First Record of Myxobolus Species (Myxosporea: Myxobolidae) in Grey Mullet Mugil cephalus (Teleostei, Mugilidae) from Syria

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Abstract:
Four different species of the genus Myxobolus Butschli, 1882 were recorded for the first time from Mugil cephalus in Syrian coast. Myxobolus episquamalis was identified as infecting scales and caudal fins, in prevalence 4.12%. Myxobolus ichkeulensis was found in the gill archesand the base of gill filaments, in prevalence 9.15%. Myxobolus raibauti was found in liver andgonadsin prevalence 24.77% while Myxobolus spinacurvatura wasidentified as infecting mesentery and mesenteric vesselsin prevalence 22.62%. Myxobolus raibauti and Myxobolus spinacurvatura recorded the highest level of infection during winter, while Myxobolus ichkeulensis and Myxobolus episquamalis recorded the highest level of infection during spring and summer.

Keywords: *Fish parasites, Myxozoa, Myxosporea, Myxobolus spp., Mugil cephalus, Syria.*

I INTRODUCTION

The grey mullets (Osteichthyes: Mugilidae) are a family of euryhaline fish widely distributed in the world's tropical, subtropical and temperate regions, and particularly the family type species *Mugil cephalus* Linnaeus, 1758. These fish are intensively and semi-intensively farmed through the Mediterranean region [1]. Myxozoans (phylum Myxozoa) are a diverse group of microscopic obligate endoparasites with characteristic multicellular spores, distinct polar capsules and an extrudable polar filament used in the invasion of hosts. They are composed of two subgroups, the Malacosporea and the Myxosporea, which include more than 2000 species ([2]; [3]; [4]).

Myxosporeaare parasites infecting fishes, amphibians, reptiles as well as human beings [5]. Myxosporeans are believed to be a direct cause of fish mortality ([6]; [7]). The importance of grey mullets for aquaculture and the pathogenic potential of myxosporeans motivate their detailed study.

Myxobolus Butschli, 1882 is the most specious group within the phylum myxozoa, which contains 905 species primarily parasites of fish [8],although a small number of species have been found infecting amphibians and reptiles ([9]; [10]).Myxobolus species can infect diverse set of specific tissues that may include the tegument, gills, skeleton, eyes, glands, gonads, kidneys, scale epithelium, muscle, digestive tract, and nervous system, andit is now generally accepted that the myxozoa life cycle requires an alternation of vertebrate and invertebrate host [9].

II MATERIALS AND METHODS

From March 2014 to February 2016, 557 specimens of wild flathead grey mullet *Mugil cephalus* were caught in three sites of the Lattakia coast: Marine researches, Lattakia port and Alkabir Alshimali River Estuary. Fish were brought alive to the laboratory of Tishreen University – Lattakia, measured (range of total length 11-48 cm), weighed (range of total weight 13.67-872 g), and all organs were examined macroscopically and microscopically for the presence of parasitic infections.

In particular, the presence of myxozoan cysts was evaluated in scales, gills, liver, and mesentery. Cysts were removed from the infected tissues and photographed with a digital camera. Then, cysts and spores were examined under a light microscope in wet mounts, and the spores measured with an eyepiece micrometer. For permanent preparations, infected material was fixed with absolute methanol and stained with Gimsa. Fresh spore samples were stained with Lougol's iodine to determine the presence of iodinophilous vacuoles according to [11]. Descriptions and measurements were made according to ([4]; [12]).

III RESULTS

The morphological analysis of the *Myxobolus* spores allowed to identifying four different species: *M. episquamalis*, *M. ichkeulensis*,

M. spinacurvatura and *M. raibauti*. (Table 1) shows the location and prevalence of each *Myxobolus* species, jointly with the main measurements of the spores. Below, the morphological characteristics and measurements of cysts and spores are briefly described.

Myxobolus episquamalis Egusa, Maeno &Sorimachi, 1990 (on scales and caudal fin)

Compact whitish cysts, oval or irregular in shape, located on apical region of scales and caudal fin, and randomly scattered on the body surface, (Figure1a,b,c). Cysts measured 6-8 mm in length and 3-6 mm in width. Spores oval, narrowing edge anteriorly, with two pyriform polar capsules in the anterior region. No intercapsular appendix and thick mucus layer surrounding the spores was observed. There were 5 to 7 sutural marksalong the sutural edge. Sporoplasm was without iodinophilous vacuole and binucleate (Figure1d). The highest level of *M. episquamalis* was noted in spring and summer.

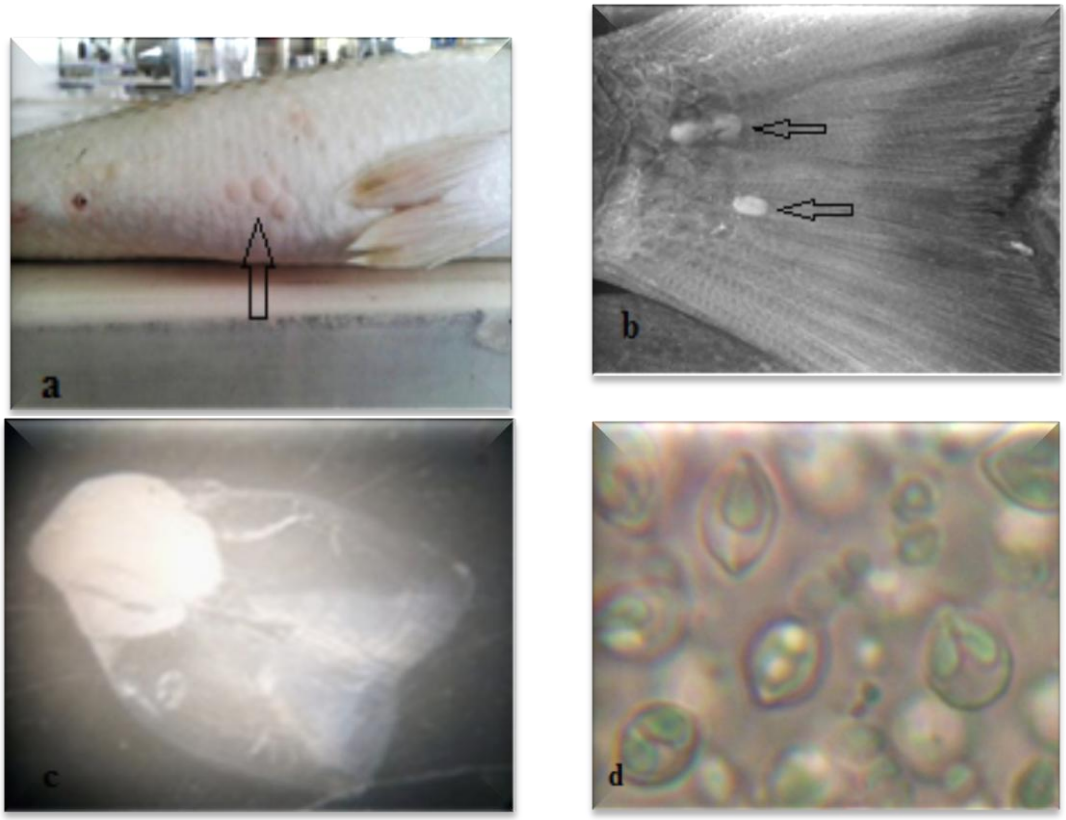

Figure 1. *Myxobolus episquamalis*: a) Cysts of *M. episquamalis* on the body surface of *Mugil cephalus* (bar = 2cm); b) cysts of *M. episquamalis* located on the caudal fin (bar = 2cm); c) cyst of *M. episquamalis* located on the distal region of scale (bar= 2mm); d) *M. episquamalis* spores with 2 polar capsules (7µm)

Myxobolus ichkeulensis Bahri & Marques, 1996 (on gill arches)

This species infected the gill arches and the base of gill filaments of *M. cephalus*. Cysts elongated, forming irregular clumps of 2-5 cysts. Single cysts were also found. In some cases cysts were located on both sides of the same gill arches (Figure2a,b). Spores were rounded with 2 oval polar capsules, and reached with their posterior end more than the half length of the spores. Spores had 9-11 sutural marks along the sutural edge. No intercapsular appendix was visible. No iodinophilous vacuoles in the spores treated with Lugol's iodine (Figure2c). The highest prevalence of infection was recorded during summer.

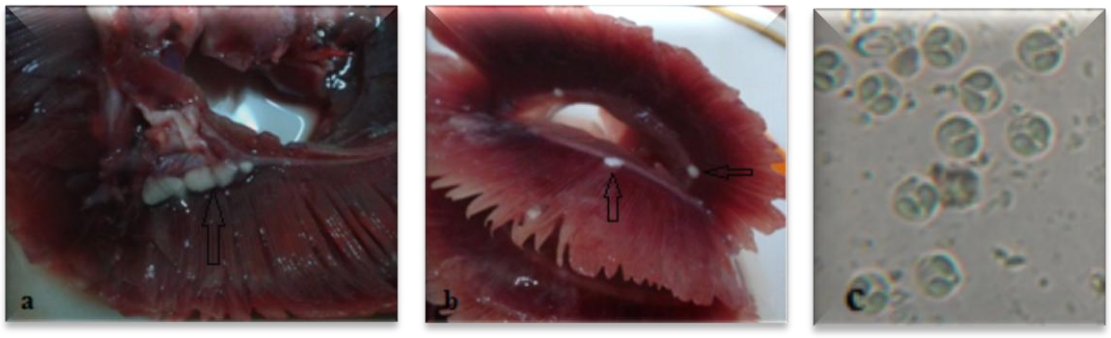

Figure 2. *Myxobolus Ichkeulensis*: A) Cystic Masses on the Base of Gill Arches (Bar= 4mm); B) Single Cysts On The Gill Arches (Bar= 3.5mm); C) Spores Of *M. Ichkeulensis*with 2 Polar Capsules (Bae= 4µm).

Myxobolus raibauti Fall et al, 1997 (in liver and gonads)

This species formed oval or spherical cysts of various size, located in the liver (Figure3a) and female and male gonads (Figure3b,c) of *M.cephalus*. Spores were ovoid, with the anterior region slightly wider than the posterior one, no sutural marks along the sutural edge, and covered with a thick mucus layer. Polar capsules were pear-shaped; the posteriorend approached the midpoint of the spore length. A small intercapsular appendix between the anterior ends of the polar capsules was observed. Sporoplasm filled the half posterior cavity of the spore, without iodinophilous vacuole (Figure3d). The highest prevalence of infection recorded in winter.

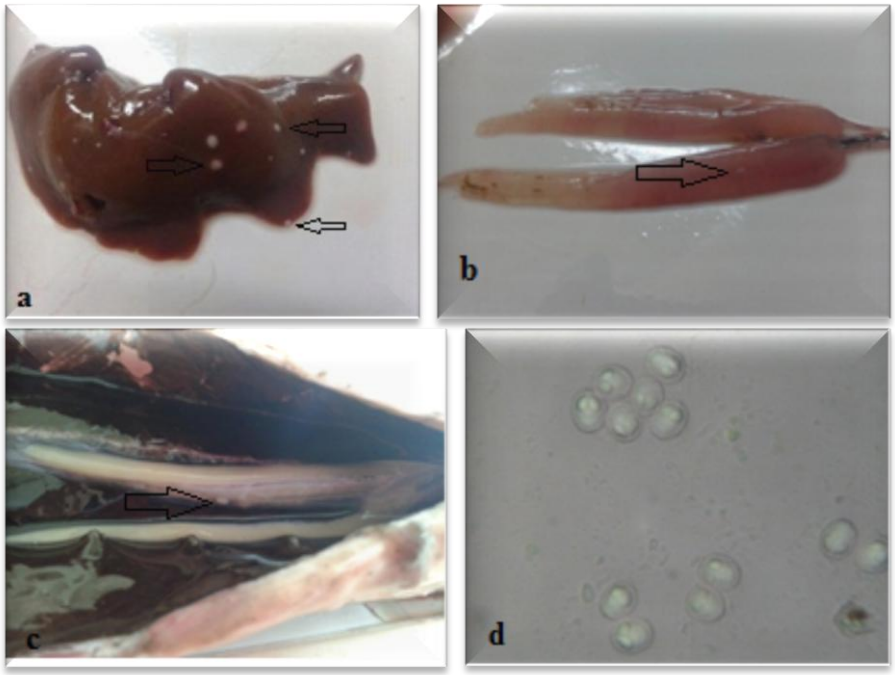

Figure 3. *Myxobolus Raibauti:* A) Numerous Cysts in Liver (Bar= 4mm); B) Cyst in Female Gonad (Bar= 1mm); C) Cyst in Male Gonad (Bar= 1mm); D) Spores Of *M. Raibauti* (Bar= 7µm)

Myxobolus spinacurvatura Maeno et al, 1990 (on mesentery and mesenteric vessels)

Whitish ovoid cysts located on the mesentery and the walls of mesenteric vessels of *M. cephalus* (Figure4a,b). Cysts measured 0.3-7mm in length, and 0.2-4 mm in width. Spores regularly ellipsoidal in frontal view. Small numerous sutural marks (12-14) were observed along the sutural edge. Polar capsules oval in shape, and their posterior ends didn't reach the midpoint of the spore length. Sporoplasm filled more than the half of the sporal cavity. No intercapsular appendix and no iodinophilous vacuole were observed (Figure4c,d). The highest prevalence was recorded in winter.

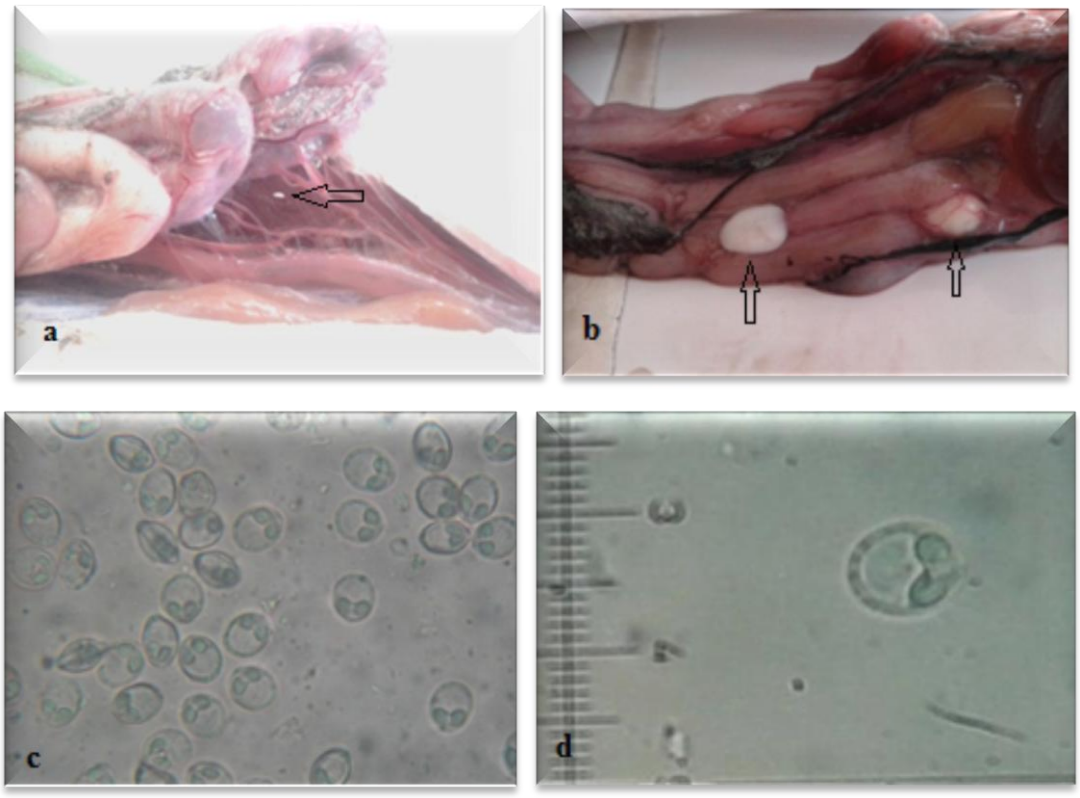

Figure 4. *Myxobolus Spinacurvatura*: A) Small Cyst on Mesentery (Bar = 1mm); B) Big Cysts on Mesenteric Vessels Wall (Bar= 2mm); C) Spores of *M.Spinacurvatura* with 2 Polar Capsules (Bar= 3µm), D) Spore of *M.Spinacurvatura* Shows Numerous Sutural Marks (Bar= 3.5 µm)

Table 1. Comparison of Spore Dimensions (µm) Between the Four Myxobolus Species Infecting Mugil Cephalus

species		*M.ichkeulensis*	*M.raibauti*	*M.spinacurvatura*	*M.episquamalis*
Infected organs		Gill arches	Liver, gonads	Mesentery	Scales, caudal fins
prevalence		9.15%	24.77%	22.62%	4.12%
spores	shape	round	oval	ellipsoidal	Oval with narrowing edge anteriorly
	length	13.2 (12.5-14)	14.7 (13.5-16)	12.5 (12-13)	8.2 (7-9.5)
	width	13 (12.5-13.5)	12.5 (12.1-13)	10 (9.4-10.5)	6.2 (5.5-7
Polar capsule	shape	oval	oval	Oval	pyriform
	length	5.8 (5.2-6.5)	5.5 (5-6)	4.8 (4-5.6)	4.2 (3.5-5)
	width	4 (3.5-4.5)	3.7 (3.2-4.3)	2.8 (2-3.7)	2.2 (1.5-3)
Iodinophilous vacuole		-	-	-	-
Intercapsular appendix		-	+	-	-

IV DISCUSSION

This is the first report of Myxosporean presence in *M. cephalus* in Syrian marine waters. Three Myxobolus species were previously recorded from freshwater fish (common carp) in Syria [13]. In the present study, four *Myxobolus* species: *M. raibauti*, *M. spinacurvatura*, *M. episquamalis* and *M. ichkeulensis*, were found in different organs of *M. cephalus*. Scales, mesentery, mesenteric vessels, brain, gill arches, gonads, and liver were the sites of infection.

The morphological characteristics and dimensions of our finding spores, Host and sites of infection overlapped with those described by other authors. *Myxobolus raibauti*, located in liver and gonads, was previously recorded in Senegal [14]. Liver has been reported to be infected with other *Myxobolus* species, such as *M. spinacurvatura*, recorded in Japan [15]. In Tunisia authors[6] recorded *Myxobolus* sp. in the liver of *Mugil cephalus*. *Myxobolus ichkeulensis* found had the same characteristics recorded by [17], site of infections (gill arches, and the base of gill filaments) lead to definition this species as *M. ichkeulensis*. This species can have different pathogenic effects, mainly weakening of cartilaginous tissues of the gill arches [18].

The gills are the primary and most common site of infection for most of the parasites found, though they can also be found at other sites: gill arches, kidney, fins, gall bladder, eyes and other tissues. *Myxobolus spinacurvatura*, found in the mesentery and mesenteric vessels had previously reported in different localities such as brain [17]and all characteristics of the specimens found were similar to those recorded by other authors ([15]; [16]; [17]). *Myxobolus spinacurvatura* infects other organs, as brain, liver, spleen, and pancreas of *M. cephalus* [15]. *Myxobolus episquamalis* has previously been recorded on the scales of *M. cephalus* from different geographical localities, such as Tunisia, Australia, Senegal, Russia, Turkey, and New Zealand ([17]; [19]; [20]; [21]; [22]; [23]). All characters described by these authors corresponded with those of the present specimens. The prevalence of *M. episquamalis* was relatively low (4%), similar values (5-8%) were recorded in Australia [19], whereas higher values in Senegal (13-17%) [20], and turkey (18%)[22]. No mortalities have been associated with this species, but fish infected with *M. episquamalis* greatly reduces the market value of the product [23].

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