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Original Article

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

Henneguya sacacaensis no. sp (Myxozoa: Myxosporea) parasitando brânquias do acará bicudo *Satanoperca jurupari* (Osteichthyes: Cichlidae) na Amazônia oriental

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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 *S. 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 (41.3-56.92) µm. The fusiform body of the *Henneguya* measured 16.5 (13.16-20.01) µm long and 5.1 (3.91-6.12) µm in width, the two polar capsules had a taper of 3.83 (3.4-4.32) µm and a width of 1.68 (1.4-1.99) µm, and the tail measured 30 (22.47-41.67) µm in length, containing a polar filament coiled seven to nine times. Morphogical and phylogenetic analysis allowed the preposition of a new 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 branquiais tinham formatos ovais e esbranquiçados. Seus esporos apresentaram um comprimento médio de 46,5 (41,3-56,92) µm, corpo fusiforme medindo 16,5 (13,16-20,01) µm de comprimento e 5,1 (3,91-6,12) µm de largura, suas duas cápsulas polares apresentam uma conicidade de 3,83 (3,4-4,32) µm e sua largura 1,68 µm (1,4-1,99), a cauda 30 (22,47-41,67) µm de comprimento, contento um filamento polar de 7 à 9 voltas. Análises morfológicas e filogenéticas permitiram a preposição de uma nova espécie, *Henneguya sacacaensis* n. sp, que pertence à família Myxobolidae e ao gênero *Henneguya.*

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

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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, 2012).

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 et al., 2018). Recently Zatti et al. (2018) claim that 55 species of *Henneguya* spp. infect fish in Brazil. Of these, about 20 were found infecting fish species in the Amazon region (Velasco et al., 2016; Naldoni et al., 2018; Abrunhosa et al., 2018; Zatti 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 et al., 2016). When they infect the gills, they can cause filament destruction and respiratory failure (Lom & Dyková, 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 *S. 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 (0°8'43.6"N, 51°2'30.3"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).

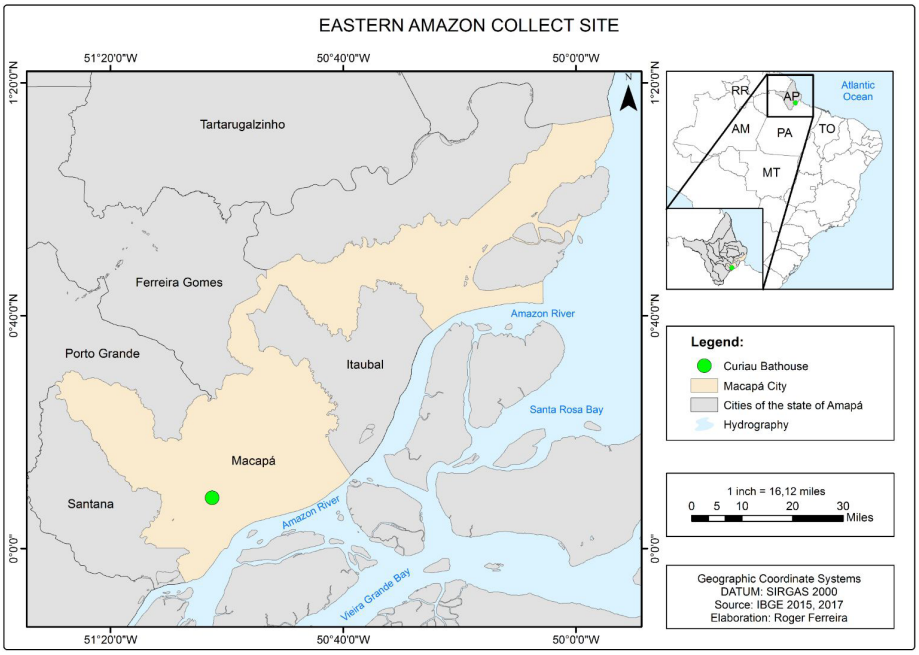

Figure 1. Map of the collection site in Macapá, Amapá: Rio Curiaú Environmental Protection Area, Eastern Amazon region.

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.

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

All molecular analyses were based on the 18S rDNA sequences, which were amplified using MC5/MC3 primers (Molnár, 2002). The final volume of the polymerase chain reaction (PCR) was of 25 µL using taq DNA polymerase Master Mix (PROMEGA, Madison, USA), with 0.5 µM of each primer and 2 µL of the DNA sample. With the initial amplification being initiated by denaturation of 10 s at 95 °C, followed by 40 cycles of denaturation at 95 °C for 1 min, hybridisation at 48 °C for 2 min, and polymerisation for 4 min 30s at 72 °C, with final polymerisation of 10 min at 92 °C.

The amplicons were subjected to electrophoretic analyses through 1.5% agarose gel purified using GFX™ PCR DNA and Gel Band Purification Kit reagents were used to purify the material after 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 (Molnár, 2002). Stereomicroscope analysis under incident light showed whitish cysts and ellipsoids in a thin layer of connective tissue (Figure 2A).

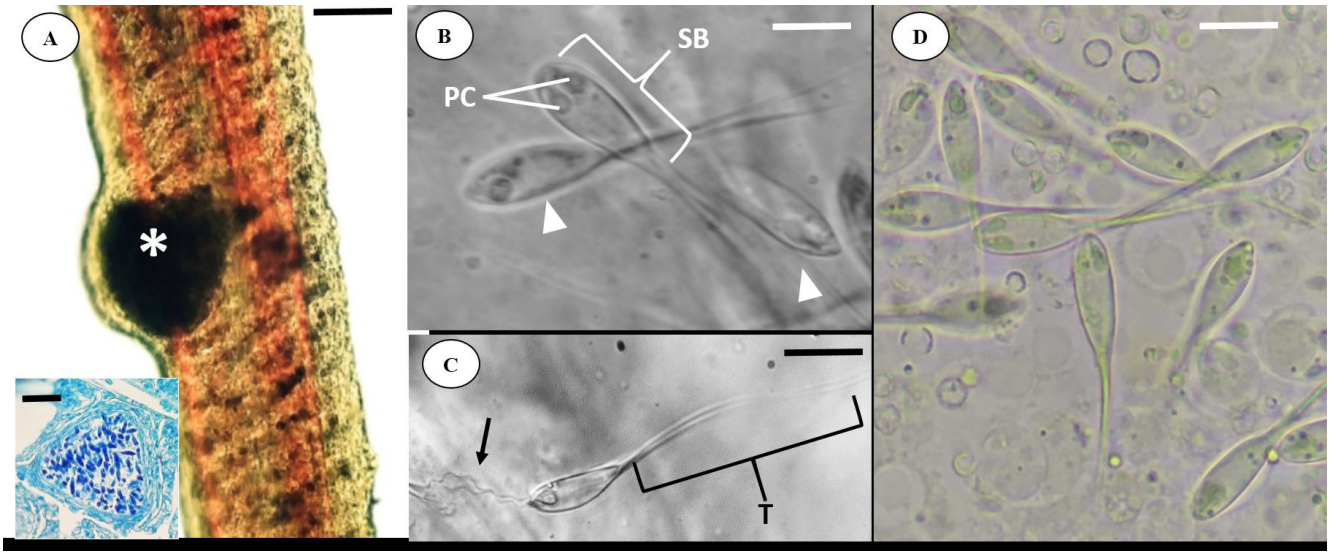

Figure 2. (A) Light microscopy of the *Satanoperca jurupari* gill filament with a myxosporid cyst *Henneguya sacacaensis* n. sp. (asterisk). Scale bar: 10 µm. Inset: Histological section of a cyst in the Ziehl-Neelsen-stained gill filament. Scale bar, 5 µm; (B) *H. sacacaensis* n. sp. spores highlighting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

Spores

Fresh spores were measured (n=31) (Figure 2B, C, 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).

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 in the gill filaments.

Collection site: Rio Curiaú Environmental Protection Area, Macapá, Amapá (0°8'43.6" N, 51°2'30.3" 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).

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 MT137652). For phylogenetic analysis, were used 38 South American sequences of *Myxobolus* spp. and *Henneguya* spp. available on Genbank. 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 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.

Species	Host	Locality	IS	SPORE (µm)				POLAR CAPSULE (µm)		PF	References
				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	Present study
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	gills	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 plagiozonatus*	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 nerve	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. schizodon	*Schizodon 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)

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.

Table 1. Continued...

Species	Host	Locality	IS	SPORE (µm)				POLAR CAPSULE (µm)		PF	References
				TL	TA	BL	BW	PL	PW		
H. astyanax	*Astyanax 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	Vita 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	*Moenkhausia oligolepis*	Pará	testicules	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	*Crenicichla 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)

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.

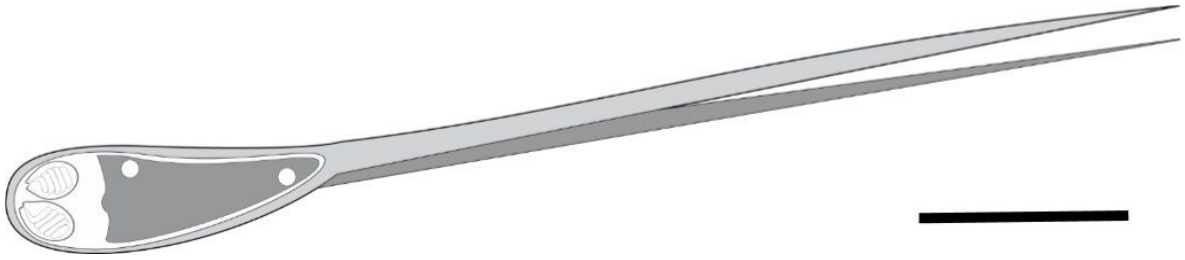

Figure 3. Drawing of *Henneguya sacacaensis* n. sp. Apical view of spore and polar capsules. Scale bar, 10 µm.

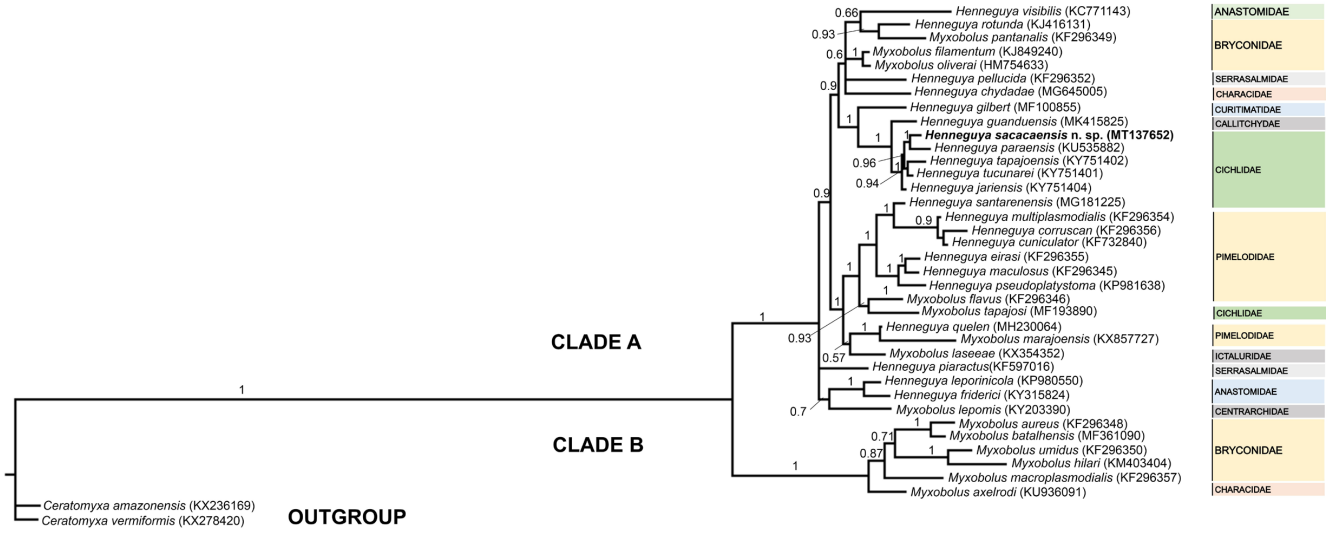

Figure 4. Phylogenetic tree generated by Bayesian inference (IB) through partial alignment of *Henneguya sacacaensis* n. sp. with SSU rDNA gene sequences of selected *Henneguya* and *Myxobolus* species. GenBank accession numbers are shown next to species names. Node numbers are indicated for posterior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

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

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, about 100 species of the myxosporidian have been recorded in freshwater, being most of these species belonging to the Myxobolidae family, and the genera *Myxobolus* and *Henneguya* 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%.

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 myxosporidian species described in Amapá.

The *Henneguya* spp. have been described to infect various sites in Amazonian fish including gills, kidneys, gallbladder, medulla, testicles, and brain. Velasco et al. (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; Abrunhosa et al., 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 *Henneguya amazonica* stands out (Rocha et al., 1992), with the longest total length (59.3 µm) and tail length (45.4 µm). Being that *Henneguya sacacaensis* n. sp. has a total length of 46.5 µm and tail length of 30 µm, only *Henneguya astyanax* (Vita et al., 2003) approaches it in relation to the total length (47.8 µm) and *Henneguya paraensis* (Velasco et al., 2016) is similar in tail length (29.5 µm).

Regarding spores, the total length of *Henneguya sacacaensis* n. sp. (16.5 µm) closely resembles *Henneguya curimata* (16.6 µm) (Azevedo & Matos, 2002), *Henneguya tapajoensis* (16.4 µm) (Zatti et al., 2018), *Henneguya quelen* (15.5 µm) (Abrunhosa et al., 2018), *Henneguya melini* (15.5 µm) (Mathews et al., 2016), and *Henneguya aequidens* (15.5 µm) (Videira et al., 2015). *Henneguya sacacaensis* n. sp. spore width (5.1 µm) was similar to *Henneguya rhamdia* (5.2 µm) (Matos et al., 2005).

Regarding polar capsule length, it is clear that *Henneguya malabarica* (Azevedo & Matos, 1996) is similar to *Henneguya 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*. *Henneguya 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, *Henneguya 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), *Henneguya schtzodon* (8-10 coils) (Eiras et al., 2004), *Henneguya friderici* (7-8 coils) (Casal et al., 2003), *H. astyanax* (8-9 coils), and *H. malabarica* (6-7 coils). *Henneguya 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 *Henneguya 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 *Henneguya 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 2A and 5A); according to the classification by Molnár (2002), large cysts deform and press secondary lamellae laterally on both sides (Figure 5B).

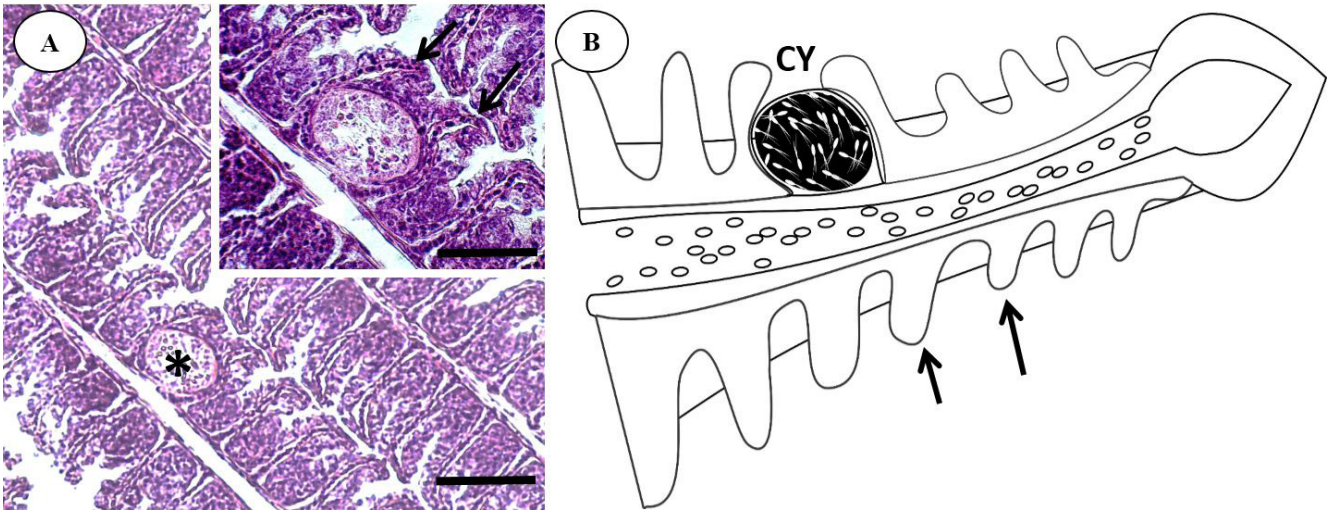

Figure 5. Gill tissue section of *Satanoperca jurupari* parasitized by *Henneguya sacacaensis* n. sp. (A) Cyst (*) between secondary lamellae with slight filamentous (intralamellar) hyperplasia. Insert: Cyst between secondary lamellae (arrows). Section stained with H&E. Scale bar, 10 µm; (B) Drawing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

Similar changes have been described previously in other Amazon hosts such as *Astyanax keithi* (Vita et al., 2003), *Cichla temensis* (Velasco et al., 2016), *Phractocephalus hemioliopterus* (Naldoni et al., 2018), and *Metynnis hypsauchen* (Figueredo et al., 2020).

Conclusion

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

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References

Abrunhosa J, Sindeaux-Neto JL, Hamoy I, Matos E. A new species of Myxosporea, *Henneguya quelen*, from silver catfish *Rhamdia quelen* (Siluriforme: Pimelodidae) in the Amazonian region. *Parasitol Res* 2018; 117(12): 3809-3820. http://dx.doi.org/10.1007/s00436-018-6086-1. PMid:30255237.

Adriano EA, Arana S, Cordeiro NS. An ultrastructural and histopathological study of *Henneguya pellucida* n. sp. (Myxosporea: Myxobolidae) infecting *Piaractus mesopotamicus* (Characidae) cultivated in Brazil. *Parasite* 2005; 12(3): 221-227. http://dx.doi.org/10.1051/parasite/2005123221. PMid:16218209.

Adriano EA, Carriero MM, Maia AAM, Silva MRM, Naldoni J, Ceccarelli PS, et al. Phylogenetic and host-parasite relationship analysis of *Henneguya multiplasmodialis* n. sp. infecting *Pseudoplatystoma* spp. in Brazilian Pantanal wetland. *Vet Parasitol* 2012; 185(2-4): 110-120. http://dx.doi.org/10.1016/j.vetpar.2011.10.008. PMid:22051071.

Adriano EA, Oliveira OMP. Myxosporea. In: Jardim Botânico do Rio de Janeiro – JBRJ. *Catálogo taxonômico da fauna do Brasil*. Rio de Janeiro: PNUD; 2018.

Azevedo C, Casal G, Matos P, Alves A, Matos E. *Henneguya torpedo* sp. nov. (Myxozoa), a parasite from the nervous system of the Amazonian teleost *Brachyhypopomus pinnicaudatus* (Hypopomidae). *Dis Aquat Organ* 2011; 93(3): 235-242. http://dx.doi.org/10.3354/dao02292. PMid:21516976.

Azevedo C, Casal G, Matos P, Matos E. A new species of Myxozoa, *Henneguya rondoni* n. sp. (Myxozoa), from the peripheral nervous system of the Amazonian fish, *Gymnorhamphichthys rondoni* (Teleostei). *J Eukaryot Microbiol* 2008; 55(3): 229-234. http://dx.doi.org/10.1111/j.1550-7408.2008.00317.x. PMid:18460160.

Azevedo C, Corral L, Matos E. Light and ultrastructural data on *Henneguya testicularis* n. sp. (Myxozoa, Myxobolidae), a parasite from the testis of the Amazonian fish *Moenkhausia oligolepis. Syst Parasitol* 1997; 37(2): 111-114. http://dx.doi.org/10.1023/A:1005793930837.

Azevedo C, Matos E. *Henneguya adherens* n. sp. (Myxozoa, Myxosporea), parasite of the Amazonian fish, *Acestrorhynchus falcatus. J Eukaryot Microbiol* 1995; 42(5): 515-518. http://dx.doi.org/10.1111/j.1550-7408.1995.tb05898.x. PMid:7581326.

Azevedo C, Matos E. *Henneguya malabarica* sp. nov. (Myxozoa, Myxobolidae) in the Amazonian fish *Hoplias malabaricus. Parasitol Res* 1996; 82(3): 222-224. http://dx.doi.org/10.1007/s004360050099. PMid:8801553.

Azevedo C, Matos E. Fine structure of the Myxosporean, *Henneguya curimata* n. sp., parasite of the Amazonian fish, *Curimata inormata* (Teleostei, Curimatidae). *J Eukaryot Microbiol* 2002; 49(3): 197-200. http://dx.doi.org/10.1111/j.1550-7408.2002.tb00522.x. PMid:12120984.

Barassa B, Adriano EA, Cordeiro NS, Arana S, Ceccarelli PS. Morphology and host-parasite interaction of *Henneguya azevedoi* n. sp., parasite of gills of *Leporinus obtusidens* from Mogi-Guaçu River, Brazil. *Parasitol Res* 2012; 110(2): 887-894. http://dx.doi.org/10.1007/s00436-011-2571-5. PMid:21842391.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 1997; 83(4): 575-583. http://dx.doi.org/10.2307/3284227. PMid:9267395.

Carriero MM, Adriano EA, Silva MR, Ceccarelli PS, Maia AA. Molecular phylogeny of the *Myxobolus* and *Henneguya* genera with several new South American species. *PLoS One* 2013; 8(9): e73713. http://dx.doi.org/10.1371/journal.pone.0073713. PMid:24040037.

Casal G, Matos E, Azevedo C. Light and electron microscopic study of the myxosporean, *Henneguya friderici* n. sp. from the Amazonian teleostean fish, *Leporinus friderici. Parasitology* 2003; 126(4): 313-319. http://dx.doi.org/10.1017/S0031182003002944. PMid:12741510.

Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 2012; 9(8): 772. http://dx.doi.org/10.1038/nmeth.2109. PMid:22847109.

Dyková I, Lom J. Histopathological changes in fish gills infected with myxosporidian parasites of the genus *Henneguya. J Fish Biol* 1978; 12(3): 197-202. http://dx.doi.org/10.1111/j.1095-8649.1978.tb04165.x.

Eiras JC, Adriano EA. A checklist of new species of *Henneguya* Thélohan, 1892 (Myxozoa: Myxosporea, Myxobolidae) described between 2002 and 2012. *Syst Parasitol* 2012; 83(2): 95-104. http://dx.doi.org/10.1007/s11230-012-9374-7. PMid:22983797.

Eiras JC, Malta JC, Varela A, Pavanelli GC. *Henneguya schizodon* n. sp. (Myxozoa, Myxobolidae), a parasite of the Amazonian teleost fish *Schizodon fasciatus* (Characiformes, Anostomidae). *Parasite* 2004; 11(2): 169-173. http://dx.doi.org/10.1051/parasite/2004112169. PMid:15224578.

Feist SW, Longshaw WM. Phylum Myxozoa. In: Woo PTK, editor. *Fish diseases and disorders: protozoan and metazoan infections*. 2nd ed. Oxfordshire: CAB International; 2006. p. 230-296. http://dx.doi.org/10.1079/9780851990156.0230.

Felsenstein J. *Inferring phylogenies*. Sunderland: Sinauer Associates; 2004.

Ferguson JA, Atkinson SD, Whipps CM, Kent ML. Molecular and morphological analysis of *Myxobolus* spp. of salmonid fishes with the description of a new *Myxobolus* species. *J Parasitol* 2008; 94(6): 1322-1334. http://dx.doi.org/10.1645/GE-1606.1. PMid:19127969.

Fiala I. The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. *Int J Parasitol* 2006; 36(14): 1521-1534. http://dx.doi.org/10.1016/j.ijpara.2006.06.016. PMid:16904677.

Figueredo RTA, Oliveira JEF, Vilhena MPSP, Berredo J, Santos WJP, Santos E, et al. Henneguyosis in gills of *Metynnis hypsauchen*: an Amazon freshwater fish. *J Parasit Dis* 2020; 44(1): 213-220. http://dx.doi.org/10.1007/s12639-019-01183-7. PMid:32174727.

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

Holzer AS, Bartošová-Sojková P, Born-Torrijos A, Lövy A, Hartigan A, Fiala I. The joint evolution of the Myxozoa and their alternate hosts: a cnidarian recipe for success and vast biodiversity. *Mol Ecol* 2018; 27(7): 1651-1666. http://dx.doi.org/10.1111/mec.14558. PMid:29575260.

Kaur H, Attri R. Morphological and molecular characterization of *Henneguya bicaudi* n. sp. (Myxosporea: Myxobolidae) infecting gills of *Cirrhinus mrigala* (Ham.) in Harike Wetland, Punjab (India). *Parasitol Res* 2015; 114(11): 4161-4167. http://dx.doi.org/10.1007/s00436-015-4647-0. PMid:26239799.

László B, Csaba S, Kalman M. Atuais conhecimentos sobre Myxosporea (Myxozoa), parasitas de peixes: um estágio alternativo dos parasitas no Brasil. *Braz J Vet Res Anim Sci* 2002; 39(5): 271-276. http://dx.doi.org/10.1590/S1413-95962002000500010.

Lom J, Dyková I. *Protozoan parasites of fishes*. Amsterdam: Elsevier; 1992.

Lom J, Dyková I. Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol* 2006; 53(1): 1-36. http://dx.doi.org/10.14411/fp.2006.001. PMid:16696428.

Mathews PD, Maia AAM, Adriano EA. *Henneguya melini* n. sp. (Myxosporea: Myxobolidae), a parasite of *Corydoras melini* (Teleostei: Siluriformes) in the Amazon region: morphological and ultrastructural aspects. *Parasitol Res* 2016; 115(9): 3599-3604. http://dx.doi.org/10.1007/s00436-016-5125-z. PMid:27206653.

Matos E, Casal G, Matos P, Corral L, Azevedo C. Microrganismos parasitos de animais aquáticos da Amazônia. In: Ranzani-Paiva MJ, Takemoto RM, Lizama MAP, editors. *Sanidade de organismos aquáticos*. São Paulo: Varela; 2004. p. 158-178.

Matos E, Tajdari J, Azevedo C. Ultrastructural studies of *Henneguya rhamdia* n. sp. (Myxozoa) a parasite from the Amazon teleost fish, *Rhamdia quelen* (Pimelodidae). *J Eukaryot Microbiol* 2005; 52(6): 532-537. http://dx.doi.org/10.1111/j.1550-7408.2005.00063.x. PMid:16313446.

Molnár K. Taxonomic problems, seasonality and histopathology of *Henneguya creplini* (Myxosporea) infection of the pikeperch *Stizostedion lucioperca* in Lake Balaton. *Folia Parasitol* 1998; 45(4): 261-269. PMid:9868790.

Molnár K. Site preference of fish myxosporeans in the gill. *Dis Aquat Organ* 2002; 48(3): 197-207. http://dx.doi.org/10.3354/dao048197. PMid:12033706.

Moreira GSA, Adriano EA, Silva MRM, Ceccarelli PS, Maia AAM. Morphology and 18S rDNA sequencing identifies *Henneguya visibilis* n. sp., a parasite of *Leporinus obtusidens* from Mogi Guaçu River, Brazil. *Parasitol Res* 2014; 113(1): 81-90. http://dx.doi.org/10.1007/s00436-013-3629-3. PMid:24100607.

Morsy K, Abdel-Ghaffar F, Bashtar AR, Mehlhorn H, Al Quraishy S, Abdel-Gaber R. Morphology and small subunit ribosomal DNA sequence of *Henneguya suprabranchiae* (Myxozoa), a parasite of the catfish *Clarias gariepinus* (Clariidae) from the River Nile, Egypt. *Parasitol Res* 2012; 111(4): 1423-1435. http://dx.doi.org/10.1007/s00436-012-2976-9. PMid:22699967.

Naldoni J, Arana S, Maia AAM, Ceccarelli PS, Tavares LER, Borges FA, et al. *Henneguya pseudoplatystoma* n. sp. causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: histopathology and ultrastructure. *Vet Parasitol* 2009; 166(1-2): 52-59. http://dx.doi.org/10.1016/j.vetpar.2009.07.034. PMid:19695782.

Naldoni J, Maia AAM, Correa LL, Silva MRM, Adriano EA. New myxosporeans parasitizing *Phractocephalus hemioliopterus* from Brazil: morphology, ultrastructure and SSU-rDNA sequencing. *Dis Aquat Organ* 2018; 128(1): 37-49. http://dx.doi.org/10.3354/dao03210. PMid:29565252.

Pote LM, Hanson LA, Shivaji R. Small subunit ribosomal RNA sequences link the cause of proliferative gill disease in channel catfish to *Henneguya* n. sp. (Myxozoa: myxosporea). *Aquat Anim Health* 2000; 12(3): 230-240. http://dx.doi.org/10.1577/1548-8667(2000)012<0230:SSRRSL>2.0.CO;2.

Reis RE, Albert JS, Di Dario F, Mincarone MM, Petry P, Rocha LA. Fish biodiversity and conservation in South America. *J Fish Biol* 2016; 89(1): 12-47. http://dx.doi.org/10.1111/jfb.13016. PMid:27312713.

Rocha E, Matos E, Azevedo C. *Henneguya amazonica* n. sp. (Myxozoa, Myxobolidae), parasitizing the gills of *Crenicichla lepidota* Heckel, 1840 (Teleostei, Cichlidae) from Amazon River. *Eur J Protistol* 1992; 28(3): 273-278. http://dx.doi.org/10.1016/S0932-4739(11)80233-6. PMid:23195230.

Ronquist F, Huelsenbeck JP. MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 2003; 19(12): 1572-1574. http://dx.doi.org/10.1093/bioinformatics/btg180. PMid:12912839.

Swofford DL. *PAUP*: Phylogenetic analysis using parsimony (*and other methods*). Sunderland: Sinauer Associates; 2003.

Velasco M, Videira M, Nascimento LC, Matos P, Gonçalves EC, Matos E. *Henneguya paraensis* n. sp.(Myxozoa; Myxosporea), a new gill parasite of the Amazonian fish *Cichla temensis* (Teleostei: Cichlidae): morphological and molecular aspects. *Parasitol Res* 2016; 115(5): 1779-1787. http://dx.doi.org/10.1007/s00436-016-4916-6. PMid:26847632.

Videira M, Velasco M, Azevedo R, Silva R, Gonçalves E, Matos P, et al. Morphological aspects of *Henneguya aequidens* n. sp. (Myxozoa: Myxobolidae) in *Aequidens plagiozonatus* Kullander, 1984 (Teleostei: Cichlidae) in the Amazon region, Brazil. *Parasitol Res* 2015; 114(3): 1159-1162. http://dx.doi.org/10.1007/s00436-014-4295-9. PMid:25573695.

Vita P, Corral L, Matos E, Azevedo C. Ultrastructural aspects of the myxosporean *Henneguya astyanax* n. sp. (Myxozoa: Myxobolidae), a parasite of the Amazonian teleost *Astyanax keithi* (Characidae). *Dis Aquat Organ* 2003; 53(1): 55-60. http://dx.doi.org/10.3354/dao053055. PMid:12608569.

Zatti SA, Atkinson SD, Maia AAM, Bartholomew JL, Adriano EA. Novel *Henneguya* spp. (Cnidaria: Myxozoa) from cichlid fish in the Amazon basin cluster by geographic origin. *Parasitol Res* 2018; 117(3): 849-859. http://dx.doi.org/10.1007/s00436-018-5762-5. PMid:29374784.