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FISH PARASITOLOGY - ORIGINAL PAPER

Co-existence of *Myxobolus* spp. (Myxozoa) in gray mullet (*Mugil cephalus*) juveniles from the Mediterranean Sea

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Abstract
Gray mullet (Mugilidae) occur in all seas and are farmed widely around the world, and thus, the risk of their parasites spreading through transport of aquaculture seed is a serious concern. Among others, gray mullets typically host a diversity of myxosporeans, a group in which spore morphometrics of genera has been consistently shown to be inadequate for determination of species. In this study, we investigated *Myxobolus* Bütschli 1882 (Myxosporea) species found in two fingerling stocks of *Mugil cephalus* caught in the wild off the coasts of the eastern (Israel) and western (Spain) Mediterranean. Although we observed similar morphological features, significant dissimilarities in spore size and differences in *Myxobolus* species SSU rDNA sequences were noted. Genetic analyses demonstrated that multiple *Myxobolus* species, some with SSU rDNA sequences new to GenBank, infected the stock from Spain. In addition, *Myxobolus* DNA was found associated with several types of host tissue (gill, tail, and internal organs), and sequence analyses indicated that multiple species of *Myxobolus* were also present, sometimes in different tissues from the same fish. The results suggest that the gray mullets supported a collection of several different *Myxobolus* species with similar morphology.

Keywords Fish · Parasite · Myxosporea · Aquaculture

Introduction

Mugil cephalus, gray mullet, is a catadromous, euryhaline fish widely distributed in coastal areas, commonly inhabiting estuaries and lower river systems (Lane et al. 2015). For centuries, it has been an important human food resource, and throughout the Mediterranean region, *M. cephalus* is farmed both intensively and semi-intensively. Culture ponds are frequently stocked with wild caught gray mullet fry collected in shallow coastal and estuarine sites (Pruginin et al. 1975; Merella and Garippa 2001; Kim et al. 2013a).

Gray mullets are infected by diverse parasites, including myxosporeans (Lom and Dyková 1992; Kent et al. 2001), some of which are serious pathogens (Yurakhno and Ovcharenko 2014). To date, 26 different *Myxobolus* species have been documented from gray mullet (Eiras et al. 2005, 2007), of which 22 have been found in two or more different host tissues (Shvedko and Aseev 2008). The genus *Myxobolus* Bütschli 1882 (Myxosporea) includes some important pathogens of marine and freshwater fish (Maíllo-Bellón et al. 2011), many of which are known to directly cause host mortality (Lom and Dyková 1995; Rigos et al. 1999; Brown and Bruno 2006). Several hundred species have been described to date (Eiras et al. 2014). Early studies and identification of *Myxobolus* species, as in other myxosporea, were typically based on spore morphology as well as host and organ/tissue specificity. As molecular sequencing of SSU rDNA gene became the accepted standard approach for differentiating between myxosporean species with close spore morphology, this method which is now widely used, enabling effective comparison of different fish tissues that may be infected with closely related species (Székely et al. 2009). Indeed, in the last 20 years, the taxonomy of *Myxobolus* has been subjected to extensive phylogenetic analyses (Liu et al. 2010). In this study, we investigated *Myxobolus* spp. infections in gray mullet fingerlings that originated from two distinct sources: (a) wild gray mullet captured as fry in a river estuary in northern Israel, and (b) wild gray mullet captured as fry in the Ebro

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River Delta in Spain, and imported to Israel. These stocks, originating from the extreme west and east sides of the Mediterranean Sea, were introduced into the facilities of Israel Oceanographic and Limnological Research, National Center for Mariculture (IOLR-NCM) in Eilat, for experimental culture purposes. The *Myxobolus* spp. found parasitizing these fish are presented.

Materials and methods

Fish

The Israeli Mediterranean (IM) and Spanish Mediterranean (SM) stocks arrived in two separate batches at IOLR-NCM, Eilat. Both were examined for parasites and other disease agents upon arrival.

The imported SM gray mullets, upon initially arriving in Israel in mid-March 2015, were stocked in outdoor ponds in a fish farm in northern Israel, after which on March 29, 2015, 6000 of these fish were transported to the Reproduction Department at IOLR-NCM, Eilat, for experimental purposes (Project no. 9207/11; study approved by the Animal Experimentation Ethics Committee, Agricultural Research Organization, Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel). All fish were subjected upon arrival to veterinary inspection and then a routine quarantine procedure. The fish were placed in 6-m^3 containers with artificial aeration fed with UV-treated flowing seawater (40 ± 0.5 ppt salinity) at a flow rate of ten water changes per day, water temperature average was 25 °C ± 1, and water quality parameters (dissolved oxygen, pH, salinity, and temperature) were monitored constantly. A subsample of 200 fish 6.33 g avg. weight, 84 mm total length (TL), were examined. Some individuals displayed minute, cyst-like lumps on their caudal fins. These fish were separated, and microscopical examination indicated presence of parasite plasmodia. Accordingly, individual fish were visually scored as having light (1–2), medium (3–5), or heavy (> 6) infection. After approximately 4 months on July 23, 2015, a second shipment of gray mullet fingerlings (for the same project no. 9207/11) of approximately the same size and weight was received at NCM. This stock (IM) was comprised of 3000 wild gray mullet fingerlings caught in a river estuary in northern Israel during the first week of July 2015 and kept for 2 weeks in a separate outdoor holding pond at the same farm where the SM batch was kept. In the IM stock too, plasmodia were visible on fish caudal fins. Visibly infected fish were isolated and the number of plasmodia per caudal fin was recorded and infection intensity scored as above.

Microscopic examination of fish infected with *Myxobolus* spp.

Fish were sedated with 50 mg/ml clove oil and euthanized by decapitation. Fresh smears of plasmodia removed from the caudal fin were examined under a light microscope and measured. Air-dried smears were fixed in absolute methyl alcohol and stained with Giemsa, Hemacolor, or Gram stain. Photographs were taken with an Olympus 4040 digital camera mounted on phase contrast and Nomarski interference microscopes. For histology, infected tissue bits were fixed in 10% buffered neutral formalin, dehydrated, and embedded in paraffin blocks. Five micron sections were stained with hematoxylin and eosin, Gram, or Ziel–Neelsen. For transmission electron microscopy (TEM), the plasmodia were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated in a graded ethanol series, and embedded in Epon 812 blocks. Grids were stained with uranyl acetate and lead citrate and examined in a Jeol 100CX transmission electron microscope at 60 kV.

Extraction, purification, and precipitation of DNA

Whole plasmodia from both IM and SM batches were stored at − 80 °C until processed. From SM fish no. 6, a parasite plasmodium was carefully removed from the caudal fin and in addition, two infected scales were removed from SM#6 and SM#7. These samples were processed for rDNA sequencing. In addition, random organ samples from five heavily infected fish from the SM batch were sampled; these included caudal fin, intestine, spleen, liver, kidney, skin, mesentery fat, muscle, and gill. From the IM batch samples were taken from the caudal fin, intestine, skin, and gills. All samples were stored in − 80 °C until processing.

Plasmodia and/or tissue were ground with 300 ml of grinding buffer (100 mM Tris-HCl pH 9, 100 mM EDTA, and 1% SDS). The homogenate was incubated for 30 min at 70 °C. Forty-two microliters of 8 M potassium acetate was then added; the mixture was placed on ice for 30 min, then centrifuged at 12,000 *g* for 15 min at 4 °C. To eliminate traces of pellet, the supernatant was transferred to a fresh tube and centrifuged again for 5 min. DNA was precipitated with 1 volume of isopropanol and left for 15 min at room temperature. Pelleted DNA was washed twice in 70% ethanol, and the air-dried pellet was dissolved in 50 μl of double-distilled H_2O. DNA quantity and quality (260:280 ratios) were estimated in a microplate spectrophotometer (PowerWave™ XS, BioTek, Winooski, VT). PCRs were performed with a Programmable Thermal Controller (PTC-100; MJ Research, San Francisco, CA) in a final volume of 50 μl containing 1 U of *Taq* DNA polymerase (Promega, Madison, WI) following Diamant et al. (2005).

Amplification, visualization, and sequencing of PCR products

The small-subunit ribosomal DNA (SSU rDNA) gene cluster was targeted for amplification using PCR previously described by Hallett and Diamant (2001). A combination of primers designed on the basis of SSU rDNA sequences of myxozoan species (ACT1f, ACT1r, MYX4r) (Hallett and Diamant 2001) and the universal primers (18e, 18g) (Hillis and Dixon 1991) were used for PCR and sequence reactions. As control for DNA quality, the SSU rDNA gene was also amplified using the universal primers KuF1/KuR1 (Diamant et al. 2005). PCR products were analyzed in 1.2% agarose gels containing gel stain RedView™ (Applied BioProbes, GeneCopoeia, Inc. Rockville, MD) alongside a molecular weight standard (50-bp DNA ladder, GeneDireX, Bio-Helix Co Ltd. Taoyuan County, Taiwan) and visualized under UV light. Amplified products were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and the quantity and purity (260:280 ratio) of the double-stranded PCR products were estimated in a microplate spectrophotometer (PowerWave™ XS, BioTek, Winooski, VT). Purified PCR products from template DNA from fish SM#1, SM#5, SM#6, and SM#7 of the SM batch, and two fish samples from the IM batch were sent for DNA sequencing which was performed at Hy Laboratories Ltd. (Hylabs, Rehovot, Israel). In each sample, both DNA strands were sequenced at least three times to confirm reading accuracy. Forward and reverse sequences were visualized, using BioEdit v7.2.5, 1997–2013, Tom Hall). The ambiguous bases were clarified by sight using corresponding ABI chromatograms, or were noted as polymorphisms. Template DNA from SM#1, SM#5, SM#6, and SM#7 as well as two fish samples from the IM batch, were sequenced. The sequences (Table 1) were deposited in GenBank.

Phylogenetic analysis

The SSU rDNA partial sequences of the isolates in this study were compared and aligned with those of other *Myxobolus* spp. available in the GenBank database (NCBI/BLAST), using nucleotide BLAST protocol (Altschul et al. 1990) as presented in Table 1. Alignments were obtained by using the Clustal W program. A phylogenetic tree was constructed by UPGMA analysis with the MEGA 6.06 software. Bootstrap proportions were used to assess the robustness of the tree with 1000 bootstrap replications. Taxa included in the alignments were based on similarities to BLAST results including congeners of the novel species and representatives from each of the major clades reported in previous studies (Fiala 2006).

Results

Dark, spherical plasmodia typical of the genus *Myxobolus* (Myxosporea) were observed embedded in the dorsal and caudal fins of both IM and SM mullet stocks (Fig. 1a, b). A total of 40 out of 200 examined SM fish (20%) had visibly affected fins. The intensity of infection was variable ranging from 1 to 10 plasmodia/fish. Other organs examined revealed no evidence of the parasite.

Spore morphology

All spores found in the gray mullets were examined fresh and displayed a morphology consistent with the genus *Myxobolus*.

SM gray mullets

Spore dimensions ($N = 29$; mean size ± SD, in μm) length 12 ± 0.55, width 9.8 ± 0.47. Spores presented two elliptical smooth polar capsules, equal in size, positioned in close proximity to the anterior end, the polar capsules did not extend past the mid-length of the spore. Polar capsule dimensions: length 4.2 ± 0.32, width 3 ± 0 with 5–6 coils. The intact polar filament stained strongly with Giemsa-stained in air-dried smears, as did the discharged polar filaments (Fig. 1d, e).

IM gray mullets

Spore dimensions ($N = 29$) length 6 ± 0.75, width 5 ± 0.63. Two elliptical, smooth polar capsules equal in size and positioned in close proximity to the anterior end of the spore. Polar capsule dimensions: length 3 ± 0.16, width 2 ± 0.16.

A comparison between the SM and IM *Myxobolus* sp. spore dimensions is summarized in Table 2.

Light microscopy and Histology

Multiple plasmodia were embedded within the connective tissue between the dorsal fin rays in both SM and IM mullets. The plasmodia were generally spherical, and frequently clusters of two or three neighboring plasmodia were observed (Figs. 1a and 2a). The plasmodial cytoplasm contained sporoblasts and spores at various stages of development (Fig. 2b, c). There was no evidence of any cellular immune response or fibrosis in the tissue surrounding the plasmodia (Figs. 2d and 3a, b).

Transmission electron microscopy

The *Myxobolus* plasmodia were separated from the host connective tissue by a single membrane. The core of the plasmodium contained mature spores, while sporoblasts were situated at the periphery (not shown). The fine structure of the

Table 1 *Myxobolus* sequences from GenBank database used in the present comparative study

GenBank no.	Isolates	Fish host	Organ	Localization origin
MF118769	Spain5-t	*M. cephalus*	Tail	Ebro River delta, Spain
MF118772	Spain5-i	*M. cephalus*	Intestine	Ebro River delta, Spain
MF118770	Spain5-sk	*M. cephalus*	Skin	Ebro River delta, Spain
MF118771	Spain5-k	*M. cephalus*	Kidney	Ebro River delta, Spain
MF118773	Spain5-m	*M. cephalus*	Muscle	Ebro River delta, Spain
MF118767	Spain1-g	*M. cephalus*	Gills	Ebro River delta, Spain
MF118766	Spain1-t	*M. cephalus*	Tail	Ebro River delta, Spain
MF118763	Spain7-s	*M. cephalus*	Scale	Ebro River delta, Spain
MF118768	Spain1-m	*M. cephalus*	Muscle	Ebro River delta, Spain
MF118764	Spain6-tp	*M. cephalus*	Tail, plasmodium	Ebro River delta, Spain
AF378337	*Myxobolus ichkeulensis*	*M. cephalus*	Gills, muscle, skin, scale	Lake Ichkeul Tunis; Ebro River delta, Spain; Ukraine; Atlantic Ocean; Senegal
KC733438	*Myxobolus* sp. WSK-2013	*M. cephalus*	Intestine	South Korea
AF378341	*Myxobolus spinacurvatura*	*M. cephalus*	Intestine, liver, intrahepatic bile duct, gall bladder, spleen, mesentery, brain, gills, pancreas	Mediterranean (Tunisa, Spain), Russia, Australia, Japan
AY129317	*Myxobolus exiguus*	*M. cephalus, C. labrosus, L. aurata, L. saliens, L. ramada*	Gills, pyloric caeca, heart, stomach, gall bladder, intestine, kidney, mesentery, spleen, fins	France; Italy; Montenegro; Tunisian lagoons; Russia; Caspian Sea (Ukraine); Senegal
AY129314	*Myxobolus muelleri*	*M. cephalus, L. aurata, L. saliens, L. ramada*	Gills, mesentery, intestine, kidney, fins, heart, muscle, gonads, liver, gall& urinary bladder	Italy; Tunisia; Russia; Ukraine; Senegal
MF118765	IsraelMS gipt	*M. cephalus*	Gills, intestine, pasmodium, tail	Mediterranean Sea, Northern Israel
AY129318	*Myxobolus bizerti*	*M. cephalus*	Gills	Senegal
JF810537	*Myxobolus episquamalis*	*M. cephalus*	Gills, scales, fins	Bizerte; Tunisia; Korea; Japan; Australia; Turkey; Spain; Senegal
AY129312|	*Myxobolus episquamalis*	*M. cephalus*	Scales	Ichkeul Lake, Tunisia
MF118774	IsraelRS-i	*D. noct*	Intestine	Red Sea, Gulf of Eilat, (Israel)
KC711053	*Myxobolus khaliji*	*A. bifasciatus*	Intestinal wall	Arabian Gulf (Saudi Arabia)
EF370481	*Myxobolus cerebralis*	Salmonid fish: *S. trutta, and O. mykiss*	Head	USA; Alaska; Sakhalin Islands, Russia; British Columbia, Canada

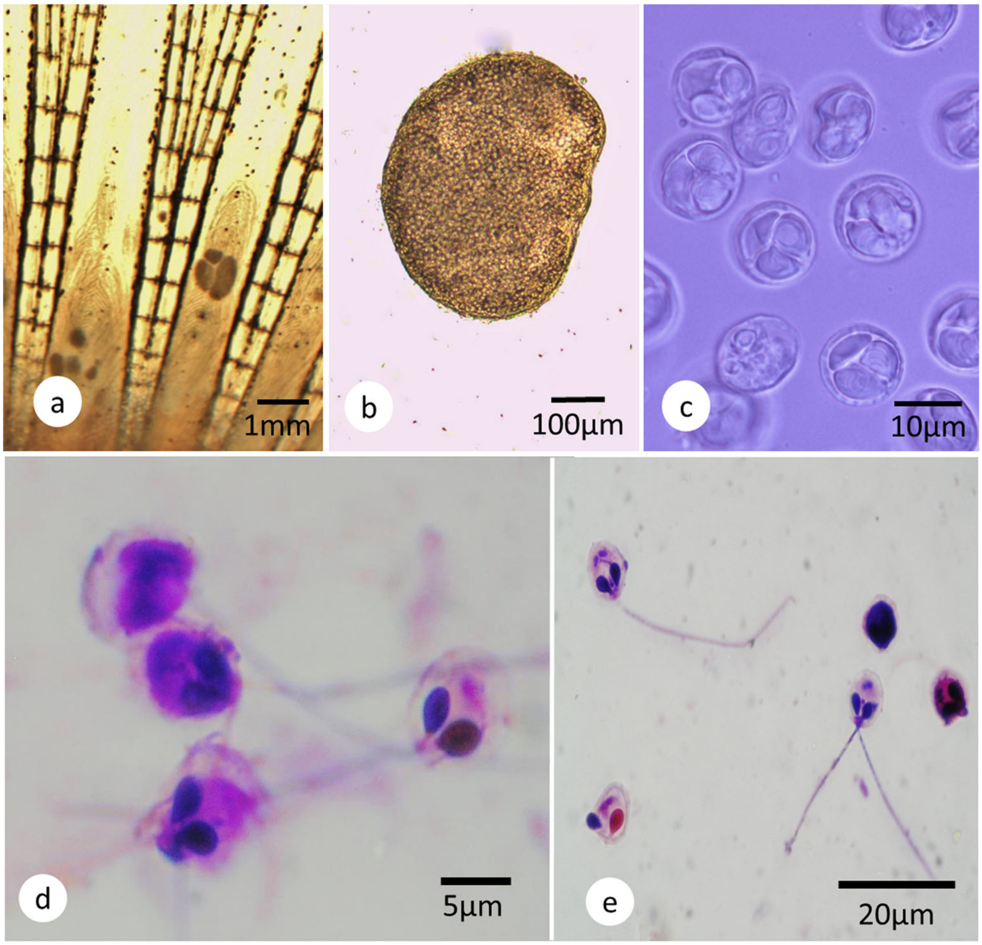


Fig. 1 Light micrographs of *Myxobolus* sp. plasmodia isolated from tail fins of gray mullet, *Mugli cephalus.* **a** Plasmodia as seen in situ, embedded in tail fin connective tissue. **b** Single plasmodium containing sporoblasts and spores. **c** Wet mount of fresh sporoblasts and spores at various stages of development. **d**, **e** Giemsa-stained air-dried smears showing spores; polar capsules and extruded polar filaments stain strongly

developing sporoblast presented two primordial polar capsules, each with 5–6 coils and a thick, laminar outer envelope and relatively translucent internal matrix, valve cells, and sporoplasm (Fig. 3c). In more advanced staged of sporoblasts, the development, the body exhibited two equally sized, elongated polar capsules, now with a thinner wall and more electron-dense than before. The polar filament coils maintained five to six turns (Fig. 3d). The sporoplasm was positioned near the posterior pole of the spore and the endoplasm contained several electron dense bodies and a single nucleus. The suture between the two outer valve cells of the spore could be clearly discerred (Fig. 3e).

Table 2 Spore and polar capsule measurements of *Myxobolus* spp. found on Spanish Mediterranean (SM) and Israeli Mediterranean (IM) gray mullet. Measurements were obtained from live material in wet mounts

Dimensions (µm)	Spain (SM) (*N* = 29)	Israel (IM) (*N* = 29)
Spore length	11.5 ± 0.55	6 ± 0.75
Spore width	9.8 ± 0.47	5 ± 0.63
Polar capsule length	4.2 ± 0.32	3 ± 0.16
Polar capsule width	3.0	2 ± 0.16

Phylogenetic analysis

The results of the organ samples from seven SM and two IM gray mullet fish which were analyzed by PCR and DNA sequences are summarized in Table 3. A phylogenetic tree constructed from the SSU rDNA gene sequences of the gray mullet isolates confirmed that all the sequenced samples belonged to the genus *Myxobolus* (Table 1). The topology was constructed by both UPGMA and neighbor joining, which yielded similar results; therefore, only one (UPGMA) is presented here (Fig. 4). The data summary for the various isolates of *Myxobolus* spp. sequences available in GenBank that were used in our study as references for comparison are given in Table 1. Based on the sequence analysis, there is a clear variability in *Myxobolus* spp. not only from different geographical regions but also among fish from the same batch and even within different organs of the same fish. The SM sequence analyses yielded six different sequences of *Myxobolus* (Fig. 4). DNA extractions taken from various organs (intestine, tail, skin, kidney, and muscle) of SM#5 that had a visibly infected fin displayed 97–100% similarity in the SSU rDNA gene sequences among themselves and were distinct from those isolated from other SM isolates. SM#5 isolates from intestine and

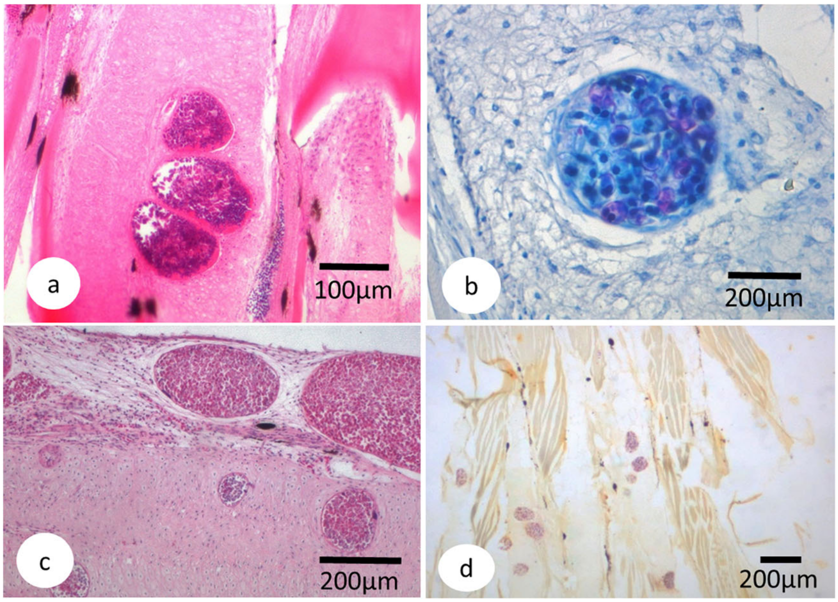


Fig. 2 Histological sections of *Myxobolus* sp. infected SM gray mullet. **a** Multiple plasmodia in fish dorsal fin connective tissue, flanked by fins rays (H&E). **b** A single plasmodium stained with ZN, showing developing sporoblasts and spores. **c** Tail fin of IM gray mullet, showing multiple plasmodia in the connective tissue between two fin rays (H&E). **d** Multiple plasmodia in the tail fin of SM gray mullet (Gram stain)

tail (Spain5-it) were 100% identical, while isolates from the kidney and muscle (Spain5-k and Spain5-m) had 99% similarity between them, and only 95% similarity was found between the two groups. Sequences of DNA extracted from skin (Spain5-sk) showed 97–98% similarity to kidney and muscle and tail and intestine groups, respectively. While, 100% similarity was found between SM#1 tail and muscle (Spain1-m and Spain1-t) and from the scale of SM#7 (Spain7-s), these had only 97% similarity to sequences from DNA sample extracted from gills of SM#1 (Spain1-g). SM#6 exhibited 100% sequences similarity between amplicons from its tail and the plasmodia (Spain 6-tp) which had 99% similarity to *Myxobolus ichkeulensis* Bahri and Marques 1996 (Fig. 4). Only 94–96% similarity was found between samples taken from SM#5 fish and those taken from SM#1 and SM#6. Overall, these sequence data results suggest there were two different *Myxobolus* spp. in SM#1. All *Myxobolus* spp. found in the SM batch excluding fish SM#6, showed only 95% similarity to *Myxobolus ichkeulensis*, Bahri and Marques 1996.

Sequences of DNA extractions from host organs (tail, gill, and intestine) and plasmodia from the IM batch (IsraelMS-gipt) had a 100% similarity among themselves (Table 3). Nevertheless, the degree of similarity between *Myxobolus* sp. from the IM mullets to that of *Myxobolus exiguus* and *Myxobolus muelleri* from GenBank was only 95%. Similarities between the sequences of DNA extractions taken from the different organs of the SM and IM gray mullet's ranged between 85 and 86% (Fig. 4).

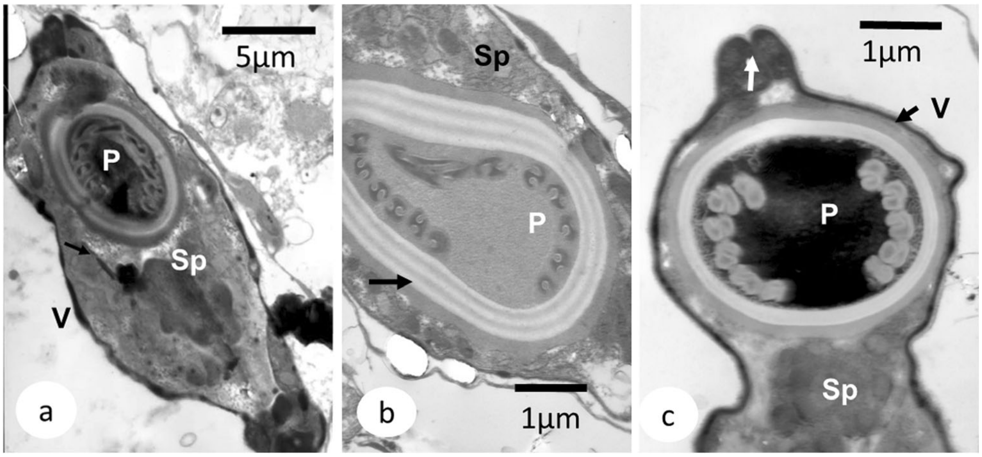


Fig. 3 Transmission electron micrographs (TEM) of SM *Myxobolus* sp. **a** A maturing sporoblast, showing valve cells (V), and polar capsule (P) at a stage where it is becoming increasingly electron-dense; the turns of the polar filament can be seen inside; the large sporoplasm is visible (SP). **b** Developing sporoblast showing sporoplasm (Sp), one of the two pyriform polar capsules (P) and the prominent tri-laminar wall of the forming capsule (arrows). **c** An electron-dense polar capsule (P) containing the polar filament, with its five coils; a section of the suture between the two spore valves (V) can be seen (white arrow)

Table 3 A summary of the PCR and sequence results from all SM and IM fish tissues examined by molecular analysis

Fish#	Tail+scale	Skin	Mesentery	Muscle	Kidney	Intestine	Gills
SM-1	A	–	–	A	–	–	+
SM-2	+	–	+	+	+	+	–
SM-3	+	+	+	+	+	–	–
SM-4	+	–	–	+	–	+	–
SM-5	C	D	+	F	E	C	–
SM-6	G						
SM-7	A						
IM-1	H					H	H
IM-2	H					H	H

(+) Tissue that yielded a positive PCR

(−) Tissue that yielded a negative PCR

Sequence codes: A, MF118763/68/66; B, MF118767; C, MF118772; D, MF118770; E, MF118771; F, MF118773; G, MF118764; H, MF118774

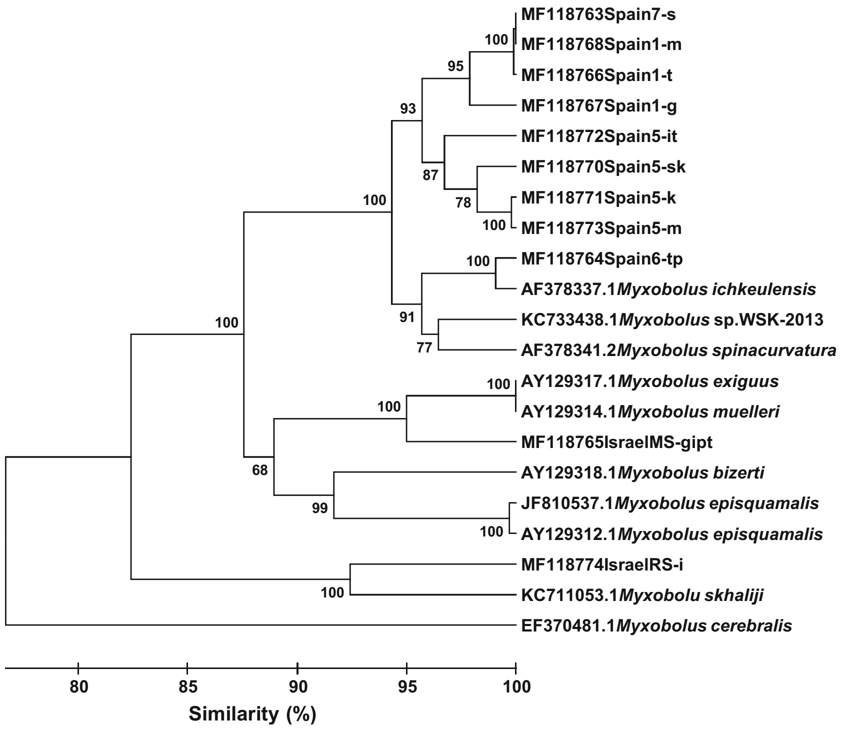
Discussion

Spore morphology of *Myxobolus* originating from the same or similar stocks of fish, have been a constant source of confusion and misidentification (Kent et al. 2001). SSU rDNA sequences enable a much more efficient separation between *Myxobolus* species that are morphologically indistinguishable (Kent et al. 2001; Fiala 2006; Liu et al. 2010; Carriero et al. 2013). While a 100% similarity suggests identical species, values below 98% have been considered to be in the "gray area," rendering it difficult to determine how much difference % is in fact necessary for a type to be called a new species (Ferguson 2002; Fiala 2006; Cech et al. 2012).

In our results, the IM gray mullet batch was clearly infected by a single species of *Myxobolus*, which was hitherto unsequenced and we thus considered it a new species. This species shows only 95% similarity to either *Myxobolus muelleri* Bütschli, 1882, or *M. exiguus* Thélohan, 1895, which are there closest related species.

In the SM stock, the SSU rDNA sequence results are much less straightforward. At least six distinct *Myxobolus* sequences appear to be present there, as evidenced by the phylogenetic tree (Fig. 4). Only 94–96% similarity was found between the *Myxobolus* spp. from SM#5 and those from SM#1 fish, regardless of the tissue from which they were extracted. Moreover, PCR amplification from SM#5 organs (intestine, tail, skin, kidney, and muscles) yield results suggesting a single species. Surprisingly, the results revealed no less than five different sequences, with 97% similarity between them. Salim and Desser (2000) reported on the existence of different *Myxobolus* species with similar spore morphology in different host tissues of the same cyprinid fish host. In our SM#1 fish, a 100% similarity was found between all amplicons of DNA extracted from muscle, scale and tail, but not from the gills. The latter tissue amplicons had only 97% similarity with the other isolates. Interestingly, a relationship between two species of *Myxobolus*, *M. muelleri*, and *M. exiguus* is apparent from the sequence analysis results. Although the SSU rDNA sequences of these species differed by only three nucleotide

Fig. 4 Phylogenetic tree constructed on the basis of UPGMA analysis of SSU rDNA sequences of selected *Myxobolus* spp. Numbers at the nodes indicate the bootstrap values. t tail, i intestine, sk skin, k kidney, m muscle, g gills, s spleen, p plasmodium



substitutions, their spore morphism is quite different and they undergo sporogenesis in different organs, supporting that they are different species, diverging at the level of the ribosomal gene (Kent et al. 1994; Siddall et al. 1995). Following this line of reasoning, it may be argued that five out of six isolates of our SM gray mullet batch are new species since the closest identity to a registered *Myxobolus* sp. was to *Myxobolus ichkeulensis* Bahri and Marques 1996, but with only 95% similarity. The *Myxobolus* sp. found on the tail of SM#6 was 99% similar to *Myxobolus ichkeulensis* and is thus presumed to belong to this species. An unequivocal species differentiation was observed between the SM batch *Myxobolus* spp. and the IM *Myxobolus* sp.

Numerous non-identified distinct *Myxobolus* spp. are likely to exist in fish species that have already been studied, adding to the diversity of *Myxobolus* species which were already described (Cech et al. 2012). In the SM mullets, the morphologically indistinguishable *Myxobolus* spores displayed considerable molecular variability. Gray mullets are well-known hosts of *Myxobolus*, and multiple species infection has been reported from a variety of geographical locations (Kim et al. 2013a, b). To better understand the differences and the evolutionary relationship between the different *Myxobolus* species the analysis of marker genes other than ribosomal RNA gene sequences, the SSU and LSU, separated by shorter regions (ITS1, 5.8S and ITS2), or other molecular chronometers such as HSP70 and COX-1, may be necessary (Bahri et al. 2003; Fiala 2006; Atkinson et al. 2015). On the other hand, these different *Myxobolus* DNA isolates may belong to the same species, which the differences in tissue tropism and small molecular diversities are wrongly interpreted; that said, these changes may also be attributed to host habitat microenvironment, but this hypothesis should be further evaluated (see Bahri et al. 2003).

In summary, the results of this study indicate that *Myxobolus* species found in the two examined mugilid batches differ from all other previously reported *Myxobolus* spp. in GenBank. Our results provide evidence of a mixed infection in gray mullets, in agreement with Andree et al. (1999) and Kim et al. (2013a). Our study underlines the importance of wild caught imported fish in contributing to the diversity of *Myxobolus* infections through spreading to new geographical regions.

Whether wild caught or farm reared, aquaculture species transportation to different growing areas can lead to accidental introduction of parasites with devastating results. A frequently cited example of myxozoan parasitism is *Myxobolus cerebralis*, a myxozoan responsible for whirling disease in salmonids. This parasite is native to Germany; its natural host presumed to be European brown trout (Hoffman 1990; Bartholomew and Reno 2002). It is likely that through transportation of rainbow trout stocks from Germany (Hedrick et al. 1998), whirling disease spread to at least 26 countries around the world, including North America, New Zealand, and South Africa (Bartholomew and Reno 2002). Another example is the myxozoan *Enteromyxum leei*, a parasite that has spread, presumably with sea bream stocks, to coastal farms in practically all countries bordering the Mediterranean Sea (Montero et al. 2007). Today, stringent regulations exist in most developed countries that restrict the movement of cultured species between areas. Transferred stocks must be certified SPF (specific pathogen free) before importation and as a result of such improved management practices spread of diseases in this manner has declined, although outbreaks due to novel parasitic agents still do occur (Lafferty et al. 2015).

Although reports of mortalities associated with *Myxobolus* infections are rare, the regular presence of this parasite in gray mullet has been shown to affect the overall health of the fish (Lane et al. 2015). Thus, there is a clear need to better understand the implications of host–parasite interactions and the diversity of the genus *Myxobolus* Bütschli, 1882 on gray mullet in aquaculture.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403–410

Andree KB, Szekely C, Molnár K, Gresoviac SJ, Hedrick RP (1999) Relationships among members of the genus Myxobolus (Myxozoa; Bivalvulida) based on small subunit ribosomal DNA sequences. J Parasitol 85:68–74

Atkinson SD, Bartošová-Sojková P, Whipps CM, Bartholomew JL (2015) Approaches for characterising Myxozoan species. In: Okamura B, Gruhl A, Bartholomew JL (eds) Myxozoan evolution, ecology and development (part I). Springer International Publishing, Cham, pp 111–123

Bahri S, Marques A (1996) Myxosporean parasites of the genus *Myxobolus* from *Mugil cephalus* in Ichkeul lagoon, Tunisia: description of two new species. Dis Aquat Org 27:115–122

Bahri S, Andree KB, Hedrick RP (2003) Morphological and phylogenetic studies of marine *Myxobolus* spp. from mullet in Ichkeul Lake, Tunisia. J Eukaryot Microbiol 50(6):463–470

Bartholomew JL, Reno PW (2002) The history and dissemination of whirling disease. In: American fisheries society symposium. American fisheries Society, pp 3–24

Brown LL, Bruno DW (2006) Infectious diseases of Coldwater fish in fresh water. In: Woo PTK, Bruno DW, Lim LHS (eds) Diseases and disorders of finfish in cage culture. CAB International, Wallingford, pp 107–170

Carriero MM, Adriano EA, Silva MRM, Ceccarelli PS, Maia AAM (2013) Molecular phylogeny of the Myxobolus and Henneguya genera with several new South American species. PLoS One 8(9): e73713

Cech G, Molnár K, Székely C (2012) Molecular genetic studies on morphologically indistinguishable *Myxobolus* spp. infecting cyprinid fishes, with the description of three new species, *M. alvarezae* sp. nov., *M. sitjae* sp. nov. and *M. eirasianus* sp. nov. Acta Protozool 57(4):354–366

Diamant A, Ucko M, Paperna I, Colorni A, Lipshitz A (2005) *Kudoa iwatai* (Myxosporea: Multivavulida) in wild and cultured fish in the Red Sea: Redescription and molecular phylogeny. J Parasitol 91(5): 1175–1189

Eiras JC, Molnár K, Lu YS (2005) Synopsis of the species of Myxobolus Bütschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). Syst Parasitol 61:1–46

Eiras JC, Abreu PC, Robaldo R, Pereira Junior J (2007) Myxobolus platanus n. sp. (Myxosporea, Myxobolidae), a parasite of Mugil platanus Gunther, 1880 (Osteichthyes, Mugilidae) from Lagoa dos Patos, RS, Brasil. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 59(4):895–898

Eiras JC, Zhang J, Molnar K (2014) Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea, Myxobolidae) described between 2005 and 2013. Syst Parasitol 88:11–36

Ferguson JWH (2002) On the use of genetic divergence for identifying species. Biol J Linn Soc 75(4):509–516

Fiala I (2006) The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. Int J Parasitol 36:1521–1534

Hallett SL, Diamant A (2001) Ultrastructure and small-subunit ribosomal DNA sequence of *Henneguya lesteri* n.sp. (Myxosporea), a parasite of sand whiting *Sillago analis* (Sillaginidae) from the coast of Queensland, Australia. Dis Aquat Org 46:197–212

Hedrick RP, Adkison MA, El-Matbouli M, MacConnell E (1998) Whirling disease: re-emergence among wild trout. Immunol Rev 166(1):365–376

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66(4):411–453

Hoffman GL (1990) Myxobolus cerebralis, a worldwide cause of salmonid whirling disease. J Aquat Anim Health 2(1):30–37

Kent ML, Margolis L, Corliss JO (1994) The demise of class protists: taxonomic and nomenclatural revision proposed for the protest phylum Myxozoa Grassé, 1970. Can J Zool 72:932–937

Kent ML, Andree KB, Bartholomewc JL, El-Matbouli M, Desser SS, Devlin RH, Feist SW, Hedrick RP, Hoffmann RW, Khattra J, Hallett SL, Lester RJG, Longshaw M, Palenzeula O, Siddall ME, Xiao C (2001) Recent advances in our knowledge of the Myxozoa. J Eukaryot Microbiol 48(4):395–413

Kim WS, Kim JH, Oh MJ (2013a) Morphologic and genetic evidence for mixed infection with two *Myxobolus* species (Myxozoa: Myxobolidae) in gray mullets, *Mugil cephalus*, from Korean waters. Korean J Parasitol 51(3):369–373

Kim WS, Kim JH, Jang MS, Jung SJ, Oh MJ (2013b) Infection of wild mullet (*Mugil cephalus*) with *Myxobolus episquamalis* in Korea. Parasitology Research 112:447–451

Lafferty KD, Harvell CD, Conrad JM, Friedman CS, Kent ML, Kuris AM, Saksida SM (2015) Infectious diseases affect marine fisheries and aquaculture economics. Annu Rev Mar Sci 7(1):471–496

Lane HS, Booth K, Pande A, Jones JB (2015) First report of the myxozoan parasite *Myxobolus episquamalis* infecting grey mullet (*Mugil cephalus*) from New Zealand. N Z J Mar Freshw Res 49(2):173–177

Liu Y, Whipps CM, Gu ZM, Zeng LB (2010) *Myxobolus turpisrotundus* (Myxosporea: Bivalvulida) spores with caudal appendages: investigating the validity of the genus *Henneguya* with morphological and molecular evidence. Parasitol Res 107:699–706

Lom J, Dyková I (1992) Protozoan parasites of fishes. Development in aquaculture and fisheries sciences, Elsevier, 26: 160–235

Lom J, Dyková I (1995) Note on the ultrastructure of two myxosporean (Myxozoa) species, *Zschokkella pleomorpha* and *Ortholinea fluviatilis*. Folia Parasitol 43:189–202

Maíllo-Bellón PA, Marques A, Graciaroyo MP (2011) Myxosporean infection of grey mullet in the Ebro Delta: identification and ultrastructure of *Myxobolus ichkeulensis* Bahri & Marques, 1996. Infecting the gills of *Mugil cephalus* L. Acta Protozool 50:67–71

Merella P, Garippa G (2001) Metazoan parasites of grey mullets (Teleostea: Mugilidae) from the Mistras lagoon (Sardinia, western Mediterranean). Sci Mar 65(3):201–206

Montero FE, Cuadrado M, Padros F, Crespo S, Raga JA (2007) *Cryptocaryon irritans* and *Enteromyxum leei*, two threats for the culture of *Diplodus puntazzo* in the Mediterranean. Bull Eur Assoc Fish Pathol 27(6):242–249

Pruginin Y, Shilo S, Mires D (1975) Grey mullet: a component in polyculture in Israel. Aquaculture 5:291–298

Rigos G, Christophilogiannis P, Yiagnisi M, Andriopoulou A, Koutsodimou M, Nengas I, Alexis M (1999) Myxosporean infections in Greek Mariculture. Aquaculture International 7(5):361–364

Salim KY, Desser SS (2000) Descriptions and phylogenetic systematics of *Myxobolus* spp. from cyprinids in Algonquin Park, Ontario. J Eukaryot Microbiol 47:309–318

Shvedko MB, Aseev NL (2008) Parasites on mullets from southern Far East Russia. In Proceedings of the Scientific Conference on the Current Status of Aquatic Bio Resources, Vladivostok pp 316–320

Siddall EM, Martin SD, Bridge D, Desser SS, Cone KD (1995) The demise of a phylum of protists: phylogeny of Myxozoa and other parasitic Cnidaria. J Parasitol 81(6):961–967

Székely CS, Hallett SL, Atkinson SD, Molnár K (2009) Complete life cycle of *Myxobolus rotundus* (Myxosporea: Myxobolidae), a gill myxozoan of common bream *Abramis brama*. Dis Aquat Org 85:147–155

Yurakhno V, Ovcharenko MO (2014) Study of Myxosporea (Myxozoa), infecting worldwide mullets with description of a new species. Parasitol Res 113:3661–3674