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Short Communication

Infection of *Henneguya* sp. on the gills of *Metynnis lippincottianus* from Curiaú River, in eastern Amazon region (Brazil)

Infecção de *Henneguya* sp. nas brânquias de *Metynnis lippincottianus* do rio Curiaú, na region da Amazônia oriental (Brasil)

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Abstract

Infection of fish gills by *Henneguya* causes greater contact between the secondary gill lamellae, thereby giving rise to decreased absorption surface area at the end of the filaments. This ectoparasite can cause damages on the gills infected fish. In the present study, fresh gills of *Metynnis lippincottianus* were analyzed using optical microscopy techniques. The myxoporean *Henneguya* sp. was found to be infecting 80% of the gills of this host fish. Presence of this parasite caused hyperplasia and fusion of the gill lamellae, but without inflammation in the parasitized organ.

Keywords: Myxosporea, fish, parasitosis.

Resumo

A infecção de *Henneguya* nas brânquias de peixes causam o maior contato entres as lamelas branquiais secundárias. Provoca diminuição da superfície de absorção na extremidade dos filamentos, podendo ocasionar danos as brânquias dos peixes infectados. Neste estudo foram analisadas a fresco e com técnicas de microscopia de luz as brânquias de *Metynnis lippincottianus*. Foi determinada a presença de mixosporídeos *Henneguya* sp. infectando 80% das brânquias dos peixes hospedeiros. A presença desse ectoparasito causou hiperplasia e fusão das lamelas branquiais, porém sem inflamação no órgão parasitado.

Palavras-chave: Myxosporea, peixe, parasitoses.

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Metynnis lippincottianus Cope, 1870, a fish that is popularly known as pratinha, is widely distributed in the rivers of the Brazilian and French Guiana basins system. It belongs to the family Serrasalmidae and feeding basically on seeds and phytoplankton. It is characterized by silver color and has commercial importance for the aquarium trade and for the food chain, within which it occupies the second trophic level (Ota, 2015).

The subphylum Myxozoa encompasses the cnidarians, a group that parasitizes both vertebrates and invertebrates (Teixeira et al., 2018). This subphylum includes more than 2400 species, classified into 17 families and 64 genera (Zhang, 2011; Fiala et al., 2015). The genus *Henneguya* Thélohan (1892) comprises more than 200 species, among which 55 have been described as occurring in Brazil, and 16 have been reported in the Amazon basin (Zatti et al., 2018). Regarding morphology, this genus has two prolonged valves and each of these contains a polar capsule with a spiral filament and a sporogenic cell, usually binucleated, which forms the spores of this genus (Matos et al., 2001).

According Mólnar (2002), *Henneguya* sp. infection on fish gills causes greater contact between the secondary gill lamellae, thereby decreasing the absorption surface area at the end of the filaments, due to gill hyperplasia and hypertrophy. Species-level identification for myxosporeans based only on spore morphology and histopathology should be assessed with caution, since multiple reported species can occur in the same tissue/organ (Stilwell et al., 2020).

The fish gills constitute the first contact with the external environment, and therefore they are easily damaged by parasitic infections. These infections affect the main functions of the gills, such as osmoregulation, breathing and excretion. In addition, this organ can be used as a sentinel for parasitism levels, based on histopathological changes, respiratory disorders and electrolyte imbalances (Flores-Lopes & Thomaz, 2011).

Given the scarce studies relating to the Curiaú river basin, state of Amapá, Brazil, on myxosporeans in fish, the main aim of the present study was to evaluate the histopathological damage caused by high levels of infection on the gills of *M. lippincottianus*, caused by *Henneguya* sp.

Thirty specimens of *M. lippincottianus* were caught in the Curiaú river floodplain, in municipality of Macapá (0º08'N, 51º02'W). The hosts were collected and transported alive, in thermal boxes with the aid of a battery-operated pump for aeration, to the Laboratory of Morphophysiology and Animal Health (LABMORSA) of the University of the State of Amapá (UEAP), located in Macapá-AP. In the laboratory, they were placed in aquariums containing water from their habitat, which were equipped with an electric pump and filter, until the time of parasitological analysis.

At the beginning of the analysis, the fish were desensitized by means of medullary sectioning and biometric data such as total length (cm) and weight (g) were measured. Then, the operculum was cut to expose the gills. Macroscopically, no signs of parasitism (presence of cysts) were observed. Small fragments of the gills were removed, placed between a slide and cover slip and analyzed under an optical microscope.

The analysis on these slides showed the presence of parasitism (cysts). Fragments of these tissues/organs were removed and fixed in Davidson's solution (neutral formalin, acetic acid, 95% alcohol and distilled water) for 24 hours. They were then processed for embedding in paraffin and histological slices of thickness 5μm were cut. These thin sections were stained using hematoxylin and eosin (H&E) and a special Ziehl-Neelsen (ZN) technique at the Carlos Azevedo Research Laboratory (LPCA) of the Federal Rural University of the Amazon (UFRA).

From the gill fragments that were placed between a slide and cover slip with a drop of water and observed under an optical microscope, the presence of parasite cysts could be seen. Cysts that had ruptured exhibited spores with morphological characteristics of the genus *Henneguya*. Out of the 30 specimens analyzed, 24 presented *Henneguya* sp., i.e. with prevalence of 80%.

Staining with hematoxylin-eosin (H-E) was enough to show the presence of some parasite cysts. However, use of the ZN technique enhanced the visualization of the cysts, such that cysts that had not been observed using H&E now became marked.

Stilwell et al. (2020), showed the importance of marking specific spore characteristics using various immunohistochemistry techniques, including Ziehl-Neelsen used in this work, of Myxozoa (Cnidaria) spores, as structural of mature myxospores in histological sections, corroborating with our observations in the parasite-host interaction.

The spores of *Henneguya* sp. (n = 30) found in gills filaments of *M. lippincottianus* showed 67.4 ± 6.8 μm (57.8 – 73.1) of total length; 37.8 ± 5.0 μm (30.3 – 43.5) spore body length; 7.2 ± 1.0 μm (5.3 – 8.1) spore width; 29.9 ± 4.8 μm (21.9 – 33.8) tail length; 22.3 ± 1.7 μm (18.9 – 23.4) of polar capsule length; and 2.5 ± 0.9 μm (1.6-3) of polar capsule width.

The total length and spore body length of *Henneguya* sp. found in this study was closest of *Henneguya* sp. found by Figueredo et al. (2020) in *Metynnis hypsauchen* Müller & Troschel, 1844 with 61.9 ± 1.25 µm and 38.2 ± 0.8 µm, respectively. The tail length (29.5 ± 0.7) and the polar capsule width (2.6 ± 0,08 µm) of *Henneguya paraensis* described by Velasco et al. (2016) in *Cichla temensis* Humboldt, 1821 are similar to *Henneguya* sp. of this study. The polar capsule length of *Henneguya* sp. (22.3 ± 1.7 µm) was the morphometric data that most distanced itself from all species of *Henneguya* spp. described in Table 1.

Table 1. Morphological comparison (µm) of mature spores of *Henneguya* sp. with species described from Amazon Brazilian fish hosts.

Parasite	TL	BL	BW	TAL	PCL	PCW	Sities	Hosts
Henneguya sp1. (present study)	67.43 ± 6.86	37.87 ± 5.02	7.2 ± 1.05	29.91 ± 4.88	22.3 ± 1.67	2.47 ± 0.83	Curiau River/AP (Brazil)	*Metynnis lippincottianus*
Henneguya sp2. (Figueredo et al., 2020)	61.9 ± 1.25	38.2 ± 0.8	5.8 ± 0,45	23.7 ± 0.5	6,1 ± 0.15	1.8 ± 0.1	Capim River/PA (Brazil)	*Metynnis hypsauchen*
Henneguya adherens (Azevedo & Matos, 1995)	32.3 ± 2.2	12.4 ± 1.65	5.8 ± 0.7	20.5 ± 1.85	3.1 ± 0.35	1.2 ± 0.3	Amazon River/PA (Brazil)	*Acestrorhynchus falcatus*
Henneguya aequidens (Videira et al., 2015)	41 ± 1.5	15 ± 0.9	6 ± 0.8	27 ± 0.6	3 ± 0.3	3 ± 0.3	Peixe-boi River/PA/ (Brazil)	*Aequidens plagiozonatus*
Henneguya paraensis (Velasco et al., 2016)	42.3 ± 0.65	12.8 ± 0.42	8.6 ± 0.32	29.5 ± 0.73	7.4 ± 0.16	2.6 ± 0.08	Tocantins River/PA (Brazil)	*Cichla temensis*
Henneguya rhamdia (Matos et al., 2005)	50 ± 1.8	13.1 ± 1.1	5.2 ± 0.5	36.9 ± 1.6	4.7 ± 0.4	1.1 ± 0.2	Peixe-boi River/PA (Brazil)	*Rhamdia quelen*
Henneguya astyanax (Vital et al., 2003)	47.8 ± 0.71	15.2	5.7 ± 0.71	32.6	5.0 ± 0.13	1.5 ± 0.07	Amazon River/PA (Brazil)	*Astyanax keithi*
Henneguya malabarica (Azevedo & Matos, 1996)	28.3 ± 1.6	12.6 ± 0.65	8.6	17.1 ± 1.35	3.7 ± 0.65	1.8 ± 0.3	Amazon River/PA (Brazil)	*Hoplias malabaricus*
Henneguya santarensis (Naldoni et al., 2018)	31.9 ± 3	10.8 ± 0.5	4.3 ± 0.3	21 ± 3.1	4.6 ± 0.4	1.4 ± 0.2	Tapajós River/PA (Brazil)	*Phractocephalus hemioliopterus*
Henneguya tucunarei (Zatti et al., 2018)	43.8 ± 6.75	14 ± 1.8	6.1 ± 1.45	28.1 ± 8	3.4 ± 1.05	2 ± 0.65	Tapajós River/PA (Brazil)	*Cichla monoculus*
Henneguya tapajoensis (Zatti et al., 2018)	54.6 ± 7.5	16.4 ± 2.3	7 ± 1,8	39 ± 7.4	4.2 ± 1.05	2.1 ± 0.65	Tapajós River/PA (Brazil)	*Cichla pinima*

AP: Amapá state, PA: Pará state, TL: total length, BL: body length, BW: body width, TAL: tail length, PCL: polar capsule length, PCW: polar capsule width.

Regarding the spore body morphology of *Henneguya* sp. in present study, it showed ellipsoidal shape, as well the *Henneguya paraensis* (Velasco et al., 2016) in *Cichla temensis* Humboldt, 1821, but differentiated by the occupation of the polar capsule in the sporoplasm.

Most species of the genus *Henneguya* do not cause notable diseases in their hosts. However, some are pathogenic and infection with these species can cause mortality in fish populations. If the parasites replicate at high intensity in the gills, this will cause respiratory failure (Figueredo et al., 2020). The genus *Henneguya* usually develops in the form of cysts of histozoic nature. The description of this genus includes additional characteristics, such as host identification and level of tissue tropism (Matos et al., 2001).

In the present study, the gills of *M. lippincottianus* were found to present large numbers of cysts of the genus *Henneguya*, as shown in Figure 1a. The high numbers of *Henneguya* sp. cysts in fish gills may be directly related to the feeding habits and biological and physiological behavior of the fish species affected, since these factors can affect the structure of the parasite community (Carvalho & Tavares-Dias, 2017).

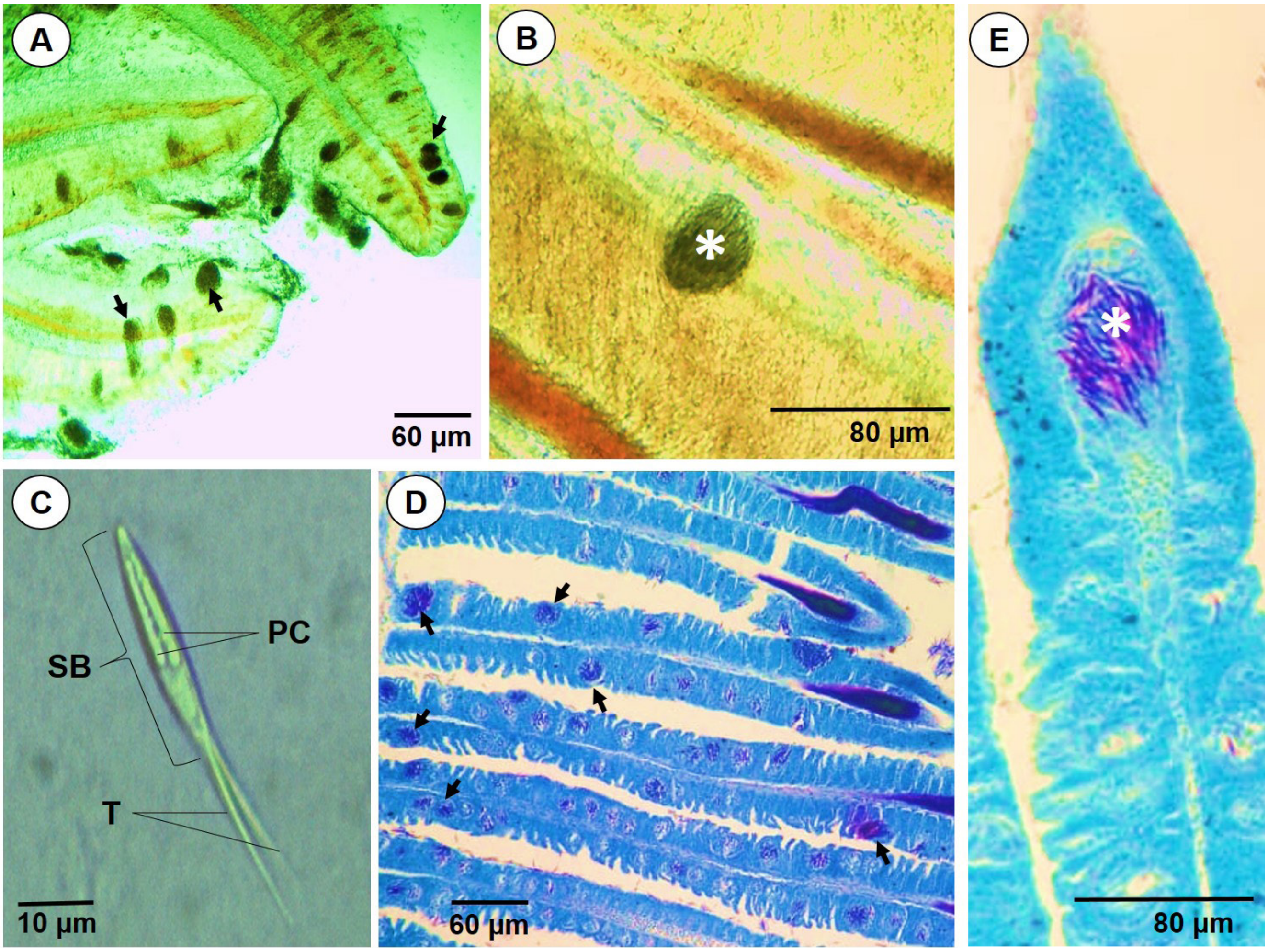

Figure 1. Cysts of *Henneguya* sp. in *Metynnis lippincottianus*: A - cysts (arrows) observed freshly on the gill filaments under optical microscopy (bar 20 µm); B - cyst (asterisk) on the gill filament (bar 20 µm); C - spore of *Henneguya* sp. (bar 10 µm), SB: spore body, PC: polar capsule, T: tail; D - cysts (arrows) of interlamellar type associated with hyperplasia and secondary lamellar fusion (bar 20 µm); E - cysts (asterisk) inside the main lamellae (bar 20 µm).

Gills are the primary site of nitrogenous waste excretion and species of *Henneguya* spp. have different way to interaction with these structures and can resulting in different levels of disease (Mólnar, 2002). In *Phractocephalus hemioliopterus* Bloch & Schneider, 1801, *H. santarensis* caused compression, deformation and fusion of adjacent lamellae (Naldoni et al., 2018), while *Henneguya astyanax* caused in *Astyanax keithi* Géry et al., 1996, surrounding tissues, moderate gill epithelial cell hyperplasia and a mild mononuclear inflammatory infiltrate within the interstitium of the gill (Vita et al., 2003).

The fresh and histological analyses on our samples showed that the cysts were surrounded by a thin layer of connective tissue (Figure 1c), with an enlarged cyst inside the main lamellae (Figure 1d), which led to slight compression of the gill filaments. No inflammation of the parasitized host tissue was observed, as was previously described in relation to the gills of *Arapaima gigas* Schinz, 1822; parasitized with cysts of *Henneguya arapaima* (Feijó et al., 2008).

While some parasite species may even develop in a filamental or lamellar location depending on fish size, plasmodium size and intensity of infection, species of *Henneguya* sp. can adapted to epithelium, cartilage, connective tissue or endothelium have not been found to develop primarily in other tissues, their possible occurrence in other tissues is the result of secondary host reaction (Mólnar, 2002).

Velasco et al. (2016) reported that the gills of *Cichla temensis* Humboldt, 1821 presented cysts of *Henneguya paraensis* cysts with thin layers of connective tissue and slight compression of the gill filaments, since the cysts grew adjacent to the filaments. Henneguyosis in gills of *Metynnis hypsauchen* Müller & Troschel, 1844 was related by Figueredo et al. (2020), causing lesions associated to ischemic necrosis, compromising drastically the host's respiratory system.

Histopathological analysis showed that the secondary lamellae had become fused, and that hyperplasia and intraepithelial cysts were present in the primary lamella, due to the numerous cysts. This had previously been reported by Velasco et al. (2015) in relation to infection by *Henneguya* sp. in the gills of *Hypophthalmus marginatus* Valenciennes, 1840. *Henneguya* sp. has high specificity for its host, and its parasitic action can cause structural damage and, in some cases, the death of its host. It can also cause infertility when located in the sexual organs (Matos et al., 2001).

The fusion of the lamellae and hyperplasia of the gill epithelium caused by *Henneguya* sp. was also previously diagnosed in *Oreochromis niloticus* Linnaeus, 1758; with lesions that compromised the lamellar structures (Teixeira et al., 2018).

The high quantities of *Henneguya* sp. that were observed in the form of interlamellar cysts in the gills of *M. lippincottianus*, through special staining techniques under an optical microscope, notably impaired the gill structures such that consequent death of the host became a possibility.

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