

Ultrastructural Morphology of *Myxobolus testicularis* sp. n., Parasite of the Testis of *Hemiodopsis microlepis* (Teleostei: Hemiodontidae) from the NE of Brazil

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Summary. *Myxobolus testicularis* sp. n. was found within cyst-like structures in the testis of the freshwater fish *Hemiodopsis microlepis* Kner, 1859 (Teleostei, Hemiodontidae) in close contact with the testis stroma. The fish were collected in the Poty River, near the city of Teresina (State of Piauí, Brazil). Large spherical to ellipsoidal plasmodia up to 0.5 mm were filled with disporic pansporoblasts and mature spores. The plasmodial wall presented a sinuous outline surrounded by several fibroblasts and a continuous layer of numerous collagen fibrils. The plasmodia contained different proliferative stages. Early sporogenic cells were present in the cortical zone. Immature and mature spores were located more internally. Ellipsoidal spores measured $8.6 \pm 0.5 \mu\text{m}$ ($n = 30$) length, $7.2 \pm 0.5 \mu\text{m}$ ($n = 15$) width, and $2.7 \pm 0.4 \mu\text{m}$ ($n = 12$) thickness. The anterior end of the spores contained two equal pyriform polar capsules measuring $3.5 \pm 0.3 \mu\text{m}$ ($n = 15$) length, $1.7 \pm 0.4 \mu\text{m}$ ($n = 10$) width, each with an isofilar polar filament with 5-6 turns obliquely to the longitudinal axis. The wall of the polar capsule was filled with a hyaline substance contrasting with the very dense internal matrix. The spores consisted of two equal valves forming the wall. The spore wall was surrounded by a dense and uniform layer with variable thickness (up to $0.6 \mu\text{m}$) consisting of a complicated network of anastomosing microfibrils closely adhered to the wall. More externally, in the plasmodial matrix, the fibrils formed a looser network. Based on the ultrastructural morphology and specificity to the host species, we propose the creation of a new species, named *Myxobolus testicularis* sp. n.

Key words: fish parasite, *Myxobolus testicularis* sp. n., Myxozoa, ultrastructure.

INTRODUCTION

Numerous descriptions of myxosporeans have been reported in fish from different geographic areas (Landsberg and Lom 1991, Lom and Dyková 1992). Myxozoa Bütschli, 1882 comprises more than 1200

available species commonly found in fish (Lom and Dyková 1992). Among them, the genus *Myxobolus* Bütschli, 1882, with 744 species described, is the largest group of the Myxobolidae family (Eiras *et al.* 2005), and has been reported as an important pathogen in freshwater fishes (Kent *et al.* 2001).

Although considerable information has already been collected on South America myxozoans, the available descriptions are based, almost exclusively, on light microscopical records and diagrammatic drawings of the spores (Aragão 1919, Nemeček 1926, Penido 1927,

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Pinto 1928, Walliker 1969, Kent and Hoffman 1984, Molnár and Békési 1993, Cellere *et al.* 2002). At the present, nineteen Brazilian *Myxobolus* species have been described from different host fishes (Walliker 1969; Kent and Hoffman 1984; Molnár and Békési 1993; Gioia and Cordeiro 1996; Casal *et al.* 1996, 2002; Molnár *et al.* 1998; Adriano *et al.* 2002; Azevedo *et al.* 2002; Cellere *et al.* 2002). However, very few represent ultrastructural studies on *Myxobolus* sp. from Brazil, of which only three species have been described (Casal *et al.* 1996, 2002; Azevedo *et al.* 2002).

The present study gives ultrastructural details of the developmental stages, including spores of a new myxosporean infecting the Amazonian teleost *Hemiodopsis microlepis* Kner, 1859 species. The morphological characteristics and taxonomic position of the new species are discussed.

MATERIALS AND METHODS

Forty specimens (27 males and 13 females) of *Hemiodopsis microlepis* Kner, 1859 (Teleostei: Hemiodontidae) known by the Brazilian common name "Flexeiro", were collected from Poty River (05°05'21"S/ 42°48'07"W), near the city of Teresina (State of Piauí), NE Brazil. The fishes were killed by over-anaesthetising in MS 222 solution (3-aminobenzoic acid ethyl ester) (Sandoz Laboratories) and dissected. Small fresh fragments of testes, ovary, gills, kidney and liver were excised and examined under light microscope with Nomarski differential interference contrast (DIC) optics. The infected testes were observed by DIC for spore measurement. For ultrastructural studies, small fragments of infected tissues were excised and fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 12 h at 4°C, washed in the same buffer for 12 h at 4°C and postfixed in 2% OsO₄ buffered with the same solution for 4 h at the same temperature. After dehydration in a graded ethanol-series followed by two changes of propylene oxide (4 h in each), samples were embedded in Epon. Semithin sections were stained with methylene blue-Azur II. Ultrathin sections were cut with a diamond knife, following double-contrast with both aqueous uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM operated at 60 KV.

RESULTS

The only organs parasitized were the testes. No other organs studied by light microscopy were parasitized. The majority of the parasitized male fish had 2-3 cyst-like structures up to 0.5 mm in diameter in different locations in contact with the seminiferous lumen (Figs 1, 2). The plasmodia contained different proliferative stages with early sporogenic cells in the cortical zone and immature and mature spores more internally (Figs 2, 3). Mature

spores were extremely numerous (several thousands). After rupture of the cysts some free spores were observed (Fig. 1 insert). The plasmodial wall was surrounded by a single thin layer of dispersed fibroblasts and numerous collagen fibres (Fig. 3). Externally, this layer appeared in contact with the seminiferous lumen. (Fig. 2).

Myxobolus testicularis sp. n. (Figs 1-10)

Type host: *Hemiodopsis microlepis* Kner, 1859 (Teleostei: Hemiodontidae) (Brazilian common name "flexeiro").

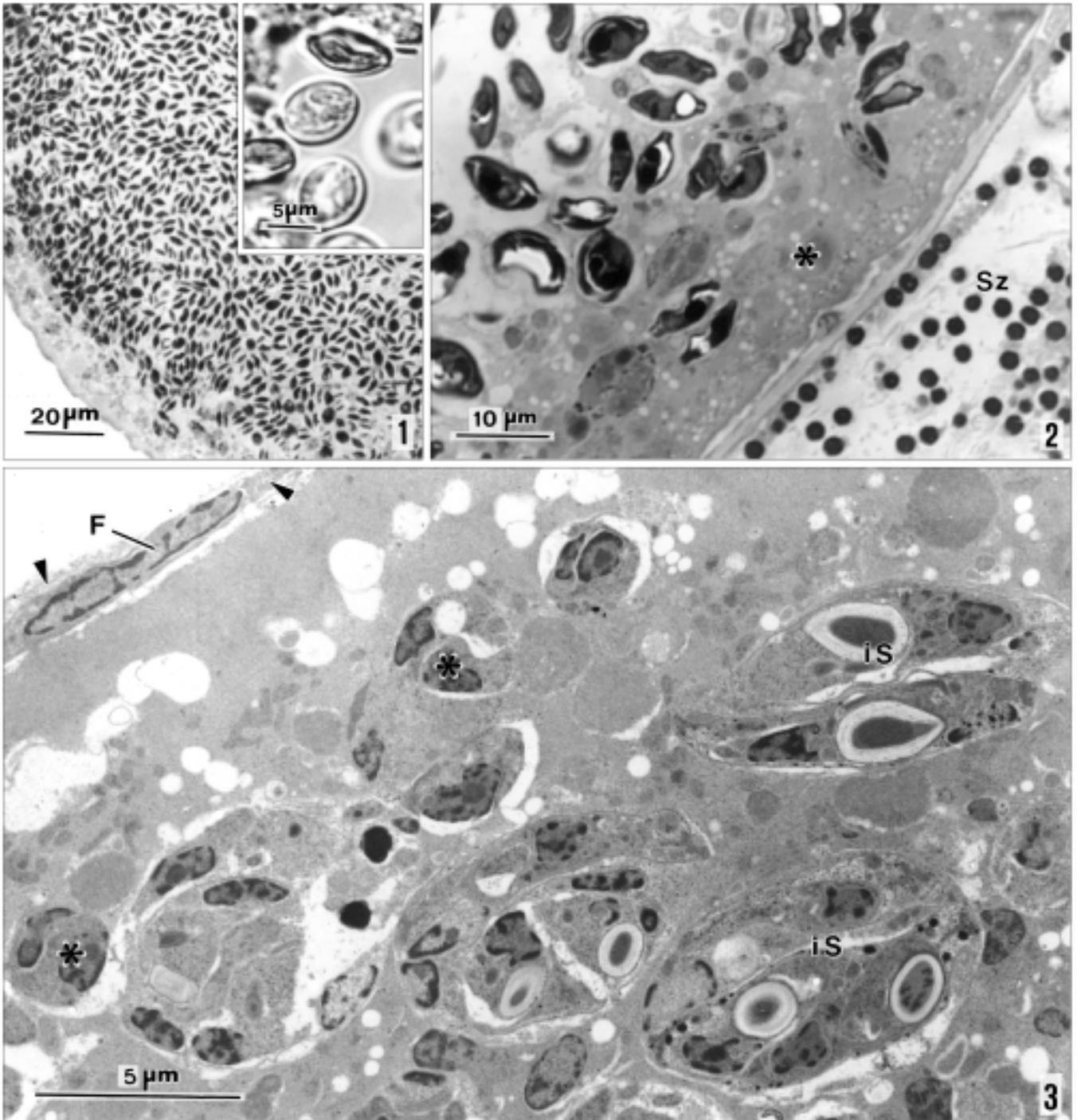
Site of infection: Plasmodium in contact with the seminiferous lumen.

Prevalence of infection: Seven of 40 (17.5 %) of the total fish or 7 of 27 (25.9 %) male fish were parasitized.

Type locality: Poty River near the city of Teresina (State of Piauí), NE Brazil (Latitude: 05°05'21"S; Longitude: 42°48'07"W).

Description of spores: The spores were typical of the genus *Myxobolus* Bütschli, 1882 because they were rounded in valvular view and biconvex in sutural view, and the shell valves were smooth and without projections. They measured 8.2-9.1 (8.6 ± 0.5) μm ($n = 30$) in length, 6.7-7.5 (7.2 ± 0.5) μm ($n = 15$) in width and 2.4-3.0 (2.7 ± 0.4) μm ($n = 12$) in thickness (Fig. 1 insert, 5-7). The spores consisted of two equal dense valves adhering together along the suture line forming the wall (Figs 5-8). Two equal smooth polar capsules, located symmetrically to the spore axis, measured about 3.3-3.8 (3.5 ± 0.3) ($n = 15$) \times 1.3-2.0 (1.7 ± 0.4) ($n = 10$) μm each and contained a polar filament with 5-6 turns in a single layer surrounded by a dense matrix (Figs 6, 7). Sporoplasm containing two nuclei was surrounded by irregular patches of dense matter (Fig. 5). The spore wall was smooth and contained a dense and uniform layer with variable thickness (up to 2 μm) of numerous anastomosed microfibrils closely adhered to the wall (Figs 5-8). More externally, these microfibrils formed a more diffused network (Fig. 5). The layer of microfibrils was absent in early sporogenic cells. It appeared during the spore maturation stage and attained maximum development in the mature spore (Fig. 4). A schematic drawing of the ultrastructure of the spore (Fig. 9) was made from serial ultrathin sections and another was obtained by DIC observations (Fig. 10).

Type specimens: One slide with paratypes and one slide with semithin sections of the cyst containing spores and surrounding tissues were deposited in the Interna-



Figs 1-3. *Myxobolus testicularis* sp. n. **1** - semithin section of a cyst-like structure, showing numerous spores; **1 insert** - some free mature spores released from the cyst-like structure observed in DIC optics; **2** - semithin section of the periphery of the cyst-like structure, showing some spermatozoa (Sz) in close contact with the wall. Near the periphery of the plasmodium some early sporogonic cells (*) are present; **3** - ultrathin section of the periphery of the cyst-like structure showing different life cycle stages (* - young sporogonic stages, iS - immature spores). In the wall, one fibroblast (F) and numerous collagen fibres (arrowheads) are present.

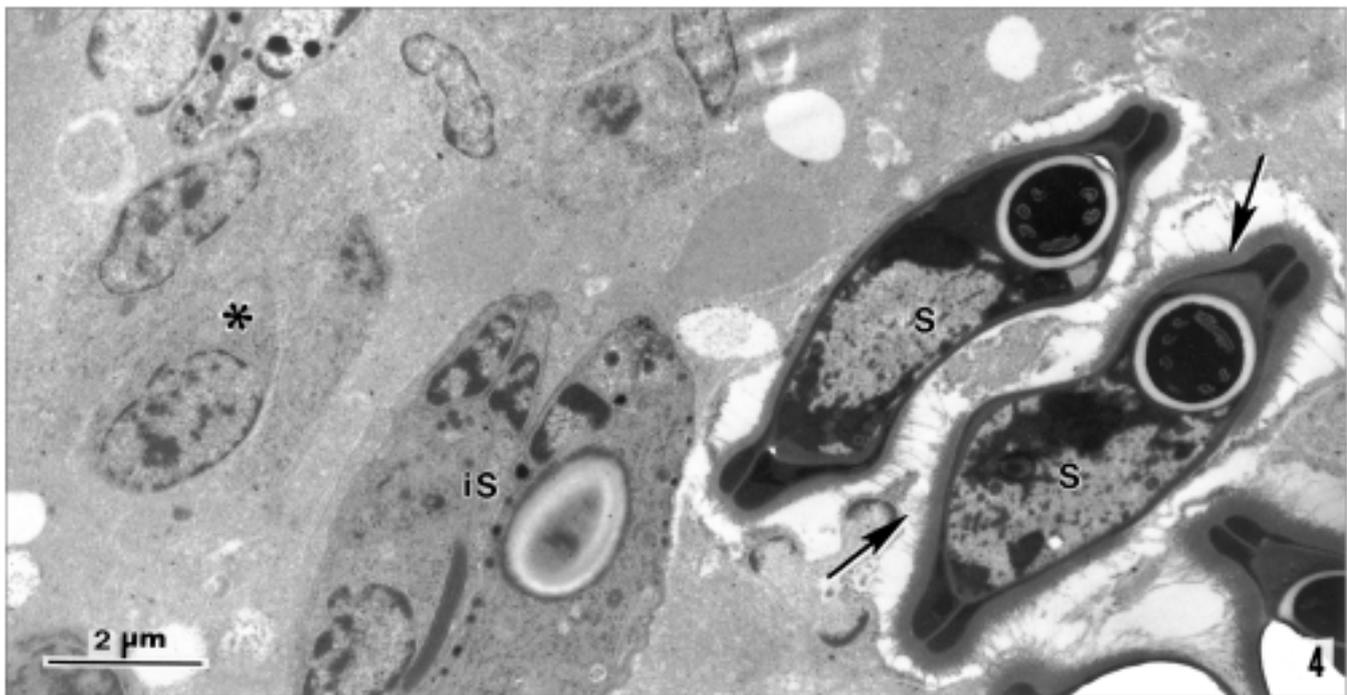


Fig 4. *Myxobolus testicularis* sp. n. ultrathin section showing a young sporogonic stage (*), two immature spores (iS) and two mature spores (S). Surrounding the mature spores, the microfibrils are observed (arrows).

tional Type Slide Collection at Smithsonian Institution, Washington, D.C. USA (USNM number 1076956)

Etymology: The specific epithet “*testicularis*” derives from the name of the infected organ of the host.

DISCUSSION

The light and ultrastructural morphology of the spores described in the present work, corresponds to those of genus *Myxobolus* (family Myxobolidae) (Lom and Noble 1984, Lom and Dyková 1992).

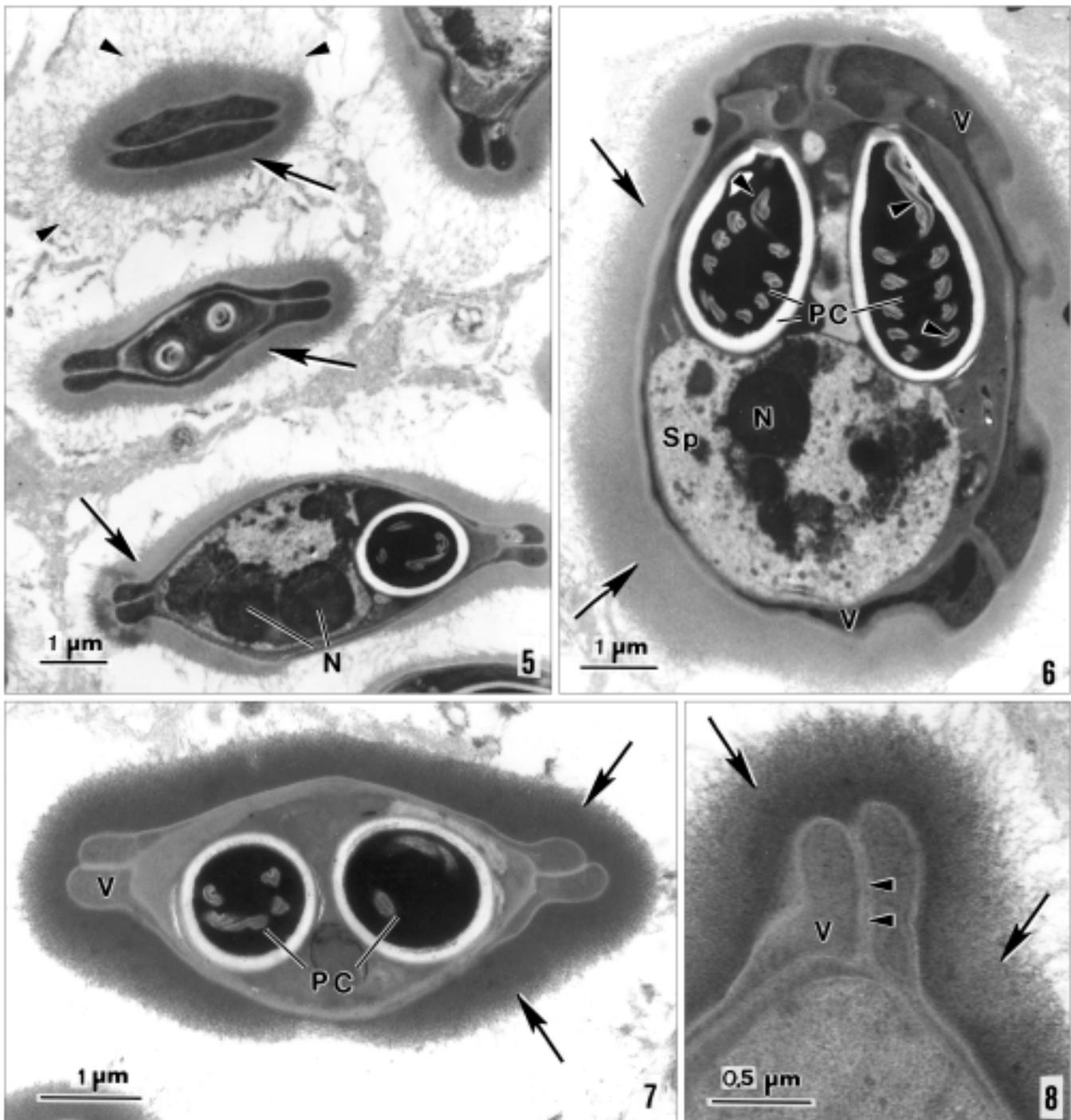
When comparing these results with those obtained for the different *Myxobolus* spp., with equal polar capsules, previously described mainly from Brazilian host fishes (Table 1), both *Myxobolus kudo*i Guimarães et Bergamin, 1938 and *Myxobolus lutzi* Aragão, 1919 show similarities with our results. The spores of *M. kudo*i and its polar capsules have very similar dimensions to those of *M. testicularis* sp. n. (Table 1). Despite the fact that the only information available on *M. kudo*i represents diagrammatic drawings of the spores, the morphology seems quite dissimilar from that

of *M. testicularis* sp. n. In *M. kudo*i, the spores appear to be pyriform shaped, while in *M. testicularis* sp. n. they are almost circular with small differences in the length and width diameters. It is particularly difficult to establish a comparison between the spores of *M. testicularis* sp. n. and *M. lutzi* because the only data available on the later is the medium length and the width of the spore (Table 1) and a diagrammatic drawing. In spite of their similar spore dimensions, a close analysis of the diagrammatic drawing of *M. lutzi* reveals that is clearly pyriform shaped unlike *M. testicularis* sp. n., which is rounder.

Myxobolus sp. are very common parasites of fresh-water fishes. In most of the fishes studied, one or more *Myxobolus* species were found. Although there have been very few studies on the host specificity of *Myxobolus* sp., new data indicates that most species are strictly host specific or only capable of developing in closely related fishes (Molnár *et al.* 1998). These studies support the distinction between *M. testicularis* sp. n. that appears in the testes of *Hemiodopsis microlepis* (Teleostei: Hemidontidae), *M. kudo*i which parasitizes the body skin except the fins of “*Nematognatha* sp.”

Table 1. Characters and dimensions (in μm) of the spores and the polar capsules in species of *Myxobolus* found in Brazil which show only one type of spore and equal polar capsules.

<i>Myxobolus</i> sp.	Host tissue	Spore			Polar Capsules			References
		Length	Width	Thickness	Length	Width	Number of coils	
<i>M. lutzi</i>	Testes	10	7	-	-	-	-	Aragão 1919
<i>M. chondrophilus</i>	Gills	6	4.5	-	-	-	-	Nemeczek 1926
<i>M. associatus</i>	Kidney	15	10	-	-	-	-	Nemeczek 1926
<i>M. pygocentris</i>	Intestinal contents	15-16	9-11	-	9-11	3-4	-	Penido 1927
<i>M. cunhai</i>	Intestinal contents and cloaca	9-11	4-6	-	-	-	-	Penido 1927
<i>M. noguchii</i>	Gills (?)	13.6	8.5	-	6.8	2.2	-	Pinto 1928
<i>M. stokesi</i>	Subcutaneous tissue of the snout	8.5	5.3	-	3.4	1.7	-	Pinto 1928
<i>M. kudoii</i>	Skin of the body except the fins	8.5-8.9	6.5-7.3	-	3.5-4.2	1.3-2.0	-	Guimarães and Bergamin 1938
<i>M. pfeifferi</i>	Esophagus, stomach, intestine, liver, gall-bladder, kidney, muscle, gills, ovary and fins	8.2	5.6	-	3.8	1.9	-	Thélohan 1895
<i>M. colossomatis</i>	Connective tissues of various organs	11.8	6.9	3.7	6.0	2.1	7-8	Molnár and Békési 1993
<i>M. braziliensis</i>	Gills	10.17	5.28	3.69	5.30	1.43	9-11	Casal <i>et al.</i> 1996
<i>M. macroplasmodialisis</i>	Abdominal cavity	11	8.5	5.2	4.5	2.8	6	Molnár <i>et al.</i> 1998
<i>M. maculatus</i>	Kidney	21	8.9	7.5	12.7	3.2	14-15	Casal <i>et al.</i> 2002
<i>M. porofilus</i>	Visceral cavity	5.7	4.8	-	1.6	1.1	3	Adriano <i>et al.</i> 2002
<i>M. testicularis</i>	Testes	8.2-9.1 (8.6 \pm 0.5) (n = 30)	6.7-7.5 (7.2 \pm 0.5) (n = 15)	2.3-3.1 (2.7 \pm 0.4) (n = 12)	3.3-3.8 (3.5 \pm 0.3) (n = 15)	1.3-2.0 (1.7 \pm 0.4) (n = 10)	5-6	Present study



Figs 5-8. *Myxobolus testicularis* sp. n. **5** - ultrathin section of some spores sectioned at different levels, showing the spore organization with special emphasis on the surrounding microfibrils (arrows). More externally, a loose network of anastomosed microfibrils (arrowheads) is seen. In the spore section located below, two nucleus (N) are observed in the sporoplasm; **6** - ultrathin longitudinal section of a spore in frontal view (slightly oblique), showing all spore elements arrows - surrounding microfibrils, V - valves, PC - polar capsules each with a coiled polar tube (arrowheads), Sp - sporoplasm, N - nucleus; **7** - ultrathin transverse section at the polar capsules (PC) level, showing their circular transverse section, the equal valves (V) and the surrounding microfibrils (arrows); **8** - ultrastructural detail of the two equal valves (V), the sutural line (arrowheads) and the complex network of anastomosed microfibrils (arrows).

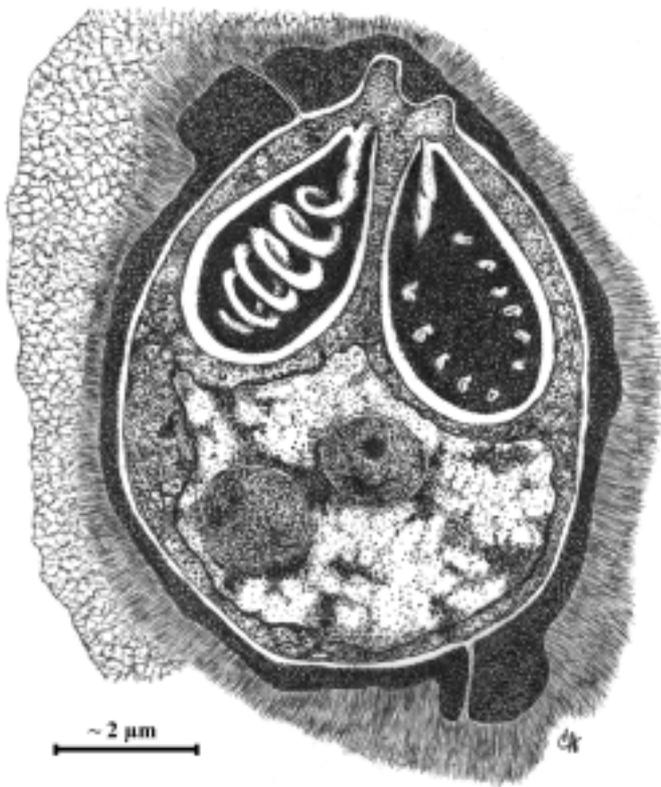


Fig. 9. Semischematic drawing of a slightly oblique longitudinal section of a mature spore showing the morphology of *Myxobolus testicularis* sp. n. emphasising the spore wall ornamentation which consists of a network of numerous anastomosed microfibrils attached to the spore wall periphery.

(Pisces) (Guimarães and Bergamin 1938), and *M. lutzi* which appears in the testes of *Poecilia vivipara* (Teleost). The British Museum (Natural History) considers that “*Nematognatha* sp.” probably refers to catfish.

Our results show several morphological differences between *M. testicularis* sp. n. and other *Myxobolus* spp. described from South America mainly in the dimension and shape of the spores and polar capsules, as well as in the number, position and structure of the coils in the polar filament. Moreover, another difference between the present species and those previously described, is in the complicated network of irregular and anastomosed surrounding microfibrils forming projections in the spore wall towards the periphery of the spore. This feature has never been observed before in other *Myxobolus* sp. from South America. Nevertheless, in *Henneguya pilosa* the spores present similar fibrils to our results (Azevedo and Matos 2003). These fibrils were only observed by

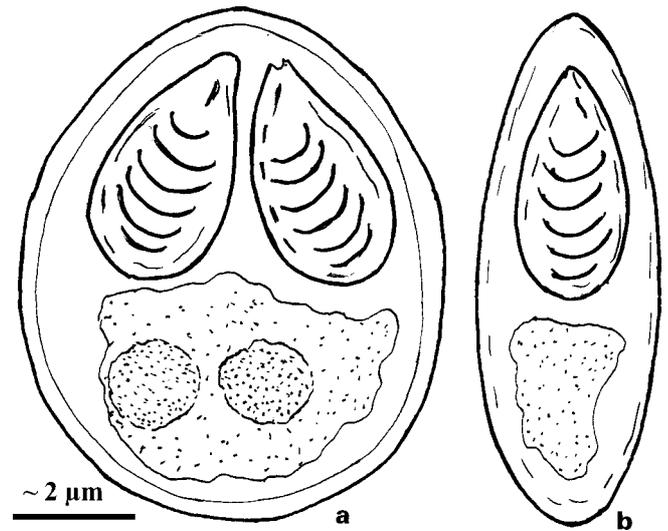


Fig. 10. Semischematic drawing of *Myxobolus testicularis* sp. n. in frontal view (a) and lateral view (b).

TEM and not by DIC because they were not distinguishable from the spore wall.

Based on these differences, and host specificity we are able to justify the establishment of a new species, named *Myxobolus testicularis* sp. n.

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