

## Ultrastructural Re-description of *Henneguya piaractus* (Myxozoa), a Parasite of the Freshwater Fish *Piaractus mesopotamicus* (Teleostei, Characidae) from the Paraguai River, Brazil

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**Summary.** Ultrastructural analyses of fish-infecting myxosporean *Henneguya piaractus* that is found in the gill lamellae of the freshwater teleost *Piaractus mesopotamicus* (Characidae) and collected from the Paraguai River, Brazil were described. The parasite occurs within large whitish spherical to ellipsoidal polysporic cysts (up to 2.5 mm long) delimited by a layer of fibroblasts generally connected with some capillaries on the gill epithelium. No external morphological signs of disease were visible in the infected fishes. The tailed spores measured  $61.5 \pm 0.91$  (60.2–62.6)  $\mu\text{m}$  in total length and ellipsoidal spore body  $21.1 \pm 0.62$  (20.6–21.9)  $\mu\text{m}$  long,  $6.7 \pm 0.40$  (6.2–7.3)  $\mu\text{m}$  wide and  $2.5 \pm 0.54$  (2.0–3.1)  $\mu\text{m}$  thick. The spore wall was about 97 nm of thickness and consisted of a thin electron-dense exospore and a thick electron-lucent endospore with about 85 nm of thickness. The tailed spores were composed of two equal-sized shell valves adhering together along the straight suture line each having in continuity a equal caudal tapering tail measuring  $40.5 \pm 1.02$  (38.7–43.1)  $\mu\text{m}$  in length. Two symmetric polar capsules measured  $9.8 \pm 0.28$  (9.3–10.1)  $\mu\text{m}$  long and  $1.9 \pm 0.37$  (1.4–2.4)  $\mu\text{m}$  wide, each having a polar filament with 10–11 (rarely 12) coils.

**Keywords:** Brazilian fish, *Henneguya piaractus*, Myxozoa, parasite, ultrastructure.

### INTRODUCTION

South America contains one of the biggest hydrographic networks in the world, in which a great variety

of ichthyofauna species inhabit (Cellere *et al.* 2002). Since the first description of genus *Henneguya* Thélohan, 1892 (Lom and Dyková 2006), the second largest genus of Myxobolidae, many species have been reported, mainly parasitizing freshwater fishes throughout the world. Thirty six myxosporidians species have been described based on light micrographs and diagrammatic illustrations from the Brazilian fauna (Jakowska and Nigrelli 1953; Kent and Hoffman 1984; Gioia *et*

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*al.* 1986; Gioia and Cordeiro 1996; Martins and Souza 1997; Barassa *et al.* 2003a, b; Eiras *et al.* 2004a, b, 2008, 2009; Martins and Onaka 2006; Abdallah *et al.* 2007). Recently, ultrastructural studies on developmental stages and mature spores supported the classification of some new species of the genus *Henneguya* (Rocha *et al.* 1992; Azevedo and Matos 1995, 1996, 2002, 2003; Azevedo *et al.* 1997, 2008; Casal *et al.* 1997, 2003; Vita *et al.* 2003; Adriano *et al.* 2005; Matos *et al.* 2005). In the present paper, we describe ultrastructural and re-describe light data on the mature spores of a myxosporean previously described as *Henneguya piaractus* which infected the gills of a commercially important teleost fish from a Southern Brazilian river.

Despite some difficulties in comparing the ultrastructural aspects of the spore described here with the corresponding light and scanning electron-microscopy studies previously reported, we found only few morphological differences between them and therefore we suggest that these two parasites belong to the same species, *Henneguya piaractus*.

## MATERIALS AND METHODS

### Light and electron microscopy

Eleven adult specimens of the freshwater fish, *Piaractus mesopotamicus* Holmberg, 1887 (Teleostei, Characidae) (Brazilian common name “pacú”) (with standard length 24–38 cm) were collected from May to July of 2009 in the “Porto da Manga” in the River Paraguai (57°14'S /19°15'W) located near the city of Corumbá (State of “Mato Grosso do Sul”), Brazil. The fishes were lightly anesthetised with MS 222 (Sandoz Laboratories), transported to the laboratory, dissected and the infected tissues, containing several whitish cysts (cyst-like plasmodia), were removed from the gill filaments and examined by a light microscope equipped with Nomarski differential interference-contrast (DIC) optics. All measurements are presented in micrometers as mean  $\pm$  SD followed in parentheses by the range.

For the ultrastructural studies, small fragments of parasitized tissues containing cysts were excised and fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4°C for 12 h. Samples were subsequently rinsed overnight in the same buffer at 4°C and post-fixed in 2% osmium tetroxide in the same buffer for 3 h at 4°C. The fragments were dehydrated through an ascending ethanol series, followed by propylene oxide and embedded in Epon. Semithin sections were stained with methylene blue-Azure II and observed under DIC optics. Ultrathin sections were double stained with aqueous uranyl acetate and lead citrate and observed under a transmission electron microscope (TEM) JEOL 100CXII, operated at 60 kV.

## RESULTS

Large whitish spherical to ellipsoidal polysporic cysts (cyst-like plasmodia) (up to 2.5 mm long) and several small cysts were observed macroscopically in the gill lamellae of the fish, *Piaractus mesopotamicus*. The cysts seen in semithin sections were of irregular form (Fig. 1) and contained several groups of juxtaposed cysts disseminated randomly in the host epithelial tissue. The cysts contained in the central position numerous mature spores surrounded by the youngest developmental stages (Figs 1, 3). After dissection and rupture of the cysts, numerous ellipsoidal tailed spores (some thousands) were observed and identified as belonging to the phylum Myxozoa and genus *Henneguya* (Fig. 2). At high magnification, it was observed that the cyst wall was surrounded by a layer of fibroblasts and contained a hypertrophic cell with a central hypertrophic nucleus surrounded by numerous spores and different developmental stages (Fig. 3).

### Diagnosis

Phylum Myxozoa Grassé, 1970  
Class Myxosporea Bütschli, 1881  
Order Bivalvulida Shulman, 1959  
Family Myxobolidae Thélohan, 1892  
Genus *Henneguya* Thélohan, 1892, according to the classification by Lom and Dyková (2006).

### Re-description of the species

**Name:** *Henneguya piaractus* Martins & Souza, 1997.

**Type host:** *Piaractus mesopotamicus* Holmberg, 1887 (Teleostei, Characidae).

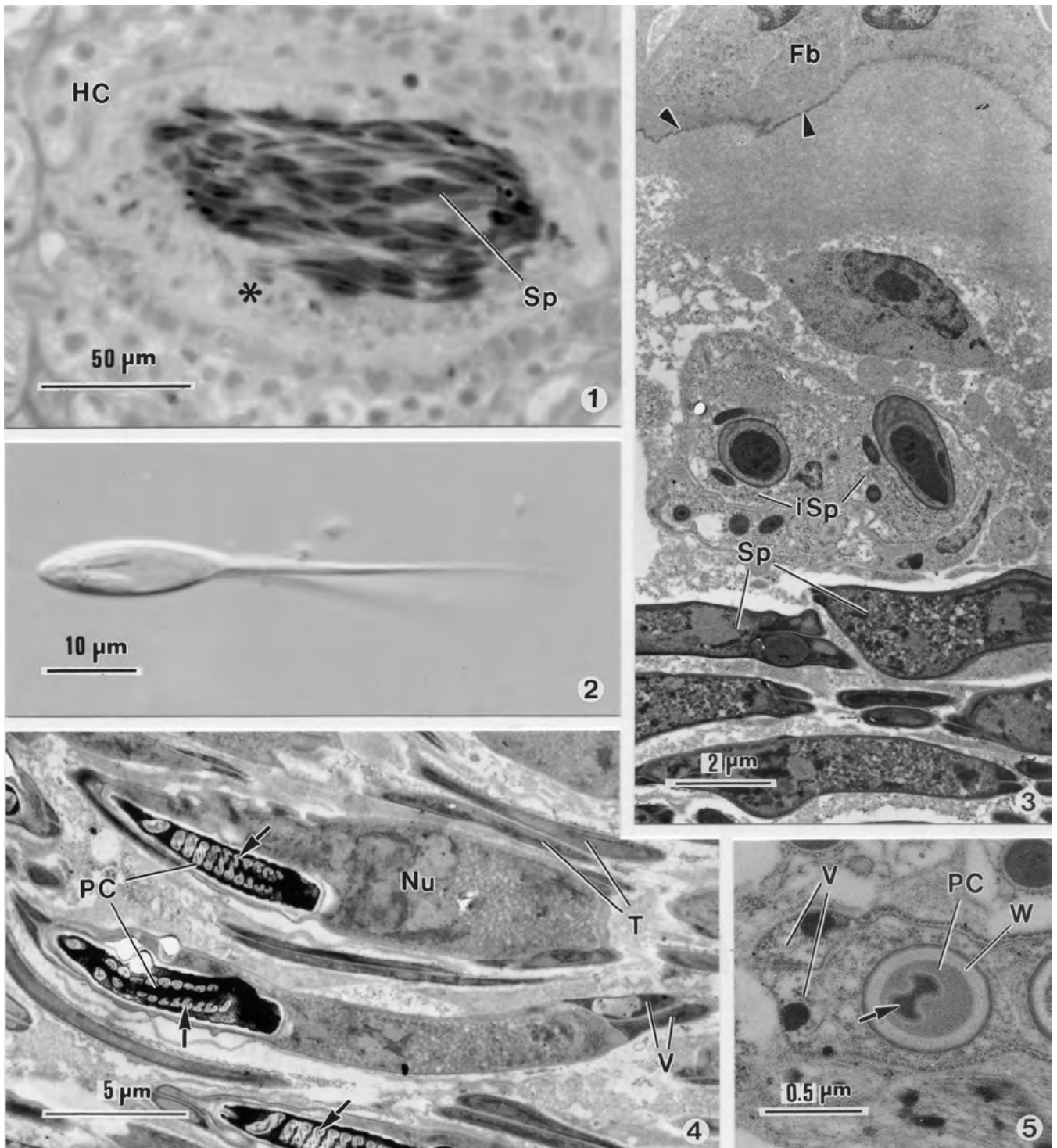
**Type locality:** “Porto da Manga” (57°14'S/19°15'W) in the River Paraguai near the city of Corumbá (State of “Mato Grosso do Sul”), Brazil.

**Location in the host:** Cysts in the gill lamellae.

**Prevalence of infection:** Five of 11 examined fishes (45.4%) were parasitized with similar rates in both sexes.

### Description of the spore

Ellipsoidal tailed spores containing all the typical characteristics of genus *Henneguya* measured  $61.5 \pm 0.91$  (60.2–62.6)  $\mu\text{m}$  ( $n = 50$ ) in total length and spore body  $21.1 \pm 0.62$  (20.6–21.9)  $\mu\text{m}$  ( $n = 25$ ) long,  $6.7 \pm 0.40$  (6.2–7.3)  $\mu\text{m}$  ( $n = 25$ ) wide and  $2.5 \pm 0.54$  (2.0–3.1)  $\mu\text{m}$  ( $n = 15$ ) thick (Fig. 2). The spores were composed of two equal shell valves adhering together along the



**Figs 1– 5.** Light and electron micrographs of the cyst and spores of *Henneguya piaractus* parasite of the gill lamellae of the teleost *Piaractus mesopotamicus* collected in the Paraguai River (Brazil). **1** – semithin section of a cysts containing internally several spores (Sp) surrounded by the youngest developmental stages (\*) and surrounding host cells (HC) observed in DIC; **2** – fresh mature spore observed in DIC; **3** – ultrathin section of the periphery of a cyst (arrowheads) showing the surrounding external fibroblasts (Fb) and internally some developmental stages: iSp-immature spores; Sp-mature spores; **4** – ultrathin section of some spores sectioned at different levels, with special evidence of the polar capsules (PC) and there polar filament sections (arrows), the nuclei of the sporoplasm (Nu), the spore shell valves (V) and some spore tail sections (T); **5** – ultrathin transverse section of one of the two polar capsules (PC) showing the capsule wall (W) and a transverse section of the polar filament (arrow). Externally the spore shell valves (V) are present.

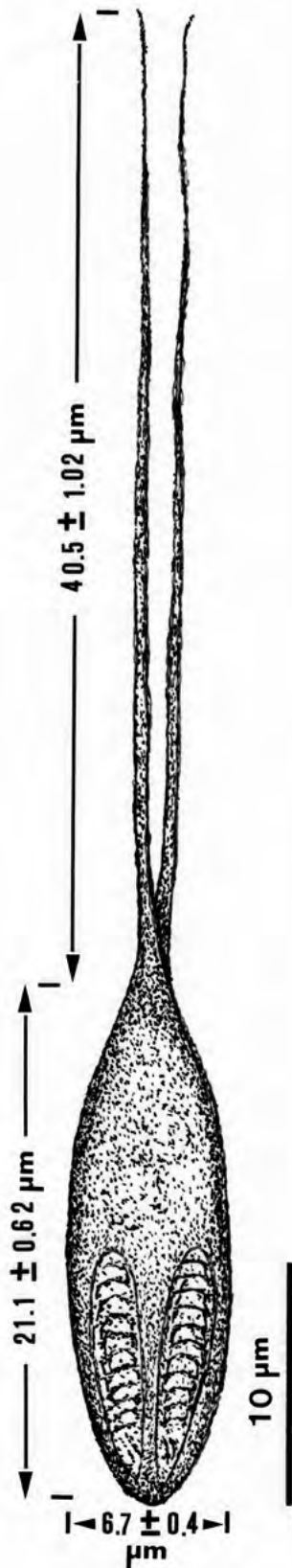


Fig. 6. Schematic drawing of *Henneguya piaractus* spore with some measurements.

straight suture line each having in continuity a equal caudal tapering tail measuring  $40.5 \pm 1.02 \mu\text{m}$  ( $n = 25$ ) in length (Fig. 2). The spore wall was about  $97 \pm 4.10$  ( $90.8\text{--}103.8$ ) nm ( $n = 20$ ) of thickness and consisted of a thin electron-dense exospore and a thick electron-lucent endospore with about  $85 \pm 2.9$  ( $82.2\text{--}89.0$ ) nm of thickness (Figs 3, 4). Two symmetric elongated and equal polar capsules measured  $9.8 \pm 0.28$  ( $9.3\text{--}10.1$ )  $\mu\text{m}$  long ( $n = 15$ ) and  $1.9 \pm 0.37$  ( $1.4\text{--}2.4$ ) ( $n = 15$ )  $\mu\text{m}$  wide, and contained a polar filament with 10–11 coils (Fig. 4), rarely 12. The polar capsules had a circular transverse section and the polar filament had irregular transverse sections (Fig. 5). A schematic drawing of the spore morphology was made from light observations (Fig. 6).

**Histopathology:** No external morphological signs of the infection were observed. The anatomical form of the infection appeared as whitish, spherical to ellipsoidal isolated cysts of the intralamellar-type located in the epithelium of the gill lamellae. The cysts were generally connected with some capillaries on the gill epithelium.

## DISCUSSION

The parasite described in this paper presents all the typical morphology and characteristics of myxozoan *Henneguya* spp. (i.e., ellipsoidal spore body formed by two shell valves, each with an elongated tail and internally two polar capsules) (Lom and Dyková 2006). Recently, 34 valid *Henneguya* species described from Brazilian fish were summarized in a table containing the spore measurements (Eiras *et al.* 2008). However, two new species, more recently described, must be added to this number: *H. rondoni* described on the basis of ultrastructural data (Azevedo *et al.* 2008) and *H. corruscans* based in light micrographs and a drawing (Eiras *et al.* 2009). Comparing the morphology and the dimensions of the spores of these species, we observed that *H. piaractus* differs mainly in the body, tails and polar capsules shape and size, as well as in the polar filament coil arrangements. All these species have different morphologic characters and host specificity when compared with the species described here.

The morphological and ultrastructural aspects of the mature spores, the host specificity and localization of the infection of this parasite were compared with other myxosporidian species of the genus *Henneguya*, mainly

**Table 1.** Comparative dimensions (in  $\mu\text{m}$ ) between two different spores from the previously described species (Martins & Souza, 1997) and from the species described here.

<i>Henneguya</i> spp.	Total length	Body length	Body width	Body thick	Tail length	PCs length	PCs width	Coil number
<i>H. piaractus</i> Martins & Souza, 1997	52.5	12.7	3.6	–	41.2	6.7	1.2	–
<i>H. piaractus</i> (present study)	61.5 $\pm$ 0.91 (60.2 – 62.6)	21.1 $\pm$ 0.62 (20.6 – 21.9)	6.7 $\pm$ 0.40 (6.2 – 7.3)	2.5 $\pm$ 0.54 (2.0 – 3.1)	40.5 $\pm$ 1.02 (38.7 – 43.1)	9.8 $\pm$ 0.28 (9.3 – 10.1)	1.9 $\pm$ 0.37 (1.4 – 2.4)	10 – 11 (rarely 12)

from different Brazilian geographical areas, that have as host freshwater fishes (Martins and Souza 1997, Eiras *et al.* 2008). The morphological similarities between the present study and the *H. piaractus* study described on the bases of light microscopy (Martins and Souza 1997), suggest that these two parasites are of the same species despite being collected from rivers of different Brazilian hydrographic network. On the other hand, important morphological taxonomic characters such as the evidence of two tails in continuity with the basal portion of the spore body, was used to distinguish the genus *Henneguya*. Taking into account the spore morphology and dimensions, we summarize in the Table 1 the comparative measurements between *Henneguya piaractus* spores described on the previous paper by Martins and Souza (1997) and those described in the present paper. Apart from some little dimensional differences between these two forms, we believe that these forms belong to the same species. Moreover, they occurred in the same organ (gills) and in the same host species (*Piaractus mesopotamicus*) and are frequently found in different Brazilian rivers. The precise location of the myxozoan cysts and location of the plasmodium development in the gill fish were described in detail by Molnár (2002). In our study, the cysts were located in the interlamellar gill epithelium, according to Molnar' classification. This location is frequent in several *Henneguya* spp., showing however, different pathological changes (Adriano *et al.* 2005, Matos *et al.* 2005, Eiras *et al.* 2008). In conclusion, on the basis of the morphological similarities, spore measurements, polar capsule sizes and shapes occurring in the same organ and location, as well as in the same host species, we believe that these arguments are sufficient to confirm that this parasite belongs to *Henneguya piaractus*, as previously described by Martins and Souza (1997) and ultrastructurally described by the first time in the present paper.

**Acknowledgments.** This work was partially supported by Eng. A. Almeida Foundation, (Porto, Portugal), Embrapa Pantanal, “CNPq,” and “CAPES,” Brazil. We would like the technical assistance of J. Carneiro (ICBAS/UP). This work is original and complies with the current laws of our countries in where it was performed. The helpful comments and suggestions of the anonymous reviewers in reviewing this manuscript are greatly appreciated.

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Received on 7<sup>th</sup> October, 2009; revised on 3<sup>rd</sup> February, 2010; accepted on 28<sup>th</sup> February, 2010