Research Note

Braz. J. Vet. Parasitol., Jaboticabal, v. 24, n. 3, p. 365-369, jul.-set. 2015
ISSN 0103-846X (Print) / ISSN 1984-2961 (Electronic)
Doi: http://dx.doi.org/10.1590/S1984-29612015021

Infection by *Henneguya* sp. (Myxozoa) in the bone tissue of the gill filaments of the Amazonian catfish *Hypophthalmus marginatus* (Siluriformes)

Infecção por *Henneguya* sp. (Myxozoa) em tecido ósseo do filamento branquial do bagre amazônico *Hypophthalmus marginatus* (Siluriformes)

Michele Velasco[1]; Marcela Videira[2]; José Ledamir Sindeaux-Neto[1]; Patrícia dos Santos[1]; Osimar Sanches[3]; Patrícia Matos[4]; Edilson Matos[1]*

[1]Laboratório de Pesquisa Carlos Azevedo, Universidade Federal Rural da Amazônia – UFRA, Belém, PA, Brasil

[2]Laboratório de Morfofisiologia e Sanidade Animal, Universidade do Estado do Amapá – UEAP, Macapá, AP, Brasil

[3]Centro de Diagnóstico Veterinário – CDAPVET, Presidente Prudente, SP, Brasil

[4]Laboratório de Pesquisa Edilson Matos, Universidade Federal do Pará – UFPA, Belém, PA, Brasil

Received December 18, 2014
Accepted February 11, 2015

Abstract

This study describes aspects of the infection caused by the myxosporean genus *Henneguya*, which forms cysts in the bony portion of the gill filaments of *Hypophthalmus marginatus*. Specimens of this catfish were acquired dead from artisanal fishermen near the town of Cametá, state of Pará, northern Brazil, between July 2011 and May 2012. They were transported in refrigerated containers to the Carlos Azevedo Research Laboratory at the Federal Rural University of Amazonia, in Belém, where analyses were performed. After confirmation of parasitism by the genus *Henneguya*, observation were made using optical and differential interference contrast (DIC) microscopy. The histological technique of embedment in paraffin was used. Ziehl-Neelsen staining was applied to the histological sections. Necropsy analyses on specimens of *H. marginatus* showed that 80% of them (40/50) had cysts of whitish coloration inside the bony portion of the gill filaments, filled with *Henneguya* spores. The present study found inflammatory infiltrate in the vicinity of the cysts. Furthermore, the special Ziehl-Neelsen staining technique made it possible to mark the *Henneguya* sp. cysts in the bone tissue and in spore isolates in the gill tissue structure. The descriptions of these histopathological findings show that this parasite is very invasive and causes damage to its host tissues.

Keywords: Myxosporean, catfish, gill, histopathology, Amazon.

Resumo

O presente estudo descreve os aspectos da infecção causada por mixosporídio do gênero *Henneguya*, formando cistos na porção óssea dos filamentos branquiais de *Hypophthalmus marginatus*. Espécimes desse bagre foram adquiridos mortos de pescadores artesanais perto da cidade de Cametá, Estado do Pará, Brasil, entre julho de 2011 e maio de 2012. Os animais foram transportados em contêineres refrigerados até o Laboratório de Pesquisa Carlos Azevedo, na Universidade Federal Rural da Amazônia, em Belém, onde se procederam as análises. Após a constatação do parasitismo pelo gênero *Henneguya,* foi realizada a observação em microscópio óptico e em microscópio de contraste de interferência diferencial (DIC). Foi realizada técnica histológica de impregnação em parafina e coloração dos cortes histológicos em Ziehl-Neelsen. As análises necroscópicas dos espécimes de *H. marginatus* revelaram que 80% (40/50) destes apresentavam cistos esbranquiçados na porção óssea dos filamentos branquiais, repletos de esporos do gênero *Henneguya*. O presente estudo revelou infiltrado inflamatório nas imediações dos cistos. Além disso, a técnica especial de coloração em Ziehl-Neelsen possibilitou marcar os cistos de *Henneguya* sp. no tecido ósseo e de esporos isolados na estrutura de tecido branquial. As descrições desses achados histopatológicos mostram que esse parasita é muito invasivo e produz danos aos seus tecidos do hospedeiro.

Palavras-chave: Mixosporídios, bagre, brânquia, histopatologia, Amazônia.

***Corresponding author:** Edilson Rodrigues Matos. Laboratório de Pesquisa Carlos Azevedo, Universidade Federal Rural da Amazônia – UFRA, Avenida Presidente Tancredo Neves, 2501, Bairro Montese, CEP 66077-901, Belém, PA, Brasil.
e-mail: edilson.matos9@gmail.com.

Introduction

The Amazon basin is home to the largest and most diverse fish fauna in the world, with over 1500 recorded species (MONTAG et al., 2008). Several species among these fish are valued in the international market, as is the case of a flatfish known as the Amazonian catfish (CUTRIM & BATISTA, 2005).

The species *Hypophthalmus marginatus* Valenciennes, 1840, is known in Brazil as mapará and stands out as a flatfish of great commercial importance in the Amazon basin, and in the markets in that region (COSTA et al., 2010). It is a midsized catfish (order Siluriformes), in the Pimelodidae family, found in rivers throughout the Amazonian basin (MARTINS et al., 2011). They are rheophilic fish and depend on the natural river flow to perform their reproductive functions (CARVALHO, 1980). They differ from other members of the Pimelodidae in their feeding habits and their location in the water column: whereas most species in this family have demersal carnivorous habits, maparás have pelagic planctophagous habits (CUTRIM & BATISTA, 2005). Because of the great acceptance of the species *H. marginatus* in consumer markets, studies are being conducted to demonstrate the potential of this species for aquaculture (COSTA et al., 2010).

The parasite fauna of *H. marginatus* has been little explored and may include parasite species that have not yet been described in the literature. Parasitic infections very frequently affect aquatic animals, causing profound structural and physiological changes and increasing the mortality rate (TAVARES-DIAS et al., 2014). Many species of economic interest can be infested by parasites and other biological agents. Myxosporeans are prominent among these parasites: these endoparasitic organisms are responsible for myxosporidiosis, a disease that affects freshwater and marine fish in many different geographical areas (AZEVEDO et al., 2009). Myxosporeans taxonomically belong to the phylum Myxozoa Grassé, 1970, and more than 2,200 species have been described. They are mostly fish parasites, both in the natural environment and in farming systems, and some species are responsible for diseases that generate high rates of mortality around the world (LOM & DYKOVÁ, 2006). The genus *Henneguya* has worldwide distribution and is considered to be one of the largest and most important groups belonging to the phylum Myxozoa, with about 190 species described around the world (EIRAS & ADRIANO, 2012) and more than 39 species in South America (AZEVEDO et al., 2009).

The importance of this genus as pathogens of freshwater fish has been described by several authors (LOM & DYKOVÁ, 1992; MOLNÁR, 1998; ALI, 1999; MARTINS et al., 1999; BARASSA et al., 2003; KAGEYAMA et al., 2009). Infection due to *Henneguya* species occurs mainly in the gills, and this leads to destruction of the gill filaments, thus causing respiratory failure (LOM & DYKOVÁ, 1992).

The gills are a very complex organ and a great number of species of myxosporeans are found parasitizing this organ. These parasites produce cysts in various locations within this organ, and the infection can be characterized as three types: lamellar, filamental and arch (in the gill arches) (MOLNÁR, 2002).

The objective of this work was to provide a histopathological description of infection due to *Henneguya* sp. in the bony portion of the gill filaments of *H. marginatus.*

Materials and Methods

Fifty specimens of the catfish *H. marginatus*, of length 30 cm (range: 28 – 34) and weight 75 g (range: 72 – 78), were acquired dead from artisanal fishermen near the town of Cametá (2° 14' S, 49° 29' W), in the northern Brazilian state of Pará, between July 2011 and May 2012. The specimens were transported in refrigerated containers to the Carlos Azevedo Research Laboratory at the Federal Rural University of Amazonia, in Belém. The fish were necropsied and the gill region was observed under a stereomicroscope appropriate for parasitological studies. For fresh examination, small tissue fragments containing suspected parasitism were placed between slides and cover slips together with a drop of water, for observation under an optical microscope and a differential interference contrast (DIC) microscope (Nomarski). For the histological procedure, small fragments (0.5 cm) of the parasitized tissue extracted from the gill region of the fish specimens were fixed in Davidson's solution (neutral-buffered formalin, glacial acetic acid, 95% ethanol and distilled water) for 24 hours and were then processed for embedding in paraffin. Sections were cut and stained with Ziehl-Neelsen (LUNA, 1968), and these were mounted on slides with a coverslip and were photographed under a Zeiss Primo Star optical microscope equipped with the Zeiss AxioCam ERc 5s photographic camera and the AxioVision 5.1 software.

Results and Discussion

The necroscopic analysis on *H. marginatus* specimens revealed that 80% of the hosts (40/50) presented cysts of whitish coloration, inside the bony portion of the gill filament (Figure 1). From fresh examination under the optical microscope, it could be seen that when the cysts burst, they were full of mature spores exhibiting the diagnostic traits of spores of the genus *Henneguya* (Figures 2-4): myxospores ellipsoidal, bilaterally symmetrical with a bifurcated caudal process extending from the posterior end (LOM & DYKOVÁ, 2006).

In other species of Siluriformes, the prevalence rates of parasitism in the gill region caused by species of the genus *Henneguya* have been observed to be lower than those described in the present study. Naldoni et al. (2011) reported that the prevalence of parasitism by *Henneguya eirasi* in *Pseudoplatystoma* spp. was 17.1%; Adriano et al. (2012) reported that the prevalence of *H. multiplasmodialis* in *Pseudoplatystoma corruscans* was 3.3% and in *Pseudoplatystoma reticulatum*, 2.6%; and El-Mansy & Bashtar (2002) described parasitism by *Henneguya suprabranchiae* with a prevalence of 35.8%.

The histopathological analyses revealed the presence of diffuse necrosis of the lamellae and several gill filaments. This was associated with necrotic foci over large numbers of cysts in the bone tissue of the gill filaments. The site of the parasitic infection caused by *Henneguya* sp. has usually been observed in the intra

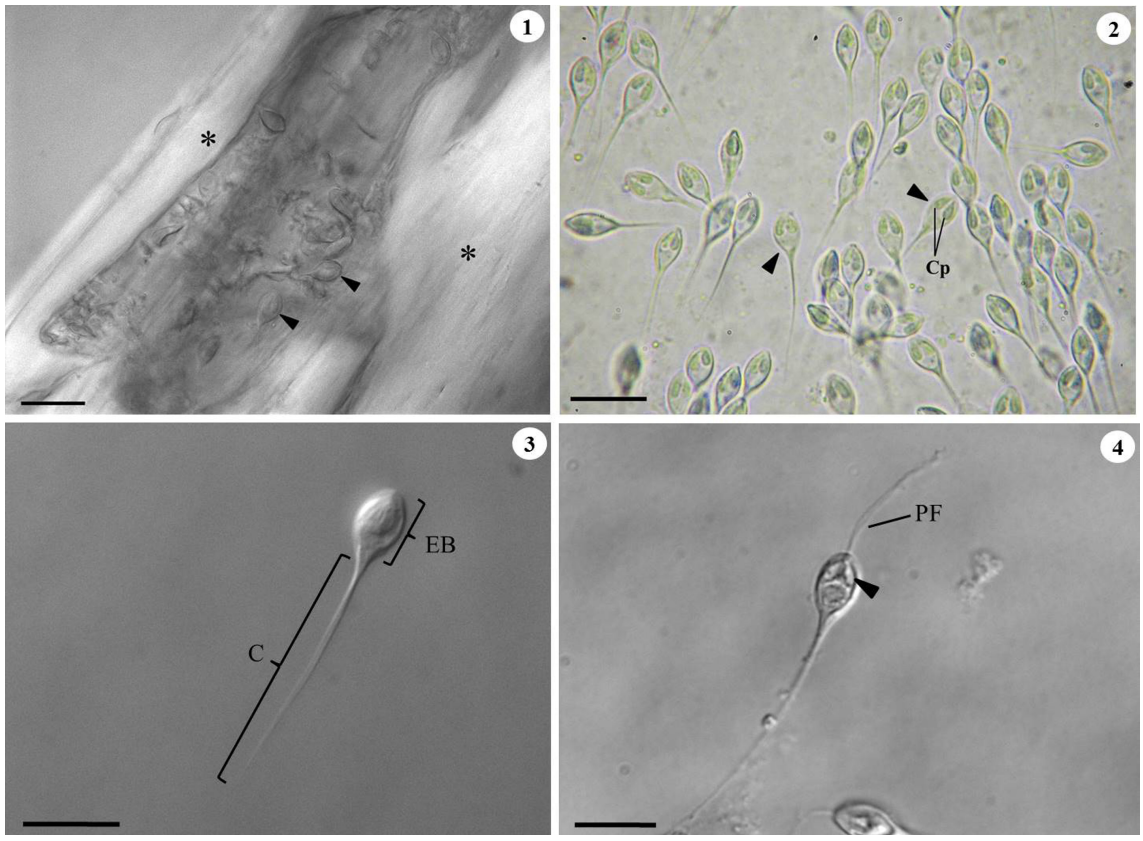

Figure 1-4. Optical micrographs of Henneguya sp. cyst and spores parasitizing H. marginatus. 1. Differential interference contrast (DIC) microscopy showing spores (arrowhead) encysted on the bony portion of a gill filament (*); bar scale 20 µm. 2. Spores (arrowhead) with the fresh observation of the polar capsules (PC); bar scale 20 µm. 3 and 4. DIC microscopy showing spores: 3. spore body (EB) and caudal extension (C); bar scale 10 µm; 4. extrusion of the polar filament (PF) and empty polar capsules (arrowhead); bar scale 10 µm.

and inter-lamellar region of the gill filaments (FEIJÓ et al., 2008; NALDONI et al., 2009; YE et al., 2012). There have not been any previous reports of infection located in the bone tissue of the gill filament. On the other hand, there have been descriptions of parasitism by *Henneguya* in cartilaginous tissue. Batueva et al. (2013) reported the presence of *Henneguya cerebralis* infection in the cranial cartilage of *Thymallus arcticus nigrescens*; Yokoyama et al. (2012) reported *Henneguya cartilaginis* infection in the cranial cartilage of *Oncorhynchus masou masou*; and El-Mansy & Bashtar (2002) reported parasitism by *Henneguya suprabranchiae* in the hyaline cartilage of the gills of the catfish *Clarias gariepinus*.

Ziehl-Neelsen staining revealed *Henneguya* sp. cysts in the bone tissue of the gill filaments (Figures 5-8), and parasite spores were released from the necrotic foci (Figures 6 and 8). Intense inflammatory infiltrate consisting of heterophils, lymphocytes and macrophages around cysts and spores was also observed. The gill filaments parasitized by this myxosporeans had become crooked and deformed, such that the cartilaginous parts had withered due to pressure caused by the development of plasmodium (Figure 7 and 8). Deformities in the gill filaments brought about by parasites of the genus *Henneguya* have often been described in the literature (ADRIANO et al., 2005; NALDONI et al., 2009; CAMPOS et al., 2011; YE et al., 2012; AZEVEDO et al., 2014).

El-Mansy & Bashtar (2002) obtained findings similar to those described in the present study, through studying parasitism by *Henneguya suprabranchiae*. This parasitism induced atrophy of the hyaline cartilage, resulting from continued growth of the plasmodium. The high intensity of infection also led to desquamation and necrosis of the tissue surrounding the suprabranchial body.

Although reports of inflammatory responses in the absence of *Henneguya* spp. infection are fairly common (ADRIANO et al., 2005; FEIJÓ et al., 2008), the present study found inflammatory infiltrates in the vicinity of the cysts. Furthermore, the special Ziehl-Neelsen staining techniques (LUNA, 1968) made it possible to mark the presence of *Henneguya* cysts in bone tissue and spore isolates in the gill tissue structure. The descriptions of these histopathological findings show that this parasite is very invasive and causes damage to its host tissues.

Given the commercial importance of *H. marginatus* and its high potential for aquaculture, the results obtained from the present study are highly relevant. Thus, they need to be disseminated since they contribute towards characterizing the parasitological profile of the host species. Moreover, this study establishes that research aimed at standardizing the methods for controlling and preventing water-borne diseases is necessary.

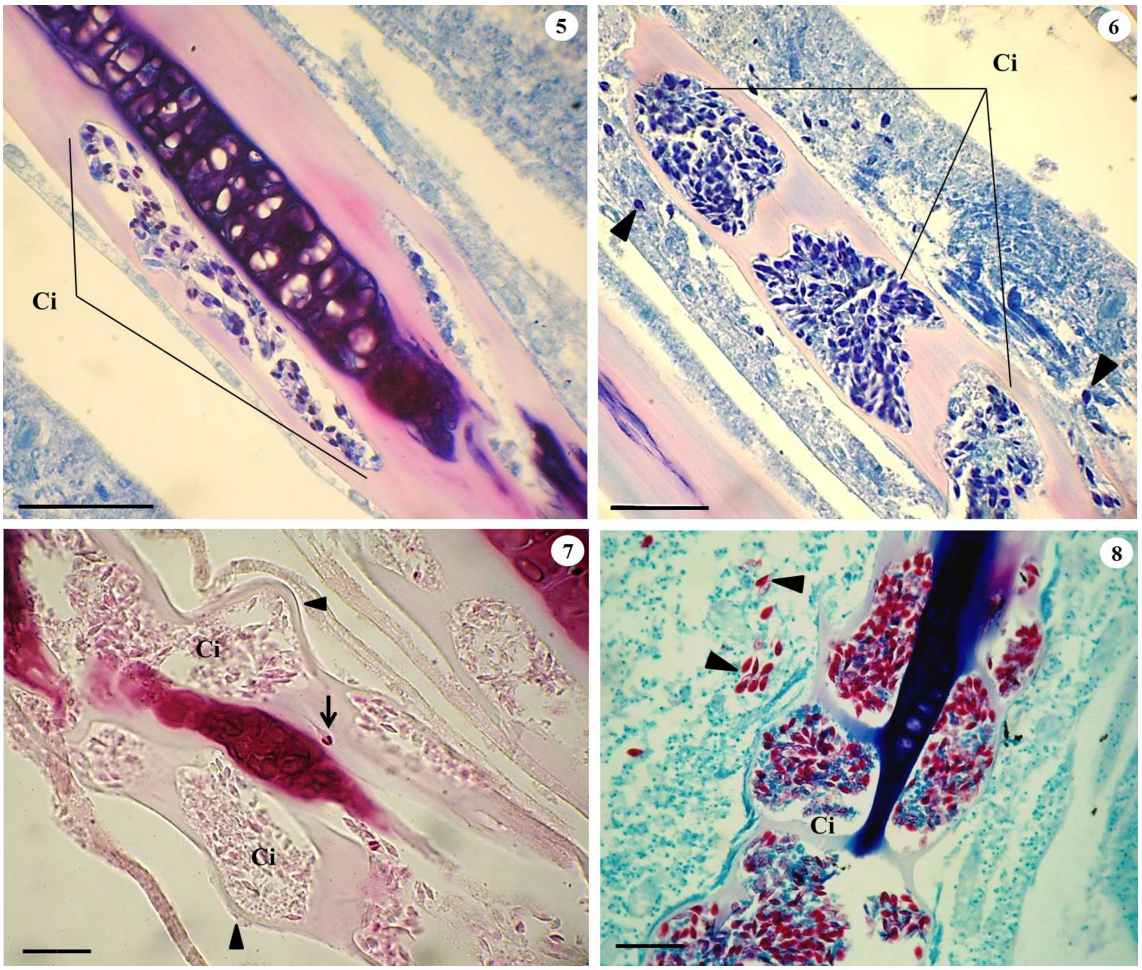

Figure 5-8. Optical micrographs showing histological sections of Henneguya sp. with Ziehl-Neelsen staining. 5. Cyst (C) in the bony portion (*) of a gill filament; bar scale 50 µm. 6. Filament (FB) presenting cysts (C) in the bony portion and free from necrotic foci of spores (arrowhead); bar scale 30 µm. 7 and 8. Gill filament of tortuous and deformed appearance (arrowhead) due to presence of cysts (C) (arrowhead); bar scale 30 µm.

Acknowledgements

We are grateful to the Coordination Office for Advancement of University-level Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES); National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq); Amazonas and Pará Research Support Foundation (Fundação Amazônia Paraense de Amparo à Pesquisa, FAPESPA); SISBIO/ICMBio-IBAMA, Licence nº. 27119 (Brazil); the electron microscopy laboratory of the Evandro Chagas Institute; the Edilson Matos Research Laboratory (LPEM–UFPA); and Mr. David Elliff for the English-language revision of the manuscript. The helpful suggestions and comments of the Associate Editor and reviewers are greatly appreciated.

References

Adriano EA, Arana S, Cordeiro NS. Histophatology and ultrastructure of *Henneguya caudalongula* sp. n. infecting *Prochilodus lineatus* (Pisces: Prochilodontidae) cultivated in the state of São Paulo, Brazil. *Mem Inst Oswaldo Cruz* 2005; 100(2): 177-181. http://dx.doi.org/10.1590/S0074-02762005000200011. PMid:16021305

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

Ali MA. *Henneguya ghaffari* sp. n. (Myxozoa: Myxosporea), infecting the Nile perch *Lates niloticus* (Teleostei: Centropomidae). *Dis Aquat Organ* 1999; 38(3): 225-230. http://dx.doi.org/10.3354/dao038225. PMid:10686673

Azevedo C, Casal G, Mendonça I, Matos E. Fine structure of *Henneguya hemiodopsis* sp. n. (Myxozoa), a parasite of the gills of the Brazilian teleostean fish *Hemiodopsis microlepes* (Hemiodontidae). *Mem Inst Oswaldo Cruz* 2009; 104(7): 975-979. http://dx.doi.org/10.1590/S0074-02762009000700006. PMid:20027463

Azevedo C, Rocha S, Matos P, Matos E, Oliveira E, Al-Quraishy S, et al. Morphology and phylogeny of *Henneguya jocu* n. sp. (Myxosporea, Myxobolidae), infecting the gills of the marine fish *Lutjanus jocu*. *Eur J Protistol* 2014; 50(2): 185-193. http://dx.doi.org/10.1016/j.ejop.2013.12.002. PMid:24457131

Barassa B, Cordeiro NS, Arana S. A new species of *Henneguya*, a gill parasite of *Astyanax altiparanae* (Pisces: Characidae) from Brazil, with comments on histopathology and seasonality. *Mem Inst Oswaldo Cruz* 2003; 98(6): 761-765. http://dx.doi.org/10.1590/S0074-02762003000600009. PMid:14595452

Batueva MDD, Katokhin AV, Pronina SV, Pronin NM. Supplementary studies and molecular data on *Henneguya cerebralis* Pronin, 1972 (Myxozoa: Myxosporea), a parasite from Kosogol grayling *Thymallus arcticus nigrescens* in Mongolia. *Parasitol Int* 2013; 62(6): 530-534. http://dx.doi.org/10.1016/j.parint.2013.07.014. PMid:23933262

Campos CM, Moraes JR, Moraes FR. Histopathology of gills of *Piaractus mesopotamicus* (Holmberg, 1887) and *Prochilodus lineatus* (Valenciennes, 1836) infested by monogenean and myxosporea, caugth in Aquidauana River, State of Mato Grosso do Sul, Brazil. *Rev Bras Parasitol Vet* 2011; 20(1): 67-70. http://dx.doi.org/10.1590/S1984-29612011000100014. PMid:21439236

Carvalho FM. Alimentação de Mapará (*Hypophthalmus edentatus* Spix, 1829) do lago do Castanho, Amazonas (Siluriformes, Hypophthalmidae). *Acta Amazon* 1980; 10(3): 545-555.

Costa TV, Oshiro LMY, Silva ECS. O potencial do mapará *Hypophthalmus* spp. (Osteichthyes, Siluriformes) como uma espécie alternativa para a piscicultura na Amazônia. *Bol Inst Pesca* 2010; 36(3): 165-174.

Cutrim L, Batista VS. Determinação de idade e crescimento do mapará (*Hypophthalmus marginatus*) na Amazônia Central. *Acta Amazon* 2005; 35(1): 85-92. http://dx.doi.org/10.1590/S0044-59672005000100013.

Eiras JC, Adriano EAA. 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

El-Mansy A, Bashtar AR. Histopathological and ultrastructural studies of *Henneguya suprabranchiae* Landsberg, 1987 (Myxosporea: Myxobolidae) parasitizing the suprabranchial organ of the freshwater catfish *Clarias gariepinus* Burchell, 1822 in Egypt. *Parasitol Res* 2002; 88(7): 617-626. http://dx.doi.org/10.1007/s00436-002-0598-3. PMid:12107453

Feijó MM, Arana S, Ceccarelli PS, Adriano EA. Light and scanning electron microscopy of *Henneguya arapaima* n. sp. (Myxozoa: Myxobolidae) and histology of infected sites in pirarucu (*Arapaima gigas*: Pisces: Arapaimidae) from the Araguaia River, Brazil. *Vet Parasitol* 2008; 157(1-2): 59-64. http://dx.doi.org/10.1016/j.vetpar.2008.06.009. PMid:18771855

Kageyama T, Yanagida T, Ohara K, Yokoyama H. *Henneguya pseudorhinogobii* n. sp. (Myxozoa: Myxosporea) parasitizing the gills of the freshwater goby *Rhinogobius* sp. OR from the Nagara River and redescription of *Henneguya rhinogobii*. *Fish Sci* 2009; 75(3): 657-663. http://dx.doi.org/10.1007/s12562-009-0096-y.

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

Lom J, Dyková I. *Protozoan parasites of fishes*. New York: Elsevier; 1992. 315 p.

Luna LG. *Manual of histologic staining methods of the armed forces institute of pathology*. 3rd ed. New York: MacGraw-Hill Book Company; 1968.

Martins JC, Juras AA, Araújo MAS, Mello Filho AS, Cintra IHA. Seletividade da rede malhadeira-fixa para a captura do mapará, *Hypophthalmus marginatus*, no reservatório da usina hidrelétrica de Tucuruí, Estado do Pará, Brasil. *Bol Inst Pesca* 2011; 37(2): 123-133.

Martins ML, Souza VN, Moraes JR, Moraes FR. Gill infection of *Leporinus macrocephalus* Garavello & Britski, 1988 (Osteichthyes: Anostomidae) by *Henneguya leporinicola* n. sp. (Myxozoa: Myxobolidae). Description, histopathology and treatment. *Rev Bras Biol* 1999; 59(3): 527-534. http://dx.doi.org/10.1590/S0034-71081999000300018. PMid:10765464

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

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.

Montag LFA, Freitas TMS, Wosiacki WB, Barthem RB. Os peixes da Floresta Nacional de Caxiuanã (municípios de Melgaço e Portel, Pará - Brasil). *Bol Mus Para Emílio Goeldi Cienc Nat* 2008; 3(1): 11-34.

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, Arana S, Maia AAM, Silva MRM, Carriero MM, Ceccarelli PS, et al. Host-parasite-environment relationship, morphology and molecular analyses of *Henneguya eirasi* n. sp. parasite of two wild *Pseudoplatystoma* spp. in Pantanal Wetland, Brazil. *Vet Parasitol* 2011; 177(3-4): 247-255. http://dx.doi.org/10.1016/j.vetpar.2010.12.008. PMid:21237571

Tavares-Dias M, Sousa TJSM, Neves LR. Parasitic infections in two benthopelagic fish from Amazon: the arowana *Osteoglossum bicirrhosum* (Osteoglossidae) and Oscar *Astronotus ocellatus* (Cichlidae). *Biosci J* 2014; 30(2): 546-555.

Ye LT, Li WX, Wu SG, Wang GT. Supplementary studies on *Henneguya doneci* Schulman, 1962 (Myxozoa: Myxosporea) infecting the gill filaments of *Carassius auratus gibelio* (Bloch) in China: histologic, ultrastructural, and molecular data. *Parasitol Res* 2012; 110(4): 1509-1516. http://dx.doi.org/10.1007/s00436-011-2655-2. PMid:21989578

Yokoyama H, Urawa S, Grabner D, Shirakashi S. *Henneguya cartilaginis* n. sp. (Myxozoa: Myxosporea) in the head cartilage of masu salmon *Oncorhynchus masou masou*. *Parasitol Int* 2012; 61(4): 594-598. http://dx.doi.org/10.1016/j.parint.2012.05.013. PMid:22664475