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Henneguya ghaffari sp. n. (Myxozoa: Myxosporea), infecting the Nile perch *Lates niloticus* (Teleostei: Centropomidae)

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ABSTRACT: Light microscopical description is presented for a new myxozoan species, *Henneguya ghaffari*, which infects the Nile perch *Lates niloticus* (Linnaeus, 1758) in Lake Wadi El-Raiyan in Egypt. The spore is characterized by a triangular thickening at the base of the caudal processes. The relatively long caudal processes run adherent to each other for two-thirds of their length, then bifurcate to very fine processes. Prevalence of infection was 34.6% and peaked during winter and early spring. The infection was concentrated along the intestinal tract, and in severe cases gills and gill rakers were also infected. Histology revealed that, in contrast to findings of previously published works on related species, intralamellar plasmodia did not develop inside the blood capillaries of the gills. Intestinal plasmodia were very pathogenic due to their large number and size. These plasmodia caused atrophy of the muscularis layer, and replaced and distended the submucosal and mucosal layers. The validity of some *Henneguya* species in Africa is discussed.

KEY WORDS: *Myxosporea* *Henneguya* · Fish parasites · Nile · Egypt

INTRODUCTION

During the last decade, myxosporean parasites of fish in Africa have received growing attention. Efforts in Cameroon, Nigeria, Benin and Egypt have resulted in the description of approximately 100 myxosporean species (Fomena & Bouix 1997). However, given the very large diversity of fish fauna in Africa (several thousand species) one would expect that a great many myxosporeans remain to be discovered. For example, the genus *Henneguya* Thelohan, 1892 is represented by only 11 species from freshwater fishes of Africa. Some of the reported infections are very pathogenic and even fatal to the hosts (Obiekezie & Enyenihi 1988). In this paper, a new species, *Henneguya ghaffari*, is described from the commercially important Nile perch *Lates niloticus* (Linnaeus, 1758) and the associated pathological changes in the infected organs are presented. Also, the validity of some described African *Henneguya* species is discussed.

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MATERIALS AND METHODS

Fish samples were collected from Lake Wadi El-Raiyan in the western desert of Egypt. Monthly fish samples were examined from November 1997 to December 1998. A total of 188 fishes of both sexes were examined and their mean length was 27.7 ± 11.7 (15–62) cm. Description and preparation of spores followed the guidelines of Lom & Arthur (1989). For histology, infected organs were fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned at 5 to 7 µm thickness and stained with haematoxylin/eosin. Measurements are presented as mean ± SD (range).

RESULTS

Plasmodia were mainly concentrated along the length of the intestine and the pyloric caeca. In cases of severe infection, the cysts were also found in the gill filaments, gill arches and rakers. In some infected fishes, up to about 1400 cysts were counted along the

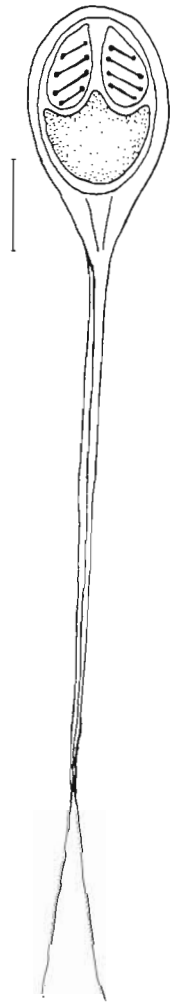
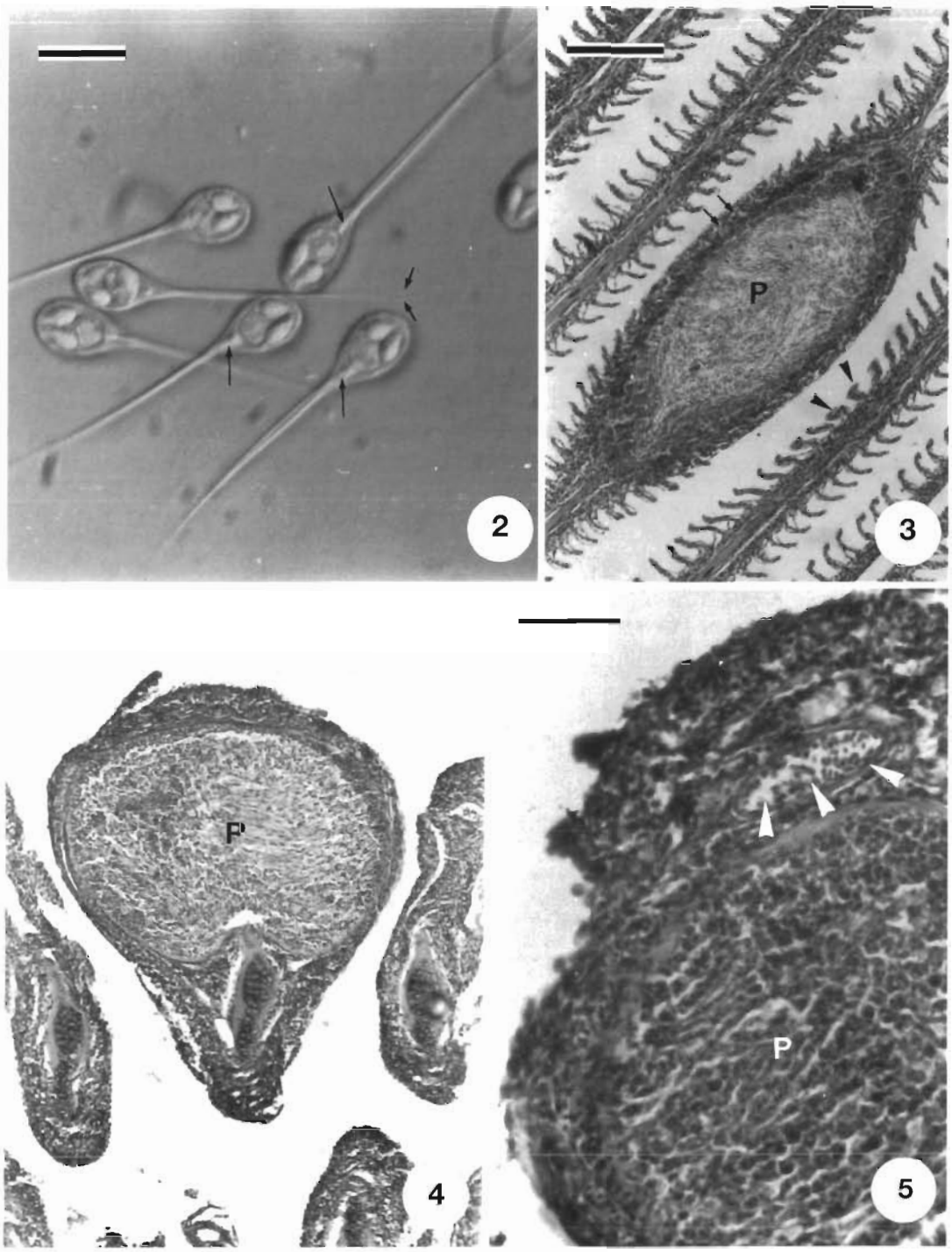


Fig. 1 *Henneguya ghaffari* sp. n. Schematic drawing of the spore. Scale bar = 5 µm



Figs. 2 to 5. *Henneguya ghaffari* from *Lates niloticus*. Light micrographs. Fig. 2. Fresh spores with triangular thickening at the base of caudal processes (long arrows) and very thin bifurcation of the tail (short arrows). Fig. 3. Intralamellar plasmodium (P) in longitudinal section of gills showing hyperplasia of epithelial cells (arrows) covering the plasmodium and curling of neighboring lamellae (arrowheads) due to plasmodial mass. Figs. 4 & 5. Transverse sections of gill filament showing (Fig. 4) the microhabitat of the plasmodium and swelling of the filament due to cyst formation; (Fig. 5) plasmodium growing outside the blood capillaries (arrowheads). Scale bars: Fig. 2, 10 µm; Figs. 3 & 4, 150 µm; Fig. 5, 40 µm

intestinal tract, while infected gill filaments had 2 to 8 cysts. Total prevalence of the infection was 34.6% (65/188) and peaked during late winter and early spring. Both sexes of *Lates niloticus* were susceptible to the infection at all lengths.

Spores

Mature fresh spores are oval in frontal view with a rounded anterior end and characteristic triangular thickening at the base of the long caudal processes. The relatively long caudal processes run adherent to each other then bifurcate at two-thirds of their length to very fine processes (Figs. 1 & 2). Dimensions (in µm), measured on 40 spores, were: total length of the spore = 57.5 ± 3.7 (48.1–66.5), spore length = 13.0 ± 0.6 (11.8–14.0), spore width = 7.5 ± 0.4 (6.9–7.9), spore thickness = 5.2 ± 0.5 (4.9–5.9), and tail length = 43.2 ± 3.5 (36.3–53.0). Polar capsules were typically equal, sometimes slightly unequal, pyriform and occupied about half of the spore length. They measured 5.2 ± 0.8 (4.8–5.9) in length and 3.2 ± 0.3 (2.8–3.9) in width. Polar filaments showed 4 or 5 coils oblique to the longitudinal axis of the capsules.

Histology

Branchial region. Intralamellar, spindle-shaped plasmodia were observed in the gill filaments (Fig. 3). Plasmodia (n = 60) measured 1.0 ± 0.09 (0.5–1.3) mm in length and 0.4 ± 0.06 (0.2–0.5) mm in width. Cysts were usually located at the distal third, or sometimes the middle, of the gill filaments. A few plasmodia were observed inside the cartilage of the gill arch and the rakers (see Fig 6).

Intralamellar plasmodia were encased in a thin wall surrounded by flattened endothelial cells. A hyperplastic interlamellar epithelial layer covered the plasmodia. Gill lamellae were atrophied and obliterated at the cyst site. The protruding plasmodial mass caused some curling of the adjacent lamellae (Fig. 3). No signs of obstruction of the blood capillaries were observed in the infected gill filaments.

In transverse sections of the spindle-shaped intralamellar plasmodia, the gill filament appeared swollen and filled with the growing cyst. Sections at the 2 ends of the plasmodium showed that it was located within the septal tissue between the efferent blood capillary and the cartilage of the filament; sections at the middle part (widest) of the plasmodium showed gradual pressure of the developing cyst mass on the adjacent blood capillary until it was completely occluded (Figs. 4 & 5).

In gill rakers, plasmodia were located between trabeculae of spongy bone (Fig. 6) and delimited by a thin plasmodial wall and flat endothelial cells with enlarged nuclei.

Intestinal tract. Plasmodia in the intestinal tract were round to oval, with a diameter of 0.8 ± 0.3 (0.3–1.4) mm (n = 100). Up to 6 plasmodia were found in 1 section of the intestine and largely constricted and narrowed the lumen. In some sections of the pyloric caeca, the whole lumen was occluded by the cyst masses.

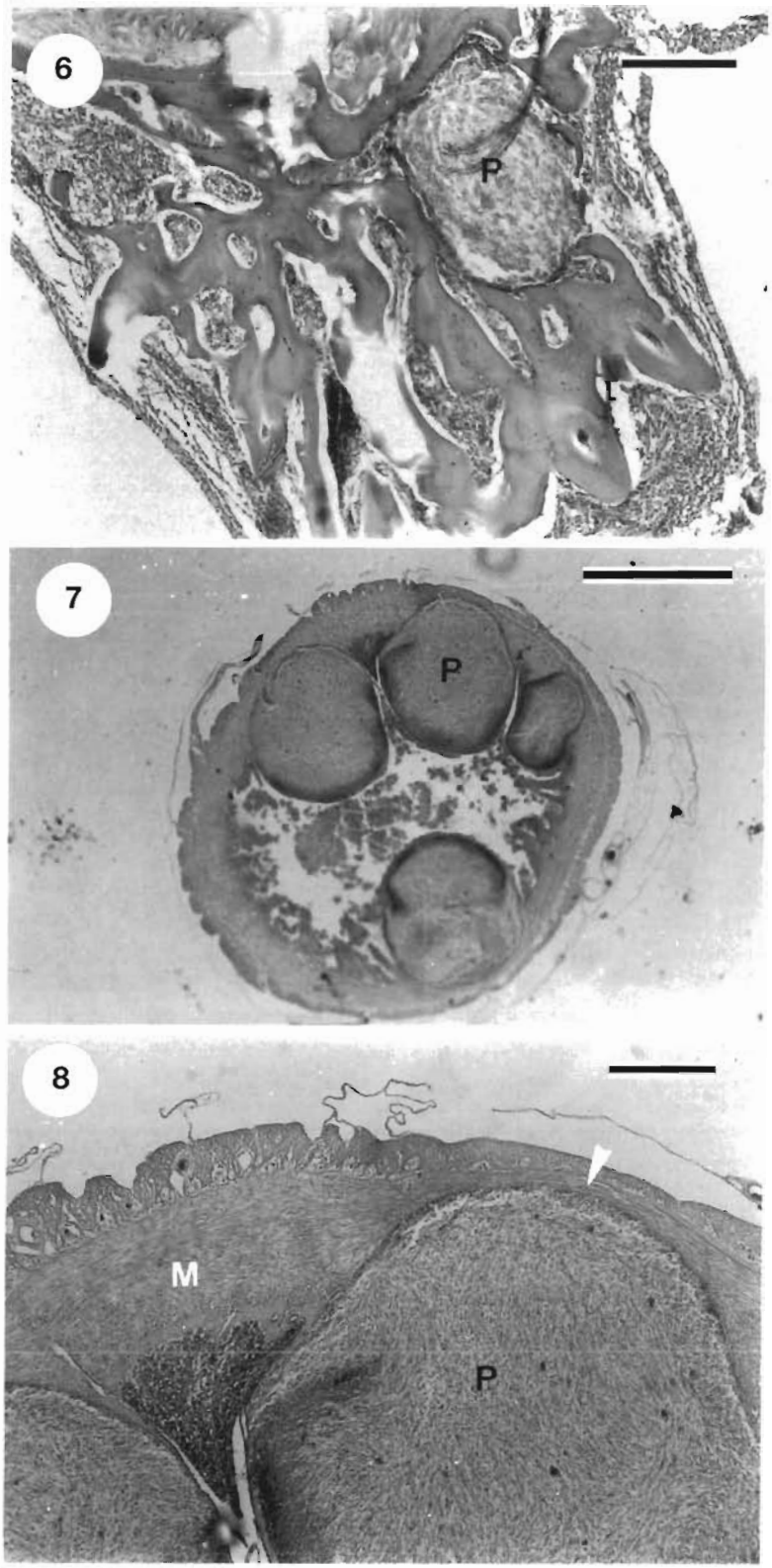
The cysts in the intestine, pyloric caeca and rectum had their origin in the circular muscle fibers of the muscularis. The growing plasmodial masses replaced and considerably distended the submucosa and lamina propria which appeared as a thin layer of loose connective tissue enveloping the cyst. In the rectum, the plasmodia extended into the muscle layer and distended the submucosal and mucosal layers (Figs. 7 & 8). A relatively thick eosinophilic plasmodial wall was observed around the cysts, but no fibrosis or cellular response was observed. Mature spores were concentrated at the center of the plasmodia while the immature spores and sporogonic stages formed a thick layer at the periphery.

DISCUSSION

Among the *Henneguya* species described in other continents (Table 1), *Henneguya dogielli* Akhmerov, 1960 (Shulman, 1966) from gills of Chinese perch and *H. amazonica* Rocha et al., 1992 from the gills of *Crenicichla lepidota* are comparable to the present material. *H. dogielli* is smaller in spore dimensions but has a longer tail and an intercapsular process. *H. amazonica* has a narrower spore, smaller polar capsules, and contains 6 filament coils compared to 4 or 5 in the present spores.

Comparing the African *Henneguya* species with the present species, 3 species were similar in shape to the present material; *Henneguya* sp. from the gills of *Ctenopoma maculatum* reported by Fomena & Bouix (1987), *H. nyongensis* Fomena & Bouix, 1996 from the gills and muscles of *Marcusenius moorii*, and *H. malapteruri* Fomena & Bouix, 1997 from the muscle of *Malapterurus electricus*. The first species has smaller overall dimensions, especially in spore width, polar capsule and tail length. *H. nyongensis* has narrower spores, a polar capsule 'neck' and a much shorter tail. *H. malapteruri* differs in its shorter total spore length and longer spore body.

Paperna (1996) illustrated stained spores and cysts from gills of *Lates niloticus* in Lake Victoria and identified them as '*Henneguya* sp.' Although he did not provide further information about this *Henneguya*, it is



Figs. 6 to 8. Plasmodia of *Henneguya ghaffari* in different organs of *Lates niloticus*. Fig. 6. Longitudinal section of gill raker infected with a plasmodium (P) between the spongy bone. Figs. 7 & 8. Transverse section of rectum showing (Fig. 7) plasmodia constricting the lumen and distending the submucosal and mucosal layers; (Fig. 8) atrophied circular muscular layer (arrowhead) due to massive plasmodial mass. M: circular muscle layer. Scale bars: Fig. 6, 150 µm; Fig. 7, 100 µm; Fig. 8, 200 µm

likely that he was dealing with the same species as that under investigation here.

The present species was also distinguished by its triangular thickening and pattern of tail bifurcation. In addition, the comparable species are reported from hosts that are not found in the Nile (except for *Malapterurus electricus*). The present species also represents the only one in Africa forming intralamellar plasmodia. For these reasons, the present *Henneguya* shows adequate criteria to be considered a new species, and the specific name is dedicated to F. Abdel Ghaffar, Professor of Parasitology, Cairo University. Type specimens of this materials are deposited at the Dept. of Zoology, Faculty of Science, Cairo University, Beni Suef branch (FWP-101,102).

Comment on the validity of some *Henneguya* species in Africa

Fomena & Bouix (1997) listed 15 *Henneguya* species in freshwater fishes in Africa. In their review, *Henneguya branchialis* Ashmawy et al., 1989 was included as a species infecting *Clarias lazera*. This species is a synonym of *H. suprabranchiae* Landsberg, 1987, which was reported from the same host and infection sites. Also, *H. bopeleti* Fomena & Bouix, 1987 from the catfish *Chrysichthys nigrodigitatus* is most likely the same species as *H. suprabranchiae*, as the only difference lies in the number of filament turns (7–9 vs 9–10) which was observed by Abdel-Ghaffar et al. (1995). The 3 *Henneguya* species mentioned by Paperna (1973) should not be considered as valid species until full descriptions are provided. On this basis, only 11 valid *Henneguya* species, including the present one, have been recorded so far in Africa.

Histology

Intralamellar plasmodia of *Henneguya ghaffari* were similar to those of *H. psorospermica*, as reported by Dykova & Lom (1978) and El-Matbouli et al. (1992). The present infection caused hyperplasia of the interlamellar epithelial cells and atrophy of the respiratory lamellae at the cyst site.

Table 1. *Henneguya* spp. comparable with *H. ghaffari* sp. n. Dimensions in µm

Species	Spore		Polar capsule (Pc)		Pc coils	Caudal process
	Length	Width	Length	Width		length
H. dogieli Akhmerov, 1960 (Shulman, 1966)	8.5–14	4.5–7	4.5–5	2–2.5	–	50–65
H. amazonica Rocha et al., 1992	13.9 (11.5–14.9)	5.7 (5.2–6.3)	3.3 (2.7–3.6)	1.5 (1.1–1.9)	6	45.4 (41.7–52.1)
Henneguya sp. Fomena & Bouix (1987)	11.65 (10–12.5)	4.51 (3.7–5)	5.26 (4.6–6.3)	1.62 (1.3–2)	–	32.51
H. nyongensis Fomena & Bouix, 1996	12.6 (10–14)	5.4 (4.5–6.5)	6.2 (5.5–7)	2.3 (2–2.8)	4–5	21 (20–23.5)
H. malapteruri Fomena & Bouix, 1997	16.2 (14–18)	9.6 (8.3–11)	5.9 (5–7.3)	3.3 (2.8–4)	4–6	30 (24–36)
H. ghafferi sp. n.	13 (11.8–14)	7.5 (6.9–7.9)	5.2 (4.8–5.9)	3.2 (2.8–3.9)	4–5	43.2 (36.3–53)

McCraren et al. (1975) classified the branchial forms of *Henneguya* infection into interlamellar and intralamellar. They defined intralamellar forms as developing within capillaries of gill lamellae or blood vessels of the gill filaments. Dykova & Lom (1978) and El-Matbouli et al. (1992) confirmed these results in *H. psorospermica* infections. Conversely, Current & Janovy (1978) indicated through ultrastructure study that the origins of the intralamellar *Henneguya* plasmodia lay within the lamellar tissues adjacent to sinuses or capillaries. Also, Molnar (1979) showed that a similar infection of *Myxobolus pavlovskii* develops within the epithelium between the gill lamellae and the plasmodia compressed against the gill capillary walls only in a later stage of growth. Price & Mellen (1980) reported a similar case of *M. microcystus* in which the plasmodia were restricted to the septal tissue of the efferent artery. The present study demonstrates that the intralamellar forms of *Henneguya* develop 'outside' the blood capillaries of the gill filament.

Infections of myxosporidia in cartilage and bone of fish are rarely reported, and to our knowledge no infection has been reported in the gill rakers. Plasmodia of *Henneguya ghaffari* in the rakers occupied areas of connective tissue in the spongy bone, and some blood capillaries were compressed by these masses. However, in the present case the low number of plasmodia in the gill filaments and rakers reduced the pathology at this site.

Gastrointestinal plasmodia of myxosporeans are usually confined to one of the tissue layers of this tract. The present plasmodia originated in the circular muscle layer of the intestinal tract and extended to the inner layers. This pattern of growth is unlike many other infections that are exclusive to the muscularis, e.g. *Kudoa intestinalis* Maeno et al., 1993, *K. ciliata* Lom et al., 1992 and *Myxobolus exiguus* reported by Abdel Ghaffar et al. (1998). This inward growth of the plasmodia is probably due to the easier passage through the loose connective tissue than through the relatively stronger circular muscle fibers outwards. Also, this pattern of growth serves the eventual purpose of spore dissemination.

The pathological changes accompanying infection by *Henneguya ghaffari* are primarily due to the large size and number of plasmodia and their pattern of growth. These changes include considerable atrophy of the intestinal muscularis leading to significant loss of muscular capacity of the intestine of this carnivorous fish, thereby increasing the probability of peritonitis. In addition, large areas of the submucosa and mucosa were replaced by plasmodia. The capacity of the host to digest food would be affected through constriction of the intestinal lumen by the plasmodial masses, which would damage the mucosal layer indirectly by the mechanical friction with food or directly when the spores are released.

Lake Wadi El-Raiyan is a new, man-made lake, and its main source of water is from agricultural drainage. *Lates niloticus* (Nile perch) is one of the well-established Nile fishes in the lake which reached this ecosystem through drainage water. Infection by *Henneguya ghaffari* in the original habitat of *L. niloticus*, the river Nile, should be investigated and compared to the present one. The new habitat of the fish might favour the infection with the present parasite due to the incomplete assembly of the ecosystem.

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