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Myxobolus saladensis sp. nov., a new species of gill parasite of *Mugil liza* (Osteichthyes, Mugilidae) from Samborombón Bay, Buenos Aires, Argentina

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ABSTRACT. Myxosporean *Myxobolus saladensis* sp. nov. in the gills of *Mugil liz*a Valenciennes, 1836 from Samborombón Bay was described by light and electron microscopy studies. Spores were pyriform and binucleated, measuring 10.63±0.36 µm (n=20) long, 9.24±0.50 µm (n=20) wide and 4.13±0.36 µm (n=20) thick, included in polysporic cyst-like plasmodia. Elongated pyriform polar capsules were of equal size (3.84±0.27 µm long and 2.30±0.12 µm wide). The sporoplasm contained some sporoplasmosomes. Each PC contained a polar filament with 4-5 coils obliquely arranged in relation to the polar capsules axis. The PC wall was composed of two layers of different electron densities. Based on the morphological and ultrastructure differences of the spore to those of previously described species of *Myxobolus*, we describe a new species, *Myxobolus saladensis* sp. nov.

KEYWORDS. Myxosporean, mullets, Myxobolidae, gills parasites.

Members of *Myxobolus* Biitschli, 1882 are considered cosmopolitan, with many species cited in mullets. According to Zatti *et al.* (2015) approximately 90 myxosporean species have been identified in South America, of which 41 are from the this genus, mostly from Brazil (Eiras *et al.*, 2010; Azevedo *et al.*, 2010; 2011; 2012). Mullets live in shallow and brackish waters and are euryhaline, ranging from hypersaline lagoons to freshwater. They utilize estuarine nursery habitats where they feed largely on plant material obtained by grubbing through bottom detritus (Cervigón *et al.*, 1993).

The Mugilidae is represented by three species along the coast of the Southwest Atlantic Ocean (Cousseau *et al.*, 2005). In Argentine waters, the only mullet permanently present is the grey mullet *Mugil liza* Valenciennes, 1836 (see Cousseau *et al.*, 2005) after synonymization of *Mugil platanus* Günther, 1880 with *M. liza* (Heras *et al.*, 2009). This species is commercially exploited in Brazil and Argentina by inshore artisanal fisheries.

Helminths parasitizing *M. liza* have been reported by many authors in the last years (Knoff & Amato, 1992; Kohn *et al.*, 1994; Knoff *et al.*, 1997; Suriano *et al.*, 2000; Carnevia & Speranza, 2003; Abdallah *et al.*, 2009; Marcotegui & Marcotelli, 2009a,b; Siquier & Ostrowski de Nuñez, 2009; Alarcos & Etchegoin, 2010; Marcotelli *et al.*, 2012). However, gill Myxozoa have not been reported from this host. Eiras *et al.* (2007) recorded *Myxobolus platanus* Eiras *et al.*, 2007 parasitizing pancreatic tissue of *Mugil platanus* in Lagoa dos Patos, Brazil.

In a survey of fish parasites in estuarine areas of Argentina a new myxosporidian species was found in *Mugil liza* collected from Samborombón Bay. A light and electron microscopic study on the new species is presented.

MATERIALS AND METHODS

A total of 206 specimens of *Mugil liza* (Valenciennes, 1836) (Mugilidae) were captured between April 2006 and April 2009. These specimens ranging in total length from 2.8 to 32.0 cm and total weight from 0.22-331.41 g were captured from the mouth of the Canal Aliviador of Salado River (35°50'12.53"S, 57°25'22.28"W, Samborombón Bay, Buenos Aires, Argentina) and examined for parasites. Live fish were transported to the laboratory in containers filled with estuarine water and were kept alive in oxygenated aquaria until examination. Skin, fins, excised gills, and intestinal tract from newly killed fishes were analyzed under a dissecting microscope to detect parasites.

Plasmodium (Pmd) containing numerous spores were found in the gill filaments and gill rakers (Fig. 1). The gills infected were observed by DIC microscopy for fresh spore measurements. For ultrastructural studies, fragments of gills infected were excised and fixed in 2.5% Glutaraldehyde in 0.2 M Sodium Cacodylate buffer (pH 7.2) for 12 h at 4°C, washed in the same buffer 12 h at 4°C and post fixed in 2% OsO_4 buffered with the same solution for 4 h at the same temperature. Ultrathin sections were observed in a JEOL 1200EX II TEM (JEOL Optical, Tokyo, Japan).

The dimensions of the spores (in µm) were expressed as the mean ±standard deviation. Length and width of spores were obtained from 20 fresh specimens. Thickness were obtained from 20 additional spores. Measurements of polar capsules length and width of the spores were obtained from ultrathin serial sections. All measurements were taken from microphotographs using Image J software (National Institutes of Health).

RESULTS

Myxobolus saladensis sp. nov.

(Figs 1-7)

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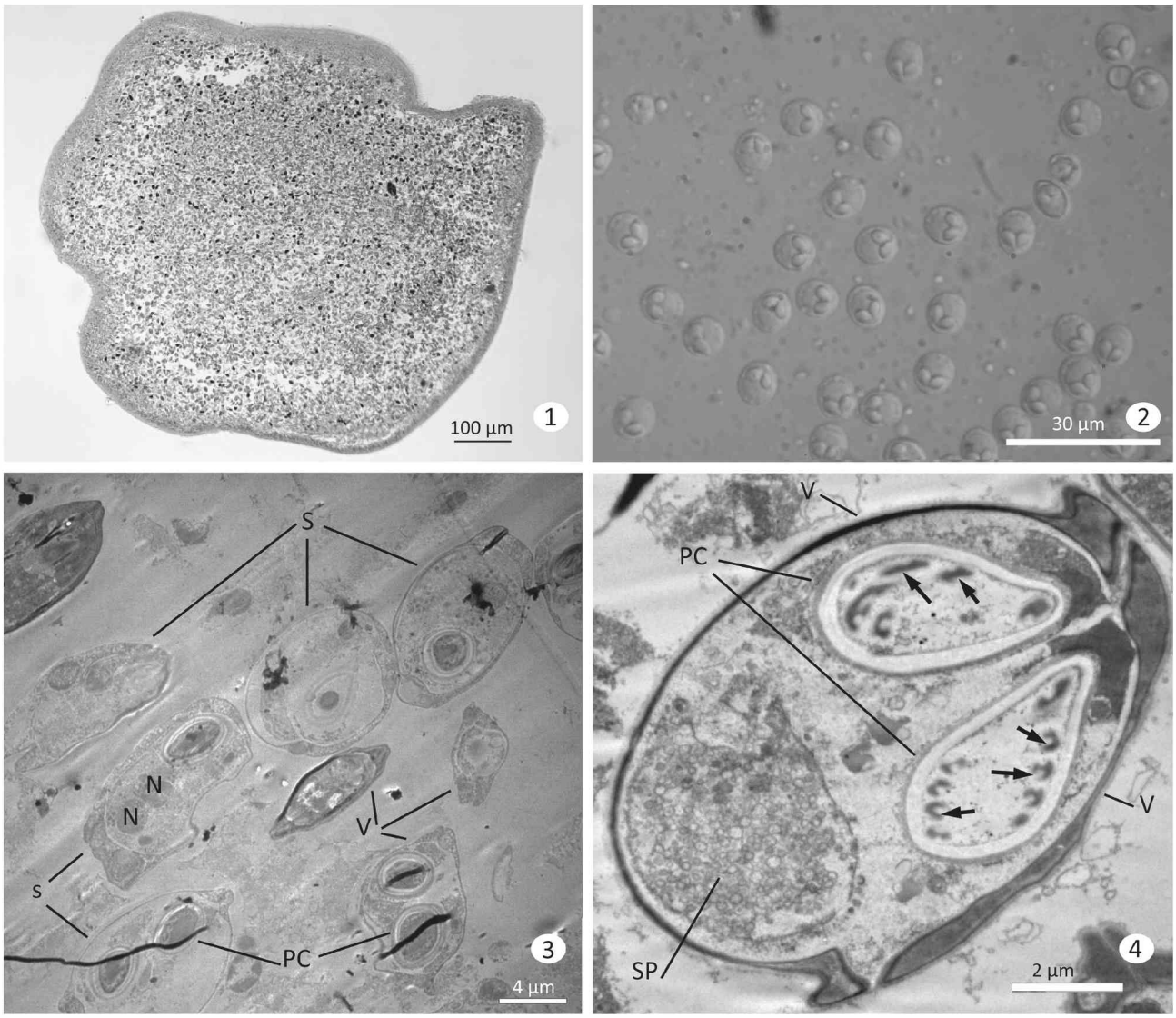
Diagnosis. Spherical Pmd containing numerous spores located in the gill filaments and rakers. Pyriform fresh spores tapering anteriorly 10.63±0.36 (range 10.05-11.13) µm long, 9.24±0.50 (range 8.42-9.79) µm wide, and 4.13±0.36 (range 2,65-4,9) µm thick. Two equal-sized pyriform PC measuring 3.84±0.27 (range 3.33-4.03) µm long and 2.30±0.12 (2.14-2.43) µm wide. Polar filament coiled in four or five turns.

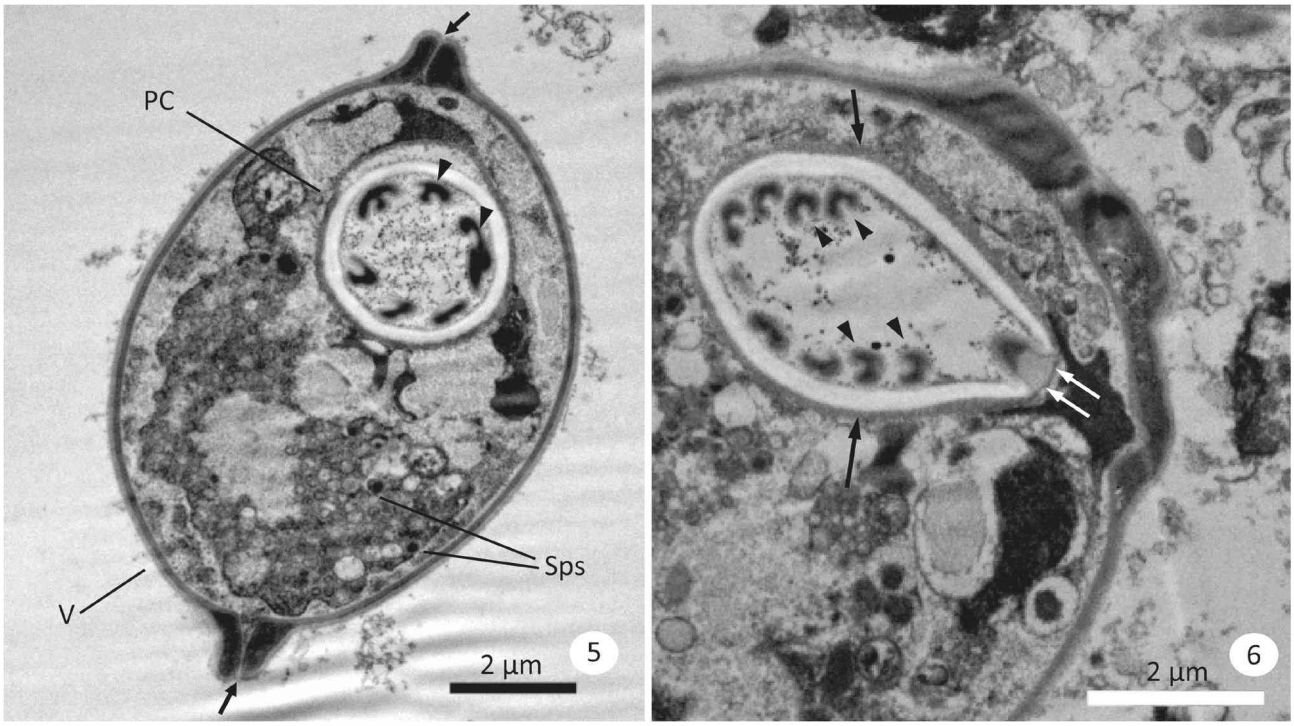
Site of infection: spores located in epithelium of gill rakers.

Etymology. The specific epithet refers to the type locality, Salado River.

Type material. One glass slide with semithin sections of the cyst containing spores (Hapantotype) was deposited in the Museo de La Plata collection, number MLP-Pr-095.



Figs 1-4. Light and transmission electron micrographs of the myxosporean *Myxobolus saladensis* sp. nov. infecting gills and gills rakers of *Mugil liza* Valenciennes, 1836: 1, semithin section of a plasmodium; 2, isolated spores observed in differential interference-contrast (DIC); 3, ultrathin section of a plasmodium showing several spores at different levels (S); 4, sectioned spore showing the shell valves (V); polar capsules (PC); Sporoplasm (SP) and transverse section of the polar filament (arrows).



Figs 5, 6. *Myxobolus saladensis* sp. nov. parasite of *Mugil liza* Valenciennes, 1836: 5, sectioned spore showing valves (V) and their suture lines (arrows), polar capsules (PC), different sections of the polar filaments (arrowheads) and some sporoplasmosomes (Sps); 6, detail of apical region of the PCs showing the PC wall (arrows) composed of two layers and the apical stopper (double arrows) and the different sections of the polar filament (arrowheads).

Type Locality. Salado River, Samborombón Bay, Buenos Aires, Argentina.

Type Host: *Mugil liza.*

Prevalence: 12.7%.

Description. Spores typical of *Myxobolus* Bütschli, 1882, rounded in valvular view and biconvex in sutural view, shell valves smooth and without projections. Fresh mature spores pyriform in shape (Fig. 2). Mean spore measurements ± standard deviations as follows: 10.63±0.36 (range 10.05-11.13) long, 9.24±0.50 (range 8.42-9.79) wide, and 4.13±0.36 (range 2.65-4.91) thick. Spore wall thin and smooth comprising two symmetrical and equal shell valves adhering together along the prominent longitudinal sutural line (Figs 3-6). Internally, two equal and elongated pyriform polar capsules (PCs), located side by side at the same level, measured 3.84±0.27 (range 3.33-4.03) long, 2.30±0.12 (range 2.14-2.43) wide (Figs 3-6). Intercapsular appendix not observed. Inside the PCs, polar filament coil displayed four or five slightly oblique to the longitudinal axis (Fig. 6). Apical end of the PCs contained a circular stopper formed by electron-lucent material (Fig. 6). At the posterior pole of the spore, a binucleated sporoplasm contained numerous light vesicles, numerous sporoplasmosomes (Figs 4, 5).

Nuclei located at the same level, contained uniform chromatin without evident nucleoli (Fig. 3). A schematic drawing of spore morphology (Fig. 7) shows the arrangements of the different structures and organelles.

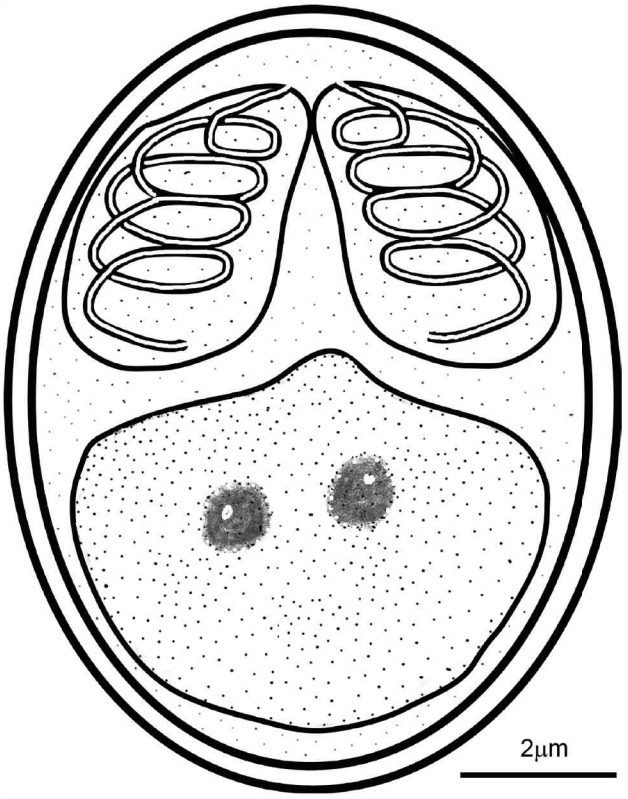


Fig. 7. Schematic drawing of the morphology of a spore of *Myxobolus saladensis* sp. nov. witch infects *Mugil liza* Valenciennes, 1836 from Argentina.

Tab. I. Comparative measurement (in μm) of the spores from *Myxobolus* spp. parasiting mugilid fishes which resembles the new species (SL, spore length; SW, spore width; PCL, polar capsules length; PCW, polar capsules width; NC, number of coils on the polar filament).

Species	SL	SW	PCL	PCW	NC	Organ	Host species
M. chiungchowensis	10.2-11.8	9.6-11	5.6-6.2	3.4-3.8	6-8	Intestine	*Mugil cephalus*
M. goensis	9.5-10.5	6-7.5	4.5-6	2-3	5	Gills archs	*Mugil cephalus*
M. goreensis	10-13	10-13	4-5	2-4	-	Gills	*Mugil cephalus*
M. mugilii	8.1-16.3	4-7.3	2.4-8.1	1.6-4	-	Gills	*Mugil cephalus*
M. rohdei	9.8-11.8	8.4-9.1	3.7-5	2.5-3.1	3-4	Kidneys	*Mugil cephalus*
M. spinacurvatura	10.5-12.5	9-11	3.5-5	2.5-3.5	-	Mesentery, brain, spleen	*Mugil cephalus*
M. platanus	10-11	10-11	7-8	3.5-4	-	Spleen	*Mugil platanus*
M. galaxii	13-15	8.8-10	-	-	-	All organs except gills	*Galaxias maculatus*
M. magellanicus	11.9 ± 0.6	12.9 ± 0.5	3	-	-	Gills	*Galaxias maculatus*
M. paranensis	12-15	7-8	6-7	2.5	-	Gonads	*Salminus maxillosus*
M. saladensis sp. n.	10.05±11.13	8.42-9.79	3.33-4.03	2.14-2.43	4-5	Gills	*Mugil liza*

DISCUSSION

Of the *Myxobolus* species described from mullet, those analyzed during the present study resemble *M. chiungchowensis* Chen, 1998, *M. goensis* Eiras & D'Souza, 2004, *M. goreensis* Fall *et al.*, 1977, *M. mugilii* Haldar *et al.*, 1996, *M. rohdei* Lom & Dykova, 1994, and *M. spinacurvatura* Maeno *et al.*, 1990 from the size of spores. Nevertheless the new species proposed can be distinguished from *M. chiungchowensis*, *M. goensis*, *M. goreensis*, and *M. mugilii*, by having smaller size of the polar capsules, and *M. spinacurvatura* and *M. rohdei* by the number of turns of the filament polar capsules (see Tab. I).

EIRAS *et al.* (2007) reported *Myxobolus platanus* parasitizing pancreatic tissue of *Mugil platanus* (actually *M. liza*) in Lagoa dos Patos, Brazil. The new species differs from *M. platanus* by having smaller polar capsules not exceeding half the length of the spore (see Tab. I). Furthermore, the infection site (pancreatic tissue) of the parasite described by EIRAS *et al.* (2007) differs from that of *M. saladensis* sp. nov., which infects gill rakers in *M. liza*.

In Argentina *M. galaxii* Szidat, 1953 and *M. magellanicus* Szidat, 1953 (FLORES & VIOZZI, 2001) have been reported from *Galaxias maculatus* Jenyns, 1842, and *M. paranensis* from *Salminus maxillosus* Bonetto & Pignalberi, 1965. The specimens studied can be distinguished from those species by having smaller spores (Tab. I). Additionally, VIOZZI (1996) has reported *Myxobolus* sp. from *Percichthys trucha* Valenciennes, 1833, *Galaxias maculatus* and *Hatcheria macraei* Girard, 1855 in Patagonian lakes, and SARDELLA *et al.* (1998) reported *Myxobolus* sp. from *Genypterus brasiliensis* Regan, 1903 in Argentine Sea. In both reports, a formal description has not been undertaken, preventing a thorough comparison with the specimens found during this study.

According to MOLNAR (2002) knowledge of the actual site of establishment of the parasite in the gill may also facilitate the identification of parasite species; therefore, indicating the precise location of plasmodium development is indispensable for species descriptions. Host and organ specificity and tissue tropism should be considered in species identification (LIU *et al.*, 2013). The new species plasmodium was found, in the epithelium of the gills rakers of all infected fish. The site of the plasmodium coincides with infections of the gill arch epithelium according to the typical localization of the plasmodium given by MOLNAR (2002).

Some gills *Myxobolus* species have been reported as pathogenic to fish (CAMUS & GRIFFIN, 2010; MILANIN *et al.*, 2010; LIU *et al.*, 2013). Heavy infections of myxozoan in gill lamellae, gills filaments or blood vessels of the gill arch, could produce severe gill changes affecting gas exchange. Although the new species is located in the epithelium of rakers this could affect the respiratory flow.

This is the second report of the genus *Myxobolus* from *Mugil liza*, and the fifth record of this genus in Argentina.

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