

# Myxosporean parasites of the genus *Myxobolus* from *Mugil cephalus* in Ichkeul lagoon, Tunisia: description of two new species

S. Bahri\*, A. Marques

Laboratoire de Parasitologie et Immunologie, Université Montpellier II, F-34095 Montpellier Cédex 5, France

**ABSTRACT:** Four species of the genus *Myxobolus* Bütschli, 1882 were recorded from *Mugil cephalus* in Ichkeul lagoon in northern Tunisia. *Myxobolus episquamalis* Egusa, Maeno & Sorimachi, 1990 was identified as infecting scales. *Myxobolus spinacurvatura* Maeno, Sorimachi, Ogawa & Egusa, 1990 was identified as parasitizing the mesenteric vessels. *Myxobolus bizerti* n. sp. was discovered in the gill filaments, while *Myxobolus ichkeulensis* n. sp. was found in the gill arches. Data concerning morphology, dimensions, scanning electron micrographs of the spores and ultrastructure of pansporoblast are presented.

**KEY WORDS:** Myxosporea · *Mugil cephalus* · Tunisia · Taxonomy · *Myxobolus ichkeulensis* n. sp. · *Myxobolus bizerti* n. sp.

## INTRODUCTION

Mullet are one of the most important resources of the Tunisian lagoons and represent a high percentage of fishing catches. These fish are appreciated not only for their flesh but also for the female gonads.

*Mugil cephalus* from Tunisia have been found to be infected with several myxosporeans belonging to the genus *Myxobolus* Bütschli, 1882. The myxosporeans show several localizations (e.g. scales, gill arches, gill lamellae, mesenteric vessels, intestine). Previously, all of them were classified by Siau (1978) as *Myxobolus exiguus*.

The importance of mullets in aquaculture in some Mediterranean countries (especially Italy, Israel, Egypt and Tunisia) and the pathogenic potential of some myxosporeans, illustrated by the epizootic caused in mullet by *Myxobolus exiguus* in the Black Sea (Shulman 1957), induced us to compare myxosporeans parasitizing *M. cephalus* and to investigate their identity.

## MATERIAL AND METHODS

Between June 1994 and May 1995, 276 mullet *Mugil cephalus* with weights ranging from 180 to 680 g were collected from the Ichkeul lagoon in northern Tunisia. Fish were brought alive into the laboratory and all tissues were examined for the presence of protozoan parasites. Measurements, micrographs and line drawings of live specimens were made.

For transmission electron microscopy (TEM), cysts were fixed in 2% OsO<sub>4</sub> in Pallade's buffer (v/v) for 1 h at 4°C. They were dehydrated and embedded in epoxy resin (Spurr), sectioned with a Reichert OMU2 microtome and stained with saturated uranyl acetate in 50% ethanol followed by lead citrate. Observations were made by a JEOL 1200-EX II transmission electron microscope.

For scanning electron microscopy, material was prefixed in 4% glutaraldehyde buffered to pH 7.4 with 0.1 M sodium cacodylate. It was dried in an atmosphere saturated with absolute ethanol and then dehydrated with acetone and dried with CO<sub>2</sub> using the critical point method. The samples were finally sputter-coated with gold and observed with a JEOL JSM-35 scanning electron microscope at the University of Montpellier II.

\*E-mail: bahri@crit.univ-montp2.fr

## RESULTS

Myxosporeans were identified in developing cysts on the scales and within gill filaments, gill arches and mesenteric vessels (Figs. 1–4).

### *Myxobolus episquamalis*

Egusa, Maeno & Sorimachi, 1990

This species forms various compact whitish masses on the distal parts of scales, with a length of 6 to 9 mm and a width of 4 to 6 mm (Fig. 1). Each cystic mass consists of numerous microcysts measuring 150 to 400  $\mu\text{m}$ . Spores (Figs. 5a, b & 9) are oval in front view and are tapered at the anterior end. The 2 polar capsules are pyriform. No intercapsular appendix was observed. Along the sutural edge there are 5 to 7 markings.

Spores are sometimes covered with a porous envelope of coagulated mucus. The sporoplasm is binucleate without any iodophilous vacuole. Within the polar capsules, 5 or 6 filament coils were observed. The 2 orifices of polar filaments are elongated and symmetrical with regard to the sutural line (Fig. 13). We identify this parasite as the species *M. episquamalis*.

**Diagnosis:** Host: *Mugil cephalus*

Locality: Ichkeul lagoon (Bizerte), Tunisia

Site of infection: scales

Prevalence: 5 of 276 specimens examined

Fresh spore measurements (n = 40)

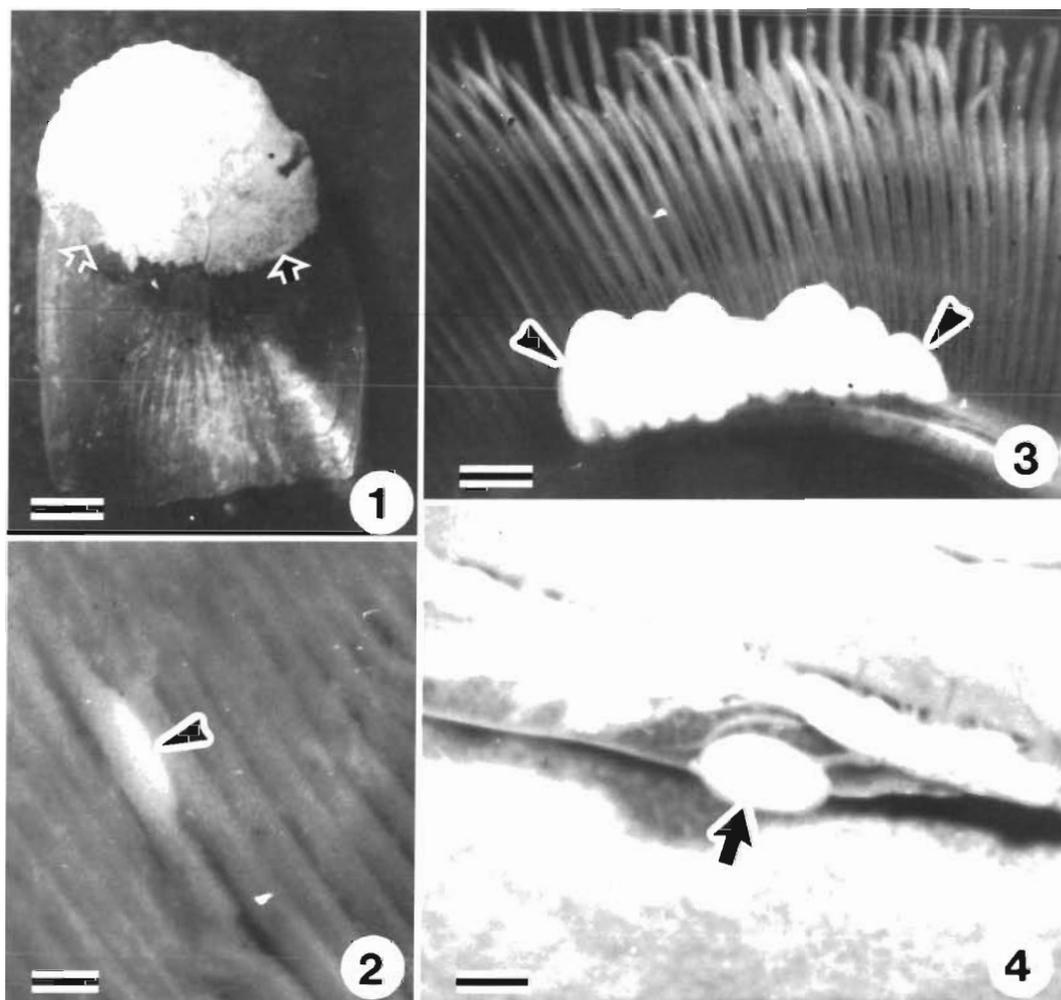
Length: 8.5 (8–9)  $\mu\text{m}$

Width: 6.5 (6–7)  $\mu\text{m}$

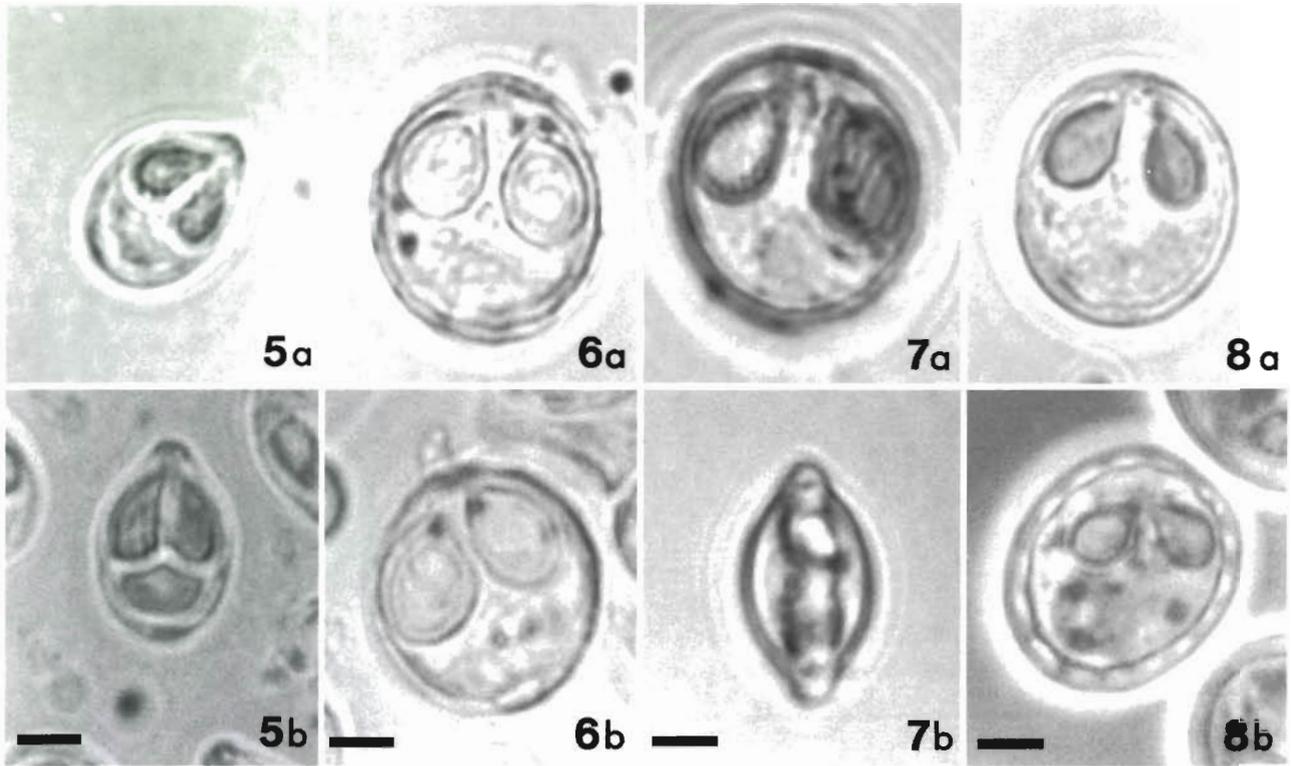
Polar capsules: length 4 (3.5–4.5)  $\mu\text{m}$

width 2 (1.5–2.5)  $\mu\text{m}$

Number of polar filament turns: 5–6



Figs. 1 to 4. *Myxobolus* spp. Different localizations of myxosporeans on *Mugil cephalus*. Fig. 1. Scale bearing a cyst (arrows) of *M. episquamalis* on the apical area (bar = 2 mm). Fig. 2. Elongated cyst (arrow) on gill filament (bar = 0.5 mm). Fig. 3. Aligned cystic masses (arrows) at the base of gill arch (bar = 4 mm). Fig. 4. Cyst (arrow) of *M. spinacurvatura* in the mesenteric vessel wall (bar = 1 mm).



Figs. 5 to 8. *Myxobolus* spp. Mature spores from fresh preparations. Fig. 5a, b. *M. episquamalis* in front view (bar = 3 µm). Fig. 6a, b. *M. ichkeulensis* n. sp. in front view (bar = 3.5 µm). Fig. 7. *M. bizerti* n. sp. in front view (a) and in side view (b) (bar = 3.5 µm). Fig. 8a, b. *M. spinacurvatura* in front view. Note the presence of numerous sutural markings (bar = 3 µm)

***Myxobolus bizerti* n. sp.**

In primary gill lamellae, the parasite forms elongated plasmodia appearing as cysts (Fig. 2) measuring 0.22 to 2.3 mm in length and 0.4 to 0.8 mm in width. Spores (Figs. 7a, b & 11) are mostly spherical. There are 8 to 11 sutural markings along the sutural edge. The polar capsules are pyriform and converge at their anterior pointed tips which do not show an intercapsular appendix. The capsules exceed the mid-length of the spore with their posterior ends. They show 6 to 7 filament coils. The discharging orifices of both polar filaments are circular and slightly displaced (Fig. 14). The sporoplasm is small and bi-

nucleate, without any iodophilous vacuole. Based upon its location and morphological characteristics, this parasite proves to be a new species. We propose the name *M. bizerti* n. sp.

**Diagnosis:** Host: *Mugil cephalus*

Locality: Ichkeul lagoon (Bizerte), Tunisia

Site of infection: gill lamellae

Prevalence: 24 of 276 specimens examined

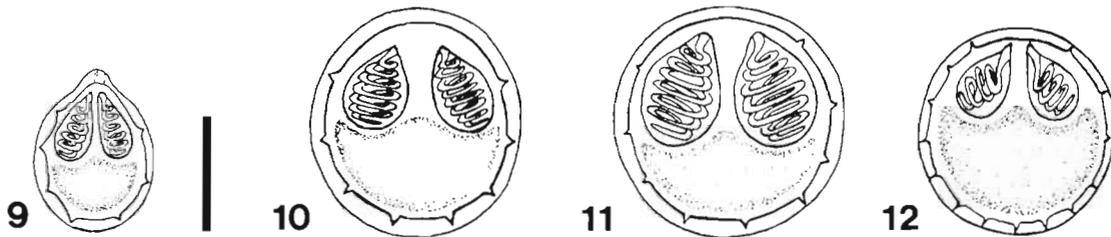
Fresh spore measurements (n = 30)

Diameter: 14.25 (14–14.5) µm

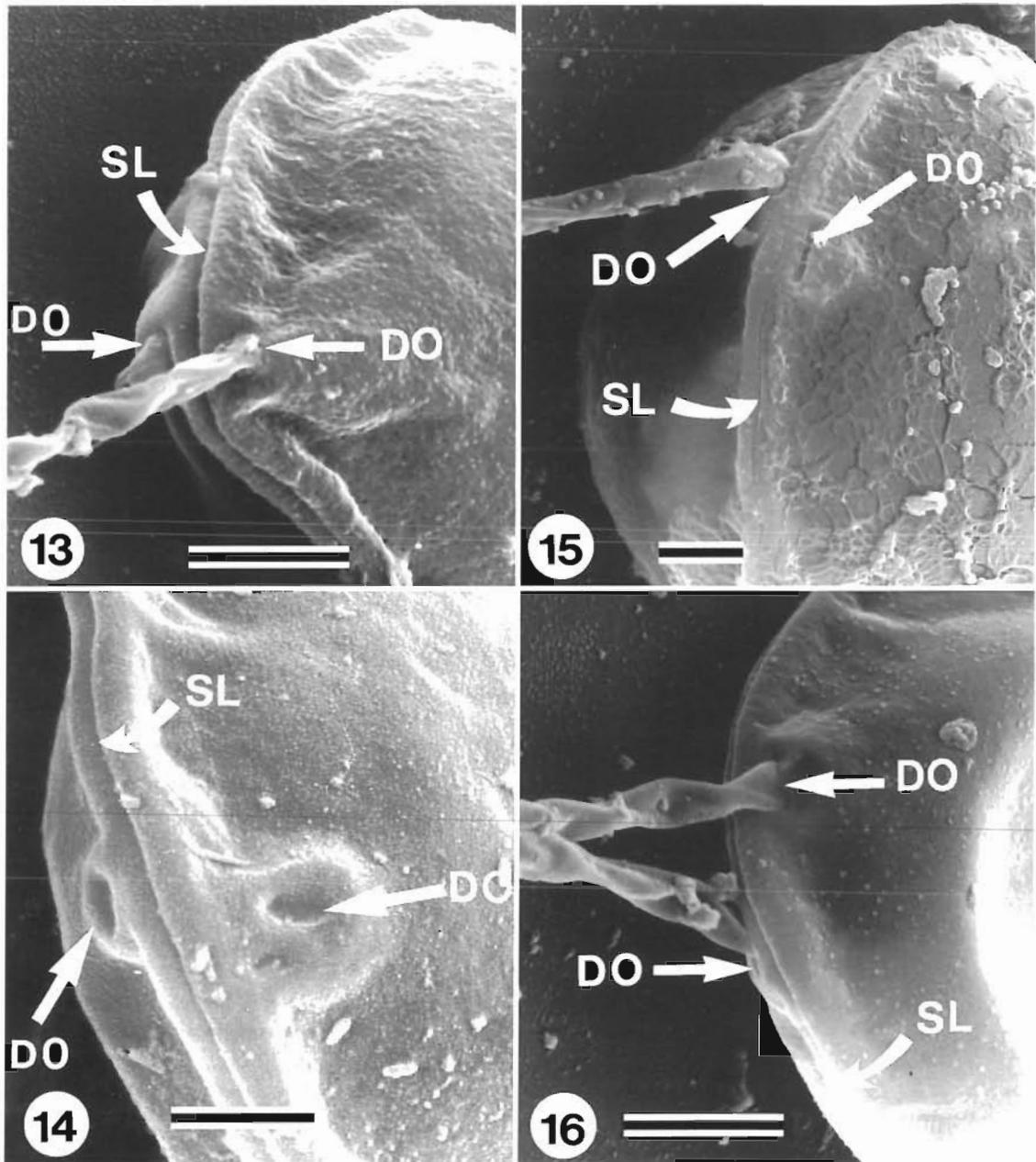
Polar capsules: length 6.5 (6–7) µm

width 5.75 (5.5–6) µm

Number of polar filament turns: 6–7



Figs. 9 to 12. *Myxobolus* spp. Line drawings of mature spores. Fig. 9. *M. episquamalis*. Fig. 10. *M. ichkeulensis* n. sp. Fig. 11. *M. bizerti* n. sp. Fig. 12. *M. spinacurvatura*. (Bar = 7 µm)

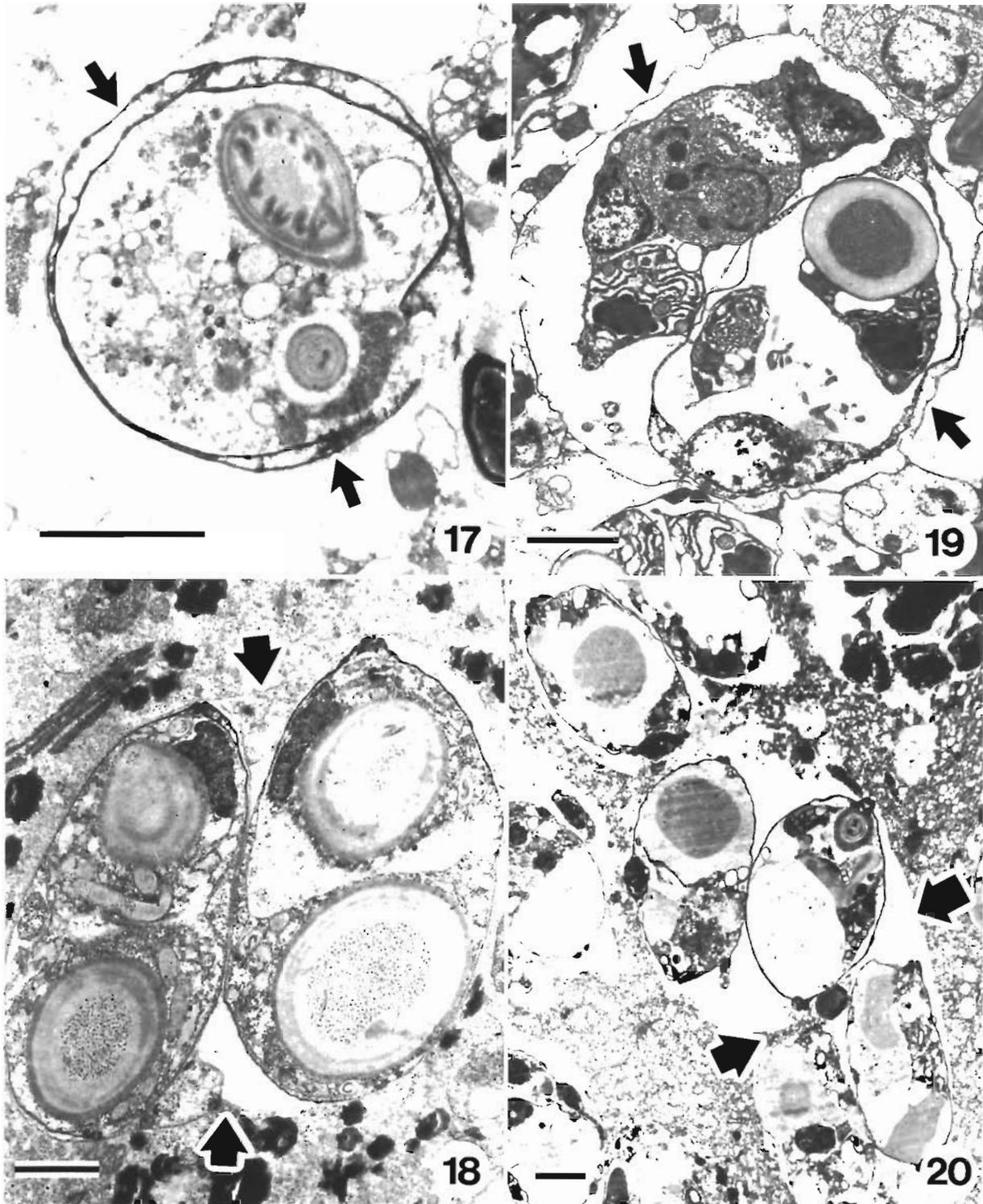


Figs. 13 to 16. *Myxobolus* spp. Scanning electron micrographs of the anterior end of the spore. Note shape, position of discharging orifices of polar filaments (DO) and sutural line (SL). Fig. 13. *M. episquamalis*. Fig. 14. *M. bizerti* n. sp. Fig. 15. *M. ichkeulensis* n. sp. Fig. 16. *M. spinacurvatura*. (Bars = 1  $\mu$ m)

#### *Myxobolus ichkeulensis* n. sp.

Myxosporeans infecting gill arches (Fig. 3) were clustered as cystic masses (2.2 to 4 mm in length and 1 to 3 mm in width) at the base of filaments. Spores (Figs. 6a, b & 10) are quite spherical; they present 9 to 11 sutural markings. Polar capsules are oval in shape and reach with their posterior end to half the length of the spore. No intercapsular appendix was visible be-

tween the anterior ends of the polar capsule. Polar filaments are coiled in 7 to 8 turns; their discharging orifices situated on both sides of sutural line are elongated and close together (Fig. 15). The sporoplasm situated at the posterior end of the spore fills half of the spore length. There is no iodophilous vacuole. Based upon its location and morphological characteristics, this parasite proves to be a new species. We propose the name *M. ichkeulensis* n. sp.



Figs. 17 to 20. *Myxobolus* spp. Ultrastructure of pansporoblast. Fig. 17. Monosporous pansporoblast of *M. episquamalis* (arrows point at the pericyte). Figs. 18 & 19. Disporous pansporoblasts (arrows) respectively of *M. bizerti* n. sp. and *M. spinacurvatura*. Fig. 20. Polysporous pansporoblast (arrows) of *M. ichkeulensis* n. sp. (Bars = 2  $\mu$ m)

**Diagnosis:** Host: *Mugil cephalus*

**Locality:** Ichkeul lagoon (Bizerte), Tunisia

**Site of infection:** gill arches

**Prevalence:** 18 of 276 specimens examined

**Fresh spore measurements** (n = 30)

Length: 13.5 (13–14)  $\mu\text{m}$

Width: 12.5 (12–13)  $\mu\text{m}$

Polar capsules: length 5.5 (5–6)  $\mu\text{m}$

width 4.15 (4–4.3)  $\mu\text{m}$

Number of polar filament turns: 7–8

### *Myxobolus spinacurvatura*

Maeno, Sorimachi, Ogawa & Egusa, 1990

The walls of mesenteric vessels in *Mugil cephalus* often bore isolated cysts of a myxosporean (Fig. 4) which were oval or spherical in shape, 0.2 to 3.8 mm in length and 0.2 to 3.3 mm in width. Spores released at maturity (Figs. 8a, b & 12) are regularly ellipsoidal in front view. Small numerous sutural markings (12 to 14 in number) are present over the sutural edge. Polar capsules are oval-shaped and their posterior ends do not reach the midpoint of the spore length. No intercapsular appendix appears. Each polar filament is coiled in 4 or 5 turns. Their discharging orifices (Fig. 16) are round in shape and slightly displaced with regard to the spore long axis. A binucleate sporoplasm fills more than a half of the sporal cavity. No iodophilous vacuole was observed. We identify this parasite as the species *M. spinacurvatura*.

**Diagnosis:** Host: *Mugil cephalus*

**Locality:** Ichkeul lagoon (Bizerte), Tunisia

**Site of infection:** mesenteric vessels

**Prevalence:** 87 of 276 specimens examined

**Fresh spore measurements** (n = 30)

Length: 12 (11–13)  $\mu\text{m}$

Width: 10 (9–11)  $\mu\text{m}$

Polar capsules: length 4.75 (4–5.5)  $\mu\text{m}$

width 2.75 (2–3.5)  $\mu\text{m}$

Number of polar filament turns: 4–5

### Sporogenesis

Data obtained from transmission electron microscopy allowed us to compare the sporogenesis of these different myxosporeans. Several plasmodia were examined for each species. The pansporoblast of *Myxobolus episquamalis* infecting the scales is monosporous (Fig. 17); those of *M. bizerti* n. sp. and *M. ichkeulensis* n. sp. parasitizing respectively the gill lamellae (Fig. 18) and the mesenteric vessels (Fig. 19) are disporous. However, the pansporoblast of *M. spinacurvatura* on gill arches is polysporous (Fig. 20).

### DISCUSSION

According to our knowledge 11 species (Table 1) belonging to the *Myxobolus* genus have been described infecting *Mugil cephalus* (Kudo 1920, Shulman 1966, Iversen et al. 1971, Egusa et al. 1990, Maeno et al. 1990, Landsberg & Lom 1991, Lom & Dykova 1992, 1994). Most of them can be clearly distinguished from each other.

*Myxobolus* species infecting the scales of *Mugil cephalus* have been reported in Tunisian coastal waters (Siau 1978), and were attributed to *Myxobolus exiguus* Thélohan, 1895. They were also mentioned along the Mediterranean coast of Israel (Overstreet & Howse 1977), in the Atlantic at Ria de Aveiro in northern Portugal (Menezes 1984), and in Japanese coastal waters (Egusa et al. 1989). Egusa et al. (1990) established a new species, *Myxobolus episquamalis*. This species seems to be similar with respect to its infection site, shape and size to our findings on *Mugil cephalus* from Tunisia (Bahri et al. 1995). Lom & Dykova (1992) considered it to be a junior synonym of *M. exiguus*. However, except for the species found on the scales in our study, the *Myxobolus* spores found in the other sites did not have morphological characteristics similar to *M. exiguus*.

Six species of *Myxobolus* have previously been identified from the gills of *Mugil cephalus*. *M. mülleri* Bütschli, 1882 has spherical spores in front view with an intercapsular appendix. Spores of *M. exiguus* Thélohan, 1895 and *M. achmerovi* Shulman, 1966 are oval and anteriorly tapered with distinct small intercapsular process. *M. branchialis* Markevitch, 1932 and *M. parvus* Shulman, 1962 have ellipsoidal spores with an intercapsular appendix.

In their shape and size (Table 1), *M. bizerti* n. sp. differs from the above *Myxobolus* species. *M. mugcephalus* Landsberg & Lom, 1991 shows similarities with *M. bizerti* n. sp. with respect to its circular shape; however, its spores (4.8 to 5.2  $\mu\text{m}$ ) are noticeably smaller than those of our species.

*Myxobolus cephalis* Iversen, Chitty & Van Meter, 1971 has been described as infecting various organs of sea mullet including gill arches in South Florida waters. The spores of this species, however, have a slightly smaller length (14 to 15  $\mu\text{m}$ ) and smaller width (10 to 11  $\mu\text{m}$ ) than those of *M. ichkeulensis* n. sp. and they have no sutural markings. In addition, the number of filament turns is lower (4 to 5) than in our species (7 to 8). These data indicate important differences from our findings.

The *Myxobolus* sp. we describe from the mesenteric vessels has approximatively the same size as *M. achmerovi* Shulman, 1966. The latter was first identified by Akhmerov (1960) from fins, gills and the mesentery

Table 1. Comparison of spore dimensions (in  $\mu\text{m}$ ) between *Myxobolus* species infecting *Mugil cephalus*

Species	Localization	Spore		Polar capsule	
		Length	Width	Length	Width
<i>M. mülleri</i> Bütschli, 1882	Gill filaments	10–12	9–11	4–5	2–3
<i>M. exiguus</i> Thélohan, 1895	Gill filaments Tissue of stomach Pyloric caeca	8–9.5	6–7.5	3–4.5	1.5–3
<i>M. branchialis</i> (Markevitch, 1932)	Gill filaments	8–7.6	6.8–8.5	4.4–4.8	2.5–4.1
<i>M. parvus</i> Shulman, 1962	Gill lamellae	6.5–7	5.5–6	4–4.2	3.8–4.2
<i>M. cheni</i> Shulman, 1962	Muscles Fins	8–8.5	6–6.5	4.5–5	2
<i>M. achmerovi</i> Shulman, 1966	Mesentery	12–14	9–10	4–5.3	2.3–3.5
<i>M. cephalis</i> Iversen, Chitty & Van Meter, 1971	Brain meninges Gill arches Buccal cavity Jaw bone	14–15	10–11	4–5	3–4
<i>M. episquamalis</i> Egusa, Maeno & Sorimachi, 1990 (junior synonym of <i>M. exiguus</i> )	Scales	7.5–9.5	6–7.5	3.8–5	2–3
<i>M. spinacurvatura</i> Maeno, Sorimachi, Ogawa & Egusa, 1990	Mesentery Brain	10.5–12.5	9–11	3.5–5	2.5–3.5
<i>M. mugcephalus</i> Landsberg & Lom, 1991	Gill filaments	4.8	5.2		
<i>M. rohdei</i> Lom & Dykova, 1994	Kidney	9.8–11.6	8.4–9.1	3.7–5	2.5–3.1

of common carp in the Amur region (eastern Russia) as *M. oviformis* Thélohan, 1892 and then reclassified as *Myxobolus* sp. by Shulman & Shtein (1962). Shulman (1966) established it as a new species, *M. achmerovi* Shulman, 1966, including in its host range *Mugil cephalus* and *M. soiuy* without further explanation. Thus we cannot compare it with our finding. However, *M. spinacurvatura* Maeno, Sorimachi, Ogawa & Egusa, 1990 observed in the mesentery and brain of *Mugil cephalus* has spores with a size and shape comparable to our finding. In order to prevent possible creation of synonyms, we have considered our finding as probably identical with *M. spinacurvatura*.

## CONCLUSION

In the past, morphological criteria of myxosporeans have been considered sufficient to group species under the same taxonomic entity infecting hosts even when they were found in different geographical locations and in different tissues, as long as the spores were similar. Currently, 2 approaches are used. The first case is that of myxosporeans parasitizing different hosts but inducing the same pathology; they should be grouped as the same entity as least until the alternate stage (actino-myxosporidian) is described. The second case is that of myxosporeans infecting the same host but in different tissues and presenting spores with distinct morphological characteristics. They probably cor-

respond to unique species. We use the latter approach to the classification of myxosporean species of the genus *Myxobolus* parasitizing Tunisian *Mugil cephalus*, species which in the past have been attributed to *Myxobolus exiguus*. It would appear from our study that different species were present, of which 2 are new.

The presence of 4 species in *Mugil cephalus* from Tunisia emphasizes the importance of tissue tropism in these species and proves again that myxosporeans are tissue-specific parasites, i.e. they always develop in a specific host tissue. An example is *Myxobolus cerebralis*, which migrates between the epidermal cells of rainbow trout to reach the cartilage via peripheral nerves and central nervous system (El-Matbouli et al. 1995). In addition, the pathogenic effects of the species we found are variable. *M. episquamalis* can invade the whole body of mullet, debilitating it and leading to its commercial rejection. *M. ichkeulensis* n. sp. weakens the cartilaginous tissue of gill arches, while the cysts of *M. bizerti* n. sp. are liable to block the lumen of the blood vessels of the gill lamellae.

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