

Fine structure of *Henneguya hemiodopsis* sp. n. (Myxozoa), a parasite of the gills of the Brazilian teleostean fish *Hemiodopsis microlepis* (Hemiodontidae)

Carlos Azevedo^{1/+}, Graça Casal^{2,3}, Ivete Mendonça⁴, Edilson Matos⁵

¹Department of Cell Biology, Institute of Biomedical Sciences ²Laboratory of Pathology, Centre for Marine and Environmental Research, University of Porto, Porto, Portugal ³Departamento de Ciências, Instituto Superior de Ciências da Saúde-Norte, Gandra, Portugal ⁴Laboratório de Saúde Animal, Universidade Federal do Piauí, Teresina, PI, Brasil ⁵Laboratório de Pesquisa Carlos Azevedo, Universidade Federal Rural da Amazônia, Belém, PA, Brasil

A fish-infecting myxosporean, Henneguya hemiodopsis sp. n., found infecting the gills of *Hemiodopsis microlepis* and collected from the Poty River near the city of Teresina, Brazil, was described based on ultrastructural studies. The parasite occurred within large whitish polysporic plasmodia (up to 200 μm in diameter) containing asynchronous developmental sporogonic stages, mainly mature spores. The spores measured $19.7 \pm 0.9 \mu\text{m}$ in total length ($n = 30$) and the ellipsoidal spore body was $10.8 \pm 0.5 \mu\text{m}$ long, $3.3 \pm 0.4 \mu\text{m}$ wide and $2.5 \pm 0.5 \mu\text{m}$ thick. The spores were composed of two equal shell valves adhering together along the straight suture line, with each valve having equal-sized caudal tapering tails measuring $8.7 \pm 0.6 \mu\text{m}$ in length. The spores were surrounded by a thin anastomosed network of microfibrils, more evident on the tails. There were two symmetric elongated bottle-like polar capsules $3.5 \pm 0.3 \mu\text{m}$ long and $1.0 \pm 0.2 \mu\text{m}$ wide, each with a polar filament with five to six coils. Given the morphological and ultrastructural differences from previously described parasites and the specificity of the host species, we propose a new species, named *H. hemiodopsis* sp. n.

Key words: ultrastructure - *Henneguya hemiodopsis* sp. n. - Myxozoa - parasite - gill - Brazilian fish

The South American Continent contains one of the biggest hydrographic networks in the world, in which a great variety of ictiofauna species reside. Since the first description of *Henneguya* Thélohan, 1892 (Lom & Dyková 2006), the second largest genus of the Myxobolidae family, many species have been reported, mainly parasitising freshwater fishes throughout the world. However, the number of myxozoan parasites described from Brazil is very low, especially considering that the country has one of the most diverse freshwater fish populations in the world with about 8,000 species (Cellere et al. 2002). Thirty-six species have been described from Brazilian fauna based on light microscopy micrographs and diagrammatic illustrations (Jakowska & Nigrelli 1953, Kent & Hoffman 1984, Gioia et al. 1986, Martins et al. 1988, Gioia & Cordeiro 1996, Martins & Souza 1997, Barassa et al. 2003a, b, Eiras et al. 2004a, b, 2008, 2009, Martins & Onaka 2006, Abdallah et al. 2007). Recently, ultrastructural studies of developmental stages and mature spores supported the classification of some new species of the genus *Henneguya* (Rocha et al. 1992, Azevedo

& 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 report light and electron microscopy-based data on the mature spores of a new parasite, designated herein as *Henneguya hemiodopsis* sp. n., infecting the gills of a teleostean fish of some economic importance found in a river in North-Eastern Brazil.

MATERIALS AND METHODS

Fifty specimens (18 males and 32 females) of the freshwater teleost *Hemiodopsis microlepis* Kern, 1858 (Teleostei: Characiformes: Hemiodontidae) (Brazilian common name *flexeiro*), endemic to this region, were recently collected from the Poty River (05°05'S 42°48'W) near the city of Teresina [state of Piauí (PI)], Brazil. The fish were lightly anaesthetised with MS 222 (Sandoz Laboratories) and dissected and the gills, containing several whitish cysts (cyst-like plasmodia), were removed from the gill lamellae and examined with a light microscope equipped with Nomarski interference-contrast (DIC) optics.

For ultrastructural studies, small fragments of the gill lamellae containing cyst-like plasmodia were excised and fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4°C for 10 h. After being 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. Semi-thin sections were stained with methylene blue-Azure II and observed by DIC optics. Ultra-thin sections were double-stained with aque-

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+ Corresponding author: azevedoc@icbas.up.pt

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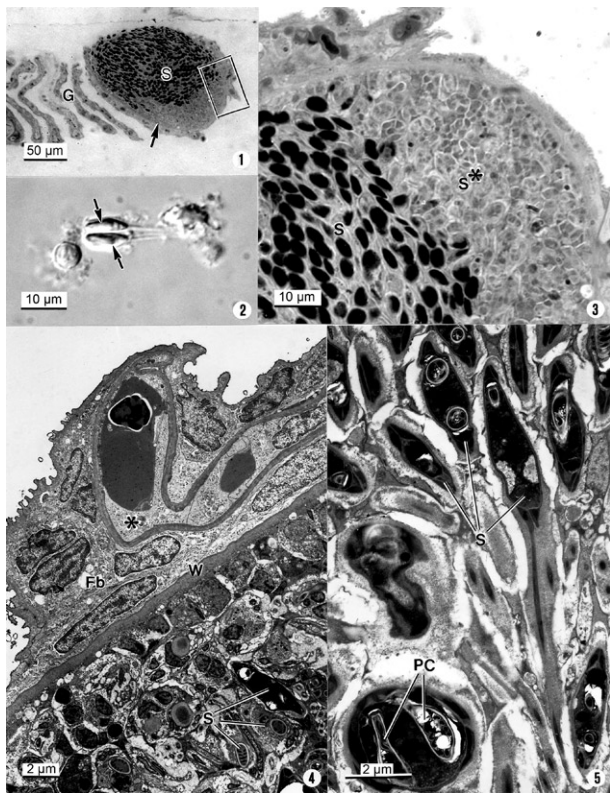
ous uranyl acetate and lead citrate and observed under a JEOL 100CXII transmission electron microscope (TEM) operated at 60 kV.

RESULTS

Of the 50 adult specimens of *H. microlepis* (Teleostei: Hemiodontidae) examined, 32 (64%) contained gills parasitised by an organism identified as follows: Phylum Myxozoa Grassé, 1970; Class Myxosporaea Bütschli, 1881; Order Bivalvulida Shulman, 1959; Family Myxobolidae Thélohan, 1892; genus *Henneguya* Thélohan, 1892, according to the classification proposed by Lom and Dyková (2006).

The morphological and ultrastructural data, mainly the shape and dimension of the spores and polar capsules (PC), as well as the polar filament coils and arrangement, indicate that this parasite is a new species, herein named *H. hemiodopsis*.

For description of the plasmodium and mature spores, light microscopy (DIC) (Figs 1-3), TEM (Figs 4-12) and a schematic drawing (Fig. 13) were used.



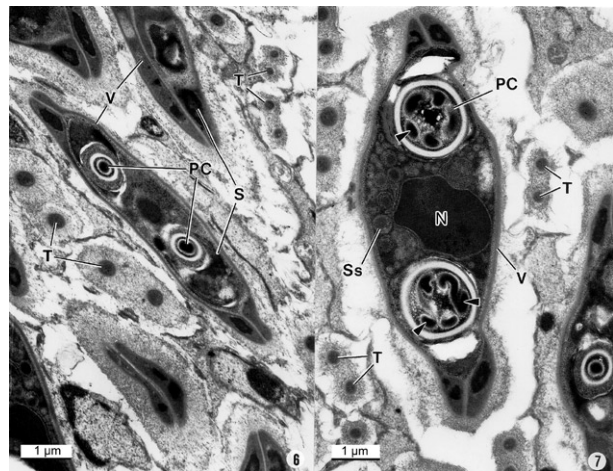
Figs 1-5: light and transmission electron micrographs of *Henneguya hemiodopsis* n.sp. 1: semithin section of a plasmodium in close contact with the gill filaments (G) containing numerous mature spores (S) and a group of immature spores (arrows); 2: two free fresh spores (arrows) observed in DIC; 3: magnification of the boxed area in Fig. 1 showing the plasmodium periphery surrounding numerous mature (S) and immature spores (S*); 4: ultrastructural details of the plasmodium periphery showing the wall (W) in the close contact with the gill cell of a filament, where some fibroblasts (Fb) and a blood vessel are visible (*). Internally several spore sections (S) are observed; 5: ultrathin sections of several spores (S) sectioned at different levels showing the sections of the polar capsules (PC).

H. hemiodopsis sp. n. (Figs 1-3)

Description - Vegetative stages: whitish round-shaped polysporic plasmodia (cyst-like plasmodia), measuring up to 200 μm contained numerous mature and immature spores, indicating asynchronous development (Figs 3, 4). The plasmodium wall was formed by a dense material that was in contact with the epithelial gill cells. The zone of contact contained several fibroblasts, collagen fibrils and some capillaries (Fig. 4). The spores were ellipsoidal with a total length of $19.7 \pm 0.9 \mu\text{m}$ ($n = 30$), body length $10.8 \pm 0.5 \mu\text{m}$ ($n = 20$), body width (frontal view) $3.3 \pm 0.4 \mu\text{m}$ ($n = 20$) and body thickness (side view) $2.5 \pm 0.5 \mu\text{m}$ ($n = 15$). The two valves were symmetrical and thin and each was prolonged by a tapering tail $8.7 \pm 0.6 \mu\text{m}$ ($n = 20$) long (Figs 2, 5-7). Elongated bottle-like PC localised side by side in the anterior pole of the spore were equal in size, measuring $3.5 \pm 0.3 \mu\text{m}$ in length ($n = 25$) by $1.0 \pm 0.2 \mu\text{m}$ ($n = 25$) in width (Figs 6-9). The apical pore of the bottle-like PC was closed by a dense stopper and an electron-dense ring surrounded the neck of the bottle-like PC. The neck of the bottle-like PC measured $1.7 \pm 0.4 \mu\text{m}$ ($n = 12$) in length and $\sim 0.5 \mu\text{m}$ in diameter (Figs 8-10). Inside the PC, the isofilar polar filament was coiled with 5-6 turns oblique to the longitudinal axis (Figs 8, 9). The internal matrix of the polar filaments was denser than the surrounding matrix of the PC (Figs 8, 9). The spores presented numerous ramified and anastomosed microfibrils at the surface (Figs 9-11), more evident in the tails (Figs 6, 7, 12).

Type host - *H. microlepis* Kner, 1858 (Teleostei: Characiformes: Hemiodontidae) (12-17 cm in length on average).

Site of infection: whitish cysts (cyst-like plasmodia) were found in secondary gill lamellae.



Figs 6, 7: transmission electron micrographs of *Henneguya hemiodopsis* n.sp. 6: some spores (S) sectioned at different levels, one of which in transverse section showing the two polar capsules (PC). The shell valves (V) and the tails (T) are observed; 7: a transverse section of a spore at PC levels showing the shell valves (V), polar filament coil sections (arrowheads), one of the two nuclei (N) and sporoplasmosomes (Ss). Some tail sections (T) are visible near the spores.



Figs 8-12: transmission electron micrographs of *Henneguya hemiodopsis* n.sp. 8: ultrastructural detail of a longitudinal section of a polar capsule (PC) showing the morphology of the capsule and the organization of the polar filament coils (arrowheads). Just near the spore another PC showing the coils of a filament tangentially sectioned. 9: longitudinal section of the anterior portion of a spore showing the position of the two nuclei (N) located between the PC. The shell valves show a surrounding network of anastomosed microfibrils (*); 10, 11: two ultrastructural details of the complex organization of the apical region of the PC and a nucleus (N). The valves show a network of anastomosed microfibrils (*); 12: detail of the transverse sections of two tails (T) showing the surrounding network of anastomosed microfibrils (arrow).

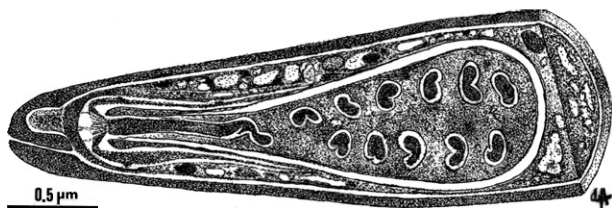


Fig. 13: semischematic drawing of a longitudinal section of a polar capsule (PC) with their filament coil sections showing the elongated bottle-like form of the PC.

Prevalence and intensity - Thirty-two out of 50 (18 males and 32 females) adult fish (64%) were parasitised, with similar rates in both sexes.

Type locality - Poty River (05°05'S 42°48'W), near the city of Teresina (PI).

Type data and depository - A glass slide containing semi-thin sections of mature spores of the hapantotype was deposited in the International Protozoan Type Slide Collection at the Smithsonian Institution Washington, DC 20560, USA, with acquisition USNM 1123997.

Etymology - The specific epithet derives from the generic name of the host (*Hemiodopsis*).

Histopathology - Our observations showed that the infected gill lamellae were associated with an increased number of fibroblasts just near the zone of contact of the epithelial gill with the wall of the cyst-like plasmodia, with local atrophy and deformation of the gill lamellae (Fig. 4).

DISCUSSION

The morphology of the myxosporean described in the present paper has the distinguishing features of the genus *Henneguya* Thélohan, 1899 (i.e., ellipsoidal spore body formed by two shell valves, each with an elongated tail and two PC internally) (Lom & Dyková 2006). The morphological and ultrastructural aspects of the mature spores, as well as the host specificity and localization of the infection with this parasite, were compared to those of other myxosporidian species of the genus *Henneguya* from different geographical areas, mainly species that parasitise Brazilian freshwater fishes.

Recently, 34 valid *Henneguya* species described from Brazilian fish were summarised in a table containing the spore measurements (Eiras et al. 2008). Two new species, more recently described, must be added to this number: *Henneguya rondoni*, described on the basis of ultrastructural data (Azevedo et al. 2008) and *Henneguya corruscan*, described based on light micrographs and a drawing (Eiras et al. 2009).

Comparing the morphology and the dimensions of the spores of these species, we observe that *H. hemiodopsis* sp. n. differs mainly in the shape and size of the body, tails and PC as well as in the arrangement of the polar filament coils. All of the previously described species have different morphological characteristics and host specificity compared to the presently described species.

On the other hand, the presence of an external adherent sheath surrounding the spore body wall and tails has been used as an important morphological taxonomic characteristic to distinguish *Henneguya* spp. Taking into account this structure, we summarise in Table the spore measurements of *Henneguya* spp, whose spores are surrounded by a sheath.

Compared to the proposed new species (*H. hemiodopsis*), several differences were observed in the external sheath of other *Henneguya* spp that contain this structure (Table). Whereas in *H. hemiodopsis* sp. n. the spores were surrounded by a thin anastomosed network of microfibrils, in *Henneguya theca*, *Henneguya adherens*, *Henneguya testicularis*, *Henneguya rhamdia* and *H. rondoni*, the spores were surrounded by a hyaline homogenous structure and in *Henneguya malabarica* and *Henneguya striolata*, the sheath was constituted by granulo-fibrillar structures organised into a complex ramified network.

TABLE
Comparative measurements (in µm) of the spore from *Henneguya* spp surrounded by a sheath and equal polar capsules described in Brazilian fish

<i>Henneguya</i> spp	Fish species/tissues	Sheath	STL	SBL	SBW	SBT	TaL	PCL	PCW	FC	References
<i>Henneguya theca</i>	<i>Eigemannia virescens</i> brain	HHL	48.0 40.6-52.6	24.8	3.5 3.0-4.1	-	23.2 20.3-24.2	11.1 9.8-12.5	1.4 1.0-1.6	-	Kent and Hoffmann (1984)
<i>Henneguya adherens</i>	<i>Acestrorhynchus falcatus</i> gill filaments	HHL	32.3 30.7-35.1	12.4 10.5-13.8	5.8 5.1-6.5	-	20.5 18.5-21.7	3.1 2.8-3.5	1.2 1.0-1.6	3-4	Azevedo and Matos (1995)
<i>Henneguya malabarica</i>	<i>Hoplias malabaricus</i> gill filaments	GFL	28.3 26.6-29.8	12.6 11.8-13.1	4.8	-	17.1 16.2-18.9	3.7 3.0-4.3	1.8 1.6-2.2	6-7	Azevedo and Matos (1996)
<i>Henneguya testicularis</i>	<i>Moenkhausia oligolepis</i> testis	HHL	27.9 27.0-28.5	14.0	6.5	-	13.5 13.0-14.5	9.0	2.0	12-13	Azevedo et al. (1997)
<i>Henneguya striolata</i>	<i>Serrasalmus striolatus</i> gill filaments	FED	42.2 39.3-45.6	15.8 14.4-17.0	5.3 4.9-5.9	-	25.9 23.6-29.8	6.8 5.1-7.0	1.2 1.1-1.3	13-14	Casal et al. (1997)
<i>Henneguya rhamdia</i>	<i>Rhamdia quelen</i> gill filaments	HHL	50.0 ± 1.8	13.1 ± 1.1	5.2 ± 0.5	-	36.9 ± 1.6	4.7 ± 0.4	1.1 ± 0.2	10-11	Matos et al. (2005)
<i>Henneguya rondoni</i>	<i>Gymnorhamphichthys rondoni</i> nervous fibres	HHL	17.7 16.9-18.1	7.0 6.8-7.3	3.6 3.0-3.9	2.5 2.2-2.8	10.7 10.3-11.0	2.5 2.2-2.7	0.85 0.79-0.88	6-7	Azevedo et al. (2008)
<i>Henneguya hemiodopsis</i>	<i>Hemiodopsis microlepes</i> gill filaments	ML	19.7 ± 0.9	10.8 ± 0.5	3.3 ± 0.4	2.5 ± 0.5	8.7 ± 0.6	3.5 ± 0.3	1.0 ± 0.2	5-6	This study

FC: number of the polar filament coils; FED: flocculent electron-dense layer; GFL: granulo-fibrillar layer; HHL: hyaline homogenous layer; HHL: hyaline homogenous layer; ML: microfibrillar layer; PCL: polar capsule length; PCW: polar capsule width; SBL: spore body length; SBT: spore body thickness; SBW: spore body width; STL: total length of the pores; TaL: tail length.

This inflammatory reaction seems to be of the same type as that observed and described in other Brazilian fishes (Martins et al. 1988, Adriano et al. 2005).

In conclusion, on the basis of the morphological differences in the size and shape of the spore and PC and the number and arrangement of the polar filament coils, we think that there are sufficient arguments for the establishment of a new species, named *H. hemiodopsis* sp. n.

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