Annals of Parasitology 2017, 63(3), 159–165
doi: 10.17420/ap6303.101

Original papers

The light microscopy and ultrastructural characteristics of *Myxobolus naffari* (Myxosporea, Myxobolidae) infecting the Nile carp *Labeo niloticus* (Cyprinidae) and its histological impact

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ABSTRACT. During a survey of myxosporean parasites infecting freshwater fishes from the River Nile at Giza Governorates, Egypt between March and September 2016, nine out of 30 specimens of the Nile carp *Labeo niloticus* (Cyprinidae) were found to be naturally infected with *Myxobolus naffari* (Myxobolidae). Small macroscopic plasmodia appeared embedded in the host gill tissue accompanied with fusion of the gill epithelia, and atrophy was observed at the site of infection. The host reaction was manifested by the encapsulation of the plasmodia with a thick layer of connective tissue. The plasmodia appeared as white, elongated rods between gill filaments with an intensity ranging from three to eight cysts/fish. The average dimensions of plasmodia were 1.2–2.0 (1.8 ± 0.2) mm long × 0.4–0.7 (0.6 ± 0.2) mm wide. The spores were oval, reaching 9.56–11.2 (10.2 ± 0.2) µm long and 6.5–7.7 (7.0 ± 0.4) µm wide with two equal-sized polar capsules regularly arranged at the anterior pole of each spore. They were 4.51–5.5 (5.1 ± 0.4) µm in length and 1.5–2.0 (1.7 ± 0.2) µm in width. Histological, semi-thin sections were taken through parasite plasmodia and transmission electron microscopic examination of ultrathin sections was performed to describe the developmental stages of the recorded parasite within the host fish.

Key words: *Myxobolus naffari*, Myxosporea, parasite, morphology, ultrastructure, *Labeo niloticus*

Introduction

With the increasing interest in fish culture and production, the awareness of the parasites that affect the health, growth and survival of fish has also increased. Early diagnosis can facilitate preventive measures, which is the best way to reduce outbreaks of disease [1,2]. Myxosporea are an economically important group of fish parasites with 52 genera [3] with a significant impact on wild and cultured fish [3–5]. Myxozoans are obligate, spore-forming parasites infecting ectothermic vertebrates; most of them are fish [6] with a few being amphibians, reptiles and some invertebrates [7]. In the class Myxosporea, vegetative stages may be coelozoic, with the parasite being found in body cavities or the cavities of organs, or histozoic: in this case, the parasite can be intercellular, or wedged between cells, or intracellular, which is more often the case [7,8]. Gross signs of histozoic infection are whitish cysts with a milky substance containing microscopic spores. Large cysts are readily traced; small cysts in tissues, viscera and connective tissue, and the muscles are detectable when tissue samples

are pressed between slides or in histological material. Spores are readily detected in aqueous methylene blue-stained smears. The so-called cyst is a plasmodium of parasite origin which forms a specialized membranous junction of pinocytotic vesicles (canals) with the surrounding host cells [9–12].

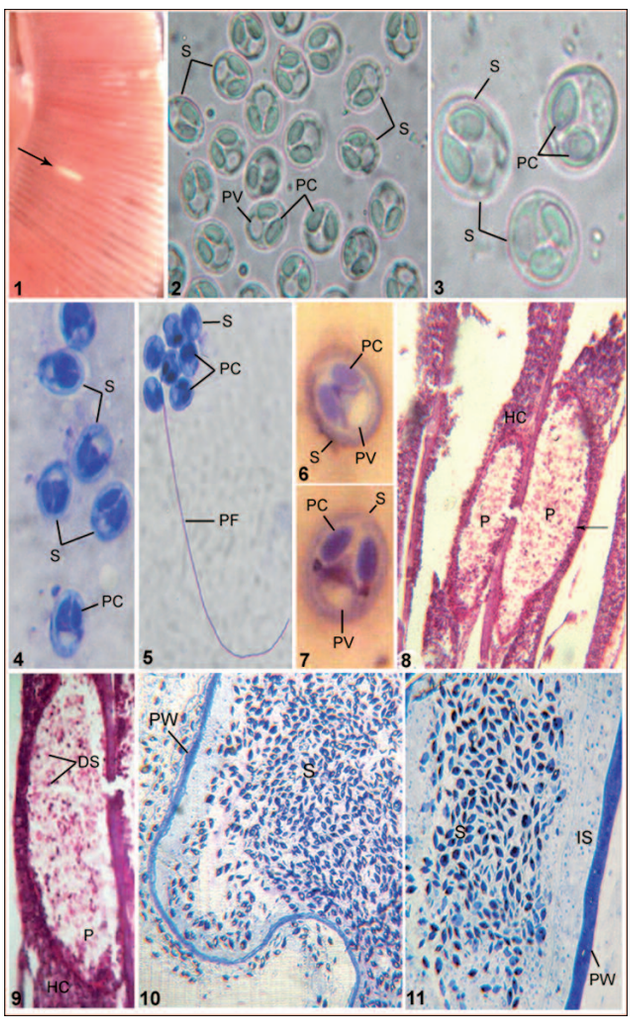
The present study examines the prevalence of myxosporean parasites infecting the gills of the Nile carp, *Labeo niloticus* (Cyprinidae) captured from the River Nile at Giza Governorates, Egypt. Also, the developmental stages of the recovered parasite plasmodia were described by light and transmission electron microscopy.

Materials and Methods

A total of 30 freshly-caught specimens of Nile carp *Labeo niloticus* were collected from the main branch of the River Nile and its tributaries at Giza Governorates, Egypt between March and September 2016. Live fish were transported to the Laboratory of Parasitology, Faculty of Science, Cairo University, Egypt, where the fish were examined for myxosporean infection.

The skin and gills of the fish were first examined by the naked eye for the presence of any macroscopically visible lesions or cysts. The gills were then scraped gently to remove excess blood from the sample and then examined. Gross microscopic examinations of internal organs were performed for myxosporidian infections. To study the morphology of the mature spores, and for taxonomic purposes, at least one fresh plasmodium was taken from each infected fish, ruptured to release their spores, spread on glass slides and photographed. Smears of parasite cysts were prepared, air dried for one hour, fixed in absolute methanol and stained with Giemsa stain in phosphate buffer (pH 7.2) for another hour. Fifty samples of fresh and stained spore preparations were recorded. The classification, measurement and description of spores were carried out according to Lom and Arthur [13].

For morphologic and morphometric study, photomicrographs were taken using a Zeiss Axiovert 135 light microscope fitted with a Canon digital Camera. For histopathological studies, highly-infected gills were fixed in 10% formalin then dehydrated in a series of alcohols, cleared in xylol, embedded in paraffin wax, and cut into 6 µm sections by a microtome. Tissue sections were stained with hematoxylin and eosin. The stained sections were examined and photographed. For TEM, small pieces of the isolated myxozoan cysts were fixed for 48 hours in 2.5% glutaraldehyde and in cacodylate buffer at 4°C for three hours and then postfixed in 2% osmium tetroxide in 0.1 M



Figs 1–11.
1: gills of *Labeo niloticus* infected with *Myxobolus naffari* plasmodia; **2-3**: fresh spores (S) of *M. naffari* with two polar capsules (PC) (Fig. 2 ×900; Fig. 3 ×1500); **4-7**: Giemsa stained spore (S) with darkly stained polar capsules (PC). One of these spores appeared with a long extruded polar filament (PF) (Fig. 4 ×1000; Fig. 5 ×600; Fig. 6-7 ×1500); **8-9**: histological sections through gill tissue of *Labeo niloticus* fish infected with *M. naffari* and stained with hematoxyline and eosin showing plasmodia (arrows) filled with developing spores (DS) establishing within the gill filaments (host cells, HC) of the infected organ. The plasmodia were surrounded by a thin layer of fibrous connective tissue (Fig. 8 ×90; Fig. 9 ×150); **10-11**: semi-thin sections through plasmodia showing the plasmodial wall (PW) surrounded a huge number of spores (S) and its immature stages (IS). (Fig. 10-11 ×160).

phosphate buffer, pH 7.2 at 4°C for two hours. After dehydration in a graded ethanol series, the specimens were embedded in Spurr's resin. Semi-thin sections (1 μm thick) were then cut on a Leica ultracut (UCT) ultramicrotome and stained with toluidine blue. The ultrathin sections were stained with uranyl acetate and lead citrate. Electron micrographs were performed with a Jeol 1220 electron microscope operating at 80 kV.

Results

Nine of 30 specimens of *Labeo niloticus* were infected with *Myxobolus naffari* Abdel-Ghaffar et al., 1998 (Myxosporea, Myxobolidae). Parasite plasmodia were located on the gills.

Description of plasmodia (Fig. 1)

They appeared as white, elongated rods between the gill filaments. The intensity of these plasmodia ranged from three to eight cysts/fish. The average dimensions of plasmodia were 1.2–2.0 × 0.4–0.7 mm.

Description of spores (Figs 2–7)

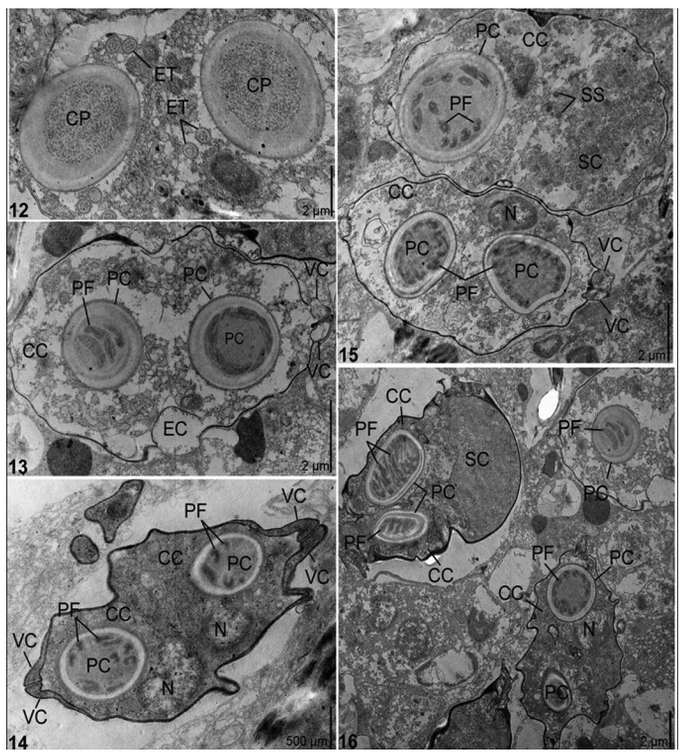
Spores were oval, measuring 9.56–11.2 (10.2 ± 0.2) μm in length, 6.5–7.7 (7.0 ± 0.4) μm in width. The two polar capsules were ovoid, equal in size and occupying nearly half of the spore length. They measured 4.51–5.5 (5.1 ± 0.4) μm in length and 1.5–2.0 (1.7 ± 0.2) μm in width.

Histopathological studies (Figs 8–11)

The histological and semi-thin sections through the parasite plasmodia revealed the presence of a plasmodial membrane covering mature spores and their developmental stages. The infection was found to cause distortion, fusion of the gill epithelia and atrophy at the site of infection. The host reaction was manifested by the encapsulation of the plasmodia with a thick layer of connective tissue.

Transmission electron microscopic study (Figs 12–23)

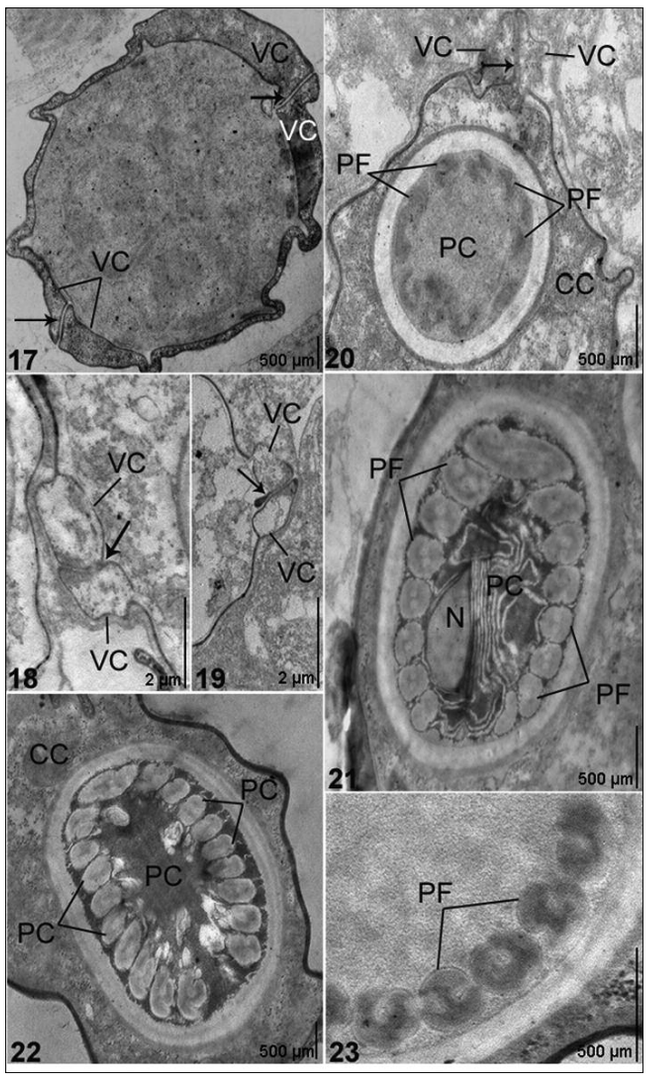
The process of capsulogenesis was characterized by the presence of capsulogenic cells with relatively large amounts of endoplasmic reticulum, several mitochondria, an elongated nucleus and the formation of polar capsules within their cytoplasm. The capsules appeared as membrane-bounded, dense, dark, bulb-like structures known as capsular primordia (Fig. 12), which were usually situated adjacent to the external tubules. An accumulation of what appeared to be polar filament-forming material was concentrated in the cortex of the capsular



Figs 12–16.
12: the capsular primoridia (CP) is the first recognizable stage. The external tubule (ET) appears adjacent to the primordium; **13-14**: two capsulogenic cells (CC) of a mature spore each with two polar capsules, one of them with a fully formed polar filament (PF) and the other one with incompletely formed filaments (asynchronous development). Terminally at the two ends of spore, there are valvogenic cells (VC) each two meet together at a suture line, (N) nucleus; **15**: two adjacent spores with capsulogenic cells (CC) containing polar capsules. One capsulogenic cell (CC) with only one polar capsule (PC) containing polar filament coils (PF); a sporoplasm cells (SC) was observed beside capsulogenic cells (CC) with sporoplasmosomes (SS) filling its cytoplasm and the other one with two completely formed filaments (asynchronous development between spores of the same plasmodia), N nucleus; **16**: top view of the internal medium of cyst showing a group of spores each marked with the presence of a capsulogenic cell (CC) and its polar capsules (PC) containing filament coils (PF), observe the asynchronous development of polar capsules, as one of them is fully developed and the other still immature.

primordium (Fig. 13). Within the cytoplasm of each capsulogenic cell, an external tube formed several loops and terminated within the developing polar filament discharge canal. The coils and folds of the developing polar filament were observed (Figs 14–16).

In mature spores, the polar capsules were elongated, consisting of an electron-lucent zone and a fine granular cortex with a polar filament coiled several times (Figs 13–14). The development of polar capsules was not always synchronized; one polar capsule may have been completely developed, while the other still had the shape of the primordium or was at an immature stage (Fig. 13). The sporoplasm filled all the space beneath both polar capsules and was partially extended between them (Figs 15–16). In relatively immature spores, thick electron-dense materials and many fibrils were observed oriented within a narrow cytoplasmic zone at the most distal part of the valvogenic cells. In the course of their development, these accumulations were not seen and the outermost membranes of the spore were somewhat thickened and extremely electron dense. Two valvogenic cells usually surrounded each diplokaryotic sporoplasm and two capsulogenic cells were observed in addition to the structural ridge between valves and the two discharge canals (Figs 17–19). Mature capsules were characterized by the presence of between seven and 11 polar filament coils (Figs 20–23).



Figs 17–23.
17-19: transmission electron micrograph of mature spore of *Myxobolus naffari* showing the process of valvogenesis. Valvogenic cells (VC) were arranged at the edges of spores where each two cells meet at the sutural line (arrow); **20-23**: high magnifications of polar capsules (PC) with polar filament coils (PF), coiled 9–11 times. High magnification of coils is shown in Fig. 23.

Discussion

This species was described firstly by Abdel Ghaffar et al. [14] from the gills of *L. niloticus* from Qarun and Wadi El Rayan lakes in Egypt, followed by Ali et al. [15] from the mouth of *Barbus bynni* and gills of *L. niloticus* caught in the River Nile. Paperna [16] recorded two *Myxobolus* spp. from the genus *Labeo* from Volta Lake (Senegal) and Rusha River (Tanzania), which differed in shape from our species; however, the study included no statistical data.

The *M. naffari* specimens recorded in the present study were compared with four species of *Myxobolus*: *Myxobolus muelleri* [7], *M. mesopo - tamiae* [17], *M. bulbocoridis* [18] and *M. noko - ueensis* [19]. It was observed that the spores of *M. muelleri* and *M. bulbocoridis* were larger than those of *M. naffari* while those of *M. mesopotamiae* were smaller. The spores of *M. nokoueensis* [19] possessed smaller polar capsules with a lower number of filament coils (5–6). However, *M. naffari* collected from the gills of *Labeo niloticus* possessed spores which were identical in their shape and dimensions to those of *Myxobolus naffari* recorded by Ali et al., and by Abdel-Ghaffar et al. [14,15] except for some minor morphometric differences (Table 1). The histopathological studies of *M. naffari* showed that the plasmodia contained immature and sporogonic stages at their periphery, while the centre was filled with mature spores, a finding in agreement with El-Mansy et al., and Ali et al. [9,20]. The presence of connective tissue encapsulating the plasmodia was one of the usual

Table 1. Comparison of morphometrical data of *M. naffari*

	Plasmodia (mm)	Spore (µm)		Polar capsule (µm)	
		length	width	length	width
Myxobolus naffari Abdel-Gaffar et al.[14]	0.75-1.18 (0.96±0.2) × 0.25-0.38 (0.33±0.05)	10.8-13.2 (11.9±0.8)	7.8-9.8 (8.8±0.4)	4.5-6.2 (5.1±0.3)	2.5-3.0 (2.9±0.1)
Myxobolus naffari Ali et al.[15]	0.8-1.5 (1.2±0.4) × 0.2-0.5 (0.4±0.2)	10.4-12.0 (11.2±0.6)	7.2-9.6 (8.5±0.8)	4.6-6.4 (5.8±0.5)	2.4-4.0 (3.3±0.4)
Myxobolus naffari (present study)	(1.2-2.0) (1.8±0.2) × (0.4-0.7) (0.6±0.2)	9.56-11.2 (10.2±0.2)	6.5-7.7 (7.0±0.4)	4.51-5.5 (5.1±0.4)	1.5-2.0 (1.7±0.2)

responses associated with myxosporean infections of fishes, as noted previously by Mitchell [21], who concluded that severe atrophy occurred in the lamellae as a result of the host reaction to the plasmodium, leading to pressure and the compression of these cells. A high intensity of infection with these parasites also induced damage and destruction of the gill filaments, resulting in respiratory failure and leading to important losses in fish aquaculture.

Capsulogenesis of the present species followed the usual pattern observed in most myxosporeans. However, the origin of the polar capsule was controversial. Lom [22] suggested that the capsular primordium was formed from smooth-walled vesicles, which might originate from the smooth endoplasmic reticulum or even the Golgi complex. Other investigators assumed that granular endoplasmic reticulum was involved in the formation of the capsular primordium [22–25]. El-Matbouli et al. [26] suggest that the polar capsule originates from a spherical body formed of a series of membranes. However, the exact origin of the polar capsules has yet to be conclusively determined. The literature suggests that the most likely organelle involved in polar capsule formation is the endoplasmic reticulum; indeed, in the present species, aggregates of rough endoplasmic reticulum were observed close to the polar capsules. Maturation of the polar capsule was also found to be asynchronous, as previously noted by Abdel-Ghaffar et al. [14]. The sporoplasmosomes observed in the present material were similar to those reported in other studies [24,27]; however, no sporoplasmosomes are present in some myxosporeans, like *Unicapsula muscularis* [23], *Sphaerospora epinepheli* [28], *Sinuolinea tetraodoni* [29] and *Myxidium trachinorum* [30].

In most myxosporean species including the present one, valvogenesis was initiated by the gradual envelopment of the capsulogenic cells and sporoplasm by valvogenic cells. In the course of development, these cells gave rise to the 2 shell valves surrounding each spore and the sutural ridge joining the valves [31]. The microtubules that are generally found among the shell valves of many myxosporean species were not observed in the present material, this is not amazing because many studies improved the absence of microtubular structures in between valve cells such as Lom et al. [31] in *M. jiroveci*, Abdel-Ghaffar et al. [14] in *Myxobolus* sp. and Canning et al. [30] in *Myxidium trachinorum*.

So, even after advanced methods for purifying and treating water resources in Egypt, parasitic myxozoans still represent an endemic infection among fish, damaging the health of these hosts. Further studies based on molecular and advanced recognition techniques are recommended to determine the exact protein composition of these parasites and identify new tools for their treatment.

Acknowledgements

We thank our colleagues from the Parasitology Group, Zoology Department, Faculty of Science, Cairo University, Egypt who provided their insight and expertise, which greatly assisted the research.

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Received 7 June 2017
Accepted 3 September 2017