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Light and electron microscopic studies on *Myxobolus egyptica* sp. nov. (Myxozoa, Myxosporea), infecting the hornlip mullet *Oedalechilus labiosus* from the Red Sea

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Abstract

A new myxosporean, *Myxobolus egyptica* sp. nov., was described from the gills of the hornlip mullet *Oedalechilus labiosus*, collected from the Red Sea at Al-Quseir city, Egypt. The prevalence of infection was 12/72 (16.66%). *Myxobolus egyptica* was identified on the basis of spore morphometry, histology and transmission electron microscopy. It was distinguished from all previously reported *Myxobolus* spp. by its shape, dimensions of the mature spore 10.0 ± 0.6 (9.5–10.5) µm in length, 8.5 ± 0.4 (8.0–9.0) µm in width and 8.7 ± 0.5 (8.4–9.2) µm in thickness, polar capsules, locality and host. The parasite formed intrafilamental cyst-like plasmodia. These plasmodia caused curling and atrophy of the gill lamellae. The ultrastructural analysis revealed a double-unit plasmodial membrane which was in direct contact with the host cells and had numerous vesicles. Some mitochondria were found below this membrane. The disporic pansporoblast was earliest recognizable stage of sporogenesis. Advanced developmental stages of spores and mature spores were reported.

Keywords

Myxosporea, *Myxobolus*, mullets, Red Sea, Egypt

Introduction

Mullets are members of the family Mugilidae found worldwide in coastal temperate and tropical waters and, for some species, also in freshwater (Randall 1992). Mullets are an important component of Egyptian fisheries and are considered as one of the most important cash crops from artisanal fisheries in the numerous lagoons throughout the country (Saleh 2008). The economic importance of the mullets increased in recent years as result of its extensive use in aquaculture (Saleh 2006). Genus *Myxobolus* is the most common fish myxosporean parasites having a wide geographical distribution and comprising a great number of species infecting both marine and freshwater fish (Lom and Dyková 2006). Eiras *et al*. (2005) listed 744 known species, while Lom and Dyková (2006) recorded 792 valid species. Genus *Myxobolus* found to be particularly relevant for the regions where mullets are important for aquaculture, as in Israel, Italy, Tunisia and Egypt (Bahri *et al*. 2003). Thus far, 26 *Myxobolus* species have reported from mullets (Eiras *et al*. 2007) and of them, 22 species were found in different tissues of *Mugil cephalus* (Shvedko and Aseev 2008). The present study is a part from ongoing research on myxosporean parasites of the Red Sea fishes during which many fish species and families were examined. This paper describes morphology, histology and ultrastructure characteristics of a new parasitic species of *Myxobolus* in the gills of hornlip mullet *Oedalechilus labiosus* Valenciennes, 1836 collected from the Red Sea, Egypt.

Materials and methods

From March 2009 till March 2010, 72 samples of *Oedalechilus labiosus* (Actinopterygii: Mugilidae) were collected from fishermen at boat landing site at Al-Quseir city (26°07′N, 34°16′E), Egypt. The collected fish ranged from 15–20 cm in size. Fish were dissected with external surface

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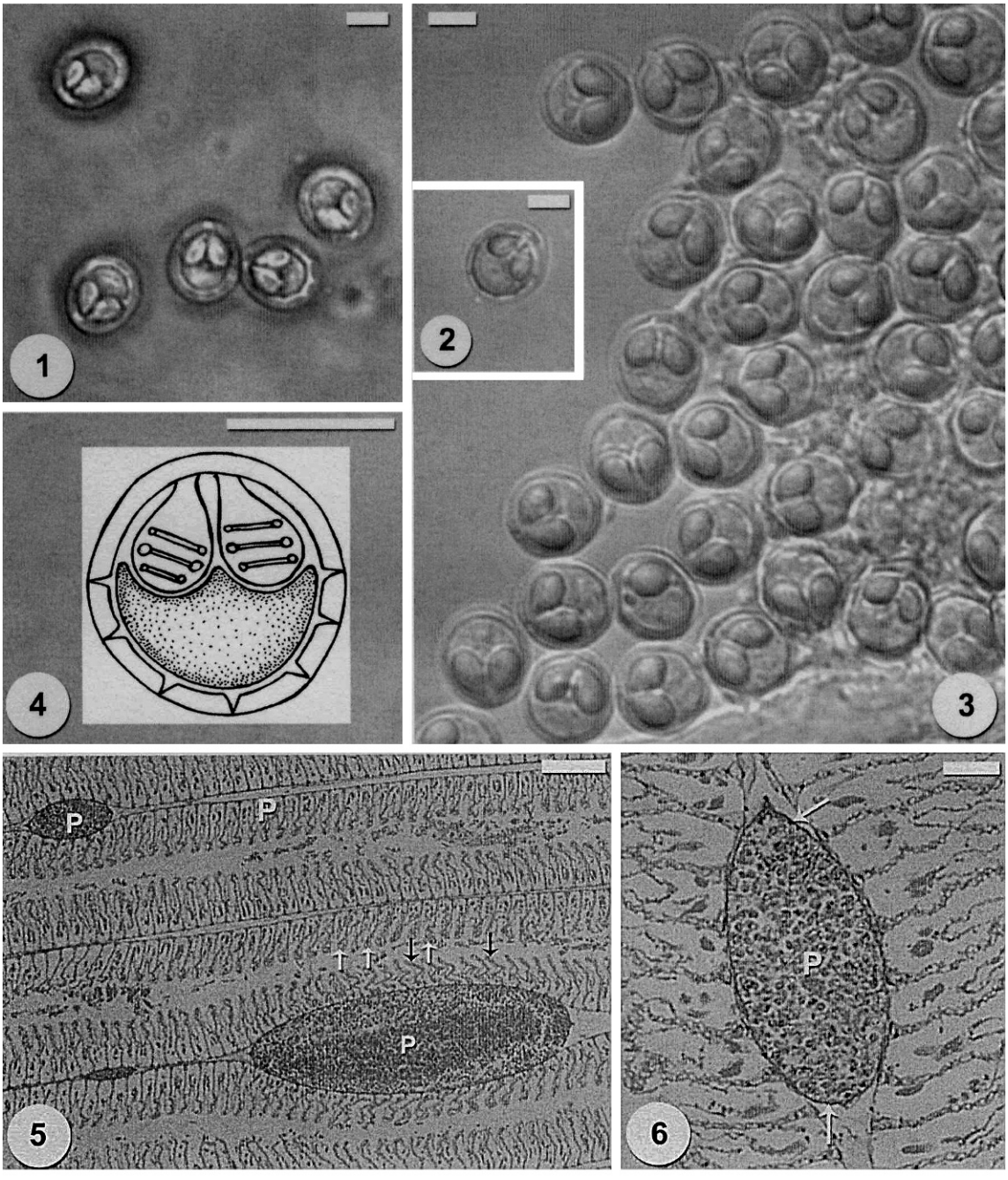
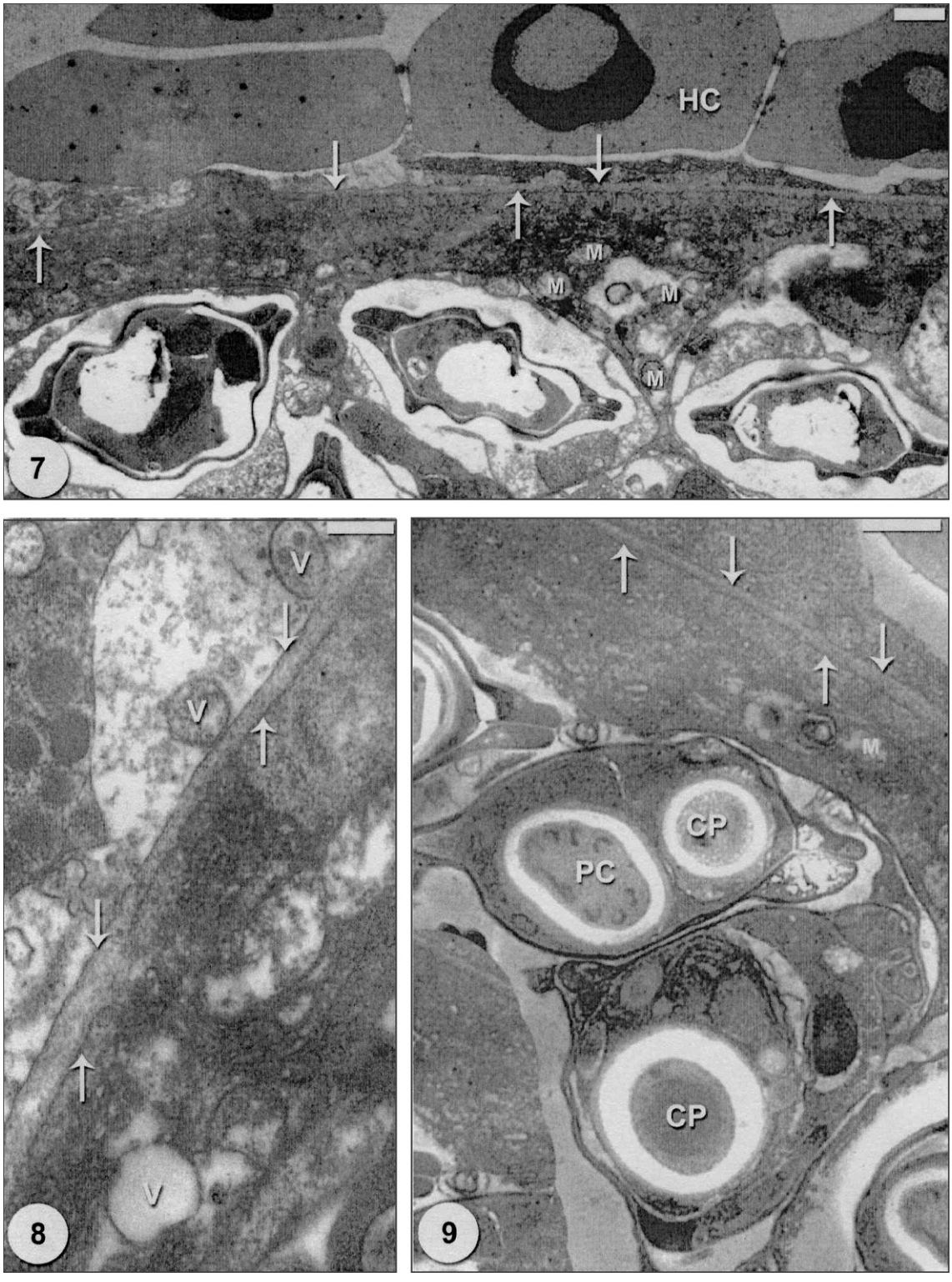
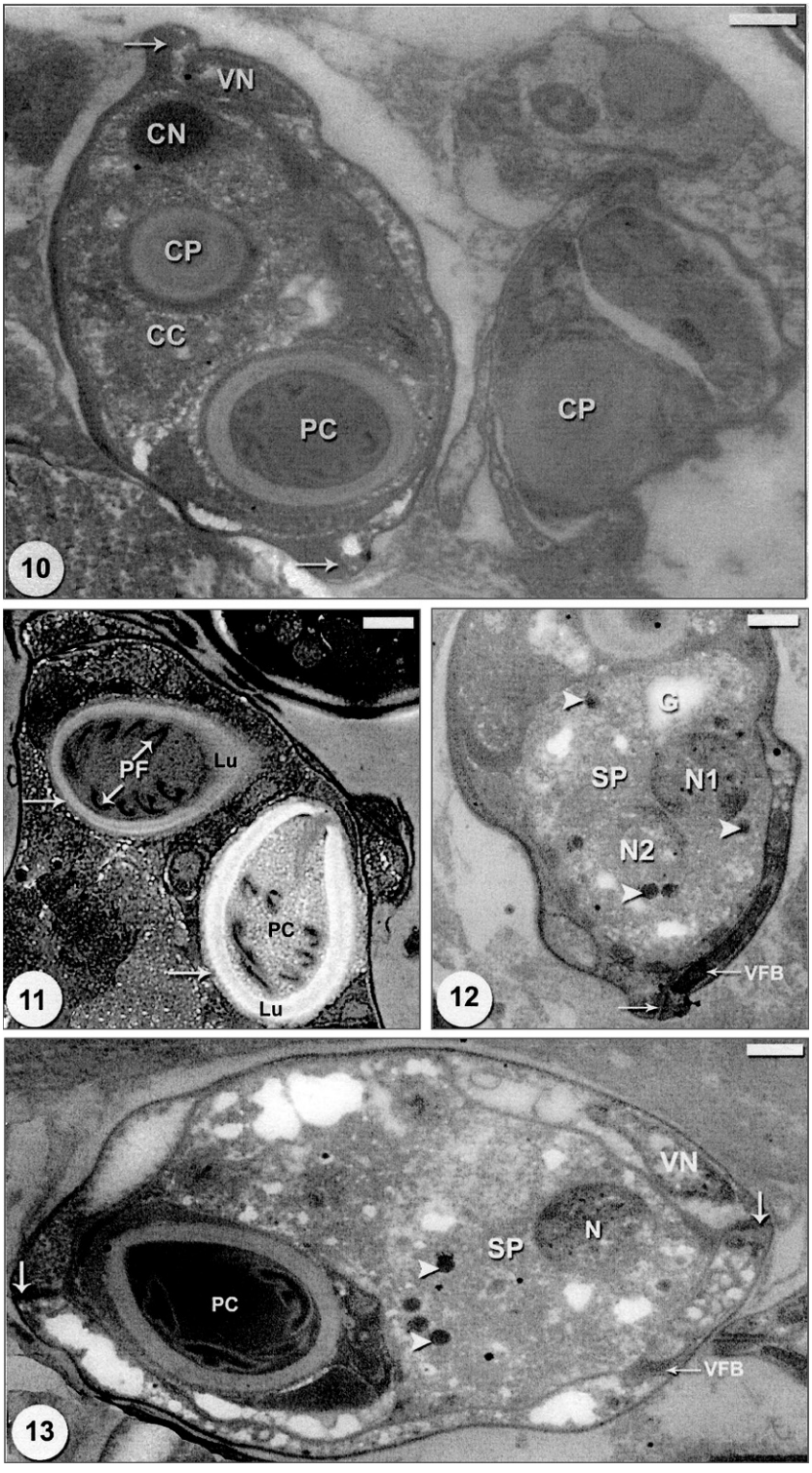
and internal organs examination for myxosporean infection. Spores studied in fresh mounts using bright field, phase contrast and Nomarski interference Zeiss Axiovert 135 microscope with Contax Camera. Descriptions and measurements of spores followed the guidelines of Lom and Arthur (1989). Measurements were based on 30 fresh spores and data were presented as mean ± SD (range). All measurements were in micrometers. Drawings were made with the aid of a camera lucida. For permanent preparation, air-dried smears were stained with Giemsa after fixation in acetone-free absolute methanol. For histology, the infected gills were fixed in 10% buffered neutral formalin and processed for paraffin blocks. Then, sec-



Figs 1–6. Light micrographs of *Myxobolus egyptica* sp. nov. from the gills of *Oedalechilus labiosus*. **1** – Fresh spores observed under phase contrast. Scale bar = 5 µm. **2** – Fresh spore observed under bright field. Scale bar = 5 µm. **3** – Fresh spores observed under Nomarski interference contrast. Scale bar = 5 µm. **4** – Schematic drawing of mature spore. Scale bar = 2 µm. **5** – Longitudinal section of gills showing intrafilamental plasmodia (P) at different stage of maturation and the gill lamellae curled (black arrows) and atrophied (white arrows) at the cyst site. Scale bar = 100 µm. **6** – Enlarged longitudinal section of gills showing intrafilamental plasmodia (P) encased with a thin wall (arrows) and no inflammatory reaction found in the infection site. Scale bar = 25 µm



Figs 7–9. Electron micrographs through plasmodia of *Myxobolus egyptica* sp. nov. from the gills of *Oedalechilus labiosus*. **7** – A part of plasmodium bounded by two membranes (arrows) in contact with the host cells (HC) and below the membrane large numbers of mitochondria (M). Scale bar = 1 μm. **8** – Enlarged part of plasmodium bounded by two membranes (arrows) to which some vesicles was attached (V). Scale bar = 0.5 μm. **9** – A part of plasmodium bounded by two membranes (arrows) and below the membrane a disporic pansporoblast was shown. Observe the asynchronous development of the polar capsules (PC) and capsule primordial (CP). Scale bar = 1 μm



Figs 10–13. Electron micrographs through spores of *Myxobolus egyptica* sp. nov. from the gills of *Oedalechilus labiosus*. **10** – Section through developing spore showing the capsulogenic cell (CC) contained capsule primordium (CP) and capsulogenic nucleus (CN) and mature polar capsule (PC). All the spore content was surrounded by two shell valves met at the suture (arrows) and contained valvogenic nucleus (VN). Scale bar = 1 μm. **11** – Transection through the apical part of mature spore showing two mature polar capsule (PC). The polar capsule composed of an electron-dense outer layer (arrow), a central translucent (Lu), and inner dense core with polar filament coils (PF). Scale bar = 1 μm. **12** – Transection through the lower part of developing spore showing the sporoplasm (SP) with two nuclei (N1, N2) and many sporoplasmosomes (arrowheads). The sporoplasm surrounded by two valves met at the suture (arrow) and contained valve forming bodies (VFB). Scale bar = 1 μm. **13** – A developing spore containing polar capsule with its filament (PC), sporoplasm (SP) with nucleus (N) and sporoplasmosomes (arrowheads), and externally surrounded by two valve cells containing valve nucleus (VN) with valve forming body (VFB) and met at the suture (arrows). Scale bar = 1 μm

tioned and stained with haematoxylin and eosin. For transmission electron microscopy (TEM), small fragments of tissue containing cysts were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) at 4°C for 24 h. Samples were subsequently rinsed in the same buffer overnight at 4°C and post-fixed with 2% OsO_4 in the same buffer for 3 h. The tissue fragments were dehydrated in ascending ethanol series and embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips (208) electron microscope at 80 kV.

Results

Vegetative stages

The prevalence of infection was 12/72 (~16.6%).The infection was recorded as tiny aggregates of whitish cysts-like plasmodia which hardly noticed within the gill filaments. In some infected fish, up to 15 cysts were counted in the infected gill.

Spore description

Spores were relatively small, subspherical in the frontal view (Figs 1–4). Five to seven sutural edge makings were easily observed around the posterior end of the fresh spores (Fig. 2). Mucous envelope was not found. In most spores no intercapsular process was seen; however, for some spores a very small knob-like thickening between capsules could be hardly observed. Spores measured 10.0 ± 0.6 (9.5–10.5) μm in length, 8.5 ± 0.4 (8.0–9.0) μm in width and 8.7 ± 0.5 (8.4–9.2) μm in thickness. Polar capsules were typically equal, pear-shaped and occupied about half of the spore length. They measured 5.2 ± 0.5 (5–6) μm length 2.3 ± 0.4 (2.0–3.0) μm in width. The polar filament showed mostly 3–4 coils oblique to the main axis of the polar capsules. There was a single binucleated sporoplasm with round iodinophilous vacuole.

Taxonomic summary

Host: *Oedalechilus labiosus* Valenciennes, 1836.

Locality: Al-Quseir, Egypt (26°07′N, 34°16′E).

Location in the host: Gills.

Prevalence: 12/72 (~16.6%).

Type material: Syntypes on slide no. Myx.-32 was deposited at the Museum of Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

Etymology: The specific epithet is proposed after the locality, Egypt.

Histology

Spindle-shaped cysts-like plasmodia were observed in the gill filaments. The plasmodia developed in the medial portion of the filament core (Figs 5, 6). This type of development was regarded as "intrafilamental type" depicted by Molnár (2002). Numerous plasmodia were reported at different stages of maturity and measured from 165 × 65 to 500 × 180 μm in size (Fig. 5). The plasmodia were encased with a thin wall (Fig. 6). No inflammatory process was found in the infection site. The gill lamellae were atrophied and curled at the cyst site (Fig. 5).

Electron microscopy

The plasmodia were bounded by two membranes in contact with the host tissue (Figs 7–10). There were some hardly visible pinocytotic channels attached to the plasmodial membrane (Fig. 8). Immediately below the membrane large numbers of mitochondria were observed (Fig. 7). Stages of spore development, including disporic pansporoblast were recognized in the present plasmodium (Fig. 9). As the spore proceeded toward maturation, there was a structural progress in the capsulogenesis, sporoplasm maturation and valvogenesis (Figs 9–13). The earliest capsule primordia were spherical or oval in the cytoplasm of the capsulogenic cell (Figs 9, 10). It was observed that the maturation of the two polar capsules was asynchronous (Fig. 10). The mature polar capsules were ovoid and consisted of an electron dense outer zone, followed by an immediately electron translucent zone and a dense cortex containing 3 to 4 polar filament coils (Fig. 11). During the sporoplasm maturation, it showed one or two nuclei. The sporoplasm contained the normal constituents like glycogen vacuoles and electron dense sporoplasmosomes (Figs 12, 13). As the spore proceeded toward maturation, the valvogenic cells appeared very distinctly. These cells were characterized by their peripheral location, their flattened nuclei and presences of valve forming bodies (Figs 10, 13). Later in course of development, these cells gave rise to the two valves surrounding each spore and the sutural ridge joining the valves (Figs 10, 12, 13).

Discussion

Oedalechilus labiosus is a Red Sea endemic fish which belongs to the family Mugilidae. All mullets are often found in brackish water; some live as adults in purely freshwater; except *Crenimugil crenilabis* and *Oedalechilus labiosus* are purely marine inhabitants (Randall 1992). The fact that only *Oedalechilus labiosus* was infected within a sample survey which including many fish species of different families, obtained from the same sampling site, cannot be matter of coincidence. This result allowed us to conclude that our species could consider as specific parasite of *Oedalechilus labiosus*. A similar finding was reported by Eiras and D'Souza (2004) in the same fish family. Therefore, our material was compared with closely related mullet's *Myxobolus* species. According to the available literatures 26 *Myxobolus* species were described from mullets. However, on the basis of morphological and di-

mensional comparison of the spores, only eight species of them could be compared with our species (Table I). *M. rohdei* (Lom and Dyková 1994) and *M. goreensis* (Fall *et al*. 1997) can be easily distinguished from the present species by having larger spore, shorter polar capsules and lacking of the sutural makings. Also, *M. chiungchowensis* (Chen and Ma 1998) can be separated from the present species in having wider spores, higher number of polar filament turns (6–8 *vs* 3–4) and lacking the sutural markings. The spore measurements of *M. exiguus* (Bahri *et al*. 2003), *M. episquamalis* (Diamanka *et al*. 2008) and *M. muelleri* (Umur *et al*. 2010) were distinctly smaller than those of our species. Moreover, *M. exiguus* and *M. episquamalis* have higher number of polar filament turns (5–6 *vs* 3–4). Also, *M. muelleri* have large intercapsular process. *M. goensis* (Eiras and D'Souza 2004) differed from the present species by having larger spores and unequal polar capsules with higher number of polar filament coils (6–7 *vs* 3–4). In the same way, *M. platanus* can be distinguished from the present species by having larger polar capsules with higher number of polar filament coils (5–6 *vs* 3–4) and having conspicuous intercapsular process. Furthermore, the combination of different hosts, different sites of infection and geographic location separate all the compared species from the present form (Table I). It appeared clearly that the present species did not satisfy the characters of any of the species discussed above, and thus it was suggested as a new species. We proposed *Myxobolus egyptica* sp. nov. as specific name after locality.

Histology

The infection with the present *Myxobolus* associated with the gill filament "intrafilamental development". This type of development proved to be one of the most common types of plasmodium development and numerous *Myxobolus* species including *M. bramae*, *M. macrocapsularis* and *M. dispar* developed in this way (Molnár 2002). Regardless of the absence of inflammatory response, the development of the present plasmodia caused atrophy and curling of the respiratory lamellae at the cyst site. A similar finding was reported by Ali (1999) and Naldoni *et al*. (2009).

Electron microscopy

The plasmodial wall of the present species consisted of two membranes with pinocytotic channels, which was similar to that of *Henneguya exilis* (Current and Janovy 1977) and *Myxobolus* sp. (Desser and Paterson 1978). However, the plasmodia of other myxosporean species may exhibit a single-unit membrane like *Myxobolus exiguus* (Pulsford and Matthews 1982), *Kudoa lunata* (Lom and Dyková 1988) and *Zschokkella helmii* (Abdel-Ghaffar *et al*. 2008). Current and Janovy (1978) concluded that the differences in the nature of the plasmodium wall are tissue-dependent. Capsulogenesis of the present species followed the usual pattern observed in most myxosporeans. The capsules first appeared as primordia in the

Table I. Comparative description of the *Myxobolus egyptica* sp. nov. with morphologically similar species (measurements in μm)

***Myxobolus* spp.**	**Host**	**Site of infection**	**Locality**	**Spore**		**Polar capsules**	
				Length	Width	Length	Width
M. rohdei (Lom and Dyková 1994)	*Mugil cephalus*	Intestine	Off Australia	11 (9.8–11.8)	8.9 (8.4–9.1)	4.3 (3.7–5)	2.8 (2.5–3.1)
M. goreensis (Fall *et al*. 1997)	*Mugil cephalus*	Gills	Off Senegal	10.9 (10–13)	10.9 (10–13)	4.1 (4–5)	3.1 (2–4)
M. chiungchowensis Chen, 1998 (Chen and Ma 1998)	*Mugil cephalus*	Intestine	Off China	10.8 (10.2–11.8)	10.5 (9.6–11)	6 (5.6–6.2)	3.6 (3.4–3.8)
M. exiguus Thelohan, 1895 (Bahri *et al*. 2003)	*Liza ramada*	Gill filament	Tunisia	8–9.5	6–7.5	3–4.5	1.5–3
M. goensis (Eiras and D'Souza 2004)	*Mugil cephalus*	Gill rankers	India	9.7 (9.5–10.5)	6.6 (6–7.5)	4.5–6 2–3	2–3 1.5–3
M. platanus (Eiras *et al*. 2007)	*Mugil platanus*	Spleen	Brazil	10.7 (10–11)	10.8 (10–11)	7.7 (7–8)	3.8 (3.5–4)
M. episquamalis (Diamanka *et al*. 2008)	*Mugil cephalus*	Skin and scales	Off Senegal	8.2 (8–9)	5.9 (5–6)	4.03 (4–4.5)	2.2 (2–3)
M. muelleri Bütschli, 1882 (Umur *et al*. 2010)	*Mugil cephalus*	Gill filament	Turkey	8.3 (7.2–9.0)	7 (6.4–7.4)	3 (2.5–3.5)	1.8 (1.5–2.5)
M. egyptica (the present study)	*Oedalechilus labiosus*	Gill filaments	Red Sea, Egypt	10.0 (9.5–10.5)	8.5 (8.0–9.0)	5.2 (5–6)	2.3 (2.0–3.0)

capsulogenic cells and the development was asynchronous. Similar findings were obtained by other investigators (Desser and Paterson 1978, El-Matbouli *et al*. 1990). In addition, mature polar capsules of the present material were pyriform in shape with an electron dense outer zone, followed by an immediately electron translucent zone and a dense cortex which was similar to several myxosporeans (Current and Janovy 1978, El-Mansy and Bashtar 2002, Matos *et al*. 2005). Genus *Myxobolus* was characterized by a single binucleated sporoplasm (Pulsford and Matthews 1982, Casal *et al*. 1996). A similar feature was encountered in this study. The glycogen accumulation noticed in the sporoplasm of the present species was similar to that reported by El-Matbouli *et al*. (1990) and Ali *et al*. (2007). A few rounded dark inclusions (sporoplasmosomes) were infrequently observed in the present sporoplasm similar to those reported by some authors (Canning *et al*. 1999, Ali *et al*. 2007). The spores of Bivalvulida are essentially composed of two shell valves. The typical flat valve cell with its flat nucleus was observed during the present valvogenesis. In mature spores, electron dense bodies were noticed in the cytoplasm of the valvogenic cells of the present species, these bodies are thought to be precursor materials for valve wall formation (Current *et al*. 1979, Stehr 1986, El-Matbouli *et al*. 1990).

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