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Lymphocytic meningoencephalomyelitis associated with *Myxobolus* sp. (Bivalvulidae: Myxozoa) infection in the Amazonian fish *Eigenmannia* sp. (Sternopygidae: Gymnotiformes)

Meningoencefalomielite linfocitária associada à infecção por *Myxobolus* sp. (Bivalvulidae:Myxozoa) em peixe amazônico *Eigenmannia* sp. (Sternopygidae: Gymnotiformes)

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Abstract

The genus *Myxobolus*, parasites that infect fishes, which cause myxobolosis, includes spore organisms belonging to the phylum Myxozoa and represents approximately 36% of all species described for the entire phylum. This study describes lymphocytic meningoencephalomyelitis associated with *Myxobolus* sp. infection in the brain and spinal cord (the central nervous system, CNS) of *Eigenmannia* sp., from the Amazon estuary region, in the Administrative District of Outeiro (DAOUT), Belém, Pará, Brazil. In May and June 2015, 40 *Eigenmannia* sp. specimens were captured from this region and examined. The fish were anesthetized, slaughtered and dissected for sexing (gonad evaluation) and studying parasites and cysts; after diagnosing the presence of the myxozoans using a light microscope, small fragments of the brain and spinal cord were removed for histological processing and Hematoxylin-Eosin and Ziehl-Neelsen staining. Histopathological analysis of the brain and spinal cord, based on histological sections stained with Hematoxylin-Eosin, pronounced and diffuse edema in these tissues, and congestion, degeneration, and focal necrosis of the cerebral cortex. The present study describes lymphocytic meningoencephalomyelitis associated with infection by *Myxobolus* sp. in the central nervous system of *Eigenmannia* sp.

Keywords: Knifefish, Amazonia, myxosporean, histopathology, microscopy, nervous-system.

Resumo

O gênero *Myxobolus* é composto por parasitas esporais que podem infectar peixes e causar a "myxobolose". São organismos pertencentes ao filo Myxozoa e representam cerca de 36% do total de espécies descritas para todo o Filo. Este estudo descreve meningoencefalomielite linfocitária, associada à infecção por *Myxobolus* sp. no cérebro e medula espinhal (SNC) de *Eigenmannia* sp, oriundo de região estuarina amazônica, no Distrito Administrativo de Outeiro (DAOUT), município de Belém, Pará, Brasil. Foram capturados e examinados 40 espécimes de *Eigenmannia* sp. entre os meses de maio e junho de 2015. Os peixes foram anestesiados, abatidos e dissecados para sexagem (avaliação das gônadas) e pesquisa de parasitos e cistos. Após o diagnóstico da presença dos mixosporidios, utilizando-se microscópio de luz, pequenos fragmentos do cérebro e da medula espinal foram removidos para processamento histológico e coloração por Hematoxilina-Eosina e coloração especial em Ziehl-Neelsen. A análise histopatológica do cérebro e da medula espinhal, com base em cortes histológicos corados com Hematoxilina-Eosina, mostrou edema difuso nesses tecidos, e congestão, degeneração e necrose focal do córtex cerebral. O presente estudo descreve meningoencefalomielite linfocítica, associada à infecção por *Myxobolus* sp., no sistema nervoso central de *Eigenmannia* sp.

Palavras-chave: Ituí, Amazônia, mixosporidios, histopatologia, microscopia, sistema-nervoso.

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Introduction

Fishes of the genus *Eigenmannia* (Order Gymnotiformes), have a serpentiform morphology and neotropical distribution (ALBERT, 2001) and are commonly known as "knifefishes" due to their long and slender bodies, which have undulatory movement, directly linked to their long anal fins (CAMPOS-DA-PAZ & ALBERT, 1998). Another important characteristic of these fishes is that they continously emit low-voltage electric discharges (KRAMER, 1999; ALVES-GOMES, 2001).

In their natural habitat, fishes can be infected by various parasites, even without showing clinical signs of parasitosis. However, when environmental changes occur due to anthropogenic action or natural reasons (which interfere with immunity), these fishes begin to show clinical signs of certain diseases that can vary from a reduction in body size to the death of the host (DEAN et al. 2001; PAVANELLI et al., 2002).

Among the parasites that infect fishes, we highlighted the genus *Myxobolus*, which causes myxobolosis (MOLNÁR & BÉKÉSI, 1992). They are parasitic spore organisms belonging to the phylum Myxozoa, having approximately 800 described species, which is equivalent to approximately 36% of all species described for the entire phylum (LOM & DYKOÁ, 2006). They have two valves, a criterion that classifies them in the order Bivalvulida, and their morphology comprises an ellipsoid body, two polar capsules, and a sporoplasm with binucleate cells, usually spherical and sometimes containing a polysaccharide inclusion (KENT et al., 2001; LOM & DYKOÁ, 2002, 2006).

M. cerebralis is the most studied Myxozoa, responsible for causing central nervous system (CNS) disease in fishes, the principal symptom of which is that infected fish swim in circles, thus giving it the name "whirling disease" (GILBERT & GRANATH, 2001).

Several other species of *Myxobolus* infect the CNS, as is the case for *M. inaequus* (KENT & HOFFMAN 1984), *M. neurophilus*, Guilford, 1963 (DZULINKSY et al., 1994), *M. encephalicus*, Mulsow, 1911, (ANTYCHOWICZ & REICHERT, 2005), and *Myxobolus* sp. (FERGUSON et al., 2011).

The present study describes lymphocytic meningoencephalomyelitis associated with infection by *Myxobolus* sp. in the brain and spinal cord (CNS) of *Eigenmannia* sp. from the Amazon estuary region, in the Administrative District of Outeiro (DAOUT), Belém, Pará, Brazil.

Materials and Methods

In May and June 2015, 40 *Eigenmannia* sp. specimens were captured from the Amazon estuary region, in the Administrative District of Outeiro-DAOUT (1°14′ S; 48°26′ W), Belém, Pará, Brazil, and examined. The specimens were transported live in artificially aerated plastic bags containing water from their habitat to the Carlos Azevedo Research Laboratory at the UFRA-Belém. Here, they were kept in aquaria with water temperature ranging from 28 to 30 °C. The fish were anesthetized with tricaine methanesulfonate (MS222,) at a concentration of 50 mg.L^{-1}, the animal was anesthetized, slaughtered and dissected to evaluate the gonads (sexing) and analyze the parasites and cysts using a stereo microscope (ethics committee on animal use nº 013/2014 – UFRA). After determining the presence of cysts in the brain and spinal cord, small fragments of the organs were removed for observation under light microscopy (LM), pressed between a slide and coverslip, and analyzed to determine the presence of parasites. Images were captured using a Zeiss AxiocaCAm Erc 5 camera, appropriately coupled to a Zeiss Primo Star microscope, to measure the spores using AxioVision LE software. After diagnosing the presence of the myxosporids, small samples of the infected organs (approximately 0.5 cm thick) were removed, fixed in Davidson solution (neutral buffered formalin, glacial acetic acid, 95% ethanol alcohol, and distilled water) for 24 h. Samples were then processed using the paraffin embedding method, and 5 µm-thick sections were stained with Hematoxylin-Eosin and Ziehl-Neelsen (LUNA, 1968).

Results and Discussion

Necropsy of the *Eigenmannia* sp. specimens enabled us to study the parasites and sex the animals; 70% (28/40) of the specimens were infected by *Myxobolus* sp. in the brain and spinal cord. 60% (24/40) of the fish evaluated were male and 40% (16/40) were female.

More specific prevalence data demonstrated that of the 28 infected animals, 16 were male and 12 were female; 66% of the males and 75% of the females exhibited infection symptomatic of *Myxobolus* sp., revealing potential differences in infections for males and females. Adriano et al. (2012) found no difference between males and female in the prevalence of infection by Myxobolidae in *Zungaro jahu*, a fish from the Mato Grosso Pantanal, Brazil.

The spores exhibited morphological characteristics of the genus *Myxobolus*, according to descriptions by Lom & Dykoá (2006), with ellipsoid bodies, two asymmetrical polar capsules (Figures 1, 2 and 3), similar to those described by Kent & Hoffman (1984), Cellere et al. (2002) for *M. inaequus* in *E. virescens* and *M. absonus* in *Pimelodus maculatus* and Azevedo et al. (2002) for *M. desaequalis* in *Apteronotus albifrons* respectively.

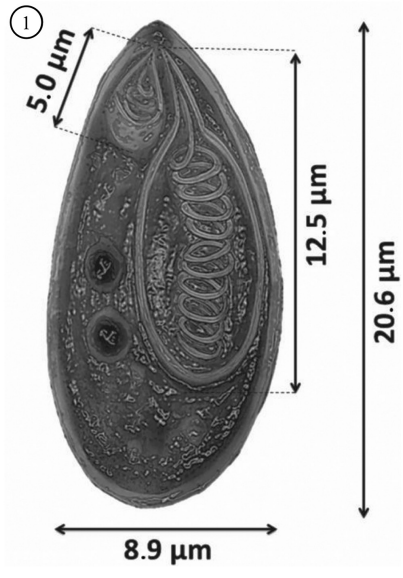

Figure 1. *Myxobolus* sp. in semi-schematic drawing of a valvar view of the spore of the myxosporean parasite from the nervous system.

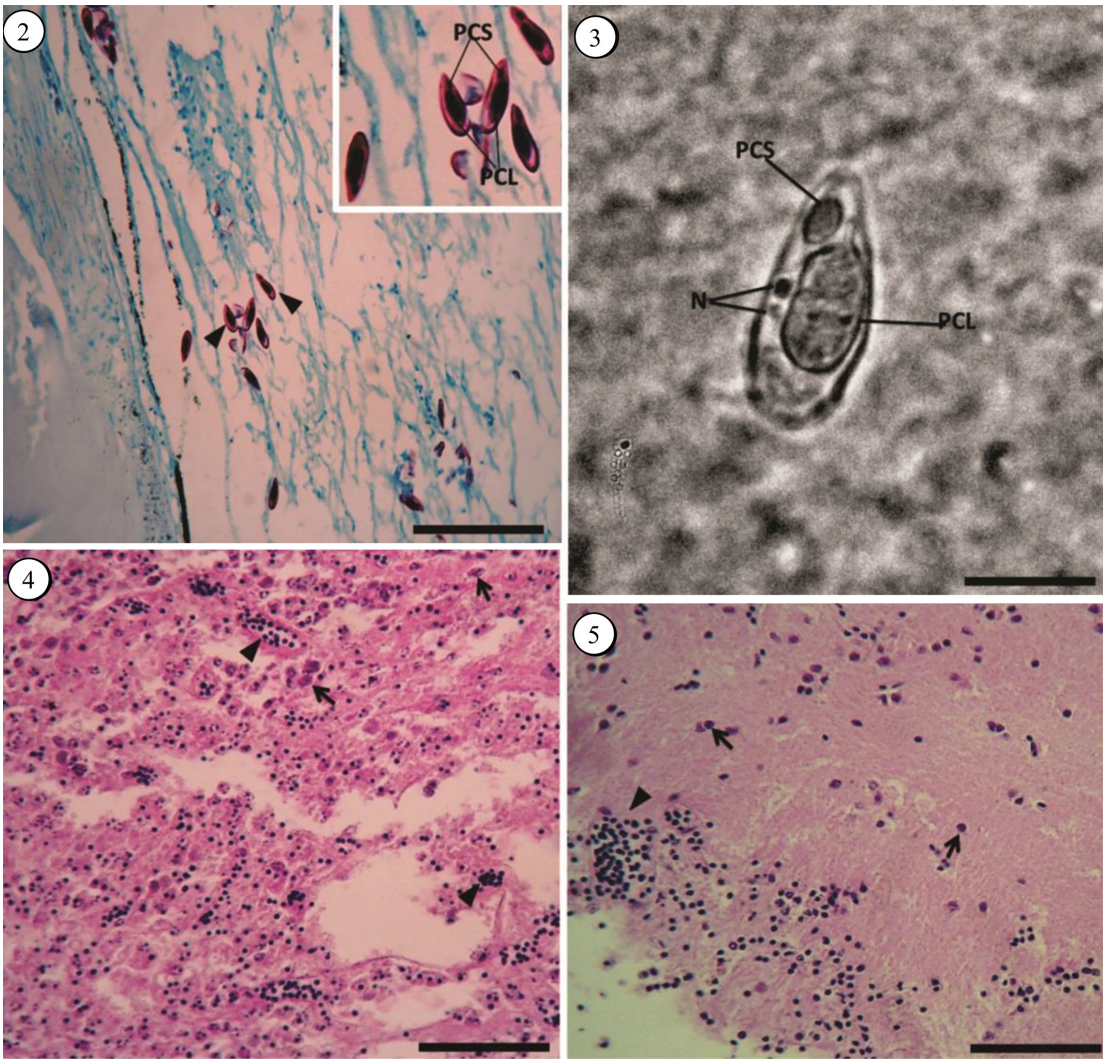

Figure 2. Spinal cord histological section stained in Ziehl-Neelsen, showing spore cluster (arrowhead) in the subarachnoid space of the spinal cord, highlighting spores (inset) with large polar capsules (LPC) and small polar capsules (SPC) Scale bar: 80 µm. **Figure 3.** *Myxobolus* sp. spore, with large polar capsule (LPC) and small polar capsule (SPC), with the presence of nuclei (n) in the lateral space below the smaller polar capsule 10 µm. **Figures 4 and 5.** Photomicrograph of brain histological sections stained in Hematoxylin and Eosin, **4.** Inflammatory infiltrate agglomerations (arrowhead), with the presence of astrocytes (arrow). Scale bar: 310 µm. **5.** Pyramidal layer, highlighting inflammatory infiltrate agglomerations (arrowhead) and pyramidal neurons (arrow). Scale bar: 300 µm.

Results of the measurements of length (L) and width (W) of the spores and polar capsules and morphometric comparisons with other *Myxobolus* spp. with different capsules are shown in Table 1.

We found a correlation between the fish cells and parasitic spores via the hematoxylin-eosin (HE) histological technique (Figures 4 and 5), which was used by Molnár & Baska (1999) to redescribe *M. hungaricus*, Jaczó 1940, in *Abramis brama*.

Ziehl-Neelsen staining, used in the histopathological analyses, highlighted the polar capsules of the spores to identify spores with asymmetrical polar capsules dispersed throughout the nervous tissue (Figure 2); Kaur & Singh (2009) used a similar histological technique to highlight the polar capsules of *M. eirasi* in *Cirrhina mrigala.*

Histopathological analysis of the brain and spinal cord, based on histological sections stained with Hematoxylin-Eosin, allowed us to analyze pronounced and diffuse edema in these tissues, and congestion, degeneration, and focal necrosis of the cerebral cortex. In the cerebral cortex, there was also gliosis and marked satellitosis, a focal area of malacia, and marked lymphocytic inflammatory infiltrate in the brain parenchyma and meninges (Figures 4 and 5). We also observed a pronounced quantity of *Myxobolus* sp. spores forming cysts dispersed throughout the spinal cord. There was marked edema, malacia, and moderate lymphocytic inflammatory infiltrate in the brain and spinal cord (the central nervous system, CNS) associated with the area of parasitism in the spinal cord (Figure 2). Using histopathological analysis, Campos et al. (2008) noted large quantities of inflammatory infiltrate caused by *Myxobolus* sp. infection, showing that parasites of this genus are able to cause inflammatory immune responses in their host.

Table 1. Comparison of measurements of spores of *Myxobolus* sp. LS, total length of the spore; WS, width of the spore; PC, polar capsules (length x width). All measurements are provided in micrometers.

	Myxobolus inaequus **Kent & Hoffman (1984)**	***Myxobolus absonus*** **Cellere et al. (2002)**	***Myxobolus desaequalis*** **Azevedo et al. (2002)**	***Myxobolus* sp.** **Present study**
Host	*Eigenmannia virescens*	*Pimelodus maculatus*	*Apteronotus albifrons*	*Eigenmannia* sp.
Site of infection	Brain	Opercular cavity	Brânquias	Brain
LS	19.8	15.7	18.3	20.6
WS	8.6	10.2	8.6	8.9
Larger PC	11.8 x 3.6	6.4 x 3.6	11.2 x 4.9	12.5 x 5.7
Smaller PC	4.8 x -	4.2 x 2.5	4.6 x 2.8	5.0 x 2.65
Larger capsule PFC	14	5	11 – 12	11
Smaller capsule PFC	6	3	4-5	3
Country	**South America**	**South America**	**Brazil**	**Brazil**

The findings described in the present study enabled us to characterize a CNS infection in *Eigenmannia* sp. caused by spores of the genus *Myxobolus*, where histopathology characterized lymphocytic meningoencephalomyelitis of the brain and spinal cord. It is important to note that no clinical sign of disease was observed in the host. Studies of this nature are fundamental for understanding myxobolosis in the nervous system of Amazonian fish.

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