Light and electron microscopic study of the myxosporean, *Henneguya friderici* n. sp. from the Amazonian teleostean fish, *Leporinus friderici*

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(*Received 1 July 2002; revised 6 November 2002; accepted 20 November 2002*)

SUMMARY

A new histozoic species of myxosporean was found to infect the gill filaments, gut, kidney and liver of the freshwater teleost *Leporinus friderici*, collected from the estuarine region of the Amazon, near the city of Belém, Brazil. The plasmodia show asynchronous development, at any one time composed of mature spores and all sporogonic stages. The ellipsoidal spore body, measuring 10·4 μm long and 5·7 μm wide, consists of 2 equal shell valves adhering together along the straight suture line. Each valve has a caudal process measuring 23·3 μm in length. There are 2 symmetric polar capsules, without intercapsular appendix, measuring 5·0 μm × 2·1 μm, and each has a polar filament with 7–8 coils. In general, ultrastructural details of sporoblast and spore development are in agreement with previously described myxosporeans. Some ultrastructural aspects such as cellular alterations of the pericyte in the different organs infected and characterization of the sporoplasmosomes during the sporoplasm maturation are described. This parasite was studied under light and electron microscope and compared with others species of the genus *Henneguya*, considering also host specificity. From our observations we propose the creation of a new species, *Henneguya friderici* n. sp.

Key words: ultrastructure, Myxozoa, *Henneguya friderici* n. sp., parasite, Amazonian fish.

INTRODUCTION

Since the first description of *Henneguya* Thélohan, 1892 (Lom & Dyková, 1992), the second largest genus of Myxobolidae family, many species have been reported, mainly parasitizing freshwater fishes throughout the world. In total 27 species have been described from Brazilian fauna, by light microscopy photos and diagrammatic illustrations (Walliker, 1969; Kent & Hoffman, 1984; Gióia & Cordeiro, 1996; Eiras, 2002). More recently, ultrastructural studies on developmental life-cycle stages and on mature spores, supported the classification of 8 of those species (Rocha, Matos & Azevedo, 1992; Azevedo & Matos, 1995, 1996, 2002, 2003; Azevedo, Corral & Matos, 1997; Casal, Matos & Azevedo, 1997; Vita *et al*. 2003).

In the present paper, we report light and electron microscopical data on the sporogenesis and mature spores of a new parasite, designated herein as *Henneguya friderici* n. sp., infecting several organs of a teleost fish of some economical importance from the river Amazon.

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MATERIALS AND METHODS

Fish, location of infection and prevalence

Several infected adult specimens of the freshwater teleost *Leporinus friderici* Bloch, 1794 (Teleostei, Anostomidae) (Brazilian common name 'aracú'), were collected from the estuarine region of the River Amazon (01° 11′ 30″ S/47° 18′ 54″ W) near the city of Belém, Brazil. The prevalence of infection was 30% (9 fishes in 30 examined) in both sexes. The fishes were dissected and the infected gills, gut, kidney and liver containing numerous cyst-like plasmodia were removed and examined by a light microscope equipped with Nomarski interference-contrast (DIC) optics.

Electron microscopy

For ultrastructural studies, small fragments of the parasitized tissues were excised and fixed in 3% glutaraldehyde in 0·2 M sodium cacodylate buffer (pH 7·2) at 4 °C for 5 h. After washing in the same buffer and post-fixation in 2·0% osmium tetroxide in the same buffer both for 2 h at 4 °C, the fragments were dehydrated through a graded ethanol series, followed by propylene oxide and embedded in Epon. Ultra-thin sections were contrasted with aqueous uranyl acetate and lead citrate and observed with

Parasitology (2003), **126**, 313–319.
DOI: 10.1017/S0031182003002944 Printed in the United Kingdom

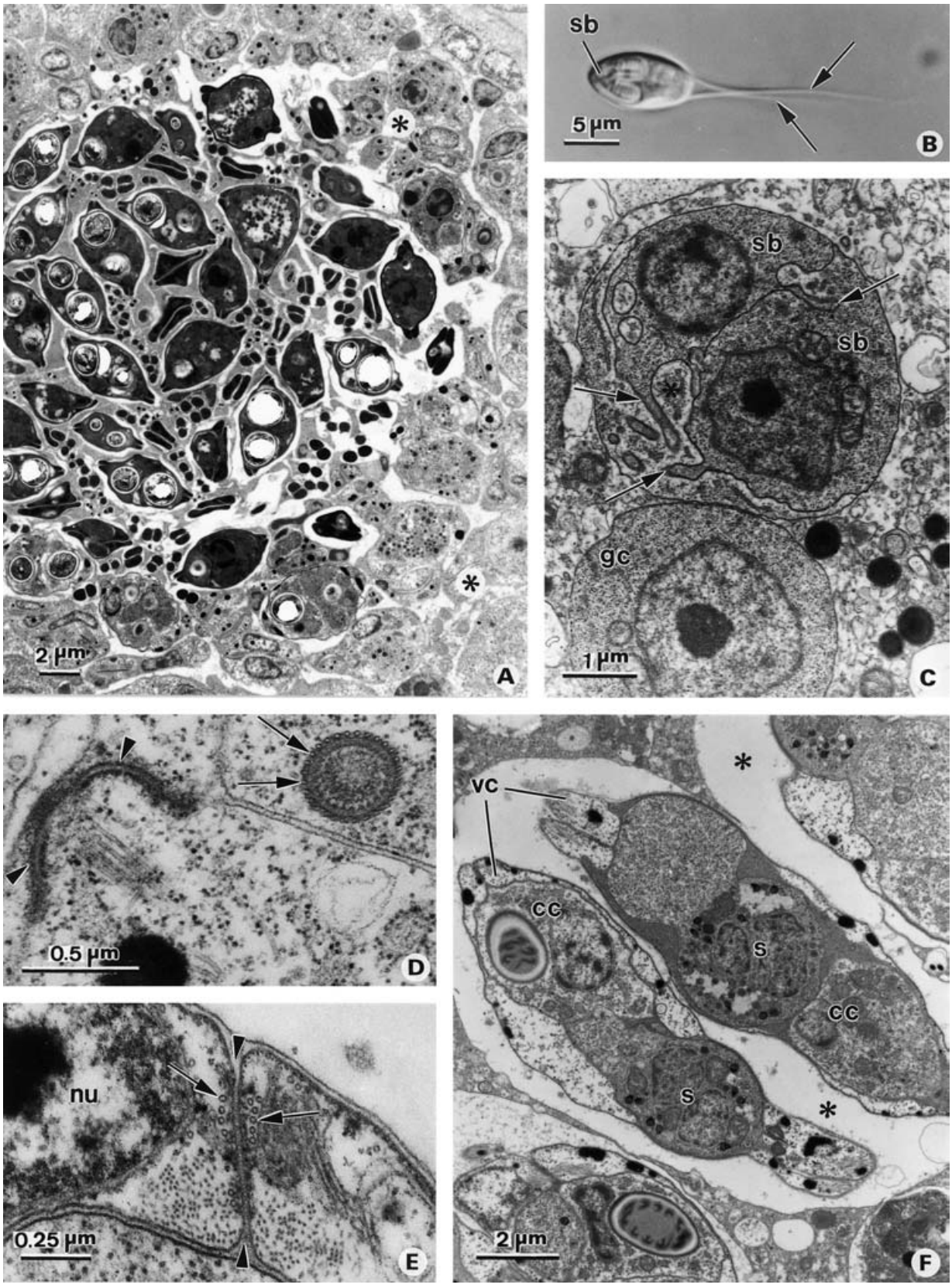

Fig. 1. (A) Ultra-thin section of a plasmodium of *Henneguya friderici* localized in *Leporinus friderici* gill filaments showing sporogonic stages (*) and mature spores in the central zone. (B) Isolated mature spore observed by Nomarski differential interference contrast photomicrography. Note the spore body (sb) and the bifurcated tail (arrows). (C–F) Transmission electron microscopy images of *H. friderici* infecting the gut showing different sporogonic stages of the life-cycle. (C) Ultra-thin section showing 1 generative cell isolated (gc) and 2 sporoblast cells (sb) into the pericyte (*). Note several pseudopodia simultaneously projected to both cells (arrows). (D) Details of the 2 capsulogenic cells showing increased membrane density (arrowheads) in the discharge channel region and several

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a transmission electron microscope (TEM) JEOL 100CXII operated at 60 kV.

For scanning electron microscopy (SEM), isolated spores removed from mature plasmodia, were fixed as described above. Then, the material was dehydrated in ethanol, gold coated and examined in a JEOL 35 SEM operated at 15 kV.

RESULTS

Systematic position

Phylum Myxozoa, Class Myxosporea, Order Bivalvulida, and Family Myxobolidae, according to the classification proposed by Lom & Noble (1984).

Description of the species

Henneguya friderici n. sp.
Type host: *Leporinus friderici* Bloch, 1794 (Teleostei, Anostomidae).
Host size: 15 cm of the length in average.
Type locality: estuarine region of the river Amazon (01° 11′ 30″ S/47° 18′ 54″ W), near Belém (Pará), Brazil.
Location in the host: histozoic infecting several organs, such as gills, gut, kidney and liver.
Prevalence and intensity: 9 out of 30 adult fishes were parasitized and in equal % in both sexes.
Type specimens: 2 slides containing matures spores of the holotypes were deposited in the International Protozoan Type Slide Collection at Smithsonian Institution Washington, DC. 20560, USA with acquisition number (USNM n° 1007181). The histological semi-thin sections showing varied developmental stages were deposited at the laboratory of the senior author.
Etymology: the specific name is derived from the name of the host species ('friderici').
Description of spores: for description of the mature spores scanning electron microscopy (SEM) (Fig. 2A), light microscopy (DIC) (Fig. 1B) and a schematic drawing (Fig. 3) were used. Variability in shape and size of the spores on the different organs was not observed and the measurements were done with plasmodia obtained from the gut tissue. The spores were ellipsoidal with a total length 33·8 (28·7–39·3) μm ($n=25$), body length 10·4 (9·6–11·8) μm ($n=25$), body width (frontal view) 5·7 (4·8–6·6) μm ($n=25$) and body thickness (side view) 4·9 (4·6–5·2) μm ($n=25$). The valves, symmetric and thin, are each prolonged by a caudal process 23·3 (19·1–28·7) μm ($n=25$) long. The spores presented neither a mucous envelope nor particular details on the surface when observed by SEM (Fig. 2A). Elongated polar capsules localized in the anterior pole of the spore were of equal size, measuring 4·98 (4·25–5·90) μm in length ($n=25$) by 2·14 (1·59–2·62) μm ($n=25$) in width and there is not an intercapsular appendix. Inside the polar capsules, the polar filament is coiled 7–8 turns obliquely to the longitudinal axis (Fig. 2B).

Ultrastructural observations

Several organs of the freshwater teleost, *L. friderici*, were found to be parasitized by a myxosporidian of the genus *Henneguya* Thélohan, 1892. Whitish and round-shaped polysporic plasmodia, measuring about 0·5–1·0 mm, indicated an asynchronous development. These were composed of vegetative nuclei and different sporogenesis stages, such as generative cells and early sporogonic stages predominantly along the plasmodium periphery, while immature and mature spores were more internally localized in the centre (Fig. 1A). Pansporoblast formation was disporoblastic (gives rise 2 spores), typical of the myxobolids and comprised envelopment by a pericyte capsulogenesis, valvogenesis and sporoplasm maturation (Fig. 1F).

At the beginning of the sporogenesis phase, 2 morphologically identical rounded generative cells were frequently observed in narrow association. Initially both cells projected numerous pseudopodia inside the cytoplasm of another cell and later this association finished with the envelopment of one of them, the sporogonic cell by the pericyte (Fig. 1C). The sporogonic cell divided several times giving rise to a disporic pansporoblast stage (Fig. 1F) composed by valvogenic, capsulogenic and sporoplasm cells that later gave rise to 2 mature spores (Figs 1B and 2A, B).

Frequently, several longitudinally oriented microtubules were found in the cytoplasm of capsulogenic and valvogenic cells, surrounding the external tube (Fig. 1D) and giving form to the valve and caudal process (Fig. 1E) respectively.

The binucleate sporoplasm cell, which contained an iodinophilous vacuole, mitochondria, an extensive system of cisternae of endoplasmic reticulum and numerous electron-dense vesicles, sporoplasmosomes, was located in the spore posterior pole (Figs 1F and 2E, F). A single membrane limits the sporoplasmosomes and during the early sporogonic stages they appeared as a spherical body. After sporoplasm maturation, they changed their form and appeared like a teardrop, approximately 190–210 nm in diameter

microtubules bundle-oriented to the external tubule in transverse section (arrows). (E) Detail of a sutural line (arrowheads) of adjoining valvogenic cells with several associated microtubules (arrows). Nucleus of a valvogenic cell (nu). (F) Two immature spores lie in a vacuole in the pericyte (*) showing cellular differentiation, into valvogenic cells (vc), capsulogenic cells (cc) and sporoplasm binucleated (s).

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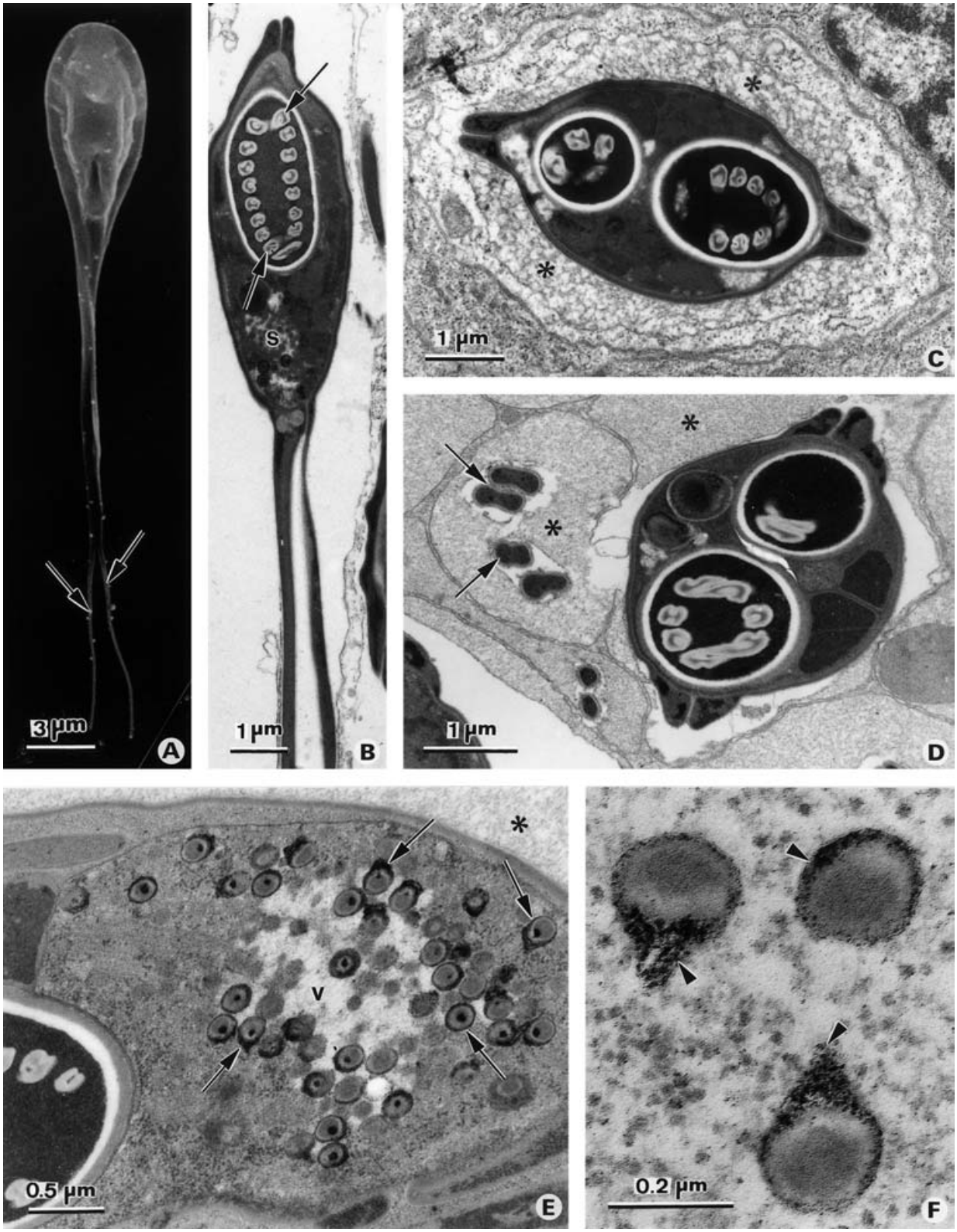

Fig. 2. (A–F) Electron micrographs of *Leporinus friderici* showing different organs infected by *Henneguya friderici*. (A) Scanning electron microscopy image of a mature spore showing 2 individual tail projections (arrows). (B–F) Transmission electron microscopy images of *H. friderici* in late sporogonic stages of the life-cycle. (B) Mature spore in lateral section showing a polar capsule in an anterior position containing 7–8 coils of the polar filament (arrows) and the sporoplasm (S) with sporoplasmosomes. (C) Transverse section of the mature spore in kidney showing 2 polar capsules side-by-side and a flocculent material surrounding the spore (*). (D) Mature spores found in the liver cut in different transverse sections showing 2 pairs of the caudal process (arrows) and the cytoplasmic preservation of the pericyte cell (*). (E) Ultra-thin section of the sporoplasm cell showing numerous sporoplasmosomes, with a characteristic form (arrows) near of the iodinophilous vacuole (v). Pericyte cell (*). (F) Detail of 3 sporoplasmosomes resembling the teardrop with an unusual electron density externally (arrowheads).

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Table 1. Comparative measurements of the spore from the Brazilian species of the genus *Henneguya* that present morphological similarities

Species	Host	TL	BL	BW	TaL	PCL	PCW	FC	References
H. leporini	*Leporinus mormyrops*	28–33	13–15	5·0	15–18	—	—	—	Nemeczek (1926)
H. fonsecai	*Leporinus copelandi*	23–27	10–12	4·5–5·0	13–15	—	—	—	Guimarães (1931)
H. santae	*Tetragnopterus santae*	21·0	9·6	5·3	11·2	2·9	—	—	Guimarães & Bergamin (1934)
H. visceralis	*Electrophorus electricus*	22–24	11–12	5·0–6·5	11–12	6·5–8	2	—	Jakowska & Nigrelli (1953)
H. electrica	*Electrophorus electricus*	35–39	11–13	6–8	24–27	5–7	2	—	Jakowska & Nigrelli (1953)
H. adherens	*Acestrorhynchus falcatus*	32·3	12·4	5·8	20·5	3·1	1·2	3–4	Azevedo & Matos (1995)
H. malabarica	*Hoplias malabaricus*	28·3	12·6	4·8	17·1	3·7	1·8	6–7	Azevedo & Matos (1996)
H. friderici	*Leporinus friderici*	33·8	10·4	5·7	23·3	5·0	2·1	7–8	Present study

(Abbreviations: TL, total length; BL, body length; BW, body width; TaL, tail length; PCL, polar capsule length; PCW, polar capsule width; FC, number of the polar coil.)

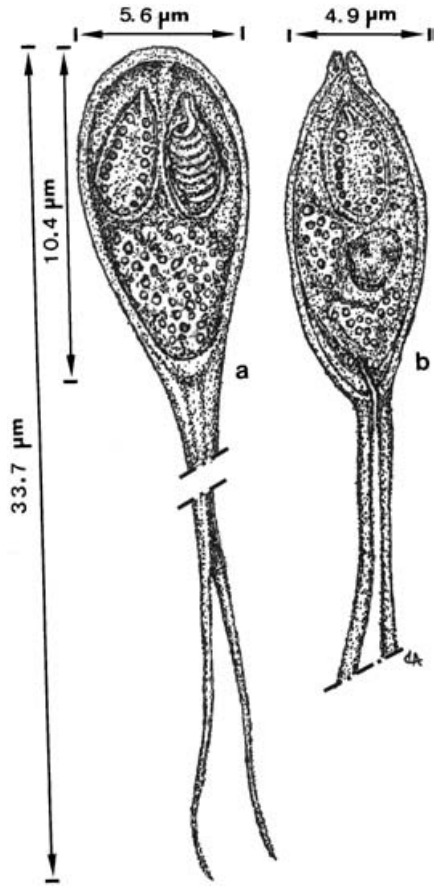

Fig. 3. Schematic drawing in 2 longitudinal sections, frontal (a) and lateral (b) view, of the spores *Henneguya friderici* n. sp.

(Fig. 2E, F). At the periphery, there was unusual deposited material with high electron density and which was uniformly distributed except on the peaked side of the vesicle where it was accumulated in greater quantity. Internally, many have a central dense dot (Fig. 2E) and in the protuberance region there was a small channel differentiated with strong electron density (Fig. 2E, F).

In the different organs, heterogeneous ultrastructural aspects during sporogenesis concerning the pericyte, were observed. In the kidney infection pericyte cellular integrity persisted until late sporogenesis, characterized by organelle preservation. Simultaneously, flocculent material appeared between mature spores and pericyte, that was gradually compressed in later sporogenesis stages (Fig. 2C). In other organs, all pericyte cytoplasm was occupied by thinly granular material as seen in the liver (Fig. 2D) and, in an opposite situation, early degeneration of the enveloping cell in the gut infection was also observed (Fig. 1E).

DISCUSSION

Sporogenesis of this parasite presents many similarities with other species previously described, such as *H. psorospermica* (Lom & Puytorac, 1965),

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H. exilis (Current & Janovy, 1977), *H. adiposa* (Current, 1979) and *H. amazonica* (Rocha *et al.* 1992). Although the differentiation and maturation process of the sporoplasm cell has been well studied, little is known about the nature and function of the electron-dense sporoplasmosomes, typical of these parasites. They have been in the sporoplasm of numerous myxosporidia and their morphological characteristics, such as form, size and inner organization have no relationship with genus or family (Lom *et al.* 1989; Lom & Dyková, 1992). In the studies of freshwater fish parasites from the River Amazon belonging to the genus *Henneguya* (Rocha *et al.* 1992; Azevedo & Matos, 2002; Vita *et al.* 2003) and *Myxobolus* (Casal, Matos & Azevedo, 1996) the sporoplasmosomes have been ultrastructurally characterized. The form and size heterogeneity of these vesicles show that it is possible to use this cellular structure as an ultrastructural parameter to differentiate parasites of the same genus, mainly when they are diagnosed in hosts from the same hydrological basin. In this case, an atypical electron-dense material surrounding the vesicle and forming a protuberance has never been described before.

The ultrastructural aspects of the enveloping cell during sporogenesis have been occasionally described as a gradual and generalized degeneration. Sometimes microfibril-forming regions (Current & Janovy, 1977) or long microfilaments resembling myosin filaments (Casal *et al.* 1997) in the cytoplasmatic space have been described. In this study we found some heterogeneity in the morphological aspects of the pericyte in the different organs infected, from a relative organelle preservation in liver tissue to an extensive degradation, as verified in the gut infection. This fact seemed indicative of the existence of some adaptation of the pericyte to the host infected tissue.

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 with other myxosporidian species of the genus *Henneguya*, from different geographical areas mainly, with those that have Brazilian freshwater fishes as host (Table 1).

Among the more than 100 non-Brazilian species described in the literature (see the revision papers Kostoïngue *et al.* 2001 and Eiras, 2002), 7 species, *H. lagodoni* (Hall & Iversen, 1967), *H. shaharini* (Shariff, 1982), *H. latesi* (Haldar, Das & Sharma, 1983), *H. mystusia* (Sarkar, 1985), *H. laterocapsulata* and *H. suprabranchiae* (Landsberg, 1987) and *H. mbourensis* (Kpatcha *et al.* 1997) present approximately the same body size. Although all have a different body shape not many of those species were ultrastructurally characterized.

Brazilian species have mostly been described by light microscopy or simply by schematic drawings (Walliker, 1969; Kent & Hoffman, 1984; Gióia & Cordeiro, 1996) and some of them show morphological similarities. The species *H. leporini* (see Nemeczek, 1926) and *H. fonsecai* (see Guimarães, 1931) parasitize fishes of the same genus *Leporinus*, but although they were caught in rivers with a distinct geographical localization, they are similar in total size and body shape, respectively. Of 3 other species with similar body shape, *H. santae* (Guimarães & Bergamin, 1934) and *H. visceralis* (Jakowska & Nigrelli, 1953) present a smaller size, while *H. electrica* (Jakowska & Nigrelli, 1953) is longer.

During the last 10 years, this group of parasites has been studied at the ultrastructural level, resulting in the description of 8 new Brazilian *Henneguya* species, all in different hosts (Rocha *et al.* 1992; Azevedo & Matos, 1995, 1996, 2002, 2003; Azevedo *et al.* 1997; Casal *et al.* 1997; Vita *et al.* 2002). Of these, only the spores of *H. adherens* (Azevedo & Matos, 1995) and *H. malabarica* (Azevedo & Matos, 1996) resemble in size the parasite studied by us in *L. friderici*. However, *H. friderici* lacks a sheath around the 2 tails and differs in the arrangement of the polar filament coil.

After a comparative study based on the morphological and ultrastructural differences with *Henneguya* species previously described, we conclude that *Henneguya friderici* is a new species.

This work was partially supported by a grant from the Engº António Almeida Foundation, Porto, Portugal. We would like to thank the iconographic work of Mr João Carvalheiro. The technical assistance provided by Mrs Laura Corral is gratefully acknowledged.

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