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SHORT COMMUNICATION

Ultrastructural description of *Myxobolus cuneus* (Myxosporea) in the skeletal muscle and kidney of tropical farmed fish *Piaractus mesopotamicus* (Characiformes: Characidae)

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Abstract This study characterizes by transmission electron microscopy (TEM) and morphometric features the myxozoan *Myxobolus cuneus* (Myxosporea) in *Piaractus mesopotamicus* and reports the skeletal muscle and kidney as site of infection. The register was based in 21 young fish from intensive fish farming in Southeast Brazil and the spores were analyzed in fresh-mounted slides of the infected organs stained with Toluidine blue and processed as usual for TEM. It differs from *Myxobolus cunhai* from the fish host and different polar capsule size, and from *Myxobolus serrasalmi* on the pyriform spore shape and an oval macrospore, differently to that reported in this study. Morphometric characteristics and TEM study confirmed the present material as *M. cuneus*.

Keywords Freshwater fish · Pacu · Myxozoa · Transmission electron microscopy

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Introduction

Piaractus mesopotamicus (Characidae: Myleinae) commonly known as "pacu" is a native omnivorous fish of great economic importance in Brazil (Manrique et al. 2015a). High stocking density and inadequate handling are responsible for increased stress that negatively affect the fish health causing more susceptibility to diseases including the myxozoan parasites. These parasites may develop intra and intercellular (histozoic) or located in the organs and body cavity (coelozoic) (Lom and Dyková 1992). They can be found in the gills, skin, liver, spleen, kidney, gallbladder, intestines, swim bladder, cartilage, and muscle, but some of them may be found in the subcutaneous tissue provoking deformations visible in naked eye (Brites-Neto and Thatcher 1994). In the skin, the plasmodia are easily visible (Baska 1986) and *Myxobolus* genus was also recorded in the internal organs (Adriano et al. 2006), blood (Maciel et al. 2011), brain (Baldwin et al. 2000), and kidney (Manrique et al. 2012).

Some species of the genus *Myxobolus* parasiting the skeletal muscle were described as *Myxobolus* sp. in *P. mesopotamicus* and did not present neither inflammation signs nor degree of myofibrillar degeneration (Manrique et al. 2015b). Other authors (Székely et al. 2009) described the infection of *Myxobolus omari* in the sutchi catfish *Pangasianodon hypophthalmus* and *Myxobolus leptobarbi* in the mad barb *Leptobarbus hoevenii* causing host cell damages with engulfed spores by melanomacrophages. Hypertrophy and vertebral fusion were also observed in *Myxobolus buckey* infection in the skeletal muscle of cyprinid fishes *Leuciscus cephalus*, *Rutilus rutilus*, and *Abramis brama* (Longshaw et al. 2003).

Molnár and Kovács-Gayer (Molnár and Kovács-Gayer 1985) and Baska (1986) have also reported *Myxobolus cyprini*

and *Myxobolus pseudodispar*, spores in the skeletal muscle. Ogawa et al. (1992) observed high infections of *Myxobolus artus* in the muscle of common carp, *Cyprinus carpio* affecting the fish growth. Pernicious anemia of carp caused by *M. cyprini* and muscle infection in *Stizostedion lucioperca* caused by *Myxobolus sandrae* were considered the most important pathogenic species for freshwater fish (Shulman 1966; Azevedo et al. 2012).

The conventional diagnosis of myxozoan is based mainly on their microscopical observation or *imprinting* of tissues. On the other hand, it reduces the possibility of correct identification of developmental stages of spores (Manrique et al. 2013). Ultrastructural identification has been used to establish the similarities among the species (Matos et al. 1999; Abdel-Ghaffar et al. 2005) but comparatively few studies consider the histopathology to complement the myxozoan diagnosis and host consequences (Martins et al. 1997; Ali et al. 2002; Adriano et al. 2005, 2006, 2009; Campos et al. 2011).

The aim of this study was to characterize by transmission electron microscopy the morphology of the myxozoan *Myxobolus cuneus* in the skeletal muscle and kidney of a Neotropical freshwater fish *P. mesopotamicus*, in Brazil.

Materials and methods

Twenty-one young fish with 120.0 ± 5.0 g mean weight and 18.4 ± 3.2 cm standard length were captured during July 2014 from the intensive fish farming in Southeast Brazil, São Paulo State; the place of study is private and is not characterized as a reserve, and has been approved by the owner of the property. During this period, the water quality was kept as follows: dissolved oxygen 5.0 ± 0.8 mg/L, temperature 29.4 ± 0.3 °C, pH 7.6 ± 0.8, electrical conductivity 117.9 ± 1.8 μS/cm, measured with an YSI Model MPS 556 equipment. The studied fish species are not endangered and are species of commercial production.

Immediately after each collection, the fish were euthanized in a benzocaine solution 1:500 *v*/*v* and then necropsied. The procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and the experimental protocol was approved by the Committee of Ethics in the Use of Animals, CEUA – "Comissão de Ética no uso de Animais" (protocol n° 020092/09) from the São Paulo State University.

The fish were dissected and the cysts were removed from the skeletal muscle and spores from kidney tissue for examination in a light microscope equipped with differential interference contrast microscopy (Olympus BX51) with image capture in a DP73 Olympus camera (software cell Sens v. 1.5) and a camera lucida.

For ultrastructural studies, fragments of infected skeletal muscle and kidney were fixed in 3 % (*v*/*v*) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 20 h at 4 °C, washed three times in the buffer for an hour at 4 °C and post-fixed in 2 % (*w*/*v*) OsO_4 buffered with 0.2 M sodium cacodylate for 2 h at the same temperature. The fragments were dehydrated in an ascending ethanol and propylene oxide series and embedded in Epon. Fresh-mounted slides of the infected organs were stained with Toluidine blue (TB). Ultrathin sections (60 nm) were cut with diamond knife, contrasted with uranyl acetate and lead citrate and observed in a JEOL JEM-1010 TEM (JEOL Optical, Tokyo, Japan) operated at 70 kV.

Results

Description The spore's morphology were the same in both tissues. The morphometry of *M. cuneus* in the present study was compared with other species of *Myxobolus* identified in Brazilian fish (Table 1). Mature fresh spores ($n = 126$) had an oval body with a length 8.9 ± 0.3 μm, spore width 5.7 ± 0.3 μm, two equal polar capsules 4.3 ± 0.3 μm long and 1.9 ± 0.1 μm wide (Fig. 1). Schematic drawing of matures spores shows the arrangement of the structures and organelles (Fig. 2). The spores wall thin and smooth measured 65.0 ± 0.3 nm thickness comprising two valves joint by a suture line (Figs. 3 and 4). Polar filaments were coiled in 7–8 turns perpendicularly to the axis of the capsule (Fig. 5). In the median region of the spore a binucleated sporoplasm provided by several electron-dense vesicles, sporoplasmosomes, and a single nucleus was observed, this latter measuring 346.0 ± 0.3 nm in diameter with a dense chromatin with no evidence of nucleolus (Figs. 6 and 7). Espores in different maturations stages (Fig. 8).

Type host Teleost fish *P. mesopotamicus* (Holmberg, 1887) (Osteichthyes: Characidae).

Site of infection Plasmodia located in the striated skeletal muscle and spores in the posterior kidney.

Prevalence Fourteen of 21 fish (66.7 %) had spores in the striated skeletal muscle and 17 of 21 fish showed spores in the posterior kidney (80.9 %).

Type locality Center for the Research and Management of Continental Fishing Resources (Cepta, Ibama), Pirassununga, São Paulo State, Brazil.

New locality Fish farm in the region of Bauru, São Paulo State, Brazil (22° 7′ 18″ S, 47° 27′ 13″ W).

Type material Slides with hematoxylin-eosin and Ziehl-Neelsen stained spores deposited in the histopathological collection (number 35785) of Veterinary Pathology Department, São Paulo State University (UNESP), Jaboticabal, SP, Brazil.

Table 1 Comparative measurements (mean values in μm) of *Myxobolus* sp. spores and those found in the skeletal muscle and kidney (n=126) of *P. mesopotamicus*

Hosts	Site of infection	Species	SL	SW	ST	PCL	PCW	PFC	Authors
C. heckelii	Gills	*M. heckelii*	12.7	6.6	4.0	2.9	1.7	4.0–5.0	Azevedo et al. (2009)
Myleus rubripinnis	Gallbladder	*M. myleus*	19.3	8.3	4.0	13.2	3.0	19–21	Azevedo et al. (2012)
Zungaro jahu	Skin	*M. cordeiroi*	10.9	7.5	5.6	5.3	1.4	5.0–6.0	Adriano et al. (2009)
Pimelodus ornatus	Cardiac muscle	*Myxobolus* sp.	8.0	5.8	3.4	3.6	1.2	–	Matos et al. (2014)
P. mesopotamicus	Kidney	*Myxobolus* sp.	8.9	5.5	–	4.4	2.4	–	Manrique et al. (2015a, b)
Serrasalmus sp.	Spleen, kidney	*M. serrasalmi*	9.7	4.7	–	4.3	1.6	–	Walliker (1969)
Pygocentrus piraya	Intestine	*M. cunhai*	10	5.0	–	–	–	–	Penido (1927)
P. mesopotamicus	Kidney	*M. cuneus*	8.9	5.5	–	4.4	2.4	–	Present study
P. mesopotamicus	Skeletal muscle	*M. cuneus*	8.9	5.6	8.3	4.5	2.4	7.0–8.0	Present study

SL spore length, *SW* spore width, *ST* spore thickness, *PCL* polar capsule length, *PCW* polar capsule width, *PFC* polar filament coils

Histopathology Plasmodia provoked dissociation of muscular fibers. No inflammatory reaction. Spores were found to be situated in the peritubular interstitial space of kidney causing reduction of tubule lumen and those located in the glomerulus caused reduced Bowman capsule and increased glomerular tuff.

Discussion

The TEM of the spores from the muscle and kidney of *P. mesopotamicus* showed morphology and ultrastructure that resembles the spores of *M. cuneus* from *P. mesopotamicus* (Manrique et al. 2012, 2013, 2015b) but no information on ultrastructural description of this species in kidney was found.

The present specimens resemble the morphology of *M. serrasalmi* (Walliker 1969), *Myxobolus inaequus* (Kent and Hoffman 1984), *M. cunhai* (Gioia and Cordeiro 1996), *M. cunhai* (Molnár and Békési 1992), *M. maculatus* (Casal et al. 2002), and *M. cuneus* (Adriano et al. 2006), but only spores of *M. cunhai*, *M. serrasalmi*, and *M. cuneus* were morphometricaly similar to the present material. However, *M. cunhai* was described in *Pygocentris piraya* and the spores showed different polar capsule size (Gioia and Cordeiro 1996).

On the other hand, the spores of *M. serrasalmi* were pyriform with an oval macrospore (Molnár and Békési 1992) differently to that reported in this study. Nevertheless, morphometric characteristics of *M. cuneus* from *P. mesopotamicus* (Adriano et al. 2006; Manrique et al. 2012) were too close suggesting that this material belongs to this species.

Spores of *Myxobolus* sp. were also registered in the hematopoietic organs such as *M. serrasalmi* in *Serrasalmus* sp. (Walliker 1969); central nerve system of rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta* (Baldwin et al. 2000), in the blood of tambaqui *Colossoma macropomum* (Adriano et al. 2006); in the connective tissue of the swim

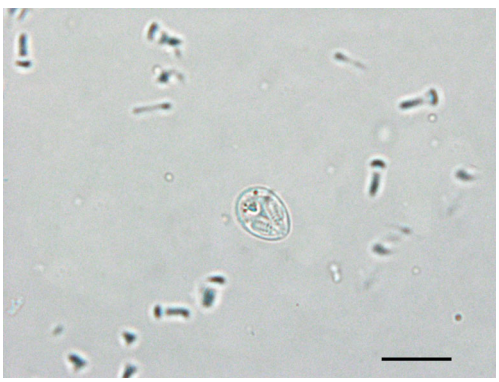


Fig. 1 Mature spores of *M. cuneus* light photomicrographs. *Scale bar* = 10 μm

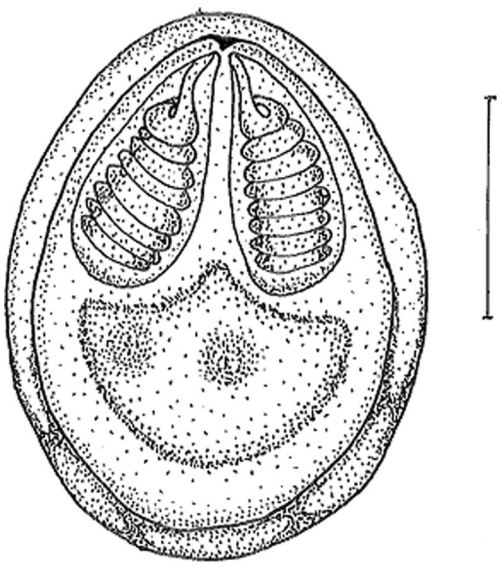
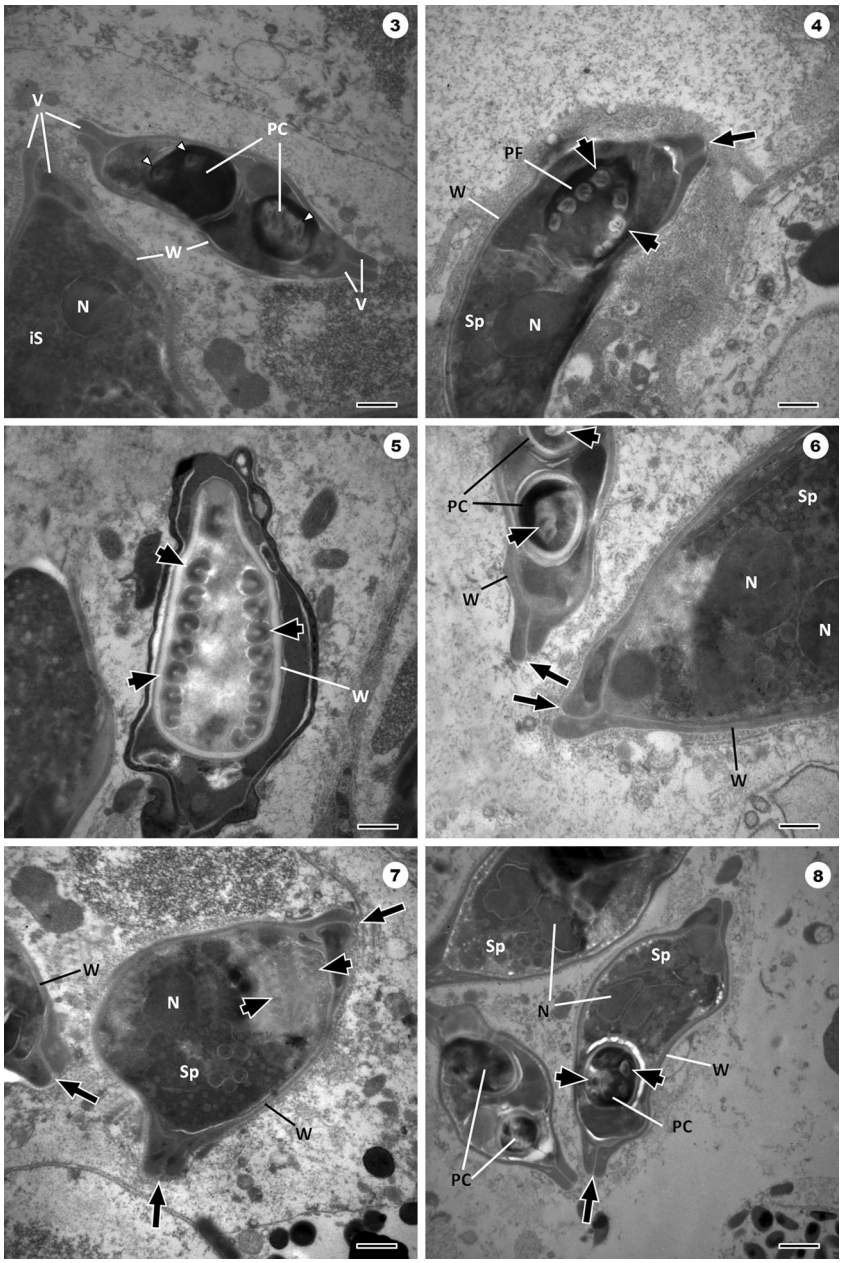


Fig. 2 Schematic drawing of mature spores of *M. cuneus*. *Scale bar* = 5 μm



Figs. 3–8 Transmission electron microscopy of *M. cuneus* parasite from the skeletal muscle and kidney of *P. mesopotamicus*. (*3*) Transversal section of the spore em maturation process showing the valves (*V*), polar capsule (*PC*), polar filaments (*arrowhead*), spore wall (*W*), nucleus (*N*) and sporoplasm in development (*iS*). (*4*) Mature spore separated by a thin wall (*W*), observe the polar filaments developed (*PF*) in different electondense degrees (*arrow end*), sporoplasm (*Sp*), nucleus (*N*), and the suture line of the valves (*arrow*). (*5*) Detail of polar capsule and wall (*W*) and polar filaments (*arrow*). (*6*) Mature and immature spore. In mature spore note the polar capsule (*PC*) wall spore (*W*) polar filaments (*arrow end*) and suture line in valves (*arrow*). In immature note the nuclei (*N*), sporoplasm (*Sp*). (*7*). Immature spore, note the nucleus (*N*), polar filaments (*arrow end*), sporoplasm (*Sp*), spore wall (*W*), and suture line (*arrow*). (*8*) Mature and immature spores. Note polar capsules (*PC*), nuclei (*N*), sporoplasm (*Sp*), polar filaments (*arrow*), and spore wall. *Scale bar* = 500 nm

bladder, urinary bladder, gills, spleen, fin rays, liver, heart, and subcutaneous tissue of the head of *P. mesopotamicus* (Adriano et al. 2006), *Myxobolus stomum* in the oral cavity and lips of the blackspotted grunt *Plectorhynicus gaterinus* (Ali et al. 2003), and *Myxobolus lubati* in the wall of the intestine of haffara seabream *Rhabdosargus haffara* (Ali et al. 2007). Manrique et al. (2015a, b) reported spores of *Myxobolus* sp. in the skeletal muscle of *P. mesopotamicus* in which shows the importance of such finding on this host.

Myxobolus sp. spores located in the posterior kidney were found by Manrique et al. (2012) and other species were described in the muscular skeletal tissue in teleost fishes such as *M. fryeri* in coho-salmon, *Oncorhynchus kisutch* (Ferguson et al. 2008), *M. omari* in *P. hypophthalmus* and *M. leptobarbi* in *L. hoevenii* (Székely et al. 2009), *M. artus* in *C. carpio* (Ogawa et al. 1992). Although these species provoke pathological alterations and decreased survival (Longshaw et al. 2003), they are not considered the most pathogenic parasites differing form *M. cyprini* in *C. carpio* that produce the pernicious anemia of carp and *M. sandrae* in the muscle of perch, *S. lucioperca* (Shulman 1966).

In conclusion, the morphological and ultrastructural study contributes to the identification of the species *M. cuneus* and complements its original description with a new site of infection herein registered in the type host *P. mesopotamicus*.

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