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Ultrastructural aspects of the myxosporean *Henneguya astyanax* n. sp. (Myxozoa: Myxobolidae), a parasite of the Amazonian teleost *Astyanax keithi* (Characidae)

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ABSTRACT: This study reports light and electron microscopical aspects of a myxosporean found in the gills of the freshwater teleost *Astyanax keithi* Géry, Planquete & Le Bail, 1996 (family Characidae), collected from the estuarine region of the Amazon River, near Belém, Brazil. The prevalence of infection was 23%. In interlamellar spaces of the gills, ellipsoidal whitish cyst-like plasmodia structures were present, which contained spores. The spores had a spermatozoa-like appearance (47.8 ± 0.71 µm in total length) with a fusiform body (15.2 ± 0.77 µm in length, 5.7 ± 0.71 µm in width and 4.2 ± 0.31 µm in thickness), and each of the 2 valves presented a tapering tail (32.6 ± 1.11 µm in length). The valves surrounded a binucleate sporoplasm cell and 2 polar capsules (5.0 ± 0.13 µm in length, 1.5 ± 0.07 µm in width) that contained 8 to 9 coils of the polar filament. In the sporoplasm, several unique sporoplasmosomes were visible. A synoptic table of spore measurements of known Brazilian *Henneguya* species is presented. The spores differed from those of previously described species. Based on spore morphology, it is concluded that this species belongs to the family Myxobolidae, genus *Henneguya*, and that it constitutes a new species: *H. astyanax* n. sp.

KEY WORDS: Ultrastructure · *Henneguya* · Myxozoa · Parasite · Teleost

INTRODUCTION

The genus *Henneguya* Thélohan, 1892 (phylum Myxozoa), one of the largest of the 9 genera in Myxobolidae, includes about 120 species (Lom & Dyková 1992). Their importance as pathological agents of freshwater and marine fishes is well documented (Dyková & Lom 1978, Current 1979, Lom & Dyková 1992).

First studies on Brazilian myxosporidosis caused by *Henneguya* spp. (for review see Gioia & Cordeiro 1996) consisted only in diagrammatic illustrations of light microscopy. In addition, some ultrastructural studies on mature spores and different life cycles stages were carried out (Azevedo & Matos 1989, 1995, 1996, Rocha et al. 1992, Casal et al. 1997, Azevedo et al. 1997). There is 1 scanning electron microscopy study (Martins & Souza 1997).

Serious pathogenic potential was recorded in a large number of myxosporeans that can affect any organ of the fish body and cause various lesions (Lom & Dyková 1992). Species of the genera *Henneguya* and *Myxobolus* can cause pressure atrophy of surrounding organs through the growth of large plasmodia (Dyková & Lom 1978, Lom & Dyková 1992). However, more than 1330 myxosporean species have been recorded, and only relatively few are known to cause serious or fatal infections, since both myxosporeans and their hosts are well adapted to each other (Lom & Dyková 1992).

The present work describes light and electron microscopical aspects of a new myxosporean species,

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Henneguya astyanax n. sp., found in the gills of the Amazonian teleost fish *Astyanax keithi* (fam. Characidae).

MATERIALS AND METHODS

A total of 30 specimens of the teleost fish *Astynax keithi* Géry, Planquete & Le Bail, 1996 (fam. Characidae), known by the Brazilian common name 'piaba transparente', were obtained from the estuarine region of the Amazon River (01° 11′ 30″ S, 47° 18′ 54″ W) near Belém, Brazil.

Fragments of gill filaments, containing some cyst-like plasmodia, were teased apart to release spores, which were observed by Nomarski differential interference contrast optics (DIC), for measurement and description.

For transmission electron microscopy (TEM), isolated spores and fragments of cysts were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 5 h at 4°C, washed overnight in the same buffer at 4°C, and postfixed in 2% OsO_4 buffered with the same solution for 4 h at the same temperature. Following ethanol-series and propylene oxide dehydration, samples were embedded in Epon. Semithin sections were stained with methylene blue-Azur II. Ultrathin sections were cut with a diamond knife, contrasted with uranyl acetate and lead citrate, and observed in a JEOL 100CXII TEM at 60 kV.

RESULTS

The parasites appeared as ellipsoidal whitish cyst-like structures (~250 µm in size), located on the gills (Fig. 1) and composed of a mass of spores, each ~47.8 µm in total length, possessing 2 refractile bodies (polar capsules). According to these criteria, the parasite belongs to the phylum Myxozoa (Lom & Noble 1984).

The spores are spindle-shaped with 2 asymmetrical valves (see Fig. 7) and 2 caudal projections (tails), slightly divergent (Fig. 2). There are 2 polar capsules per spore close to each other. The polar capsules are located in the apex of the spore and are set in the suture plane (see Fig. 9). These characteristics suggest that this parasite belongs to the genus *Henneguya* Thélohan, 1892.

Sporoblasts

Typically, the sporoblast cells are surrounded by 2 pericytes, and are divided into valvogenic, capsulogenic and sporoplasm cells (Figs. 3 & 4). During sporogenesis, the valvogenic cells became elongated and denser, differentiating into valves each of which continues posteriorly as a caudal projection (Fig. 4).

Spores

Measurements of mature fresh spindle-shaped (fusiform) spores (Fig. 2) are as follows: total length 47.8 ± 0.71 µm (n = 30); body length 15.2 ± 0.77 µm (n = 30), width 5.7 ± 0.71 µm (n = 30), and thickness 4.2 ± 0.31 µm (n = 30); tail length 32.6 ± 1.11 µm (n = 30); polar capsule length 5.0 ± 0.13 µm (n = 30) and width 1.5 ± 0.07 µm (n = 30); number of polar filament coils 8 or 9 (Fig. 5).

Electron microscopy of the binucleated sporoplasm revealed the presence of dense bodies, the sporoplasmosomes (Fig. 6). They have a globular appearance (~275 nm diameter) and seem to be single-membrane-bound. Inside the envelope there is a dense inner medulla surrounded by a thin lucent cortex. There is also an eccentric aggregation of dense material (double V-shaped cortical granular layer, denser than the medulla) (Fig. 8). A schematic drawing of a spore is given in Fig. 9.

Diagnosis

Host: Teleost fish *Astyanax keithi* Géry, Planquete & Le Bail, 1996 (fam. Characidae).

Locality: Estuarine region of the Amazon River (01° 11′ 30″ S, 47° 18′ 54″ W) near Belém, Brazil.

Site of infection: Spores were located in the gills.

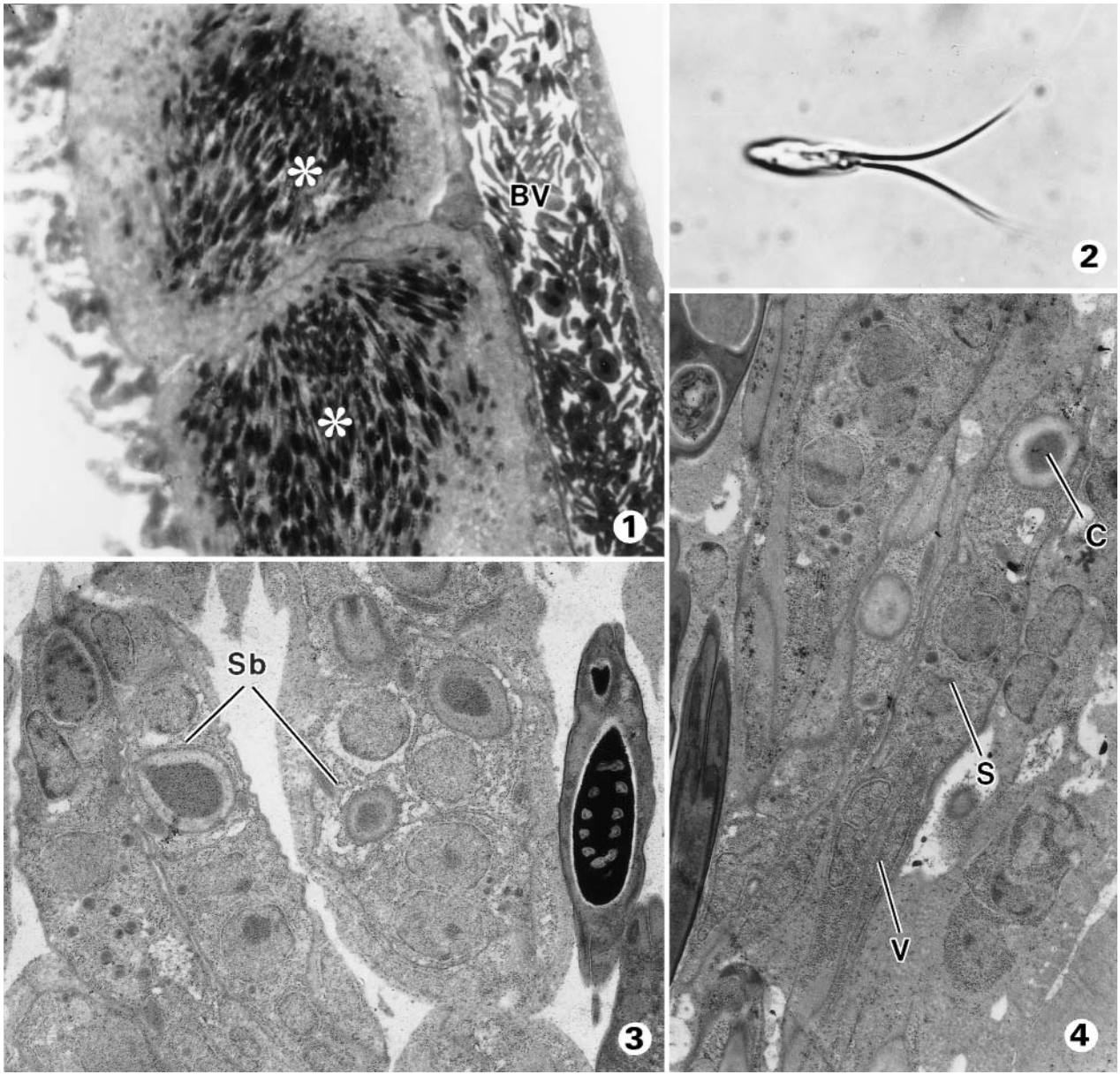
Prevalence and intensity: 7 of 30 (23%).

Fresh spore measurements (n = 30)

Length	47.8 ± 0.71 µm
Width	5.7 ± 0.71 µm
Thickness	4.2 ± 0.31 µm
Polar capsule: length	5.0 ± 0.13 µm
width	1.5 ± 0.07 µm
Number of polar filament turns:	8 to 9

Specimens deposited: Slides with holotype are deposited in the International Protozoan Type Slides Collection at the Smithsonian Institution, Washington, DC 20560, USA (USNM# 1004430) and in the collection of C.A.

Etymology: The specific name is derived from the name of the host species.



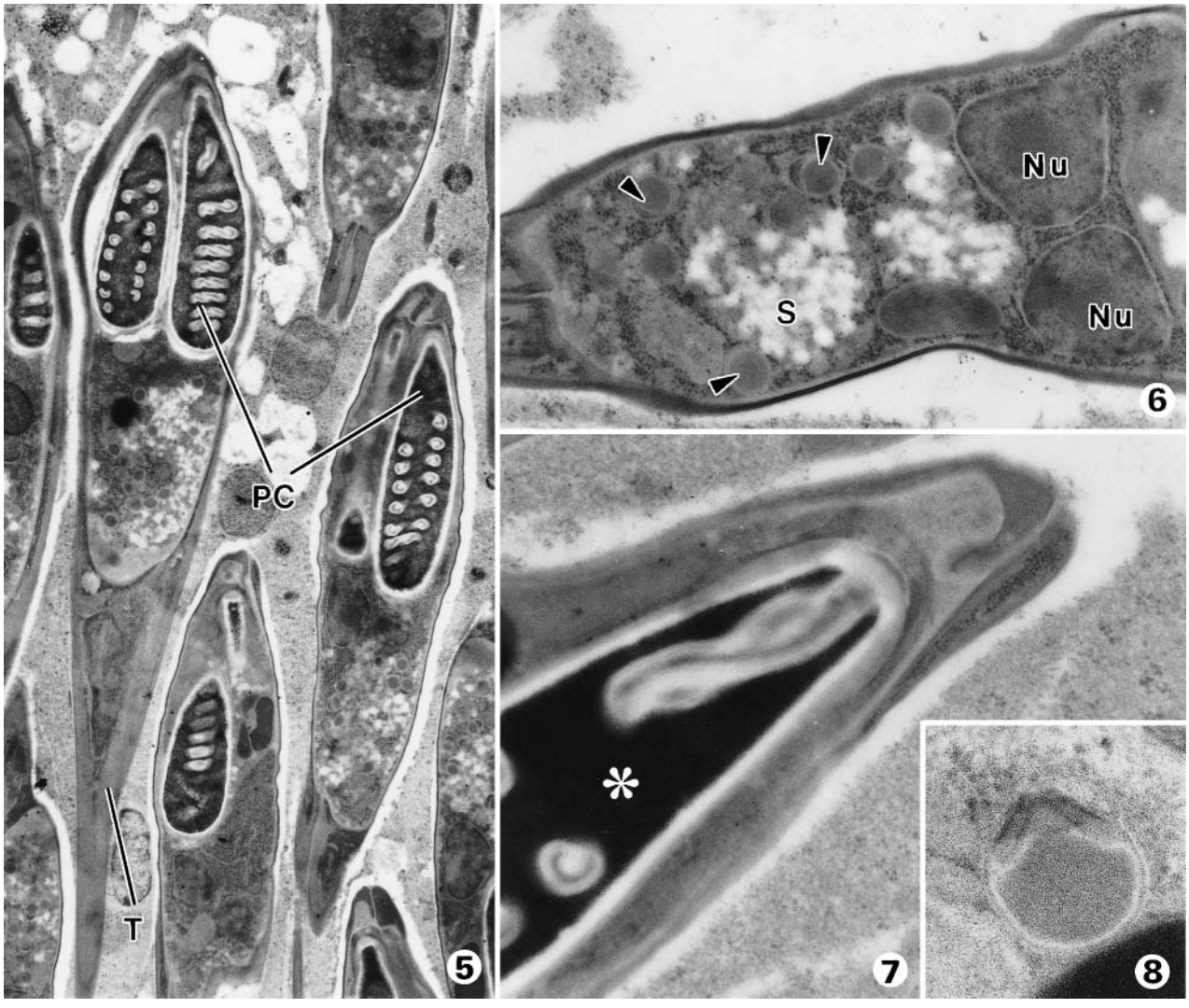
Figs. 1 to 4. Myxosporean *Henneguya astyanax* n. sp. Fig. 1. Semithin section of a gill filament of *Astyanax keithi*, showing 2 cysts (✻), each containing numerous spores, in close contact with a blood vessel (BV) (×230). Fig. 2. Nomarski DIC observations of isolated living spore (×1200). Fig. 3. Ultrathin section of sporoblasts (Sb) differentiation (×7950). Fig. 4. Longitudinal and oblique ultrathin sections of sporoblasts showing valvogenic (V), capsulogenic (C) and sporoplasm (S) cells during differentiation (×7950)

Pathogenicity

Interlamellar spaces of gills of affected fish contained intercellular cyst-like structures full of mature parasite spores with long spermatozoa-like tails (Fig. 1). In the surrounding tissues, there was moderate gill epithelial cell hyperplasia and a mild mononuclear inflammatory infiltrate within the interstitium of the gill, without signs of significant pressure atrophy.

DISCUSSION

The diagnosis of the specimens was based on spore size, shape and ultrastructural arrangement of its components, the major set of taxonomic criteria for myxosporean classification (Lom & Dyková 1992). The general organisation of this parasite is similar to that of previously described species of the genus *Henneguya* (Current 1979, Lom & Dyková 1992, Rocha et al. 1992,



Figs. 5 to 8. Spores of *Henneguya astyanax* n. sp. Fig. 5. Longitudinal section of mature spores showing polar capsules (PC) and spore tails (T) (×8000). Fig. 6. Longitudinal section of binucleated (Nu) sporoplam (S), showing several sporoplasmosomes (arrowheads) (×26 000). Fig. 7. Detail of spore apex showing polar filament coiled within the polar capsule (✻), note asymmetry of valves (×32 000). Fig. 8. Detail of a sporoplasmosome showing its peculiar shape (×80 000)

Azevedo & Matos 1995, 1996, Casal et al. 1997, Azevedo et al. 1997).

To identify the present species, Table 1 was constructed founded on a thorough analysis of the spore parameters of 24 *Henneguya* spp. from published papers on Brazilian species, based on the guidelines for species description proposed by Lom & Arthur (1989). Although the presence of an iodinophilous vacuole is an inconstant feature within the same species (Podlipaev & Schulman 1978, Lom & Noble 1984, Mitchell 1988) and without any real taxonomic value, it is included as a variable (Table 1) only because it is included in several studies. Note that *Henneguya* sp. (Azevedo & Matos 1989) and *H. amazonica* (Rocha et

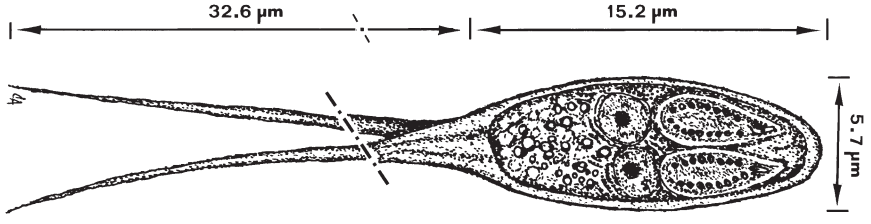


Fig. 9. *Henneguya astyanax* n. sp. Schematic drawing of a spore, showing its measurements

Table 1. *Henneguya* spp. Comparative data (mean measurements in µm) of described spores from different Brazilian species. TL: total length; BL: body length; BW: body width; TaL: tail length; PCL: polar capsule length; PCW: polar capsule width; FC: number of polar filament coils; VT: valve type; IV: iodinophilous vacuole; –: no data

Species	TL	BL	BW	TaL	PCL	PCW	FC	VT	IV	Host	Site of infection	Source
H. linearis	BL 3× or 4× the BW, very narrow				–		–	–	–	*Rhamdia sebae*	Branchial cavity	Gurley (1893), Kudo (1920)
H. lutzi	11		7	–	6–7		–	–	Present	*Pseudopimelodus zungaro*	Gall blader	Cunha & Fonseca (1918)
H. occulta	36–46	16	8	20	8		–	–	–	*Loricaia* sp.	Gills	Nemeczek (1926)
H. leporini	28–33	13–15	5	15–18	5–8		–	–	–	*Leporinus mormyrops*	Urinary duct	Nemeczek (1926)
H. iheringi	22		6	–	3.1	2	–	–	–	*Serrasalmo spilopleura*	Gills	Pinto (1928a,c)
H. wenyoni	21	11–12	5.2	10.8	3.7	1.5	–	–	Present	*Tetragonopterus* sp.	Gills	Pinto (1928b,c)
H. fonsecai	23–27	10–12	4.5–5	13–15	4–4.2		–	–	–	*Leporinus copelandi*	Fin tissues	Guimarães (1931)
H. cesarpintoi	13–14	5.5–6	4–4.5	7.5–8	2.5	0.8	–	–	–	*Astyanax fasciatus*	Gills	Guimarães (1931)
H. bergamini	17–19	7–8	2–2.5	10–11	–		–	–	Present	*Astyanax fasciatus*	Body cavity	Guimarães (1931)
H. travassosi	27.3	10.6	4.3	16.7	3.6		–	–	–	*Astyanax fasciatus*	Muscle	Guimarães & Bergamin (1933)
H. santae	21.0	9.6	5.3	11.2	2.9		–	–	Present	*Tetragnopterus santae*	Gills	Guimarães & Bergamin (1934)
H. visceralis	22–24	11–12	5–6.5	11–12	6.5–8	2	–	–	–	*Electrophorus electricus*	Various	Jakowska & Nigrelli (1953)
H. electrica	35–39	11–13	6–8	24–27	5–7	2	–	Equal	–	*Electrophorus electricus*	Electric organs	Jakowska & Nigrelli (1953)
H. pisciforme	20.4	–	6.1	10.7	4.3	1.7	–	–	Present	*Hyphessobrycon anisitsi*	Gills	Cordeiro et al. (1984)
H. theca	48.0	24.8	3.5	23.2	11.1	1.4	–	Equal	Present	*Eigemannia virescens*	Brain	Kent & Hoffman (1984)
H. intracornea	42.4	–	6.7	24.3	8.6	2.4	–	–	Present	*Astyanax scabripinnis*	Eye (cornea)	Gioia et al. (1986)
H. hoimba	24.7	–	7.5	–	4.4	1.9	–	–	Present	*Astyanax fasciatus*	Gills	Cordeiro & Gioia (1987)
H. artigasi	16.4	–	4.4	–	3.3	1.5	–	–	–	*Astyanax scabripinnis*	Gills	Gioia & Cordeiro (1987)
H. sp.	58.7	13.5	5.3	45.2	–	–	–	–	Present	*Hoplosternum littorale*	Gills	Azevedo & Matos (1989)
H. amazonica	59.3	13.9	5.7	45.4	3.3	1.5	6	–	–	*Crenicichla lepidota*	Gills	Rocha et al. (1992)
H. adherens	32.3	12.4	5.8	20.5	3.1	1.2	3–4	Unequal	–	*Acestrorhynchus falcatus*	Gills	Azevedo & Matos (1995)
H. malabarica	28.3	12.6	4.8	17.1	3.7	1.8	6–7	Equal	–	*Hoplias malabaricus*	Gills	Azevedo & Matos (1996)
H. piaractus	52.5	12.7	3.6	41.2	6.7	1.2	8–9	–	Present	*Piaractus mesopotamicus*	Gills	Martins & Souza (1997)
H. testicularis	27.5	14.0	6.5	13.5	9.0	2.0	12–13	Unequal	–	*Moenkhausia oligolepis*	Testes	Azevedo et al. (1997)
H. striolata	42.2	15.8	5.3	25.9	6.8	1.2	13–14	–	–	*Serrasalmus striolatus*	Gills	Casal et al. (1997)
H. curimata	35.4	16.6	6.2	19.1	6.5	1.2	10–11	Equal	–	*Curimata inormata*	Kidney	Azevedo & Matos (2002)
H. astyanax	47.8	15.2	5.7	32.6	5.0	1.5	8–9	Unequal	–	*Astyanax keithi*	Gills	Present study

al. 1992) are considered to be the same species.

The species presenting lengths over 40 µm are *Henneguya occulta* (Nemeczek 1926), *H. theca* (Kent & Hoffman 1984), *H. intracornea* (Gioia et al. 1986), *H. amazonica* (Rocha et al. 1992) and *H. striolata* (Casal et al. 1997). The body of *H. occulta* is wider, the tail shorter and the polar capsule larger than in the species described in this study. In *H. theca*, the body is longer and narrower, the tail segment is shorter and the polar capsule twice as long. The total length of *H. intracornea* is shorter, the body wider, the tail shorter and the polar capsule longer and wider. There is a marked difference between the total length of *H. amazonica* and that of the species described in this study (59.3 vs 47.8 µm, respectively), the tail of the former is longer, the polar capsule shorter and the polar filament has 6 turns instead of 8 or 9. Although *H. striolata* has a similar total length, body length and width, its tail is significantly shorter, its polar capsule larger and its polar filament has 13 or 14 turns.

The present species was also compared with the spore parameters of 146 described species of *Henneguya* (Eiras 2002). The main criteria for comparison were length of the spore, spore body and polar capsules. Only 4 species present a spore length between 42.8 and 52.8 µm, a spore body length between 13.2 and 17.2 µm and a polar capsule length between 4.0 and 7.0 µm (Eiras 2002). These species are *H. diversis* Minchew, 1977; *H. malapteruri* Fomena & Bouix, 1996; *H. nanhaiensis* Chen, 1998; and *H. postexilis* Minchew, 1977. The bodies of *H. diversis* and of *H. postexilis* spores are narrower and the tails longer than those of the present species. In *H. malapteruri*, the body and the polar capsule are wider and the polar filament has only 4 of 5 coils. The spore body and the polar capsule are also wider in *H. nanhaiensis*.

Differences in the dimensions and shape of the body, tail and polar capsule, as well as in the number of coils of the polar filament, show that none of the above species are identical with that described in the present study.

Sporoplasmosomes are dense inclusions that are almost invariably present in the sporoplasm of most myxosporeans. They exhibit a great diversity in size and are generally globular, sometimes elongated (Lom et al. 1989). The limited data available indicate that their structure varies irrespective of genus (Lom et al. 1989). Nevertheless, taking into account this general diversity, the sporoplasmosomes described herein present a morphology that differs from those of all previously described species of this genus.

In the light of these findings, the species reported here is considered as new, and the name *Henneguya astyanax* n. sp. is proposed.

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