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ORIGINAL PAPER

**Mohammed A. Ali · Khaled A.S. AL-Rasheid
Thabit Sakran · Abdel-Azeem Abdel-Baki
Fathy A. Abdel-Ghaffar**

Some species of the genus *Myxobolus* (Myxozoa: Myxosporea) infecting freshwater fish of the River Nile, Egypt, and the impact on their hosts

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Abstract Six *Myxobolus* species are described from Nile fish, five of which are new and one is redescribed: *M. naffari* Abdel Ghaffar et al., 1998 was recovered from the gills of *Labeo niloticus* and the mouth of *Barbus bynni*; *M. caudatus* sp. n. was observed in the tail fin of *B. bynni*; *M. fahmii* sp. n. occurred in the gills of *B. bynni*; *M. imami* sp. n. was found in the kidney of *L. niloticus*; *M. intestinalis* sp. n. was recorded from the intestine of *B. bynni*; and *M. perforata* sp. n. was found in the internal surface of the operculum of *Hydrocynus forskalii*. The histological effects of some of the *Myxobolus* infections present are described.

Introduction

Myxosporea are fish parasites which form an abundant and diversified group. Many species have been recorded, but relatively few have so far been identified as serious pathogens (Lom and Dykova 1992). In Africa, the work in the field of Myxosporea is still rudimentary with about 100 species currently known from the continent (Fomena and Bouix 1997). River Nile fish in Egypt were first examined for myxosporean parasites by Fahmy et al. (1975), then by Imam et al. (1987), Abdel Ghaffar et al. (1995a, b; 1998), Ali (1998, 1999, 2000) and Ali et al. (1999). During this study, a survey of Myxosporea was carried out on some fish from the River Nile. In addition, the potential threats of the detected parasites to their hosts were examined.

M.A. Ali
National Institute of Oceanography and Fisheries,
Cairo, Egypt

K.A.S. AL-Rasheid (✉) · F.A. Abdel-Ghaffar
Department of Zoology, College of Science,
King Saud University, P. O. Box 2455,
Riyadh 11451, Saudi Arabia
E-mail: krasheid@ksu.edu.sa
Tel.: +966-1-4675758
Fax: +966-1-4678514

T. Sakran · A.A. Abdel-Baki
Zoology Department, Beni-Suef Faculty of Science,
Cairo University, Egypt

F.A. Abdel-Ghaffar
Zoology Department, Faculty of Science,
Cairo University, Egypt

Materials and methods

Live or freshly caught fish specimens were collected from the River Nile at Beni-Suef (120 km south of Cairo) from May 1996 to December 1997. A total of 411 fish, representing 7 families, was examined. The number of each species was: *Barbus bynni* (50), *Labeo niloticus* (64), *Tetradon fahaka* (100), *Malpterurus electricus* (30), *Schilbe mystus* (66), *Mormyrus kannume* (53), *Hydrocynus forskalii* (8), *Synodontis schall* (40).

Organs infected with Myxosporea were fixed in 10% phosphate buffered formalin for histological processing. Paraffin sections of 5 μm thickness were stained with hematoxylin and eosin. Guideline, measurements and description were carried out according to Lom and Arthur (1989). Measurements are given as mean length ± SD (range) × mean width ± SD (range).

Results

All the examined fish were found to harbor myxosporean infection except *Tetradon fahaka* and *Mormyrus kannume*.

Myxobolus naffari Abdel Ghaffar et al., 1998

See Figs. 1a, 4a.

Host

B. bynni and *L. niloticus*.

Site of infection

Mouth of *B. bynni* and gills of *L. niloticus*.

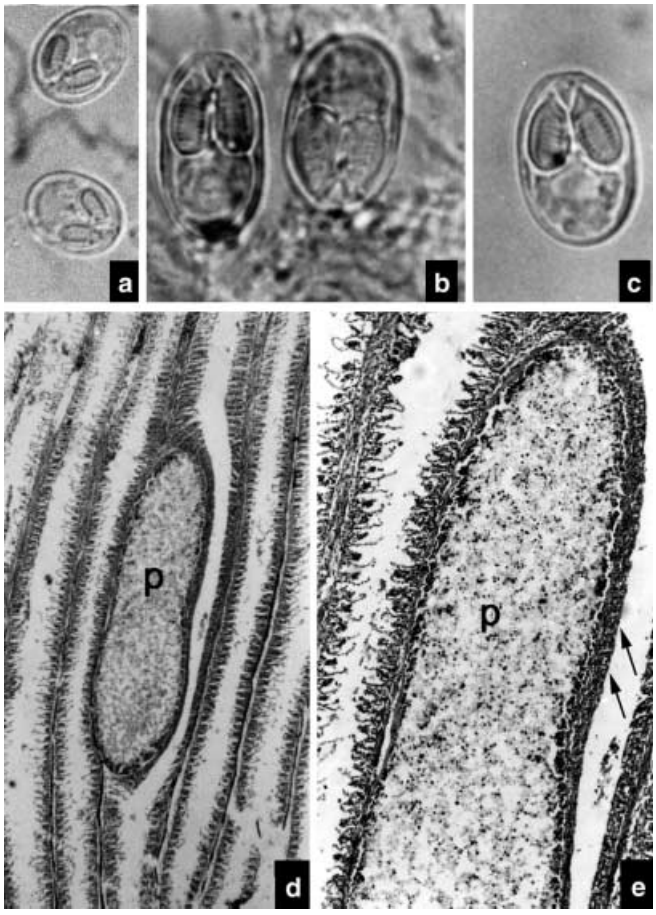

Fig. 1 **a** Photomicrograph of fresh spore of *Myxobolus naffari* Abdel Ghaffar et al., 1998 infecting the gills of *Labeo niloticus* (*bar* 5 μm = 0.8). **b, c** Fresh spore of *M. caudatus* sp. n. infecting the tail of *Barbus bynni* (*bar* 5 μm = 0.8). **d** Longitudinal section of the gill of *L. niloticus* showing the plasmodium (*P*) within the respiratory epithelia surrounded with thin layer of connective tissue. (×152). **e** Enlarged part of the plasmodium (*P*) showing atrophy of the gill lamellae along the external surface of the plasmodium and atrophy of the adjacent one (*arrows*) (× 1,520)

Prevalence

B. bynni: 20% (10/50) and *L. niloticus:* 28% (18/64). Macroscopic whitish plasmodia were scattered in the mouth, lips and the roof of the mouth of *B. bynni*. The infected fish carried 2–12 cysts. They were globular or irregular in shape, measuring 1.0 ± 0.5 (0.5–1.4) × 0.8 ± 0.3 (0.9–1.2) mm. In *L. niloticus*, plasmodia appeared as white rods usually at the proximal third of the gill filament. Fish were infected with 2–7 cysts with average dimensions of 1.2 ± 0.4 (0.8–1.5) × 0.4 ± 0.2 (0.2–0.5) mm.

Description of spores

Spores were subspherical to elliptical in frontal view measuring 11.2 ± 0.6 (10.4–12.0) × 8.5 ± 0.8 (7.2–9.6) μm. A small intercapsular process was present. Polar capsules were oval, equal in size and occupying nearly half of the spore length. They measured 5.8 ± 0.5 (4.6–6.4) × 3.3 ± 0.4 (2.4–4.0) μm. The polar filament had 6–7 coils slightly oblique along the axis of the polar capsule.

Histology

In *L. niloticus*, plasmodia were located within the lining epithelium, covering the gill filaments. Mature spores were located centrally inside the plasmodia while the different developmental stages were arranged peripherally. Developing plasmodia were extended longitudinally and occupied about one-third of the total length of the gill filament (Fig. 1d). At the site of the plasmodia, large parts of the respiratory epithelia of the gill lamellae were fused. The adjacent gill lamellae were atrophied along the plasmodia. Both ends of the plasmodia showed localized areas of hyperplasia while the lamellae at the external surface of the plasmodia were atrophied (Fig. 1e).

Myxobolus caudatus sp. n.

See Figs. 1b, 1c, 4b.

Host

B. bynni

Site of infection

Tail fin.

Prevalence

Found in 16% (8/50). Plasmodia appeared as small, white, oval spots between the rays of caudal fins and close to the caudal peduncle. The number of plasmodia per infected fish was 3–14. They measured 0.5 ± 0.1 (0.4–0.6) × 0.3 ± 0.1 (0.2–0.3) mm.

Description of spores

Spores were elliptical in shape with blunted anterior and rounded posterior ends. The dimensions of the spores were 17.5 ± 1.3 (16.0–19.2)× 12.8 ± 0.9 (11.0–13.6) μm. The sutural line was relatively thin along the boundaries of the polar capsules. The polar capsules were elliptical, equal in size and tapering anteriorly to form a prominent neck. They measured 7.4 ± 0.7 (6.4–9.0) × 3.8 ± 0.5 (3.2–4.5) μm. The polar filament coiled 8–9 times almost perpendicular to the polar capsule axis. A relatively

large intercapsular process characterizing the spores was observed. Sporoplasm was disporic.

Myxobolus fahmii sp. n.

See Figs. 2a, 4c

Host

B. bynni.

Site of infection

Gill filaments.

Prevalence

Found in 24% (12/50). Plasmodia were oval to subcircular in shape and situated mostly at the central part of the gill filaments. The infected fish harbored 2–12 cysts. They measured 0.5 ± 0.4 (0.3–1.0) $\times$ 0.4 ± 0.3 (0.2–0.7) mm.

Description of spores

Spores were pear-shaped with a characteristic nipple-like anterior tip. They measured 11.0 ± 0.5 (10.8–12.0) $\times$ 7.1 ± 0.4 (6.4–8.0) μm. Polar capsules were pyriform, equal in size and occupied more than half the spore length. They measured 6.8 ± 0.5 (6.4–7.2) $\times$ 3.2 ± 0.2 (2.8–3.8) μm. The polar filament formed 6–7 coils at right angles to the longitudinal axis of the polar capsule. Sporoplasm was disporic.

Histology

Histological observation revealed that mature plasmodia were encapsulating the gill lamellae within the epithelial layer (Fig. 2c). This epithelial layer appeared to envelop the entire plasmodium separating it from the pillar cells. Some plasmodia showed stretched and hypertrophied lining epithelial cells (Fig. 2d). In some instances of the fully developed plasmodia, the lamellae were detached from their bases with the gill filaments and appeared as transverse rods inside the cysts (Fig. 2d).

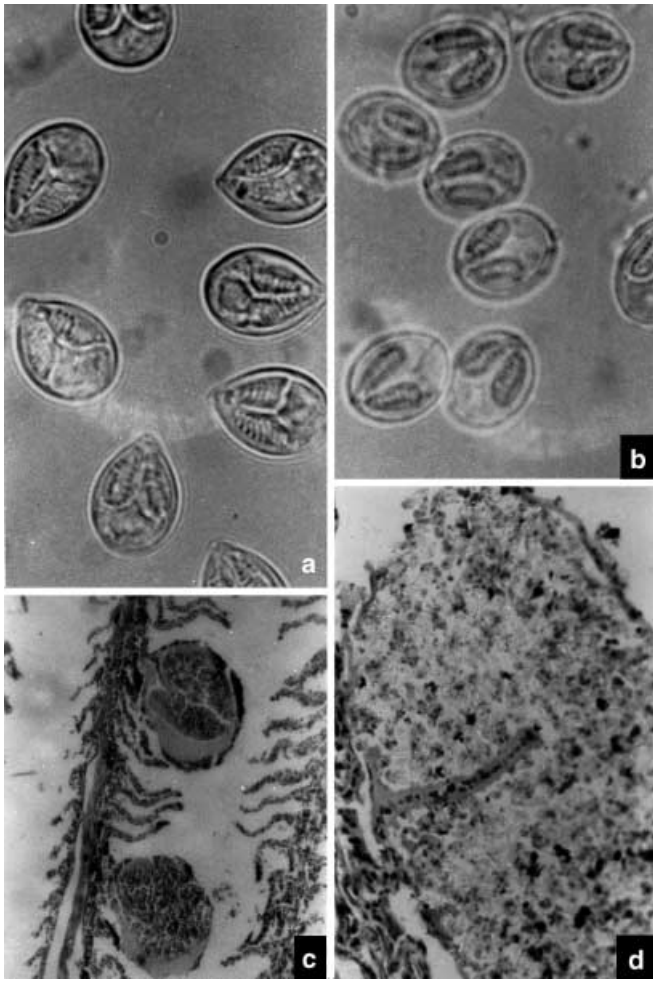

Fig. 2 **a** Fresh spores of *M. fahmii* sp. n. infecting the gills of *B. bynni*. (*bar* 5 μm = 0.9). **b** Fresh spores of *M. imami* sp. n. infecting the kidney of *L. niloticus*. (*bar* 5 μm = 0.8). **c** Longitudinal section of the infected gill of *B. bynni* showing the plasmodium (*P*) encapsulating the gill lamella. (× 380). **d** Enlarged part of the last plasmodium showing the gill lamella as transverse rod inside the plasmodium (*arrow*). (× 1,520)

Myxobolus imami sp. n.

See Figs. 2b, 4d

Host

L. niloticus.

Site of infection

Kidney.

Prevalence

Found in 15.4% (10/64). The plasmodia were observed as small, macroscopic, white nodules within the kidney. These nodules were abundant at the dorsal side of the kidney facing the vertebral column. The infected kidney had 5–7 plasmodia with average diameter of 0.23 mm.

Description of spores

Mature spores were elliptical in frontal view measuring 10.7 ± 0.6 (10.4–11.6) $\times$ 7.6 ± 0.4 (7.2–8.0) μm. Five to six notches were present at the sutural wall of the sporoplasmic part of spores. Polar capsules were nearly equal, bean-shaped and extended slightly beyond half of the spore. They measured 5.9 ± 0.4 (5.2–6.2) $\times$ 2.9 ± 0.4 (2.4–3.2) μm. Nine turns of polar filaments were slightly oblique along the longitudinal axis of the capsules.

Myxobolus intestinalis sp. n.

See Figs. 3a, 4e

Host

B. bynni.

Site of infection

Intestine.

Prevalence

Found in 4% (2/50). Only ten plasmodia were recorded and observed as tiny white spots adhered to the wall of the intestine. They measured 0.5 ± 0.4 (0.3–1.0) mm in diameter.

Description of spores

Spores were oval in frontal view with a blunt apex. The intercapsular process was very small but present. Mature spores measured 12.5 ± 0.7 (12.0–13.6) $\times$ 8.8 ± 0.7 (8.0–9.6) μm. Polar capsules were oval, elongated and measured 7.7 ± 0.4 (7.2–8.0) $\times$ 3.3 ± 0.2 (3.2–3.6) μm, occupying about two-thirds of the spore length. The polar filaments showed 5–6 coils slightly oblique along the longitudinal axis of the capsules.

Histology

The examined sections revealed that plasmodia were usually embedded in the circular muscle layer and sometimes in the adipose tissue lining the intestine (Figs. 3c, 3d). The developed plasmodia fractured the muscle layer and replaced it in the infected areas (Fig. 3d). No developmental stages were observed and only mature spores were loosely aggregated inside the plasmodia (Fig. 3d).

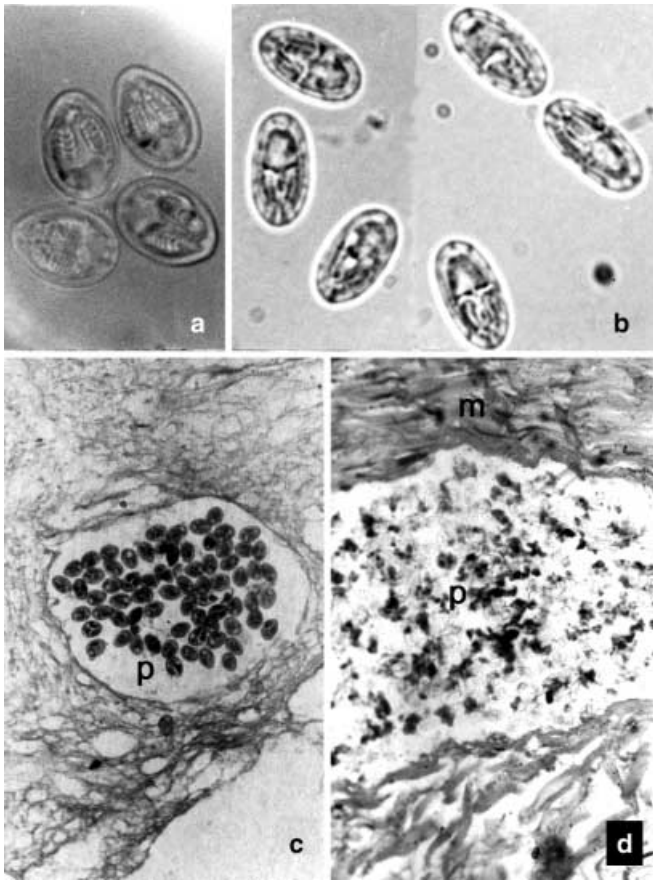

Fig. 3 **a** Fresh spores of *M. intestinalis* sp. n. infecting the intestine of *B. bynni*. (*bar* 5 μm = 0.8). **b** Fresh spores of *M. perforata* sp. n. infecting the operculum of *Hydrocynus forakalii*. (*bar* 5 μm = 1). **c** Longitudinal section of the intestine of *B. bynni* showing the plasmodium (*P*) filled with mature spores and surrounded with thin layer of connective tissue. (× 1,520). **d** Transverse section of the intestine of *B. bynni* showing the plasmodium (*P*) embedded in the circular muscle layer (*M*). (× 3,800)

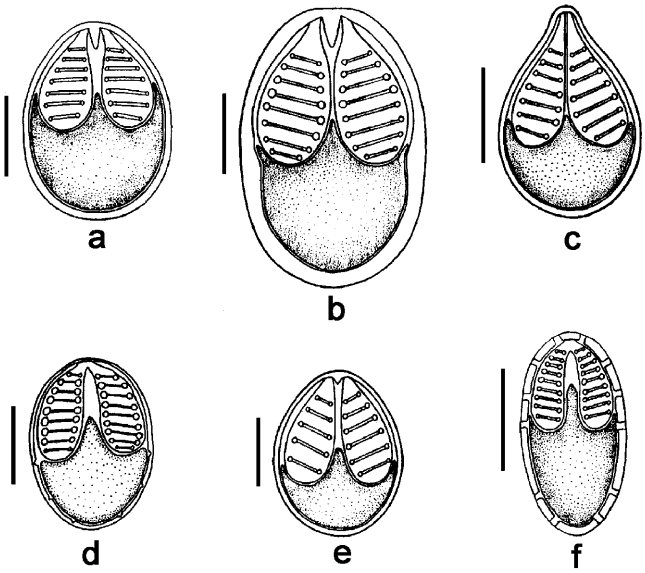

Fig. 4 Diagrammatic representation of spores. **a** *M. naffari* Abdel Ghaffar et al., 1998. **b** *M. caudatus* sp. n. **c** *M. fahmii* sp. n. **d** *M. imami* sp. n. **e** *M. intestinalis* sp. n. **f** *M. perforata* sp. n. (*bar* 5 μm)

Myxobolus perforata sp. n.

See Figs. 3b, 4f

Host

H. forskalii.

Site of infection

Internal surface of operculum.

Prevalence

Found in 12.5% (1/8). Only one cyst of this myxosporean was found. This cyst was white, elongated and measured 6.0 mm in length and 0.8 mm in width.

Description of spores

Spores were elliptical in frontal view measuring 10.4 ± 0.5 (9.9–11.3) × 5.2 ± 0.3 (4.5–5.9) μm. Polar capsules were long, oval and measured 5.2 ± 0.4 (4.0–5.4) × 2.4 ± 0.6 (1.2–2.7) μm. Polar filaments were compactly coiled to form nine turns almost perpendicular to the longitudinal axis of the polar capsule. The sutural line of the fresh spores was characterized by 10–13 marks arranged along the spore border giving the spores a perforated appearance.

Discussion

Basically, the numerous myxosporean species described outside Africa could be regarded as separate species due to the geographical barriers, and may not need to be compared with the present Myxosporea described in this investigation. However, some of these species will be compared because of a close resemblance or because of their detection on the same host family.

Myxobolus naffari Abdel Ghaffar et al., 1998

Four *Myxobolus* species can be compared with the present parasite. They are *Myxobolus muelleri* Buetschli, 1882 (Lom and Dykova1992); *M. mesopotamiae* Molnar et al., 1996; *M. bulbocoridis* (Mansoumin et al. 1996); *M. naffari* Abdel Ghaffar et al., 1998. *M. muelleri* and *M. bulbocoridis* are larger in all dimensions while *M. mesopotamiae* is smaller in all dimensions in addition to the lack of an intercapsular process. In the present material, wall notches reported by Abdel Ghaffar et al. (1998) were unclear. However, the shape of the spores is identical to that of *M. naffari*. Therefore, the present work confirms the identification of *M. naffari* as a new species and adds *Barbus bynni* as a new host and the mouth as a new site of infection.

Histology

In the gill specimens infected with *M. naffari* found in the present study, the connective tissue encapsulating the plasmodia is one of the usual responses associated with myxosporean infections of fish (Mitchell 1977). The shape and site of infection of *M. naffari* are similar to the infection of *Henneguya creplini* (Haaparanta et al. 1994). The histological changes associated with *M. naffari* mostly matched the effect of *H. creplini* in the infected tissues. Thus, the parasite mass caused lamellar fusion and hyperplasia as well as lamellar atrophy. This atrophy was due to the pressing action of the plasmodia on the neighboring tissue, the lamellae. El-Matbouli et al. (1992) reported that the primary damage of *Myxobolus* plasmodia is due to the mechanical interference with gill respiratory function caused by the sheer bulk of the plasmodia. In the present study, the plasmodia were found to occupy a large proportion of the respiratory epithelia, which could hamper the gaseous exchange. *M. naffari* can therefore impose a serious potential threat to the infected fish, as heavy infections lead to the destruction of a large proportion of the respiratory epithelia and atrophy of gill lamellae.

Myxobolus caudatus sp. n.

Four comparable *Myxobolus* species can be found, all of them infecting cyprinid hosts. They are *M. cycloides* Gurley, 1893 (Lom and Dykova 1992), *Myxobolus* sp. type 3 (Fahmy et al. 1975), *Myxobolus* sp. type 4 (Fomena et al. 1985) and *Myxobolus* sp. type 2 (Abdel Ghaffar et al. 1995b). Despite the close resemblance of *Myxobolus cycloides* to the present spores, it is smaller and has fewer polar filament turns and has 5–6 notches in the inner wall. Spore dimensions of *Myxobolus* sp. type 4 were less than the minimum ranges of the present spores. The shape as well as the dimensions of the present material are identical to *Myxobolus* sp. type 3 (Fahmy et al. 1975) and *Myxobolus* sp. type 2 (Abdel Ghaffar et al. 1995b). Furthermore, they were detected on the same host. The present study adds an important character to the description of this species, the number of polar filament turns (8–9).

In conclusion, this species was recorded twice in Egypt without specific nomenclature and should acquire a specific taxonomic order; hence, the name *Myxobolus caudatus* is suggested. It refers to the site of infection, the caudal fin, where it has occurred in all three records of this parasite.

Myxobolus fahmii sp. n.

The following *Myxobolus* species resemble the spores of this species in general shape: *M. macrocapsularis* Reuss,

1906 (Lom and Dykova 1992); *M. carassii* Klokacheva, 1914 (Lom and Dykova 1992); *Myxosoma* sp. type 1 (Fahmy et al. 1975); *M. barbi* (Fomena et al. 1985); *Myxobolus* sp. type 3 (Abdel Ghaffar et al. 1995b); and *M. iranicus* (Molnar et al. 1996).

M. macrocapsularis has wider spores and more filament turns (10–14 vs 6–7). *M. carassii* possesses significantly larger and wider spores. *M. barbi* shows slightly narrower polar capsules and a larger number of polar filament coils (6–9 vs 6–7). *M. iranicus* is larger in spore dimensions and possesses a conspicuous intercapsular process, which was not observed in the present spores.

Myxosoma sp. type 1 (Fahmy et al. 1975) is very close in shape, but has slightly smaller dimensions which might be due to the fixation and staining. *Myxobolus* sp. type 3 (Abdel Ghaffar et al. 1995b) is almost identical to our material. These last two species are therefore believed to be the same as the present spores due to their identical morphometry, site of infection and host. The salient feature of these spores is the nipple- or cap-shaped anterior tip, which could be added to the previous description in addition to the number of the polar filament coils (6–7). This species is recorded in Egypt and has not been named, hence *Myxobolus fahmii* is suggested in memory of late Professor M. A. Fahmy for his contributions to parasitology.

Histology

A review of the literature revealed that most of the *Myxobolus* infections in gills of fishes are interlamellar and sometimes intralamellar. The present site within the epithelial layer of lamellae is not often reported. Athanassopoulou and Sommerville (1993) and Lom and Dykova (1994) reported similar infections, but they did not comment on the exact site of infection. Ventura and Paperna (1984) recorded *Myxidium giardi* in many tissues of *Anguilla anguilla,* including the tip of the lamellae, and showed a similar structure in which a complete envelope of epithelial cells is present around the plasmodia. In the present species, the plasmodia attained a large mass that enclosed the whole lamellae and led to their detachment from the filament as reported by Abdel Ghaffar et al. (1998) in *M. barbi*. In the present study, some plasmodia showed hypertrophy of the enveloping epithelia, which might be an attempt of this layer to accommodate the enlarged plasmodial mass.

The current histological evaluation of the present parasite showed that the main pathological effect might result from the destruction of the respiratory surface of the gill lamellae. Combined with the severe infection, this parasite is potentially dangerous to the infected host.

Myxobolus imami sp. n.

Only two species with close general shape can be compared to the present myxosporean: *M. burei* Egus, 1985 (Lom and Dykova 1992) and *M. rohdei* (Lom and Dykova 1994).

M. burei has broader spores, relatively smaller polar capsules, smaller numbers of polar filament turns (3 vs 9) and more spore notches (9 vs 6). *M. rohdei* is dimensionally almost equal but morphologically differs markedly in: polar filament turns (3–4 vs 9) and markings (10 vs 6).

Because of these differences, the present species could be regarded as new and the name *Myxobolus imami* is suggested in memory of the late Professor A. R. Imam for his contributions to parasitology.

Myxobolus intestinaIis sp. n.

The present spores have no striking feature to compare with and none of the African species matched it. From non-African species, the following species can be compared: *M. insidiosus* Wyatt and Pratt 1963; *M. argenteus* Lewis, 1968 (Lom and Dykova 1992); *M. rhinichthidis* Cone and Raesly 1995; *M. enoblei* Lom and Cone, 1996 and *M. iranicus* Molnar et al., 1996.

M. insidiosus and *M. enoblei* are larger in all dimensions. *M. argenteus* has longer spores and smaller polar capsules. *M. rhinichthidis* shows variable spore shapes which were not observed in the present spores. In addition, they have smaller polar capsules and larger number of coils (8 vs 5–6). Spores of *M. iranicus* are close to the present species in almost all dimensions. However, the anterior of spores of *M. iranicus* is sharply tapering to a pointed end compared to the smoothly and blunt end in the present spores. Moreover, the intercapsular process is much developed in *M. iranicus*. Also, the polar filament makes 6–7 turns in *M. iranicus* compared to 5–6 in the present spores.

Therefore, the present *Myxobolus* carries enough criteria in our view to be a new species and as this species infects the intestine, the name *Myxobolus intestinalis* is proposed.

Histology

The site of infection of *M. intestinalis* is comparable with *Kudoa ciliata* (Lom et al. 1992) in the smooth muscle layer of the intestinal wall of *Sillago ciliata*. There was no discernible pathological change in the tissue because of the presence of the plasmodia. The only harmful impact of the plasmodia is the replacement of some areas of the muscles by the plasmodial masses.

Myxobolus perforata sp. n.

Three similar myxosporean species to the present parasite can be compared: *Myxosoma* sp. type 3 (Fahmy et al. 1975); *M. polycentropsi* Fomena et al., 1985; and *M. kotlani* Molnar et al., 1986 (Lom and Dykova 1992).

M. polycentropsi has larger spores, fewer polar filament turns (4–5 vs 9) and the spores lack the sutural markings. *M. kotlani* is close in dimensions except for the width of the spores and polar filament turns (7–8 vs 9).

The present spores and *Myxosoma* sp. type 3 are described from the same host, site of infection and have almost identical dimensions of cysts. Because of low infection in the present study, the full range of spore measurements reported by Fahmy et al. (1975) was not reached, which might justify the higher maxima of their spores.

As discussed earlier, the work of Fahmy et el. (1975) was not specifically allocated and hence the present species is indeed a new one. The generic status should also be amended according to Lom and Noble (1984) to the genus *Myxobolus*. We propose the new name *Myxobolus perforata,* which describes the perforated appearance of the spores.

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