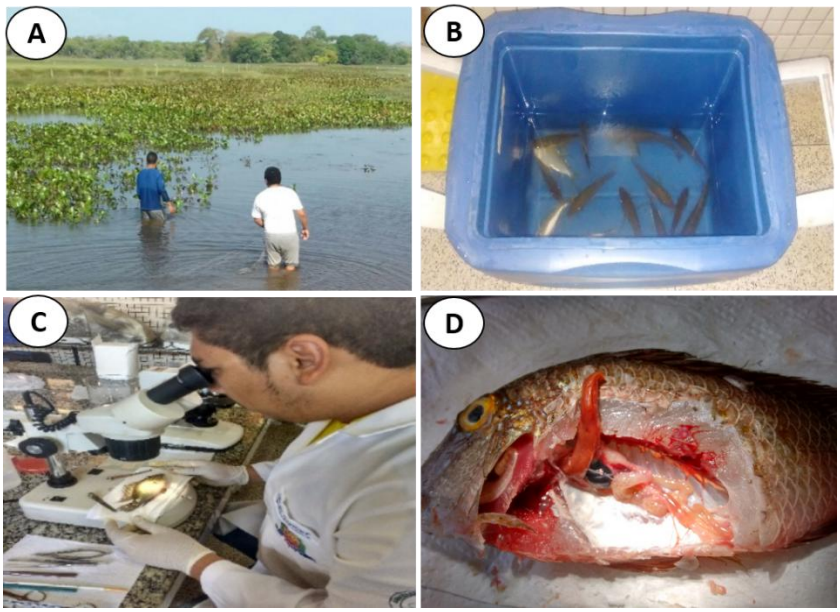

Figura 10 A. Coleta dos peixes em estudo na Área de Proteção Ambiental do Rio Curuiaú, Macapá-AP; B. Transporte dos peixes em caixa térmica com auxílio de bom a pilha; C. Análise de um Espécime em estereomicroscopio; D. Expondo as vísceras do peixe para análises

Fonte: (LABMORSA,2018)

3.2 Microscopia de luz: Convencional, técnica de histologia e diferencial de contraste de interferência (DIC)

Fragmentos de tecidos e/ou cistos foram comprimidos entre lâmina e lamínula para serem examinados em preparações a fresco e observados em Microscopia de Luz Convencional e DIC (ZEISS - Axio Cam ERC 5S) (Figura 11).

Figura 11 Microscópio trinocular com Iluminação Halogena para diferencial de contraste de interferencia (DIC) com câmera acoplada, com destaque na câmera.

Fonte: (LPCA,2018)

Nos casos afirmativos para o parasitismo, os tecidos foram fixados em Davidson (álcool 95%, formaldeído e ácido acético, água destilada) e processados pelas técnicas habituais de histologia para inclusão em parafina (ANEXO B) (Figura 12 A-C), corados com hematoxilina e eosina (H&E) (Figura 12 D) e/ou Ziehl-Neelsen (ZN) (SILVA, 2018), fotografados no LABMORSA/UEAP com a Câmera MOTICAM® 2.300 (3.0M Pixel UBS2.0) (12 E-F). Lâminas histológicas montadas, foram identificadas e remetidas por para depósito do museu do Instituto Nacional de Pesquisa da Amazônia (INPA) (Figura 12 G).

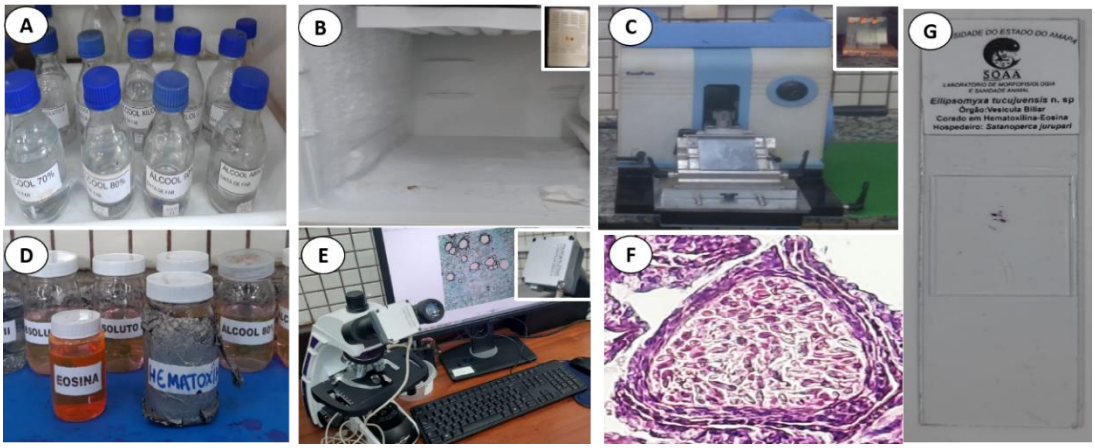

Figura 12 A. Bateria de álcool (70% ao Absoluto III) e Xilol; B. Freezer para esfriar a parafina, com destaque em um bloco; C. Micrótomo, com bloco fixado para realizar o corte, com destaque nas laminas na estufa; D. Bateria de coloração; E. Microscópio trinocula acoplado a um computador para fotografar as lâminas, com destaque na câmera; F. Foto de uma lâmina corada em H.E em objetiva de 40x. G. Lâmina identificada para depósito para tombamento.

Fonte: (LABMORSA, 2019)

3.3 Biologia molecular de microparasitos Eucariotos

Os cistos de microparasitos e/ou tecidos infectados com esporos dos microparasitos foram coletados e armazenados em álcool etílico 70%-80% à 4ºC. O DNA total de cada amostra foi extraído com uso do *Wizard® Genomic* (conforme o fabricante) (Figura 13 A). As amostras de DNA foram quantificadas por espectofometria (Biodro Duo) com comprimento de onda à 260nm (Figura 13 B).

Todas as análises moleculares basearam-se nas sequências 18S rDNA, que amplificou de acordo com as metodologias descritas por VELASCO (2016) e SILVA (2018) para Myxobolidae e Ceratomyxidae (Figura 13 C-F), respectivamente e para purificação do material após Reação em Cadeia Polimerase (PCR) foi utilizado o kit GE Healyhcare® Reagentes de purificação GFX™ PCR DNA Gel Band (100 purif), conforme descrição do fabricante (Figura 13 G). Estas etapas foram executadas no Laboratório de Genética Aplicada na Universidade Federal Rural da Amazônia (LGA/UFRA).

Os produtos das amplificações foram sequenciados no analisador automático de DNA ABI 3730 usando o BigDye v3.*1 Cycle Sequencing Ready Reaction kit* (*Applied Biosystems*) (Figura 13 H). e para confirmar as mutações observadas, cada amostra foi sequenciada com ambos os iniciadores, direto e reverso. As sequências nucleotídicas obtidas forameditadas e alinhadas no programa BioEdit (HALL, 2007).

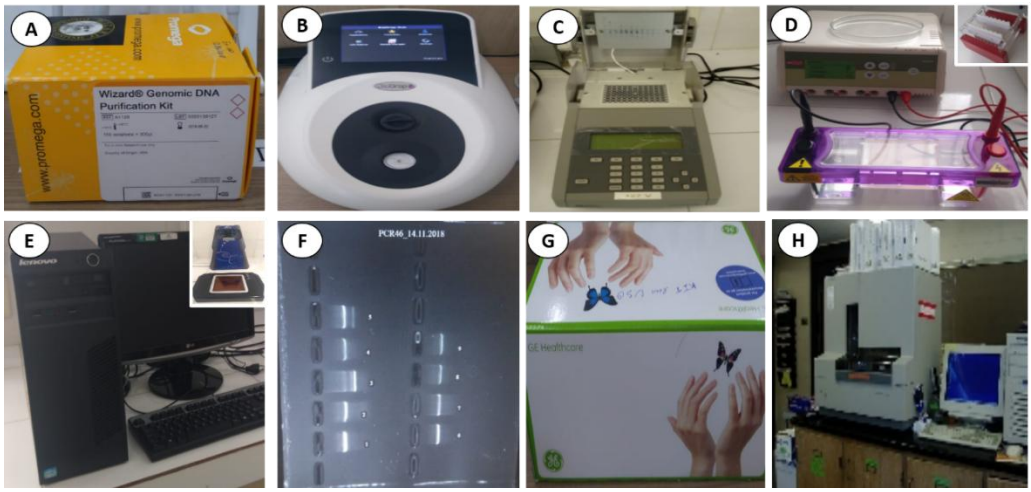

Figura 13 A. Kit para extração do DNA; B. Espectrofotômetro; C. Termociclador; D. Cuba de eletroforese horizontal; E. Computador com destaque no Fotodocumentador; F. Imagem da Reação em cadeia da polimerase; G. Kit de purificação das amostras; H. Sequenciador.

Fonte: (LGA,2018)

3.4 Análise filogenética de microparasitos Eucariotos

Os relacionamentos filogenéticos de cada táxon foram obtidos via análise de máxima parcimônia e Bayesiana, com auxílio dos programas PAUP 4.0 b10 (SWOFFORD, 2003), para a primeira e MrBayes 3.1.2 (RONQUIST & HUELSENBECK, 2003) para a última.

A análise de máxima parcimônia foi realizada com um algoritmo de busca heurística, em que foi dado igual peso para transições e transversões, enquanto inserções e deleções (indels) foram tratadas como dados perdidos. O nível de confiança dos nós da(s) árvore(s) mais parcimoniosa(s) foi avaliado por 1.000 réplicas de *bootstrap* (FELSENSTEIN, 2004).

Para a análise Bayesiana foram realizadas duas corridas paralelas de quatro buscas simultâneas, usando o método de Monte Carlo via cadeia de Markov – MCMC, por 5.000.000 gerações cada, amostrando uma árvore a cada 1.000 gerações e descartando os resultados das primeiras 1250 árvores (25% da amostra). As restantes, 3750 árvores, usadas para estimar o nível de confiança (probabilidade posterior) de cada nó na reconstrução filogenética.

Para ambas as análises, sequências de organismos relacionados a cada grupo em estudo foram obtidas diretamente do Genbank, bem como o grupo externo.

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Ellipsomyxa tucujuensis n. sp

PPGCA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AMBIENTAIS

Ellipsomyxa tucujuensis n. sp (Myxozoa: Ceratomyxidae), em *Satanoperca jurupari* (Osteichthyes: Cichlidae) no rio Curiaú, Amapá, Amazônia Brasileira

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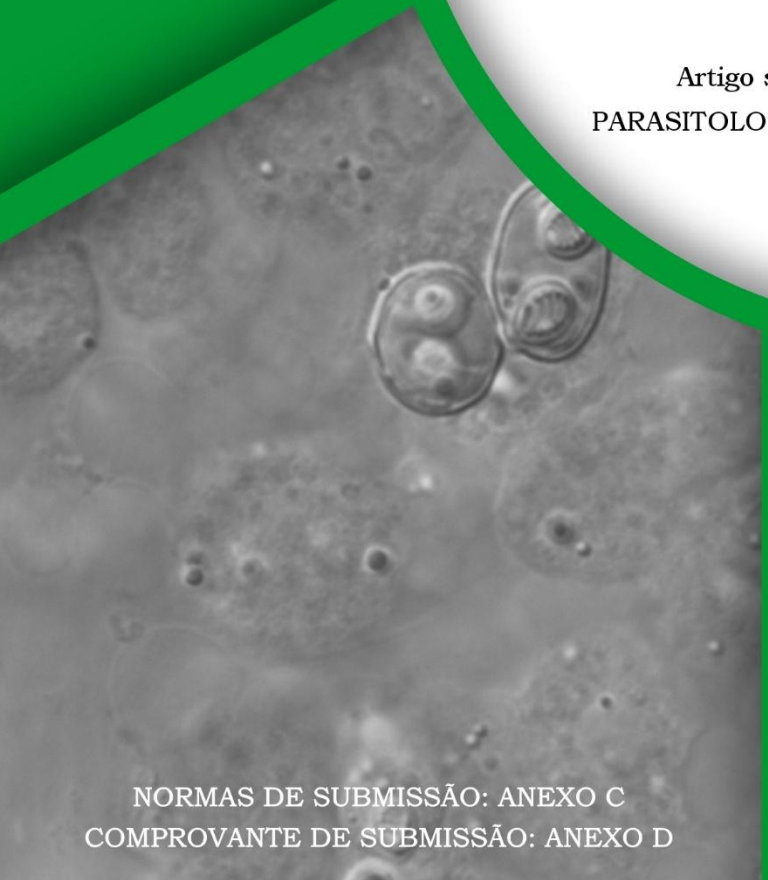
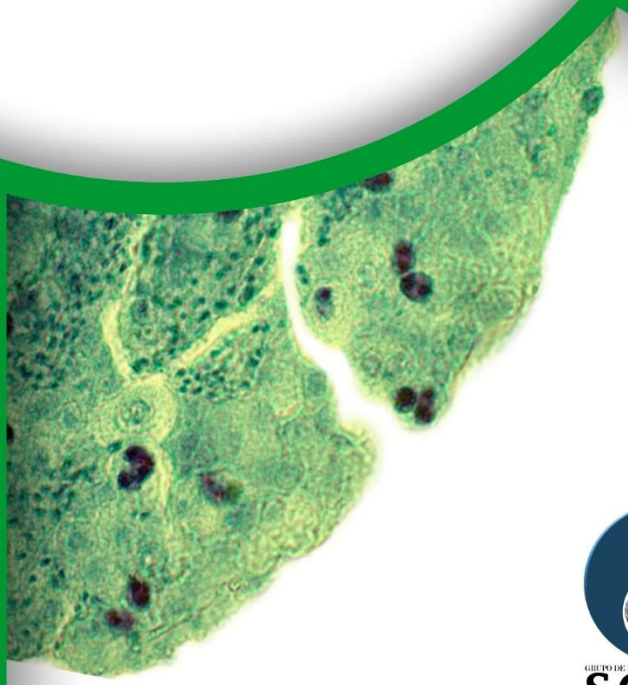
Artigo submetido ao periódico "SYSTEMATIC PARASITOLOGY" QUALIS B1 EM CIÊNCIAS AMBIENTAIS





NORMAS DE SUBMISSÃO: ANEXO C
COMPROVANTE DE SUBMISSÃO: ANEXO D

GRUPO DE PESQUISA SOAA
SANIDADE DE ORGANISMOS AQUÁTICOS NA AMAZÔNIA

Ellipsomyxa tucujuensis n. sp (Myxozoa: Ceratomyxidae), em *Satanoperca jurupari* (Osteichthyes: Cichlidae) no Rio Curiaú, Amapá, Amazônia Brasileira

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RESUMO

O *Ellipsomyxa tucujuensis* n. sp. é um novo parasito encontrado na vesícula biliar do *Santanoperca jurupari*. Uma espécie de ciclídeo de ambiente lêntico, hábito diurno e se alimenta principalmente de microcrustáceos, sementes de frutas, gramíneas e peixes pequenos, bem como larvas de insetos aquáticos e terrestres. Foram capturados na Área de Proteção Ambiental do Rio Curiaú, Amapá, Brasil. Foi observado a presença de plasmódios assimétricos, de forma irregular e esporos livres no líquido vesical, sem formação de cisto. Os esporos elípticos apresentam comprimento médio de 10.11 (8.56-10.5) µm, de largura média 7.81 (5.96-9.56) µm e paredes espessas. As cápsulas polares apresentaram formato subesférico, levemente assimétricas de comprimento médio 3.12 (2.31-3.99) µm, largura 2.5 (2.22-2.95) µm, contendo filamento polar de 5 à 6 voltas perpendiculares ao eixo longitudinal de cada cápsula. Com base nas análises das sequências do 18S rDNA do *Ellipsomyxa tucujuensis* n. sp. esporos encontrados no *S. jurupari*, da inferência bayesiana e na distância *p*, somado as análises morfológicas, confirmando assim uma nova espécie.

Palavras-chave: Peixe, água doce, parasita, Amazônia.

ABSTRACT

Ellipsomyxa tucujuensis n. sp. is a new parasite species found in the gallbladder of *Satanoperca jurupari*. It is a lentic environment species with day-long habit and feeds primarily on microcrustaceans, fruit seeds, grasses, and small fish, as well as aquatic and terrestrial insect larvae. The fish specimens were captured from the Curiaú River Environmental Protection Area, Amapá, Brazil. Asymmetric and irregularly shaped plasmodia and free spores were observed in the bladder fluid, without cyst formation. The elliptical spores had an average length of 10.11

(8.56–10.5) µm, an average width of 7.81 (5.96–9.56) µm, and thick walls. The polar capsules had a subspherical and slightly asymmetrical shape with an average length of 3.12 (2.31–3.99) µm, and a width of 2.5 (2.22–2.95) µm, containing polar filaments with 5 to 6 coils perpendicular to the longitudinal axis of each capsule. Based on analysis of the 18S rDNA sequences of the *Ellipsomyxa tucujuensis* n. sp. spores found in *S. jurupari*, Bayesian inference, *p* distance, and morphological analysis, it was recognized and confirmed as a new species.

Keywords: Fish, freshwater, parasite, Amazon

INTRODUCTION

The state of Amapá is located in the Amazon basin geographical region. Fishing in this area, as its main engaging activity, is predominantly manual, which makes it less competitive compared to the fishing practiced on the coast of Amapá by national and international boats (Silva & Tavares-dias, 2010). Located close to the urban area of the capital of Amazonas, the Environmental Protection Area of the Curiaú River (*Área de Proteção Ambiental* (APA)) is strongly influenced by anthropic actions, specifically predatory fishing, which leads to the creation of the APA (Amaral et al., 2015).

Currently, there are approximately 5,160 species of freshwater fish in South America, and the estimate for the world's freshwater ichthyofauna is between 8,000 and 9,000 different species (Reis et al., 2016).

Ichthyologic studies are still scarce considering the high diversity of fish in Brazil, known for the country's extensive series of watersheds formed by rivers and lakes (Eiras et al., 2010). Fish are parasitized by many species belonging to several phyla. The parasites are distributed worldwide, affecting all species, from tropical to polar waters involving many ecological niches and host habitats (Thatcher et al., 1991 Eiras et al., 2010).

Myxozoa are parasites characterized by a complex life cycle, alternating between hosts, fish, and invertebrates in different species. Recent genomic analyses have suggested a common origin of *Polypodium hydriform*, a cnidarian parasite of acipenseriform kinds of fishes, and Myxozoa, and it was suggested that fish are the original hosts for both lineage "companions" (Holzer et al., 2018).

The spores of the parasites in this group range from 2 to 7 valves, connected by a suture line, 1 to 2 spiroplasmas and 1 to 7 polar capsules, a complex structure with an evasive tube (or filament) that facilitates interaction with the host (Eiras et al., 2010; Chang et al., 2015). More than 2,220 species are described for Myxozoa in 64 genera and 17 families, classified mostly by spore morphology (Fiala et al., 2015). Molecular biology provides highly sensitive and specific techniques for the detection and analysis of the information present in the genome of pathogens and their hosts (Caldart et al., 2016).

The genus *Ellipsomyxa* Køie, 2003 is a taxonomic group of the metazoa of the phylum Cnidaria. It has ellipsoid (slightly oval) spores and two spherical and slightly asymmetric capsules perpendicular to the suture line, representing a small group with 12 described species, wherein only one species has not been yet analyzed on the molecular level.

The *Ellipsomyxa* of this study has asymmetric ellipsoid spores, two slightly asymmetric subspherical divergent polar capsules located at the ends of the spores, with characteristics that differ from those described in freshwater environments: *E. adlardi* (WHIPPS & FONT, 2013), *E. amazonensis (*Zatti et al., 2018a) and *E. arariensis* (Silva et al., 2018).

This study describes a new Myxozoa species of the genus *Ellipsomyxa* and its molecular characteristics using morphological analysis. The host, *Satanoperca jurupari*, known as "cará bicudo" in the Amazon region, is an essential component of human consumption and fishkeeping. Despite the importance of this fish for the Amazon region, little is known about its parasitic fauna. So far, no Myxozoa has been described in this species.

MATERIAL AND METHODS

The 42 specimens used in this study were obtained from the Curiaú River, Macapá, Amapá, Brazil, (0º8'43.6"N, 51º2'30.3"W). They were captured by the team of the Laboratory of Morphophysiology and Animal Health (LABMORSA) of the State University of Amapá (UEAP), with the help of local fishers using a 30 mm cast net between September 2018 and January 2019. The experiment was approved by the Animal Use Committee of the Brazilian Agricultural Research Company - Amapá (012–2018) and registered in the Biodiversity Authorization and Information System, IBAMA (SISBIO/ICMBIO License number 50376-1).

The captured animals were conditioned and transported alive in thermal boxes with portable pumps to LABMORSA/UEAP. They were then acclimatized and stored in glass aquariums with electric pumps for aeration. Parasitological analyses were subsequently performed.

In the aquariums, the animals were anesthetized using tricaine methanesulfonate (MS222; Sigma) at a concentration of 50 mg/L. Their entire body surface was analyzed using binocular stereoscopic microscopy to detect lesions (cysts) or loss of coating (scales and epidermis). Foci of development of parasites and their spore phase were found in the gall bladders. Small fragments were removed, placed between the slide and the glass slide, and observed by light microscopy (LM) to confirm parasitosis. Gall bladder fragments were fixed in Davidson solution (70% alcohol, formaldehyde, acetic acid, and distilled water) for 24 hours, before being histologically processed for inclusion in paraffin. They were then stained with hematoxylin and eosin (H&E) and Ziehl-Neelsen

(ZN) and photographed in the Carlos Azevedo Research Laboratory at the Federal Rural University of the Amazon (LPCA/UFRA). The methodology proposed by Bush et al. (1997) was used to calculate the prevalence.

The spore fragments of eukaryotic microparasites were collected and stored in ethyl alcohol 80% at 4 °C. The total DNA of each sample was extracted using the Wizard® Genomic DNA Purification Kit. DNA samples were quantified by spectrophotometry (Biodro-Duo).

All molecular analyses were based on the 18S rDNA sequences, which were amplified according to the methodology described by Silva et al. (2018). GE Healthcare® GFX™ PCR DNA Gel Band purification reagents were used to purify the material after Polymerase Chain Reaction (PCR), as described by the manufacturer. These steps were performed at the Laboratory of Applied Genetics at the Federal Rural University of the Amazon (LGA/UFRA).

The amplification product was sequenced on an ABI 3730 automatic DNA analyzer using the BigDye v3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems). For the confirmation of the observed mutations, the sample was sequenced with both direct and reverse primers. The obtained nucleotide sequences were edited and aligned using the BioEdit program (Hall, 1999). The phylogenetic relationship was obtained through maximum parsimony and Bayesian analyses, using the PAUP 4.0 b10 program (Swofford, 2003) for the former, and the MrBayes 3.1.2 program (Ronquist & Huelsenbeck, 2003) for the latter. Maximum parsimony analysis was performed with a heuristic search algorithm, in which equal weight was given for transitions and transversions, while insertions and deletions (indels) were treated as lost data. The confidence level of the most parsimonious tree nodes was evaluated by 1,000 bootstrap replicates (Felsenstein, 2004).

In the Bayesian analysis, two parallel runs of four simultaneous searches were performed using the Markov chain Monte Carlo method (MCMC) for 5,000,000 generations each, sampling one tree every 1,000 generations and discarding the results of the first 1,250 trees (25% of the sample). The remaining 3,750 trees were used to estimate the confidence level (subsequent probability) of each node in the phylogenetic reconstruction. In the cladogram analysis, the DNA sequences of the organisms were obtained directly from GenBank (XXX).

RESULTS

Ellipsomyxa spores and asymmetrical and irregular plasmodia were found in the gallbladder of *S. jurupari* (Fig. 1A) and insert polar filament extrusion, with a prevalence of 80.95% (n = 34). The infection was not accompanied by any cyst formation, however, disporic plasmodia (Fig. 1B) and free spores were observed. A

special staining technique, Ziehl-Neelsen, highlighted the spores and increased their visibility in the histological sections of the gallbladder (Fig. 1C).

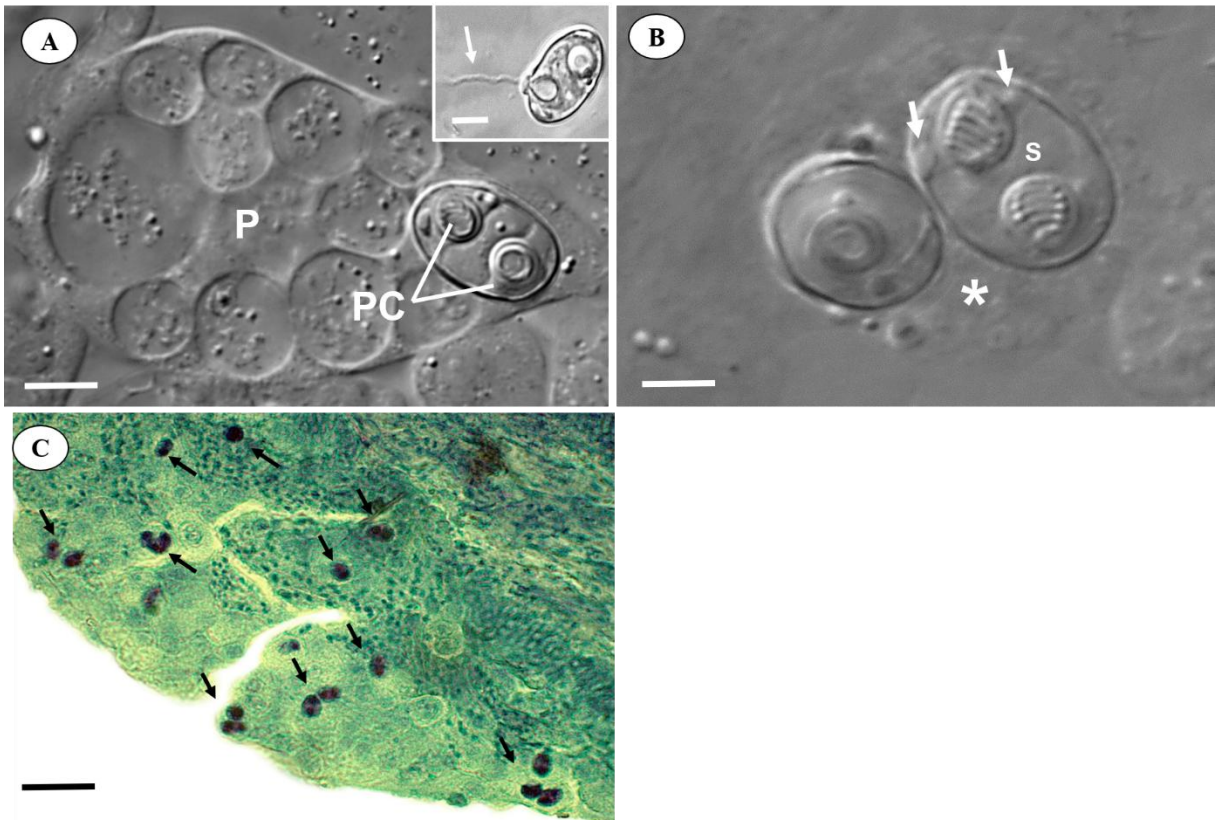

Fig. 1 Photomicrography of *Ellipsomyxa tucujuensis* n. sp. in the gallbladder of *Satanoperca jurupari*. A- Developing plasmodium (P) and mature spore with polar capsules (PC). Scale bar 10 µm. Inset: Extrusion of the polar filament (arrow) of a spore. Scale bar 5 µm; B- Plasmodium (*) with spores and suture line (arrow) (lateral view) and sporoplasm (S). Scale bar 10 µm; C - Histological section of the gallbladder stained by special Ziehl-Neelsen technique. Spores are marked with the arrows. Scale bar 10 µm

Spore description: The spores had an ellipsoidal morphology with an average length of 10.11 ± 0.86 µm (n = 30), an average width of 7.81 ± 1.14 µm (n = 30), and thick walls. The polar capsules were slightly asymmetrical and subspherical in shape, with an average length of 3.12 ± 0.53 µm (n = 30) and a width of 2.5 ± 0.32 µm (n = 30), each containing a polar filament of 5 to 6 coils perpendicular to the longitudinal axis of each capsule.

Taxonomy

Phylum Cnidaria Hatscheck, 1888

Family Ceratomyxidae Doflein, 1899

Genus *Ellipsomyxa* KØie, 2003

Ellipsomyxa tucujuensis n. sp. (Fig. 2)

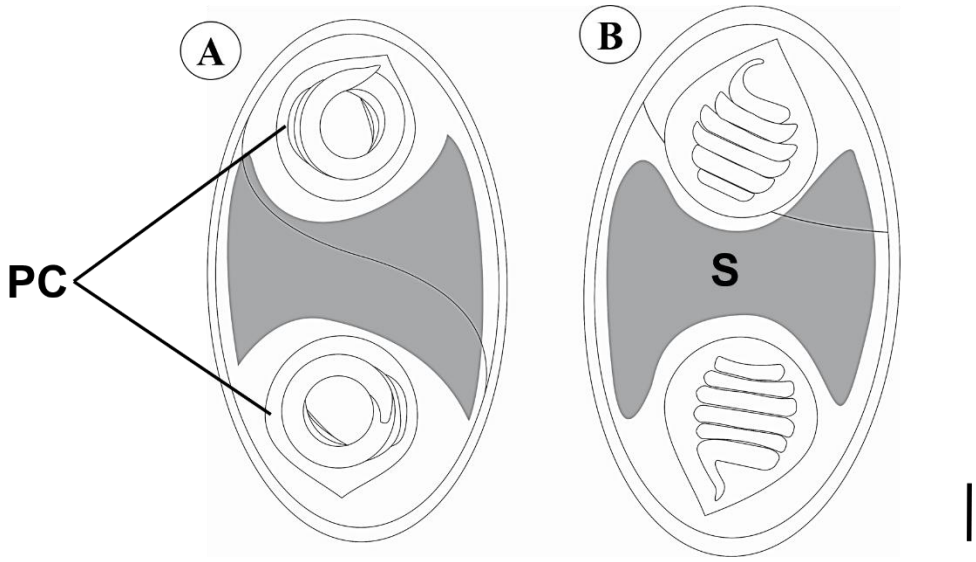

Fig. 2 Schematic representation of *Ellipsomyxa tucujuensis* n. sp. A -Apical view of spore and polar capsules (PC); B - Lateral view of spores and sporoplasm (S). Scale 10 µm

Host: *Satanoperca jurupari* Heckel, 1840

Site of infection: Gallbladder

Location: Environmental protection area of the Curiaú River, Macapá, state of Amapá.

Etymology: The species *Ellipsomyxa tucujuensis* n. sp. is named in honor of the indigenous ethnic group that inhabited the left bank of the mouth of the Amazon River, where the city of Macapá, capital of the state of Amapá, is currently located (Table 1).

Material registration: a glass slide with spores stained with H&E was deposited in the INPA collection (accession number 59). DNA sequence: The 18S rDNA (1,680) gene was sequenced and deposited in GenBank (accession number MN999871).

Taxonomic affinities: A sequence was obtained from small subunit ribosomal (SSu rRNA) through the spores of *E. tucujuensis* n. sp. found in the gallbladder of *S. jurupari* collected in the Environmental Protection Area of Curiaú. The collected spores were comprised of a total of 1,680 basepairs. The analysis of the phylogenetic tree showed two branches: clade A, where the *Ellipsomyxa* were grouped, and clade B, both with strong nodal support. The species of *Ellipsomyxa* grouped with *Ellipsomyxa tucujuensis* n. sp. are found in the Amazon region, including *Ellipsomyxa arariensis* (Silva et al. 2018), *Ellipsomyxa amazonensis* (Zatti et al. 2018a), and a species whose bases are only deposited in GenBank (Table 2), which, as described, belongs to the Amazon region. The nodal support of the "Amazon region" subclade is considered as strong (Fig. 3).

Table 1 Comparative descriptive measures (mean (μm), with ranges and ± standard deviation (SD) in parentheses (μm)) of already registered *Ellipsomyxa tucujuensis* n. sp. and all *Ellipsomyxa* spp. together with their respective authors.

SPECIES	HOST	CO	HABITAT	SM	SPORE (μm)		MPC	POLAR CAPSULE (μm)		PF	References
					SL	SW		LPC	WPC		
Ellipsomyxa tucujuensis n. sp.	*Satanoperca jurupari*	Brazil	Freswater	Ellipsoid	10.11 ±0.86	7.81 ±1.14	Subspherical	3.12 ±0.53	2.5 ±0.32	5–6	Present study
Ellipsomyxa gobii	*Pomatoschistus microps*	Dinamarca	Marine	Ellipsoid	7.0 (6.6–7.5)	8.7 (8.0–9.0)	Spherical	3.1 (3.0–3.2)	-	6–7	Køie (2003)
Ellipsomyxa arariensis	*Pigocentrus Nattereri; Pimelodus ornatus*	Brazil	Freswater	Ellipsoid	12.6 ±0.5	7.3 ±0.6	Pyriform	3.5 ±0.2	2.6 ±0.3	5–6	Silva et al. (2018)
Ellipsomyxa mugilis	*Liza saliens*	Spain	Marine	Ovoid	11.5 (10–13.5)	6.8 (5.5–8.0)	Subspherical	2.9 (2.7–4.0)	-	5	Sitjà-Bobadilla e Alvarez-Pellitero (1993)
Ellipsomyxa syngnathi	*Syngnathus rostellatus*	Denmark	Marine	Ellipsoid	6.8 (6.3–7.2)	8.1 (7.2–8.6)	Pyriform	3.6 (3.2–4.1)	2.9 (2.7–3.2)	5–6	Køie and Karlsbakk (2009)
Ellipsomyxa gobioides	*Gobioides broussonnetii*	Brazil	Estuarine	Ellipsoid	6.8 (6.5–7.0)	7.2 (6.9–7.5)	Pyriform	4.6 (4.3–4.8)	2.5 (2.1–2.7)	5–6	Azevedo et al. (2013)
Ellipsomyxa adlardi	*Gobiosoma bosc*	USA	Marine	Ellipsoid	12.4 (11.3–14.4)	7.7 (7.1–8.8)	Pyriform	4.3 (3.9–4.9)	3.6 (3.3–4.1)	5–6	Whipps and Font (2013)
Ellipsomyxa manilensis	*Arothron manilensis*	Australia	Marine	Ovoid	15.2 (13.8–17.1)	11.8 (10.2–13.3)	Subspherical	5.6 (4.6–6.6)	4.5 (4.2–5.0)	3–4	Heiniger and Adlard (2014)
Ellipsomyxa arothroni	*Arothron hispidus*	Australia	Marine	Ovoid	14.5 (11.3–16.0)	12.2 (9.4–13.8)	Pyriform	5.5 (4.5–6.7)	4.2 (3.1–5.0)	5–6	Heiniger and Adlard (2014)
Ellipsomyxa nigropunctatis	*Arothron nigropunctatus*	Australia	Marine	Ovoid	13.8 (11.9–16.3)	9.9 (8.0–12.9)	Pyriform	4.7 (3.5–5.7)	3.6 (2.8–4.6)	5	Heiniger and Adlard (2014)

Ellipsomyxa apogoni	*Apogon doederleini*	Australia	Marine	Ellipsoid	10.2 (8.8–11.1)	6.9 (6.0–9)	Pyriform	3.7 (2.9–4.8)	2.7 (2.1–3.4)	2–3	Heiniger and Adlard (2014)
Ellipsomyxa kalthoumi	*Liza saliens*	Tunisia	Marine	Ellipsoid	17.2 (13–21)	13.2 (10–15)	Spherical	5.5 (5–6)	-	9	Thabet et al. (2016)
Ellipsomyxa amazonenis	*Brachyplatystoma rousseauxii*	Brazil	Freswater	Ellipsoid	12.80 (12.3–13.6)	7.6 (6.7–8.7)	Pyriform	3.8 (3.8–4.0)	3.1 (2.5–3.4)	2–3	Zatti et al. (2018)

Subtitle: CO - Countries; SM - Spore Morphology; SL - Spore length; SW - Spore width; MPC - Morphology of the polar capsule; LPC - length of polar capsule; WPC - Polar capsule width; PF - Polar filament.

Table 2 *P* distance of *Ellipsomyxa* spp. that constitutes the clade registered in the "Amazon region."

Species	1	2	3	4
Ellipsomyxa tucujuensis n. sp (MN999871)		0.0073	0.0080	0.0074
Ellipsomyxa sp. (MH364399)	0.0791		0.0037	0.0013
Ellipsomyxa arariensis (MH183026)	0.0994	0.0203		0.0038
Ellipsomyxa amazonensis(MH193889)	0.0798	0.0023	0.0226	

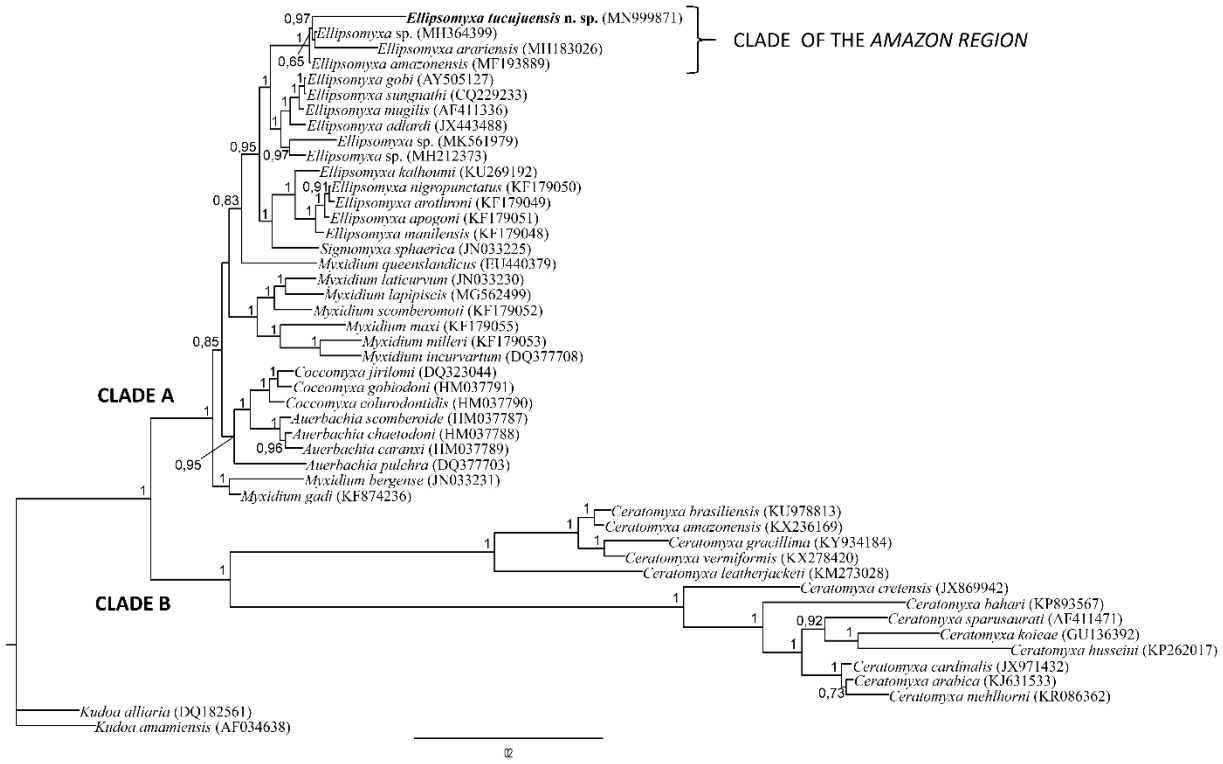

Fig. 3 Phylogenetic tree generated by Bayesian inference (IB) partial alignment of *Ellipsomyxa tucujuensis* n. SSU rDNA gene sequences. sp. and related successors. GenBank access numbers are shown next to the species names. Nodes on the nodes are the following probability values calculated by BI. The new species is highlighted in bold and within the *Ellipsomyxa* clade of the Amazon region.

DISCUSSION

Oceania is currently the leading geographic region in terms of the number of publications on the genus *Ellipsomyxa* (34%), followed by Europe and South America (25% each). With this publication, South America has equaled Oceania, highlighting the Amazon region. North America and Africa together account for 16% of these publications.

Among the species of the genus *Ellipsomyxa* reported to date, 69% are found in the marine environment (Whipps & Font, 2013), Gobiosoma *bosc*, in brackish water. Twenty-three percent of the species are found in freshwater, specifically *E. amazonensis* (Zatti et al., 2018a), which has a migratory host (*Brachyplatystoma rousseauxii*), *E. arariensis* (Silva et al., 2018), and *E. tucujuensis* n. sp. Only 8% of the species, specifically *E. gobioides* (Azevedo et al., 2013), are found in the estuarine environment, which is considered a transition environment between the marine and river environments. Under these conditions, one or more rivers flow into the sea and the forces of both environments control the dynamics and distribution of the chemical, biological, and sedimentary properties (Perillo, 1995).

Taking into account the morphology, organ specificity, and molecular/phylogenetic analysis of the identified spores, we observed a similarity between the species and the genus *Ellipsomyxa* described by KØIE, 2003. Thus, herein we describe a new species *Ellipsomyxa tucujuensis* n. sp., infecting the gallbladder of *S. jurupari*, popularly known as "cará bicudo". This is the first description of this genus of the order Cichliformes, the second description of the genus in the Amazon region, and the second Myxozoa described in the state of Amapá, the first being *Henneguya jariensis* (Zatti et al., 2018b).

Ellipsomyxa tucujuensis n. sp. was found in the form of plasmodia and mature spores. Its plasmodia are irregular in shape and are distinct from those described until now. Mature monospores were found in the numerous plasmodia in the cavity of the organs, when the plasmodium had immature spores, or in the dispores upon maturation. Although other species, such as *E. gobioides* and *E. arariensis* (Whipps & Font, 2013 and Silva et al., 2018), also have this characteristic, only *E. syngnathi* is described as monosporic (Køie, 2003).

Interestingly, 67% of mature spores were elliptical (similar to the already described species) and 33% oval in shape, showing no difference from the species described in Brazil. Although *E. tucujuensis* n. sp. is considerably larger than *E. syngnathi* and *E. gobioides* (Køie & Karlsbakk, 2009 and Azevedo et al., 2013), these species have similar widths.

With regard to the characteristics of the polar capsules of the described species, 62% were pyriform, 23% were subspherical, and 15% had spherical shape. *E. tucujuensis* n. sp. is subspherical in shape, as well as *E. mugilis* (Sitjà-Bobadilla & Alvarez-Pellitero, 1993; Whipps & Font, 2013) and *E. manilensis (*Køie & Karlsbakk, 2009).

The length of the polar capsule of *E. tucujuensis* n. sp. is close to that of *E. gobii* and differs from that of *E. arothroni* (Køie, 2003 and Heiniger & Adlard, 2014). Despite this difference in length and width, the number of coils of the polar filament is the same.

Ellipsomyxa myxosporidium was reported to have a transverse suture line with its spores having a straight or winding, perpendicular central suture line, forming an acute angle to the longitudinal axis (Køie, 2003; Køie & Karlsbakk, 2009). However, careful analysis of the described species provided evidence that the descriptions of the suture line were not consistent with the published photographs, which questioned the suture line, a marked characteristic of the species of the genera *Zschokkella* and *Ellipsomyxa* (Whipps & Font, 2013).

The suture line of *E. tucujuensis* n. sp. approaches the upper part of a polar capsule, towards the lower part of the opposite one. In an apical view, it is a winding transversal suture line, while in lateral view, the suture line passes over a polar capsule. Besides, in the apical view, it resembles *E. arothroni*, and in lateral view, it resembles *E. manilensis* and *E. arariensis*. Its suture line resembles that of *Zschokkela bicarinatis* (Whipps & Font, 2013).

Thus far, all the characteristics described above support the identification of a new species. Biomolecular characteristics and phylogenetic indicators are included in its description. We can highlight an isolated case in which a parasite belonging to the genus *Ellipsomyxa* was reclassified, and due to its detailed characteristics of morphology and phylogeny was transferred to the genus *Zschokkella*, *Z. mugilis* (Sitjà-Bobadilla & Alvarez-Pellitero, 1993; Holzer et al. Fiala, 2006; Whipps & Fonte, 2013; Fiala et al. 2015; Silva, 2018).

The phylogenetic analyses of the SSR rRNA gene indicated that *E. tucujuensis* n. sp. in the Amazon region forms a clade with all the other *Ellipsomyxa* species, especially those of the freshwater, confirming the monophyletic configuration of the genus, with subclades of species recently found in the same region.

To date, four freshwater hosts have been reported: *Brachyplatystoma rousseauxii* ZATTI (2018a), *Pigocentrus Nattereri*, *Pimelodus ornatus* Silva (2018), and *S. jurupari*, with the final host being presented in this study. There are no reports on a migratory character of the host of the *E. tucujuensis* n. sp. Parasite. Furthermore, according to the study by Matos et al. (2002) on spermatogenesis, mature sperm, and ultrastructural description of *S. jurupari*, it is a simultaneous hermaphrodite species that undergoes internal self-fertilization.

The life cycle of *E. mugilis* was described at the actinospore stage, with pansporocysts being found in marine polychaetes, similar to those found in *E. gobii* (Køie, 2003; Køie et al. 2004; Rangel et al. 2009; Silva, 2018). Silva et al. (2018) stated that polychaetes are likely to serve as a food source when entering the freshwater environment. Therefore, it seems plausible that the actinospores of *Ellipsomyxa* spp. are present in the euryhaline polychaetes ingested by freshwater fish, continuing thereby the cycle and resulting in the transfer of the parasite to this host. The region of the host of *E. tucujuensis* n. sp. is a floodplain near the mouth of the Amazon River.

Ellipsomyxa spp. are bile vesicular parasites of estuarine or marine fish (Azevedo et al. 2013; Whipps & Font 2013; Heiniger & Adlard 2014; Thabet et al. 2016; Zatti et al. 2018a), except for *E. arariensis* and *E. tucujuensis* n. sp.

CONCLUSION

The morphological, molecular, and phylogenetic characteristics of *E. tucujuensis* n. sp. correspond to the genus *Ellipsomyxa*, which is a parasite also reported in freshwater hosts. As such, this study demonstrates that this genus is also present in non-marine environments.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare no conflict of interest.

Ethical approval All applicable institutional, national, and international guidelines for the care and use of animals were followed.

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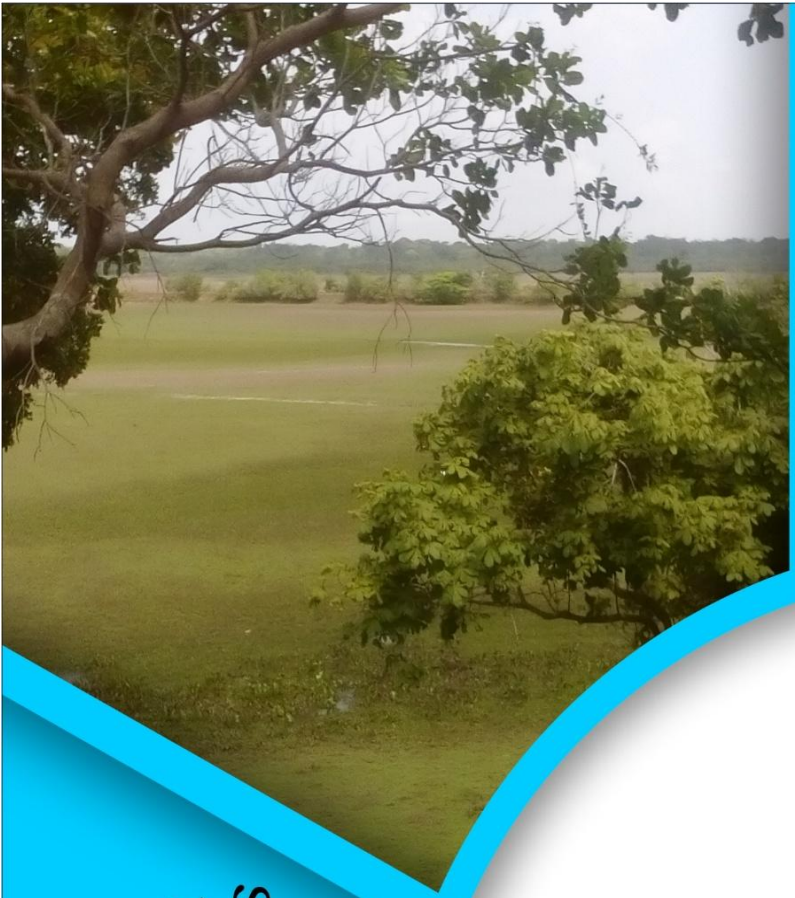

Henneguya sacacaensis n. sp

PPGCA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AMBIENTAIS

Henneguya sacacaensis n. sp (Myxozoa: Myxosporea) parasitando brânquias do acará bicudo, *Satanoperca jurupari* (Osteichthyes: Cichlidae) na Amazônia oriental

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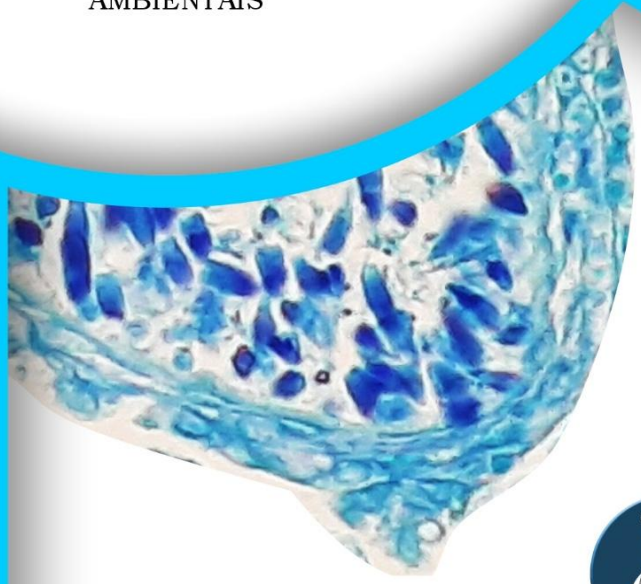

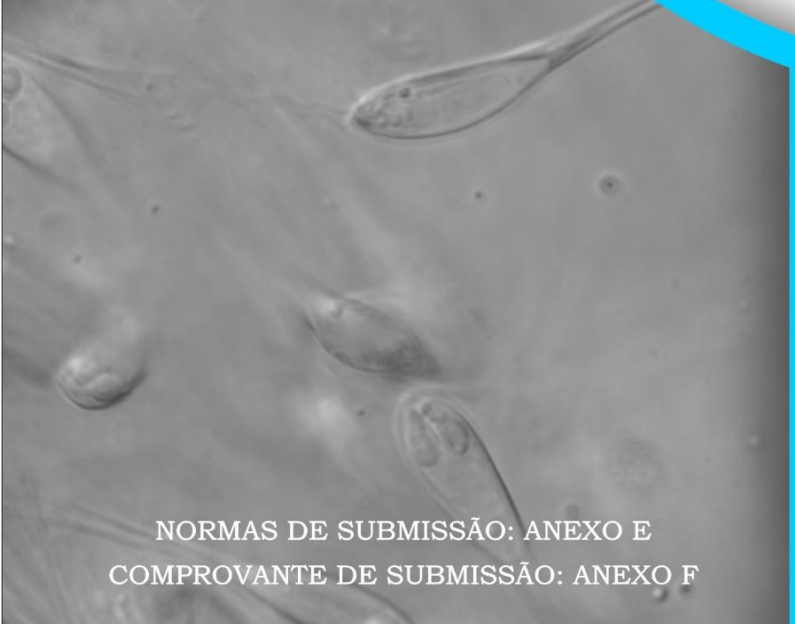

NORMAS DE SUBMISSÃO: ANEXO E

COMPROVANTE DE SUBMISSÃO: ANEXO F

GRUPO DE PESQUISA SOAA
SANIDADE DE ORGANISMOS AQUÁTICOS NA AMAZÔNIA

Henneguya sacacaensis n. sp. (Myxozoa: Myxosporea) parasitizing gills of the acará bicudo *Satanoperca jurupari* (Osteichthyes: Cichlidae) in eastern Amazonia

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Running title: *Henneguya sacacaensis* n. sp. parasitizing *Satanoperca jurupari* in eastern Amazonia

Abstract

This study describes *Henneguya sacacaensis* n. sp. in specimens of the Osteichthyes *Satanoperca jurupari* (Heckel, 1840), collected in the Rio Curiaú Environmental Protection Area in the city of Macapá, state of Amapá Brazil. Using optical microscopy and molecular analysis, these cyst-shaped parasites were analyzed. The gills of 57.14% of the analyzed *Satanoperca jurupari* contained hundreds of spores. The cysts found on the gill lamellae were oval-shaped and whitish. The *Henneguya* spores had an average length of 46.5 ± 5.47 µm. The fusiform body of the *Henneguya* measured 16.5 ± 2.64 µm long and 5.1 ± 0.94 µm in width, the two polar capsules had a taper of 3.83 ± 0.31 µm and a width of 1.68 ± 0.20 µm, and the tail measured 30 ± 6.87 µm in length, containing a polar filament coiled seven to nine times. The spores found in this study differ from others in their shape, size, and arrangement of the coiled polar filament. Therefore, we conclude that this is a novel species, *Henneguya sacacaensis* n. sp, that belongs to the family Myxobolidae and the genus *Henneguya*.

Keywords: Freshwater, Myxobolidae, fish, parasite, Amazon, gill

RESUMO

Henneguya sacacaensis n. sp. é descrito em espécimes do Osteichthyes *Satanoperca jurupari* Heckel, 1840, coletados na área de Proteção Ambiental do Rio Curiaú na cidade de Macapá no estado do Amapá, Brasil. Com auxílio de microscopia óptica e análises moleculares, esses parasitos foram analisados e observados nas brânquias em forma de cistos, contendo centenas de esporos e apresentaram a prevalência de 57,14%. Os cistos encontrados nas lamelas brânquias tinham formatos ovais e esbranquiçados. Seus esporos apresentaram um comprimento médio de 46.5 µm (±5.47), corpo fusiforme medindo 16.5 µm (±2.64) de comprimento e 5.1µm (±0.94) de largura, suas duas cápsulas polares

apresentam uma conicidade de 3.83µm (±0.31) e sua largura 1.68 µm (±0.20), a cauda 30 µm (±6.87) de comprimento, contento um filamento polar de 7 à 9 voltas. Os esporos encontrados nesse estudo diferem dos demais descritos por sua forma, tamanho e arranjo das voltas do filamento polar. Portanto, concluímos que essa espécie pertence à família Myxobolidae e ao gênero *Henneguya* que compreende em uma nova espécie: *Henneguya sacacaensis* n. sp.

Palavras-chave: Água doce, Myxobolidae, Peixe, parasita, Amazônia, brânquia.

Introduction

Myxozoa are endoparasites that can infect various organs and present high specificity (LÁSZLÓ et al., 2002). The genus *Henneguya* Thélohan, 1892 (Cnidaria: Myxobolidae) is one of the most diverse for the described species (LOM & DYKOVÁ, 1992) within the Myxosporea class and is the second largest genus, with about 190 described species worldwide (EIRAS & ADRIANO 2013).

Knowledge of parasitic biodiversity in South America is limited, especially in the Amazon region, due to the high ichthyofauna diversity and remoteness (REIS et al. 2016; ZATTI, 2018). Most species are freshwater and marine parasites and have a wide geographical distribution (LOM & DYKOVÁ 1992, 2006; VELASCO et al., 2016; SILVA et al., 2018).

The species of the genus *Henneguya* are predominantly histozoic, can infect various organs, and can cause considerable pathological changes (DYKOVÁ & LOM 1978; MOLNÁR 1998; POTE et al. 2000; ADRIANO et al. 2005; NALDONI et al. 2009; BARASSA et al. 2012; MORSY et al. 2012; MATHEWS, 2016). When they infect the gills, they can cause filament destruction and respiratory failure (LOM & DYKOVA, 1992).

Matos et al. (2004) describe that these are parasites of very different morphology and structure, found mainly in fish. The parasites are characterized by a complex life cycle, alternating between hosts, fish and invertebrates (HOLZER et al., 2018).

The morphology of this genus is characterized by the number and shape of the spore valves, the position, number, and shape of the polar capsules (round, piriform, or ellipsoid), the relative position of the suture line, the presence of grooves and superficial appendages, and the number of coiled polar filaments (FEIST & LONGSHAW 2006; LOM & DYKOVÁ 2006; KAUR & ATTRI, 2015).

This study describes the morphological characteristics and molecular aspects of a new species of Myxozoa, *Henneguya sacacaensis* n. sp., found in the gills of *Satanoperca jurupari*. To date, no Myxobolidae has been recorded to infect this host.

Material and Methods

The 63 specimens used for this study were collected from the Curiaú River, Macapá, Amapá, Brazil (0º8'43.6"N, 51º2'30.3"W) (Figure 1). They were captured between September 2018 and September 2019 by the team at the Laboratory of Morphophysiology and Animal Health (LABMORSA) of the State University of Amapá (UEAP), with the help of local fishermen using a 30 mm cast net. The experiment was approved by the Animal Use Committee of the Brazilian Agricultural Research Company - Amapá (012-2018) and registered in the Biodiversity Authorization and Information System, IBAMA (SISBIO/ICMBIO License number 50376-1).

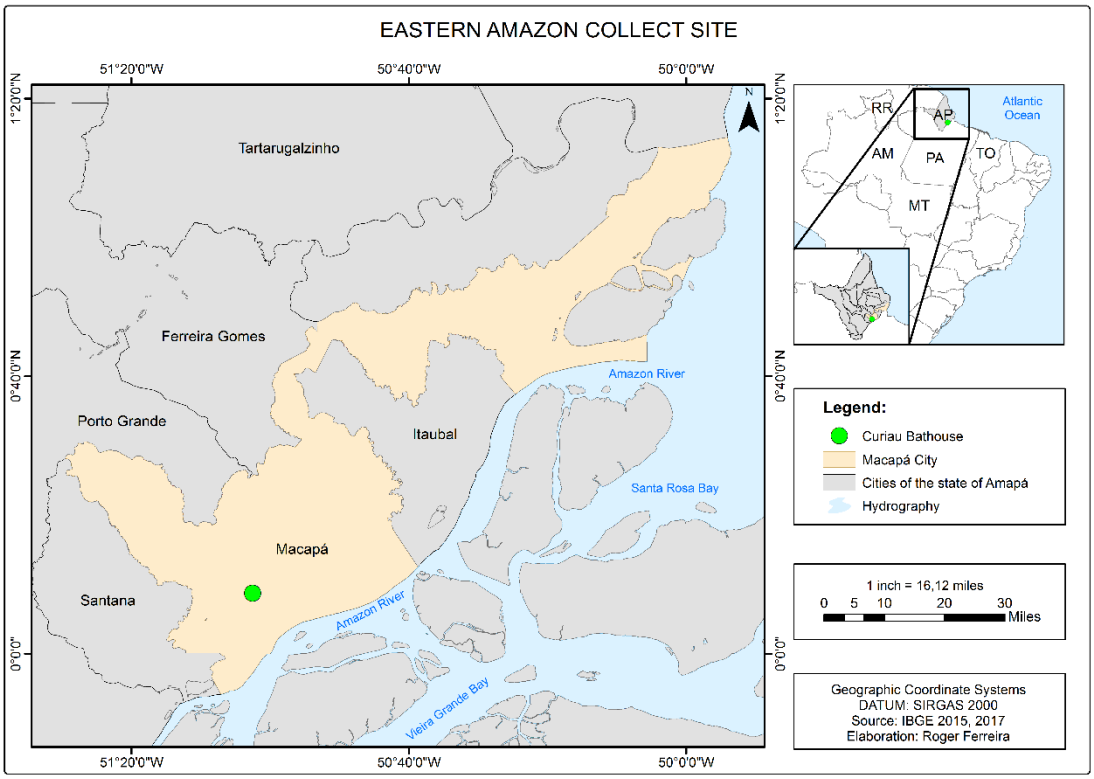


Figure 1 Map of the collection site in Macapá, Amapá: Rio Curiaú Environmental Protection Area, Eastern Amazon region.

The captured animals were conditioned and transported alive to LABMORSA/UEAP in refrigerated boxes with portable pumps. They were acclimatized and stored in glass aquariums with electric pumps for aeration. Subsequently, parasitological analyses were performed.

In the aquariums, the animals were anesthetized with tricaine methanesulfonate (MS222 SIGMA) at a concentration of 50 mg L^{-1}. Their body surface was examined with binocular stereoscopic microscopy to detect lesions (cysts) or loss of coating (scales and epidermis). Infectious foci of parasites in their developing and spore phases were found in the gall bladder. Small fragments were removed and observed with light microscopy (LM) to confirm parasitosis. Gall bladder fragments were fixed in Davidson's solution (70% alcohol, formaldehyde, acetic acid, and distilled water) for 24 hours before paraffin embedding for histopathology. They were then stained with hematoxylin and eosin (H&E) and Ziehl-Neelsen (ZN) and photographed at Carlos Azevedo Research Laboratory at the Federal Rural University of the Amazon (LPCA/UFRA). The methodology proposed by Bush et al. (1997) was used to calculate prevalence.

Spore fragments of eukaryotic microparasites were collected and stored in 80% ethyl alcohol at 4 ºC. The total DNA of each sample was extracted using the Wizard® Genomic DNA Purification Kit. DNA samples were quantified by spectrophotometry (Biodrop DUO).

All molecular analyses were based on the 18S rDNA sequences, which were amplified according to the methodology described by Velasco (2016). GFX™ PCR DNA and Gel Band Purification Kit reagents were used to purify the material after Polymerase Chain Reaction (PCR), as described by the manufacturer. These steps were performed at the Laboratory of Applied Genetics at the Federal Rural University of the Amazon (LGA/UFRA).

The amplification product was sequenced on an ABI 3730 automatic DNA analyzer using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). To confirm the observed mutations, the sample was sequenced with both forward and reverse primers. The nucleotide sequences obtained were edited and aligned using the BioEdit program (HALL, 1999). The phylogenetic relationship was obtained through maximum parsimony and Bayesian analyses, using the PAUP 4.0 b10 program (SWOFFORD, 2003) for the former and the MrBayes 3.1.2 program (RONQUIST & HUELSENBECK, 2003) for the latter. Maximum parsimony analysis was performed with a heuristic search algorithm, in which equal weight was given for transitions and

transversions, while insertions and deletions (indels) were treated as lost data. The confidence level of the most parsimonious tree nodes was evaluated by 1,000 bootstrap replicates (FELSENSTEIN, 2004).

In the Bayesian analysis (BI), two parallel runs of four simultaneous searches were performed using the Markov chain Monte Carlo method (MCMC) for 5,000,000 generations each, sampling one tree every 1,000 generations and discarding the results of the first 1,250 trees (25% of the sample). The remaining 3,750 trees were used to estimate the confidence level (subsequent probability) of each node in the phylogenetic reconstruction. In the cladogram analysis, the DNA sequences of the organisms used were obtained directly from GenBank.

As indicated by JMODELTEST 2.0.2 (DARRIBA et al. 2012), BI analysis assumed a GTR + G model of nucleotide substitution with estimated nucleotide frequencies (A = 0.2520, C = 0.1844, G = 0.2831, T = 0.2805), substitutions (A – C = 1.3916, A– G = 1.0936, A – T = 1.3916, C – G = 1, C – T = 4.3568, G – T = 1), and rates for variable sites following a gamma distribution (G = 0.4200) and *p*-inv 0.1990.

Results

VEGETATIVE PHASE

Henneguya parasites can form cysts at various sites in the gill filaments, and infection can be characterized as lamellar, filament, or arch (in gills arches) (MOLNÁR, 2002). Light microscopy analysis showed whitish cysts and ellipsoids in a thin layer of connective tissue (Figure 2-A). The cysts caused slight deformity of the gill filament (mild filamentous hyperplasia) of *S. jurupari* (Figure 2B-D).

SPORES

Fresh histozoic spores were measured (n = 31) (Figure 2 D). Average total lengths were 46.5 ± 5.47 µm; spores bodies averaged 16.5 ± 2.64 µm in length and 5.1 ± 0.94 µm in width. The two polar capsules have a taper 3.83 ± 0.31 µm in length and 1.68 ± 0.20 µm in width, with the tail measuring 30 ± 6.87 µm long. Each capsule contained a polar filament coiled seven to nine times (Table 1).

Table 1 Comparative descriptive measures (means (μm) with ranges and standard deviation (SD) in parentheses) of *Henneguya sacacaensis* n. sp. and all *Henneguya* spp. registered in the Amazon region. TL: total length; BL: body length; BW: body width; TA: tail length; PL: polar capsule length; PW: polar capsule width; PF: number of coils in the polar filament; IS: infection site.

Species	Host	Locality	IS	SPORE (μm)				POLAR CAPSULE (μm)		PF	Referências
				TL	TA	BL	BW	PL	PW		
H. sacacaensis n. sp.	*Satanoperca jurupari*	Amapá	Gills	46.5 (±5.47)	30 (±6.87)	16.5 (±2.64)	5.1 (±0.94)	3.83 (±0.31)	1.6(±0.20)	7–9	Atual
H. santarenensis	*Phractocephalus hemioliopterus*	Pará	Gills	31.9 (±3)	21 (± 3,1)	10.8 (±0.5)	4,3 (± 0,3)	4.6 (±0.4)	1.4(±0.20)	15	Naldoni et al. (2018)
H. quelen	*Rhamdia quelen*	Pará	Kidney	40.0 (±2.8)	24.3 (±2.2)	15.5 (±0.8)	4.1 (±0.3)	5.5 (±0.5)	1.68(±0.20)	-	Abrunhosa et al. (2018)
H. tucunarei	*Cichla monoculus*	Pará	Gills	43.8 (±4.1)	28.1 (±4.3)	14 (±0.8)	6.1 (±0.7)	3.4 (±0.5)	1.98(±0.3)	3-4	Zatti et al. (2018)
H. tapajoensis	*Cichla pinima*	Pará	Gills	54.6 (±3.9)	39 (±3.9)	16.4 (±1.2)	7 (±0.4)	4.2 (±0.5)	2.1 (±0.4)	4-5	Zatti et al. (2018)
H. jariensis	*Cichla monoculus*	Amapá	Fin	46.7 (±1.5)	33.16 (±1.7)	13.4 (±0.7)	6.5 (±0.5)	4 (±0.3)	2 ± (0.1)	4	Zatti et al. (2018)
H. paraensis	*Cichla temensis*	Pará	Gills	42.3 (±0.35)	29.5 (±0.73)	12.8 (±0.42)	8.6 (±0.32)	7.4 (±0.16)	2.6 (±0.08)	5-7	Velasco et al. (2016)
H. melini	*Corydoras melini*	Pará	Gills	40.8 (±0.3)	25.30 (±0.10)	15.5 (±0.2)	4.7 (±0.1)	4.8 (±0.5)	1.7 (±0.3)	5-6	Mathews et al. (2016)
H. aequidens	*Aequidens plagiozanatus*	Pará	Gills	41 (±1.5)	27 (±0.6)	15 (±0.9)	6 (±0.8)	3 (±0.3)	3 (±0.3)	4-6	Videira et al. (2015)
H. torpedo	*Brachyhypopomus pinnicaudatus*	Pará	Brain and spinal cord	48.62 (±0.51)	19.64 (±0.44)	28.53 (±0.36)	7.25 (±0.31)	6.41 (±0.26)	1.84 (±0.19)	5-6	Azevedo et al. (2011)
H. rondoni	*Gymnorhamphichthys rondoni*	Pará	Lateral nerves	17.7 (16.9-18.1)	10.7 (10.3-11)	7 (6.8-7.3)	3.6 (3-3.9)	2.5 (2.2-2.8)	0.85 (0.79-0.88)	6-7	Azevedo et al. (2008)
H. rhamdia	*Rhamdia quelen*	Pará	Gills	50 (±1.81)	36.9 (±1.6)	13.1 (±1.1)	5.2 (±0.5)	4.7 (±0.4)	1.1 (±0.2)	10-11	Matos et al. (2005)
H. schtzodon	*Schtzodon fasciatum*	Amazonas	Kidney	28.9 (27-30)	16.3 (15-17)	13.1 (12-14)	3.3 (3-4)	5.4 (5-6)	1.3 (1-1.5)	8-10	Eiras et al. (2004)
H. friderici	*Leporinus friderici*	Pará	Gills intestine and liver	33.8 (28.7-39.3)	23.3 (19.1-28.7)	10.4 (9.6-11.8)	5.7 (4.8-6.6)	4.9 (4.25-5.9)	2.1 (1.59-2.62)	7-8	Casal et al. (2003)
H. astyanaxy	*Astyanaxy keith*	Pará	Gills	47.8 (±0.71)	32.6 (±1.11)	15.2 (±0.77)	5.7 (±0.71)	5 (±0.13)	1.5 (±0.07)	8-9	Vital et al. (2003)
H. curimata	*Curimata inormata*	Pará	Kidney	35.4 (34.2-36.1)	19.1 (18.3-19.9)	16.6 (16-17.4)	6.2 (5.8-6.6)	3.33 (±0.02)	1.5 (±0.04)	10-11	Azevedo & Matos (2002)
H. testicularis	*Maenkhausia oligolepis*	Pará	Testicles	27.5 (27-28.5)	13.5 (13-14.5)	14 (14-14.5)	6.5 (6-6.5)	9 (8.5-9.5)	2 (2-2.5)	12-13	Azevedo et al. (1997)
H. malabarica	*Hoplias malabaricus*	Pará	Gills	28.3 (26.6-29.8)	17.1 (16.2-18.9)	12.6 (11.8-13.1)	4.8 x 3.6	3.7 (3-4.3)	1.8 (1.6-2.2)	6-7	Azevedo & Matos (1996)
H. adherens	*Acestrorhynchus falcatus*	Amazonas	Gills	32.3 (30.7-35.1)	20.5 (18-21.7)	12.4 (10.5-13.8)	5.8 (5.1-6.5)	3.1 (2.8-3.5)	1.2 (1-16)	3-4	Azevedo & Matos (1995)
H. amazonica	*Crenlcichla lepidota*	Amazonas	Gills	59.3 (±0.56)	45.4 (±0.61)	13.9 (±0.16)	5.7 (±0.06)	3.3 (±0.02)	1.5 (±0.04)	6	Rocha et al. (1992)

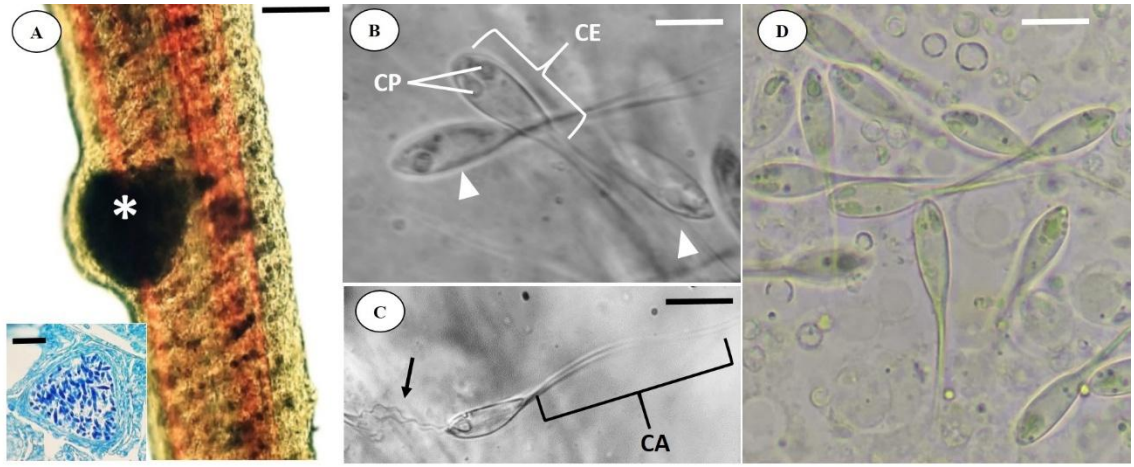


Figure 2 A - Light microscopy of the *Satanoperca jurupari* gill filament with a myxosporid cyst *Henneguya sacacaensis* n. sp. (asterisk). Scale bar: 10 µm. Inset: Histological section of a cyst in the Ziehl-Neelsen-stained gill filament. Scale bar, 5 µm. B - *H. sacacaensis* n. sp. spores highlighting the polar capsules (CP) and the sporal body (CE). Scale bar, 10 µm. C - *H. sacacaensis* n. sp. spore highlighting the fuzzy polar filament (arrow) and its tail (CA). Scale bar, 10 µm. D - Conventional light microscopy imaging of *H. sacacaensis* n. sp. spores. Scale bar, 10 µm.

TAXONOMY

KINGDOM Metazoa Linnaeus, 1758

PHYLUM Cnidaria Hatscheck, 1888

SUBPHYLUM Myxozoa Grassé, 1970

CLASS Myxosporea Bütschli, 1881

ORDER Bivalvulida Shulman, 1959

FAMILY Myxobolidae Thélohan, 1892

GENUS *Henneguya* Thélohan, 1892

SPECIES *Henneguya sacacaensis* n. sp. (Figure 3)

HOST: *Satanoperca jurupari* Heckel, 1840

Site of infection: *Henneguya* cysts and numerous spores in gill filaments.

Collection site: Rio Curiaú Environmental Protection Area, Macapá, Amapá (0º8'43.6 "N, 51º2'30.3" W).

Prevalence: Forty-three of 63 specimens analyzed (68.25%)

Species deposit: Glass slide with H&E-stained spores was deposited in the collection of the Amazon Research Institute (INPA), Manaus, in the state of Amazonas, Brazil (accession number INPA 60).

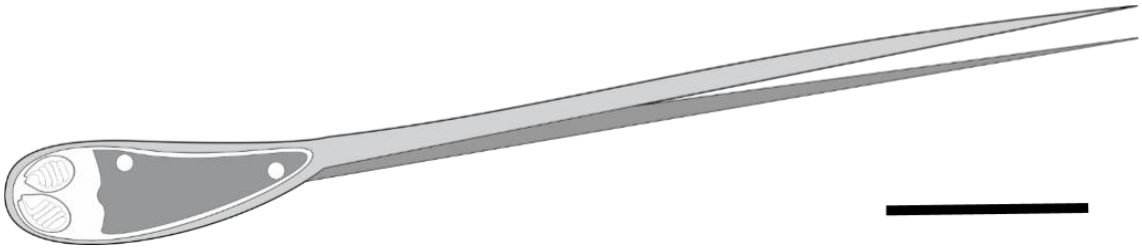


Figure 3 Drawing of *Henneguya sacacaensis* n. sp. Apical view of spore and polar capsules. Scale bar, 10 µm.

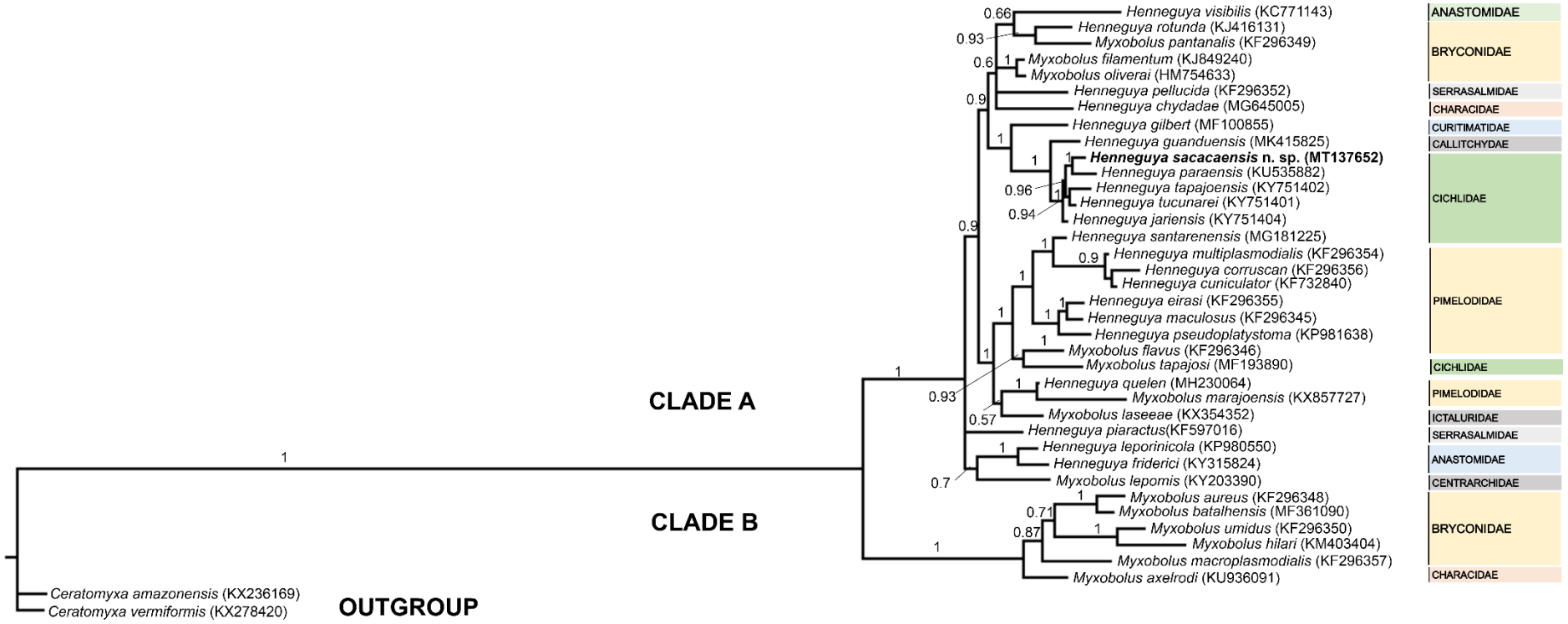
Etymology: The epithet of this genus is in honor of Raimundo dos Santos Souza, popularly known as "Sacaca," due to his contribution to Amapá popular culture.

The partial sequence of the SSU rDNA obtained from the species under study contains 1335 bp (GenBank accession number (XX)). For the phylogenetic analysis in this study, 38 South American Myxozoa sequences available from GenBank with their respective tree identifications were used. In the Bayesian inference analysis, *Henneguya* spp. formed a monophyletic group distributed in two clades, clade A, which includes *Henneguya* species that parasitize several fish species, mainly from the families Cichlidae and Pimelodidae, and clade B, which includes *Myxobolus* spp. parasites that infect organisms from the Amazon region (Figure 4). In comparisons between *Henneguya* spp. species that parasitize cichlids in the Amazon, there was a genetic *p*-distance ranging from 2.911 to 8.597% (Table 2).

Table 2 The uncorrected p-distances recorded between pairs of *Henneguya* spp. that comprise the clade of registered cichlids in the Amazon

Species	1	2	3	4
(1) *Henneguya sacacaensis* n. sp.				
(2) *Henneguya jariensis*	0,08597			
(3) *Henneguya tapajoensis*	0,08525	0,04455		
(4) *Henneguya tucunarei*	0,06861	0,02911	0,03950	
(5) *Henneguya paraensis*	0,08009	0,05006	0,07341	0,04783

Figure 4 Phylogenetic tree generated by Bayesian inference (IB) through partial alignment of *Henneguya sacacaensis* n. sp. with SSU rDNA gene sequences of selected *Henneguya* and *Myxobolus* species. GenBank accession numbers are shown next to species names. Node numbers are indicated for posterior probability values calculated by IB. The new species is highlighted in bold and within the clade of *Henneguya* spp. in Cichlidae.



Discussion

The characteristics of this myxosporidian are typical of the myxospore morphology of histozoic infection and development of cysts in host gills described by Lom & Dyková (2006). The morphology of *Henneguya sacacaensis* n. sp. in this study was compared with other species of *Henneguya* described in the Amazon region.

In South America, freshwater environments of about 100 species of Myxobolidae have been recorded, with most of the species belonging to the Myxobolidae family and the genera *Myxobolus* or *Henneguya* being the most well-studied (ADRIANO & OLIVEIRA, 2018; NALDONI et al., 2018). In the Amazon region, the most species for this genus have been recorded in the state of Pará with 75%, followed by Amazonas with 15%, and Amapá with 10%. Most species have been described by Azevedo and Matos.

In the state of Amapá, only one species of myxosporidian has been described, *Henneguya jariensis*, parasitizing the fin of *Cichla monoculus* (Agassiz, 1831) (ZATTI et al., 2018). Therefore, *Henneguya sacacaensis* n. sp. is the second described species of myxosporidian in Amapá.

The *Henneguya* spp. have been described to infect various sites in Amazonian fish including gills, kidneys, gallbladder, medulla, testicles, and brain. Velasco (2016) described that the genus *Henneguya* has a strong tendency to form clades, taking into consideration aspects such as environment, order, and family of host fish. Other researchers describe this trend in their studies as well (FIALA, 2006, FERGUSON et al., 2008, ADRIANO et al., 2012, CARRIERO et al., 2013, MOREIRA et al., 2014, and ABRUNHOSA, 2018). The *Henneguya sacacaensis* n. sp. behaves similarly and group together with others that parasitize the Cichlidae family.

Among the *Henneguya* spp. that infect fish from the Amazon, the *H. amazônica* stands out (ROCHA et al., 1992), with the longest total length (59.3 µm) and tail length (45.4 µm). Being that *H. sacacaensis* n. sp. has a total length of 46.5 µm and tail length of 30 µm, only *H. astyanax* (VITAL et al., 2003) approaches it in relation to the total length (47.8 µm) and *H. paraensis* (VELASCO et al. , 2016) is similar in tail length (29.5 µm).

Regarding spores, the total length of *H. sacacaensis* n. sp. (16.5 µm) closely resembles *H. curimata* (16.6 µm) (AZEVEDO & MATOS, 2002), *H. tapajoensis* (16.4 µm) (ZATTI et al., 2018), *H. quelen* (15.5 µm) (ABRUNHOSA et al., 2018), *H. melini* (15.5 µm) (MATHEWS, 2016), and *H. aequidens* (15.5 µm) (VIDEIRA et al., 2015). *H.*

sacacaensis n. sp. spore width (5.1 µm) was similar to *H. rhamdia* (5.2 µm) (MATOS & AZEVEDO, 2005).

Regarding polar capsule length, it is clear that *H. malabarica* (AZEVEDO & MATOS, 2006) is similar to *H. sacacanesis* n. sp., with lengths of 3.7 µm and 3.8 µm, respectively, but the capsule width most closely resembled other species of the genus *Henneguya*. *H. sacacaensis* n. sp. capsule width measure about 1.6 µm, which is more closely related to that of *H. melini* (1.70 µm) and *H. quelen* (1.68 µm).

Regarding the number of coils in the polar filament, *H. sacacaensis* n. sp. has seven to nine coils. The number of coils in the polar filament varies from species to species: *H. paraensis* (5–7 coils), *H. rhamdia* (6-7 coils) (MATOS & AZEVEDO, 2005), *H. schtzodon* (8–10 coils) (EIRAS et al., 2004), *H. friderici* (7–8 coils) (CASAL & AZEVEDO, 2003), *H. astyanax* (8–9 coils), and *H. malabarica* (6–7 coils). *H. santarenensis* (NALDONI et al., 2018) is recorded to have the highest known number of coils (15 coils).

Although the morphometric aspects are very similar to *H. astyanax*, the morphology of *H. sacacaensis* n. sp. differs in the position of the binucleated cell. The binucleated cell of *H. astyanax* is just below the polar capsules, while in *H. sacacaensis* n. sp., it is located at opposite ends in the sporoplasma. The sporoplasma also differs in its shape. Furthermore, the tail reinforces the difference in its arrangement, although there are no *H. astyanax* SSU rDNA data.

The gills of parasitized fish showed slight gill hyperplasia without lamellar fusion (Figure 5A); according to the classification by Molnár (2002), large cysts deform and press secondary lamellae laterally on both sides (Figure 5B).

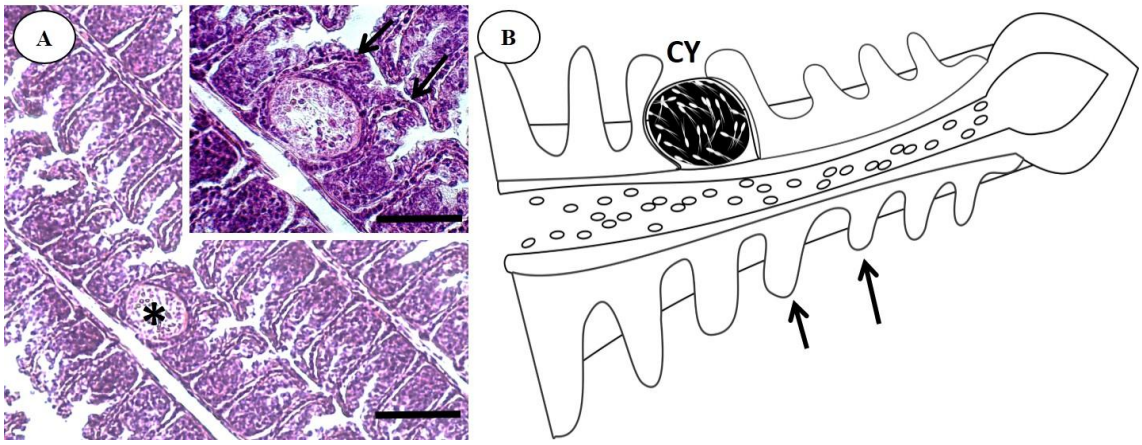


Figure 5 Gill tissue section of *Satanoperca jurupari* parasitized by *Henneguya sacacaensis* n. sp. A - Cyst (*) between secondary lamellae with slight filamentous (intralamellar) hyperplasia. Insert: Cyst between secondary lamellae (arrows). Section stained with H&E. Scale bar, 10 µm. B - Drawing of the *Henneguya* cyst (CY) between the secondary lamellae (arrows).

Similar changes have been described previously in other Amazon hosts such as *Astyanax keithi* (VITAL et al., 2003), *Cichla temensis* (VELASCO et al., 2016), *Phractocephalus hemioliopterus* (NALDONI et al., 2018), and *Metynnis hypsauchen* (FIGUEREDO et al., 2019).

Conclusion

Thorough analysis of the morphological, morphometric, and molecular aspects allow us to describe a new species, *H. sacacaensis*, with characteristics that differ from all other species previously described.

Ethics and biosafety committee

Animal Use Committee of the Brazilian Agricultural Research Corporation - Amapá (012-2018) and the Biodiversity Authorization and Information System, IBAMA (SISBIO / ICMBIO License No. 50376-1).

Declaration of conflicting interests

The authors declare no conflict of interest.

Contribution of authors

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed manuscript and approved in the final version.

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7 CONSIDERAÇÕES FINAIS

O hospedeiro estudado nesse trabalho apresenta valor econômico e serve de alimento para comunidades ribeirinhas e quilombolas, dentro e arredores da APA do Rio Curiaú. Além do seu consumo, estudos iniciados pela EMBRAPA-PA em 2008 consideram o *S. jurupari* com um grau de alta adaptabilidade para servir como peixe de forragem na aquicultura, para isso faz-se necessidade de estudos mais aprofundados para os danos e perigos aos peixes que irão se alimentar dele.

As análises morfológicas, morfométricas e filogenéticas dos microparasitos eucariotos encontrados em *S. jurupari* apresentaram presenças de diferentes famílias (Ceratomixydae e Myxobolidae) em órgãos distintos. As Gills infectadas pelo gênero *Henneguya* e a vesícular biliar pelo gênero *Ellipsomyxa*, sendo descritos dois novos trabalhos submetidos à avaliações: *Henneguya sacacaensis* n. sp. e *Ellipsomyxa tucujuensis* n. sp.

Pelas características taxonômicas, são prováveis novas espécies, o que irá contribuir com a ictiosanidade desta espécie forrageira e com a biodiversidade Amazônica.

APÊNDICE A – FICHA DE NECROPSIA

UNIVERSIDADE DO ESTADO DO AMAPÁ - UEAP
BACHARELADO EM ENGENHARIA DE PESCA - EPE
LABORATÓRIO DE MORFOFISIOLOGIA E SANIDADE ANIMAL - LABMORSA
FORMULÁRIO DE NECROPSIA DE ORGANISMOS AQUÁTICOS - FNOA

RESPONSÁVEL PELA NECROPSIA:		DATA DA NECROPSIA: / / 2018
PROJETO:	NOME CIENTIFICO:	AMOSTRA Nº:
NOME POPULAR:	SEXO/ESTADO DE MATURAÇÃO:	
LOCAL DA COLETA:	DATA DA COLETA: / /2018	
PESO (g):	COMPRIMENTO TOTAL (cm):	COMPRIMENTO PADRÃO(cm):

ÓRGÃO	PARASITA	Nº

NUTRIÇÃO				
INDICE DE REPLEÇÃO ESTOMACAL	**PESO INICIAL**	**PESO FINAL**	**PESO CONTEUDO**	**C.I**
Conteúdo:				

OBS:

ANEXO A – PROTOCOLO DO COMITE DE ÉTICA PARA USO DE ANIMAIS AQUÁTICOS

Embrapa
Amapá

Comissão de Ética para o Uso de Animais (CEUA-CPAFAP)

Resultado de Solicitação de protocolo

Número do protocolo: 012-CEUA/CPAFAP

Processo SEI: 21157.001846/2018-91

Data da entrada: 16/08/2018

Período do Projeto: Setembro de 2018 a Outubro de 2022.

Título do Projeto: Fauna parasitaria de peixes de interesse comercial em três municípios do estado do Amapá, Amazônia oriental

Prezado (a) pesquisador (a),

Em relação ao protocolo de pesquisa sob sua responsabilidade a CEUA deliberou o seguinte:

*- **APROVADO**, para o período solicitado e de acordo com a proposição do uso de animais na metodologia apresentada no projeto.*

- Por ocasião do término desse protocolo, DEVERÁ SER APRESENTADO RELATÓRIO detalhado relacionado ao uso de animais no projeto desenvolvido.

Macapá, 27/09/2018

Atenciosamente,

Dr. Marcos Tavares Dias
Presidente da CEUA

Empresa Brasileira de Pesquisa Agropecuária
Ministério da Agricultura, Pecuária e Abastecimento.
Rod.Juscelino Kubitschek, km 5, nº. 2600 – 68903-419 Macapá, AP
C. Postal 10 – Telefone (96) 3203-0200
www.embrapa.br/amapa

ANEXO B - FICHA PARA MICROSCOPIA DE LUZ

LABORATÓRIO DE PESQUISA
CARLOS AZEVEDO

Tetrauronema desaequalis
Faculdade de Ciências Agrárias do Pará

Azevedo & Matos,
1996

MATERIAL..... Nº...../..... DATA...../...../.....

( ) NORMAL
( ) ESPECIAL

OBS: ANTES DE INICIAR A COLHEITA DO MATERIAL BIOLÓGICO PREPARAR TODO O INSTRUMENTAL E MATERIAL NECESSÁRIO E INDISPENSÁVEL AO TRABALHO

OBS: PREPARAÇÃO DE "BONECA DE GAZE" COM O MATERIAL PARA SER PROCESSADO

				INÍCIO	FIM	OBS
FIXAÇÃO : TEMPERATURA AMBIENTE						
FORMOL AQ 10% / DAVIDSON / AFA / OUTRO			HS	.....	.....	.....	.....
LAVAGEM EM ÁGUA CORRENTE			HS	.....	.....	.....	.....
DUPLA FIXAÇÃO			HS	.....	.....	.....	.....
DESIDRATAÇÃO		ESPECIAL				
ALC 70 .....30'	30'		.....1H 1H	.....	.....	.....	.....
ALC 90.....30'	(*)	30'	.....1H 1H	.....	.....	.....	.....
ALC ABS I .....30'	60'		.....1H 1H	.....	.....	.....	.....
ALC ABS II .....30'	60'		.....1H 1H	.....	.....	.....	.....
ALC ABS III.....…..30'	60'		.....1H 2H	.....	.....	.....	.....
ALC XILOL	30'		.....1H 1H	.....	.....	.....	.....
DIAFANIZAÇÃO (CLAREAMENTO)						
XILOL / BENZOL I			30'.....30'.....24H	.....	.....	.....	.....
XILOL / BENZOL II						
IMPREGNAÇÃO EM ESTUFA A 60 ^{0}C						
BANHO DE PARAFINA I			30'.....30'.....24H	.....	.....	.....	.....
BANHO DE PARAFINA II			30'.....60'.....24H	.....	.....	.....	.....
BANHO DE PARAFINA III			30'(*)....60'.....24H	.....	.....	.....	.....
INCLUSÃO EM PARAFINA : TEMPERATURA AMBIENTE						
INCLUSÃO TOTAL				.....	.....	.....	.....

OBS: (*) PODE DEIXAR A NOITE NO ALCOOL, MAS NUNCA NO XILOL OU BENZOL, EM TEMPERATURA AMBIENTE, SOMENTE EM CASOS ESPECIAIS.

ANEXO C – NORMAS DA REVISTA "SYSTEMATIC PARASITOLOGY"

Instructions for Authors
Authorship Policy
Authorship should incorporate and should be restricted to those who have contributed substantially to the work in one or more of the following categories:

• Conceived of or designed study

• Performed research

• Analyzed data

• Contributed new methods or models

• Wrote the paper

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General
The following types of communication will be considered for publication:

- papers of about 6,000 words (fully illustrated)

- brief communications or research notes (about 2,000 words), not normally illustrated

- major revisions (about 24,000 words), fully illustrated.

Any communication which contains descriptions of new taxa (genera or species) should be accompanied by specimens (preferably paratypes) for scrutiny by the referees and by a statement where the holotypes are deposited. Papers and major revisions should include, at the beginning, a summary (approximately 250 words for papers and 500 words for major revisions).

Back to top
Manuscript Submission
Manuscript Submission
Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Back to top
Title page
Title Page
The title page should include:

The name(s) of the author(s)
A concise and informative title
The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
A clear indication and an active e-mail address of the corresponding author
If available, the 16-digit ORCID of the author(s)
If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract
Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Back to top
Text
Text Formatting
Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.
Use italics for emphasis.
Use the automatic page numbering function to number the pages.
Do not use field functions.
Use tab stops or other commands for indents, not the space bar.
Use the table function, not spreadsheets, to make tables.
Use the equation editor or MathType for equations.
Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).
Manuscripts with mathematical content can also be submitted in LaTeX.

LaTeX macro package (Download zip, 188 kB)

Headings
Please use no more than three levels of displayed headings.

Abbreviations
Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments
Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Back to top
Important notes on style and nomenclature
The authorities and date of all parasite taxa should be given when the names (full binomen) are first cited in the abstract and the text. Subsequently, the generic component is abbreviated except at the beginning of a sentence.

The authorities, but not the date, should be given for the hosts of described parasites.

Publications cited only as authorities for taxa should not be included in the References.

The International Code of Zoological Nomenclature will be strictly adhered to:

Online First for the articles accepted for publication in Systematic Parasitology prior to print publication is available. In order to comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012) concerning the electronic Online First publication of new scientific names and nomenclatural acts, registration of the publication on the Official Register of Zoological Nomenclature (ZooBank.org) is required. Although the Code amendment does not require registration of new names, Systematic Parasitology will also require that new names are registered in ZooBank. Authors are responsible for registration of the article and all new acts at the time of manuscript acceptance.

Museum accession numbers for all deposited type and voucher specimens are required at submission. The museum should be a recognised national or international museum which is protected by law and not a university collection.

New molecular sequences reported in manuscripts should be deposited in the GenBank database and the accession numbers included in the manuscript. For taxonomic studies based on molecular sequences, the source specimen of molecular information should be deposited in a recognised national or international museum.

Back to top
References
Citation
Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson 1990).
This result was later contradicted by Becker and Seligman (1996).
This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).
Ideally, the names of six authors should be given before et al. (assuming there are six or more), but names will not be deleted if more than six have been provided.

Reference list
The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal names and book titles should be italicized.

Journal article Harris, M., Karper, E., Stacks, G., Hoffman, D., DeNiro, R., Cruz, P., et al. (2001). Writing labs and the Hollywood connection. Journal of Film Writing, 44(3), 213–245.
Article by DOI Slifka, M. K., & Whitton, J. L. (2000) Clinical implications of dysregulated cytokine production. Journal of Molecular Medicine, https://doi.org/10.1007/s001090000086
Book Calfee, R. C., & Valencia, R. R. (1991). APA guide to preparing manuscripts for journal publication. Washington, DC: American Psychological Association.
Book chapter O'Neil, J. M., & Egan, J. (1992). Men's and women's gender role journeys: Metaphor for healing, transition, and transformation. In B. R. Wainrib (Ed.), Gender issues across the life cycle (pp. 107–123). New York: Springer.
Online document Abou-Allaban, Y., Dell, M. L., Greenberg, W., Lomax, J., Peteet, J., Torres, M., & Cowell, V. (2006). Religious/spiritual commitments and psychiatric practice. Resource document. American Psychiatric Association. http://www.psych.org/edu/other_res/lib_archives/archives/200604.pdf. Accessed 25 June 2007.
For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

EndNote style (Download zip, 4 kB)

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All tables are to be numbered using Arabic numerals.
Tables should always be cited in text in consecutive numerical order.
For each table, please supply a table caption (title) explaining the components of the table.
Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.
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Artwork and Illustrations Guidelines
Electronic Figure Submission
Supply all figures electronically.
Indicate what graphics program was used to create the artwork.
For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
Vector graphics containing fonts must have the fonts embedded in the files.
Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.
Line Art

Definition: Black and white graphic with no shading.
Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
All lines should be at least 0.1 mm (0.3 pt) wide.
Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
Vector graphics containing fonts must have the fonts embedded in the files.
Halftone Art

Definition: Photographs, drawings, or paintings with fine shading, etc.
If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
Halftones should have a minimum resolution of 300 dpi.
Combination Art

Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.
Combination artwork should have a minimum resolution of 600 dpi.
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Color art is free of charge for online publication.
If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.
If the figures will be printed in black and white, do not refer to color in the captions.
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Figure Lettering
To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
Avoid effects such as shading, outline letters, etc.
Do not include titles or captions within your illustrations.
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All figures are to be numbered using Arabic numerals.
Figures should always be cited in text in consecutive numerical order.
Figure parts should be denoted by lowercase letters (a, b, c, etc.).
If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2,

A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

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In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1

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Electronic Supplementary Material

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as electronic supplementary material, authors should read the journal’s Research data policy. We encourage research data to be archived in data repositories wherever possible.

Submission

Supply all supplementary material in standard file formats.

Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.

To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

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Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.

A collection of figures may also be combined in a PDF file.

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Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

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Captions

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Accessibility

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The manuscript contains a descriptive caption for each supplementary material

Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

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For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

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ANEXO D – SUBMISSÃO DO ARTIGO 1 NA REVISTA "SYSTEMATIC PARASITOLOGY"

Systematic Parasitology

Ellipsomyxa tucujuensis n. sp. (Myxozoa: Ceratomyxidae) in Satanoperca jurupari (Osteichthyes: Cichlidae) on the Curiaú River in the Amazon in Amapá, Brazil

--Manuscript Draft--

Manuscript Number:	SYPA-D-19-00124
Full Title:	Ellipsomyxa tucujuensis n. sp. (Myxozoa: Ceratomyxidae) in Satanoperca jurupari (Osteichthyes: Cichlidae) on the Curiaú River in the Amazon in Amapá, Brazil
Article Type:	Original Research Paper
Keywords:	Fish; freshwater; parasite; Amazon.
Funding Information:	
Abstract:	Ellipsomyxa tucujuensis n. sp. it's a new parasite species found in the gallbladder of Satanoperca jurupari . It's a lentic environment specie, with day-long habit and feeds mainly on microcrustaceans, fruit seeds, grasses and small fish, as well as aquatic and terrestrial insect larvae They were captured in the Curiaú River Environmental Protection Area, Amapá, Brazil. Asymmetric and irregularly shaped plasmodia and free spores were observed in the bladder fluid, without cyst formation. The elliptical spores had an average length of 10.11 (8.56-10.5) µm, an average width of 7.81 (5.96-9.56) µm, and thick walls. The polar capsules had a subspherical and slightly asymmetrical shape with an average length of 3.12 (2.31-3.99) µm, and a width of 2.5 (2.22-2.95) µm, containing polar filaments with 5 to 6 coils perpendicular to the longitudinal axis of each capsule. Based on the analysis of the 18S rDNA sequences of the Ellipsomyxa tucujuensis n. sp. spores found in S. jurupari , Bayesian inference, p distance, and morphological analysis, it was confirmed as a new species.
Corresponding Author:	Marcela Videira Universidade do Estado do Amapá BRAZIL
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Corresponding Author's Institution:	Universidade do Estado do Amapá
Corresponding Author's Secondary Institution:	
First Author:	Roger Ferreira
First Author Secondary Information:	
Order of Authors:	Roger Ferreira
	Diehgo Tuloza
	Abthyllane Amaral
	Igor Hamoy
	Edilson Matos
	Marcela Videira
Order of Authors Secondary Information:	
Suggested Reviewers:	Michele Velasco Universidade Federal Rural da Amazonia michele.velasco@ufra.edu.br She has great experience in the area.
	Marcos Tavares DIas marcos.tavares@embrapa.br He is a great researcher on fish parasites
	Edson Adriano

Submission ID: SYPA-D-19-00124

ANEXO E – NORMAS DA REVISTA "REVISTA BRASILEIRA DE PARASITOLOGIA VETERINÁRIA"

Introduction

The Brazilian Journal of Veterinary Parasitology is the official journal of the Brazilian College of Veterinary Parasitology (CBPV). The journal is a quarterly publication that covers topics on helminths, protozoans, arthropods and agents transmitted by arthropods, as well as other related subjects. Manuscripts can be submitted in English by researchers from any country regardless of CBPV affiliation. The Brazilian Journal of Veterinary Parasitology offers free online access to all its archives dating back to 1992, which was its first year of publication.

Editorial guidelines

Articles submitted to the Brazilian Journal of Veterinary Parasitology (RBPV) need to be original scientific articles that essentially address matters relating to parasites of animals of any kind.
The RBPV categorizes its articles as follows:

Original Article: this type of article reports data from original research that has not been published in any other periodical.

Short Communication: for this category, articles submitted will only be accepted if they have a high degree of novelty and originality, bringing new results of evident importance. The decision on whether the submission may proceed will be made by the editor-in-chief.

Review Article: submission of these articles is conditional on submission and invitation from the editor-in-chief. Reviews that were not requested will be assessed by the editor-in-chief or assistant scientific editors provided that they were written by researchers who are specialists in the topic chosen.

Paper submission:

The manuscripts submission is online by ScholarOne (https://mc04.manuscriptcentral.com/rbpv-scielo). The corresponding author needs to supply an ORCID ID (Open Researcher and Contributor ID, http://orcid.org/) at the time of submission, which should be inserted in the user's

profile in the submission system. We recommend that this should also be done for the coauthors.

Authors are required to send a signed cover letter in which they declare that they were responsible for the whole process of producing the manuscript and that it is entirely an unpublished original article. If the abstract of the manuscript has been presented in scientific meetings, this should be stated in the signed cover letter as well.

Author consent forms for manuscripts that have more than one author are required, to ensure that all authors agree with the publication. All authors need to have made substantial contributions to the study design, data acquisition, data analysis and interpretation, and drafting of the article, and need to have given final approval of the version to be submitted. Manuscripts with a number of authors that does not seem justifiable will be assessed by our assistant scientific editors in relation to the experimental research protocol. Collaborators who did not actively participate in the process described above may be listed in the Acknowledgements section. Contributions made by individuals or institutions who provided technical assistance or suggestions, or corrections or suggestions relating to writing the paper, or who in some other manner collaborated in developing the work, can be acknowledged.

The articles submitted must undergo English-language revision, done by reviewers accredited by the RBPV (http://rbpv.org.br/rbpv/guia-do-autor). A certificate of English-language revision should be sent together with the submitted article. The authors will be expected to bear the costs of the revision. If one of the coauthors is a non-Brazilian who is a native speaker of the English language, this author should review the English-language content of the study. The corresponding author needs to send correspondence to the journal confirming that this review was done by one of the authors who is a native speaker of English.

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The manuscript review process will follow the journal's Editorial Guidelines and consider the editors' and/or ad hocreviewer'sopinions. The Editor-in-chief and assistant scientific editors may make suggestions or request changes to the manuscript but the authors are ultimately responsible for the entire text content. Articles that are submitted for publication will be reviewed by at least two anonymous reviewers. The reviewers will be selected by the editor-in-chief. If the referees give conflicting opinions, the article will be sent to a third reviewer.

The reviewer should fill out the RBPV's evaluation form, which is available in the online submission system

(http://mc04.manuscriptcentral.com/rbpv-scielo). The author will receive evaluations from at least two reviewers, as statements on evaluation forms and possibly as corrections made directly in the text. The authors need to address each of the queries raised or corrections suggested by each of the referees. The reviewer may then correct the article, if necessary. After the manuscript has been accepted by the ad hoc reviewers, but before it is sent for the authors' responses, it will undergo a final analysis by one of the assistant scientific editors. It should be noted that the assistant scientific editors have autonomous authority to suggest corrections and/or reject publication of an article, even if the reviewers have already approved it. Also at this stage, if necessary, the manuscript is sent to one of the RBPV statistical method reviewers.

After the layout and editing processes, the editor-in-chief of the journal will make any final corrections.

Transfer of authorship rights:

If the article is accepted, the author(s) shall transfer their authorship rights to the journal. If any subsequent use is made of any parts of the text, figures or tables, the **Brazilian Journal of Veterinary Parasitology** (RBPV) must be cited as the source.

Ethics

Experiments using animals should be conducted following the Brazilian College of Animal Experimentation guidelines (http://www.cobea.org.br). Articles should include the protocol number approved by the Animal Ethics Committee. For non-Brazilian authors, guidelines for experiments on animals are also set forth in the "International Guiding Principles for Biomedical Research Involving Animals", issued by the "Council for the International Organizations of Medical Sciences" (https://grants.nih.gov/grants/olaw/guiding_principles_2012.pdf). These authors need to present the equivalent protocol number(s) for submission and approval of their studies.

Manuscript preparation

The following guidelines should be followed during manuscript preparation:

All articles should be submitted in United States English. Always use concise and impersonal language. Footnotes should be placed at the bottom of the corresponding page and numbered with Arabic numerals in an ascending

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Description of each item of the manuscript
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The full title and subtitle, if any, should not exceed 20 words. The title should not include any abbreviations, and species names and Latin words should be italicized. Titles that start with "Preliminary studies," "Notes about," and the like should be avoided. Do not use the author's name and date of citation in scientific names.

Author(s)/Affiliations
List all authors' full name (with no abbreviations). Affiliations should include the original institution names, not their English translations, in the following order: laboratory, department, college or school, institute, university, city, state and country. Include at the bottom of the page the corresponding author information: full address, telephone number, current e-mail and ORCID, in that order.

Abstract
Abstracts are limited to 400 words and should be structured in a single paragraph with no indentation. The abstract should not include references. Acronyms or abbreviations should be written out in full and the abbreviation given in brackets the first time they are used in abstract, for example, indirect fluorescence assay (IFA). The abstract should be informative and present the objectives, a brief description of methods, the main results, and a conclusion. All manuscripts written in English should also have the abstract and keywords written in Portuguese.

Keywords
Keywords should accurately reflect the text content. Limited to a maximum of 6 (six), and separated by comma.

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Should have a clear and concise justification of the study including its relevance and objectives and should keep the number of citations to a minimum.

Materials and Methods
A concise description including core information for the understanding and reproduction of the study. Well-established methods and techniques should

be cited and referenced but not described. Statistical analyses should be described at the end of the section.

Results
The content of this section should be informative rather than interpretative. The results should be accompanied by self-explanatory tables, figures, or other illustrations if necessary.

Discussion
Its content should be interpretative and based on the study results only. The discussion can be a single section or it can be presented together with the results and conclusions. It should emphasize the relevance of new findings and new hypotheses clearly supported by the results.

Conclusions
All conclusions may be presented in the Discussion section or in the Results and the Discussion sections when presented together, at the authors' choice. If this is the case, there is no need for a separate Conclusions section.

Acknowledgments
Should be limited to a minimum.

References
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All citations must follow the author–date system:

One author: author's name and year of publication
Levine (1985) or (LEVINE, 1985)

Two authors: authors' names and year of publication
Paim and Souza (2011) or (PAIM & SOUZA, 2011)

Three or more authors: first author's name followed by et al. and year of publication
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In the Reference section, all authors should be listed up to a limit of six authors. If more than six authors, the first six authors should be listed followed by et al.:

Reference to book
Levine JD. *Veterinary protozoology.* Ames: ISU Press; 1985.

Reference to book chapter
Menzies PI. Abortion in sheep: diagnosis and control. In: Youngquist RS, Threlfall WR. *Current therapy in large animal theriogenology*. 2nd ed. Philadelphia: Saunders; 2007. p. 667-680.

Reference to full article
Munhoz AD, Simões IGPC, Calazans APF, Macedo LS, Cruz RDS, Lacerda LC, et al. Hemotropic mycoplasmas in naturally infected cats in Northeastern Brazil. *Rev Bras Parasitol Vet* 2018; 27(4): 446-454. http://dx.doi.org/10.1590/s1984-296120180074.

Reference to thesis or dissertation
Araujo MM. *Aspectos ecológicos dos helmintos gastrintestinais de caprinos do município de patos, Paraíba - Brasil* [Dissertação]. Rio de Janeiro: Universidade Federal Rural do Rio de Janeiro; 2002.

Reference to internet URLs
Centers for Disease Control and Prevention. *Epi Info* [online]. 2002 [cited 2003 Jan 10]. Available from: http://www.cdc.gov/epiinfo/ei2002.htm.

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Figures
Figures consist of drawings, photographs, boards, charts, flow charts, and diagrams and should be supplied in .tif, .eps or .pdf format with a minimum resolution of 300 dpi. Only figures of high quality will be accepted. They should be numbered consecutively with Arabic numerals and the word "Figure" should precede the legend placed below them. List all numbered legends with their symbols and standard icons in a separate file with double spacing. Figures should be limited to a minimum. Digital pictures should be supplied in separate files. A graphic bar scale instead of a numerical one should be used in all illustrations, as it can be adjusted with size reduction.

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ANEXO F– SUBMISSÃO DO ARTIGO 2 NA REVISTA "REVISTA BRASILEIRA DE PARASITOLOGIA VETERINÁRIA"

Revista Brasileira de Parasitologia Veterinária
Brazilian Journal of Veterinary Parasitology

Henneguya sacacaensis n. sp. (Myxozoa: Myxosporea) parasitizing gills of the acará bicudo Satanoperca jurupari (Osteichthyes: Cichlidae) in eastern Amazonia

Journal:	*Revista Brasileira de Parasitologia Veterinária*
Manuscript ID	RBPV-2020-0006
Manuscript Type:	Full Article
Keyword:	Freshwater, Myxobolidae, fish, parasite, Amazon, gill

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