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Complete life cycle of *Myxobolus rotundus* (Myxosporea: Myxobolidae), a gill myxozoan of common bream *Abramis brama*

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ABSTRACT: The life cycle of *Myxobolus rotundus* Nemeczek, 1911, a myxosporean parasite of the gills of common bream *Abramis brama* L., was studied under laboratory conditions. Mature *Myxobolus* spp. spores from plasmodia in the gills of wild bream were used to infect naïve oligochaete worms in a flow-through system of aquaria. Triactinomyxon-type actinospores were released from the oligochaetes 1 yr later and allowed to continually flow into a tank containing uninfected bream fry. The gills of the fry were checked for development of plasmodia in squash preparations 3 d post-exposure, and then at weekly intervals for 8 wk. Tissue samples were fixed at each time point. Developing plasmodia were first observed 17 d post-exposure (Day 17). Mature spores were collected from plasmodia on Day 56 and were added to plastic dishes containing parasite-free *Tubifex tubifex* oligochaetes. Second-generation actinospores were released from these worms 8 mo post-exposure, and were morphologically identical to first-generation spores. Myxospores obtained from the bream fry were morphologically identical to those identified in wild bream as *M. rotundus*. Small subunit ribosomal RNA gene sequences obtained from first- and second-generation actinospores and the bream fry myxospores were 100% similar to *M. rotundus* spores from the original wild fish.

KEY WORDS: *Myxobolus rotundus* · Myxospore · Gill parasite · *Abramis brama* · Triactinomyxon · *Tubifex tubifex* · 18S rDNA · Lake Balaton

INTRODUCTION

Myxobolus rotundus Nemeczek, 1911, described from the common bream *Abramis brama*, is one of the most common myxosporeans on the gills of this fish in Lake Balaton, Hungary. While some aspects of its location and intrapiscine development have been studied (Molnár et al. 2009), nothing is known about its extrapiscine developmental stages. At least 7 *Myxobolus* species are known to infect the gills of bream and can be differentiated by spore morphology and the location of their vegetative developmental stages (Molnár & Baska 1999, Molnár & Székely 1999).

Among *Myxobolus* species that infect Lake Balaton bream, *M. bramae* appeared to have the highest prevalence over 20 yr of observation. Molnár & Székely (1999) suggested its plasmodia developed either in the afferent arteries of the gill filaments, where it formed large cysts, or in the secondary gill lamellae as several small plasmodia. Molnár et al. (2009), however, demonstrated that *M. bramae* developed in the gill filaments only, whereas spores in the gill lamellae were *M. rotundus*.

In life cycle experiments using presumedly pure *Myxobolus bramae* myxospores, resultant triactinomyxons (TAMs) did not produce *M. bramae* myxospores in naïve fish (Eszterbauer et al. 2000). It was subsequently determined that the actinospores were *M. rotundus*. In this study, we report follow-up infection experiments supported by histological and DNA sequence data, in which oligochaetes were successfully infected with *M. rotundus* myxospores, and the resultant actinospores initiated infection in the gills of bream. This study further supports distinction of the 2 plasmodial presentations and thus of separate species, *M. bramae* and *M. rotundus*.

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MATERIALS AND METHODS

We conducted 3 exposure experiments in the laboratory to follow the complete life cycle of a gill *Myxobolus* species. Expts 1 and 2 took place in a flow-through system of aquaria whereas Expt 3 used small dishes.

The flow-through system comprised 3 tiers of 60 l aquaria, with water continuously flowing from the upper tank (temperature 12 to 22°C), through the middle tank then into the lower one. The uppermost aquarium was supplied with chlorine- and parasite-free tap water at 720 ml h^{-1}; thus the water of the lower tank was exchanged completely in 3.5 d. The upper tank contained only water, whereas the middle tank contained mixed oligochaete species in a layer of mud. These originated from an environment free from myxosporean infections (from a muddy pool near the top of a hill where no fishes live), and consisted of 60% *Tubifex tubifex*, 19% *Limnodrilus udekemianus*, 17% *L. hoffmeisteri* and 4% *L. profundicola* species (as determined by oligochaete specialist Tarmo Timm). Part of this worm stock was maintained separately in flow-through water as a control population and was screened periodically for actinospores.

Parasite-free bream fry were placed into the lowermost tank upon detection of waterborne actinospores released from exposed oligochaetes (see below). The fry had been reared in a recirculation-type aquarium system as follows: roe or milt was stripped from common bream caught in Lake Balaton on 26 April 2005 (during the spawning season) and fertilised artificially; the eggs were then hatched in an infection-free environment and fry reared on brine shrimp *Artemia salina* and dry fish food.

Oligochaetes were also kept in 500 ml plastic dishes containing a 2 cm thick layer of sterilised pond mud under water that was aerated and regularly refreshed to compensate for evaporation. Oligochaetes were fed weekly with several drops of granulated fish food.

Expt 1. Exposure of oligochaetes to myxospores from wild bream. On multiple occasions, the first in 2000 and the last on 3 June 2004, the oligochaetes in the middle tank were exposed to plasmodia containing mature myxospores, freshly collected from the gills of wild common bream from Lake Balaton, Hungary. Plasmodia were removed mechanically from the gill filaments under a stereo dissection microscope and myxospores released from these were examined under a coverslip with a compound microscope. The sample was initially identified as large cysts of *Myxobolus bramae* (Molnár 2002).

At monthly intervals, about 1 l of water from the tanks was filtered through a 21 µm filter and checked for the presence of floating actinospores (first-generation TAMs). Actinospores in fresh mounts were photographed using an Olympus DP-10 digital camera, then measured and drawn on the basis of 20 spores from the digital images. Two samples of TAMs were preserved in ethanol for molecular analysis.

Expt 2. Exposure of parasite-free bream fry to experimentally obtained TAMs. Upon detection of waterborne TAMs in the middle tank (on 6 June 2005), 6 wk old specific parasite-free common bream fry (n = 150) were placed into the lowermost tank and hence exposed to the parasite. The total number of TAMs present in the system was estimated by filtering 10 l of water.

Development of plasmodial infections on the gills was studied by sampling fish starting 3 d post-exposure (Day 3, 9 June 2005) then weekly over 8 wk (to Day 58, a total of 9 samplings). On each occasion, 15 fry were sampled: 5 were dissected and examined in fresh mounts, and 10 were fixed in Bouin's solution, embedded in Paraplast® wax (Monoject Sciences), cut into 4 to 8 µm sections, and stained with haematoxylin and eosin (H&E). Myxospores in fresh mounts were photographed using an Olympus DP-10 digital camera, then measured and drawn on the basis of 20 spores from the digital images. Plasmodial infections in H&E-stained gill sections were photographed. Six samples of plasmodia, spores or tissue were preserved in ethanol.

Unexposed bream from the same source as the experimental stock were used as a control. At the end of the infection experiment, fresh mounts of the gills of 10 of these were examined by microscopy.

Expt 3. Exposure of oligochaetes to experimentally obtained myxospores. Eight weeks after the start of Expt 2 (3 August 2005), we collected 20 cysts containing mature Myxobolus spores under a stereo dissection microscope from four 14 wk old common bream fry. The spores were added to 50 *Tubifex tubifex* in ~100 ml water for 24 h, then the worms were transferred to plastic dishes.

The dish water was regularly filtered over 8 mo, until second-generation TAMs were detected (30 March 2006). Subsequently, the oligochaetes were separated from the mud using a fine cloth sieve and placed individually into cell well plates. Floating actinospores were collected with a pipette, as described by Yokoyama et al. (1991). A proportion of the spores was examined fresh under a BH-2 light microscope and photographed with a DP-10 photographic attachment, then 20 spores were measured and drawn from these photographs. Five samples of spores were fixed in ethanol for molecular analyses. The actinospore stage of *Myxobolus rotundus* was described using the terminology of Lom et al. (1997).

Molecular analysis of actinospores and myxospores. Four of the 15 preserved samples (2 actinospores and 2 myxospores) had their small subunit ribo-

somal RNA (SSU rRNA) genes sequenced: Sample 1 = TAMs collected 30 June 2005 from Expt 1; Samples 4 and 6 = myxospores collected 29 June and 21 July 2005, respectively, which had developed in small intralamellar plasmodia on the gills of the experimental common bream in Expt 2; Sample 11 = TAMs collected 5 April 2006 from Expt 3.

The ethanol-fixed samples were spun for 3 min at 14 000 rpm to pellet the spores, the ethanol removed, and the pellet air dried. Total DNA was extracted with a QIAGEN DNeasy^{TM} tissue kit (animal tissue protocol; QIAGEN) and eluted in 2 steps of 30 and 20 µl molecular grade (MG) water.

The gene was amplified using primers ERIB1 and ERIB10 (Barta et al. 1997) in a 20 µl reaction that comprised 0.5 µl extracted genomic DNA, 0.2 mM dNTPs, 0.25 µM of each primer, 2 µl 10× Taq buffer, 1.5 mM $MgCl_2$, 1 µl Rediload loading dye (Invitrogen), 1.25 U *Taq* polymerase (Promega) and MG water. The PCR cycle profile was performed in a PTC-200 (MJ Research) and consisted of an initial denaturation step of 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 45 s, 72°C for 120 s and finished with terminal extension at 72°C for 10 min, then rested at 4°C.

For sequencing, second-round PCRs were used to generate 2 overlapping templates with primer pairs ERIB1 and ACT1r (Hallett & Diamant 2001), and MyxGen4f (Diamant et al. 2004) and ERIB10. Reagent amounts were scaled up to 50 µl reactions, included 1.25 µl of the ERIB1/ERIB10 template, and the above cycling profile used with the extension step shortened to 60 s. Aliquots of the resultant PCR products were electrophoresed through a 1% agarose 1× Tris-acetate-EDTA (TAE) buffer gel stained with SYBR Safe (Invitrogen) alongside a 1 kb+ DNA ladder (Invitrogen) to confirm that only a single amplicon of expected size was present.

Products were purified using a QIAquick PCR purification kit (QIAGEN). DNA concentrations were measured with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies). The amplicons were sequenced in both directions using the amplification primers and ABI Big Dye Terminator chemistry on an Applied Biosystems Capillary 3100 Genetic Analyzer at the Oregon State University sequencing facility (Center for Gene Research and Biotechnology, Central Service Laboratory). The forward and reverse sequence reads were aligned in BioEdit (Hall 1999) and any ambiguous bases clarified using corresponding ABI chromatograms. Consensus sequences were submitted to GenBank. A standard nucleotide-nucleotide BLAST (blastn) search was conducted (Altschul et al. 1997). Sequences were compared with the type sequence of *Myxobolus rotundus* (GenBank accession number EU710583).

RESULTS

Infection in oligochaetes

The release of actinospores from oligochaetes was observed 1 yr after exposure to myxospores from the plasmodia of wild bream (Expt 1; Fig. 1a,b). Intra-oligochaete vegetative stages were not examined in detail; only the release of actinospores was followed. Prevalence and intensity of infections were studied in the oligochaetes in plastic dishes, exposed during Expt 3. From some 50 worms, 24 were alive 8 mo post-infection, when second-generation TAMs were first observed. Only 2 (8.3%) were producing the spores, both identified as *Tubifex tubifex*.

Description of experimentally obtained triactinomyxon actinospores

Description: Triactinomyxons (n = 20, Figs. 1c & 2a,c,d, Table 1) had an elongated spore axis containing 3 pyriform polar capsules and a sporoplasm at its apex, and 3 elongated caudal processes at its distal end. Average measurements of first- and second-generation TAMs were equivalent. Spore axis was 136.4 µm

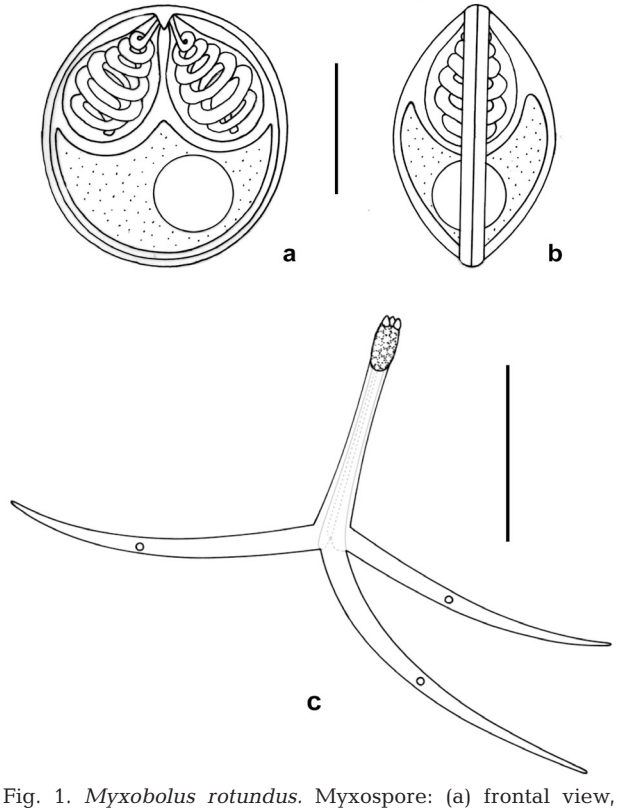

Fig. 1. *Myxobolus rotundus.* Myxospore: (a) frontal view, (b) sutural view, scale bar = 5 µm (c) Actinospore, scale bar = 100 µm

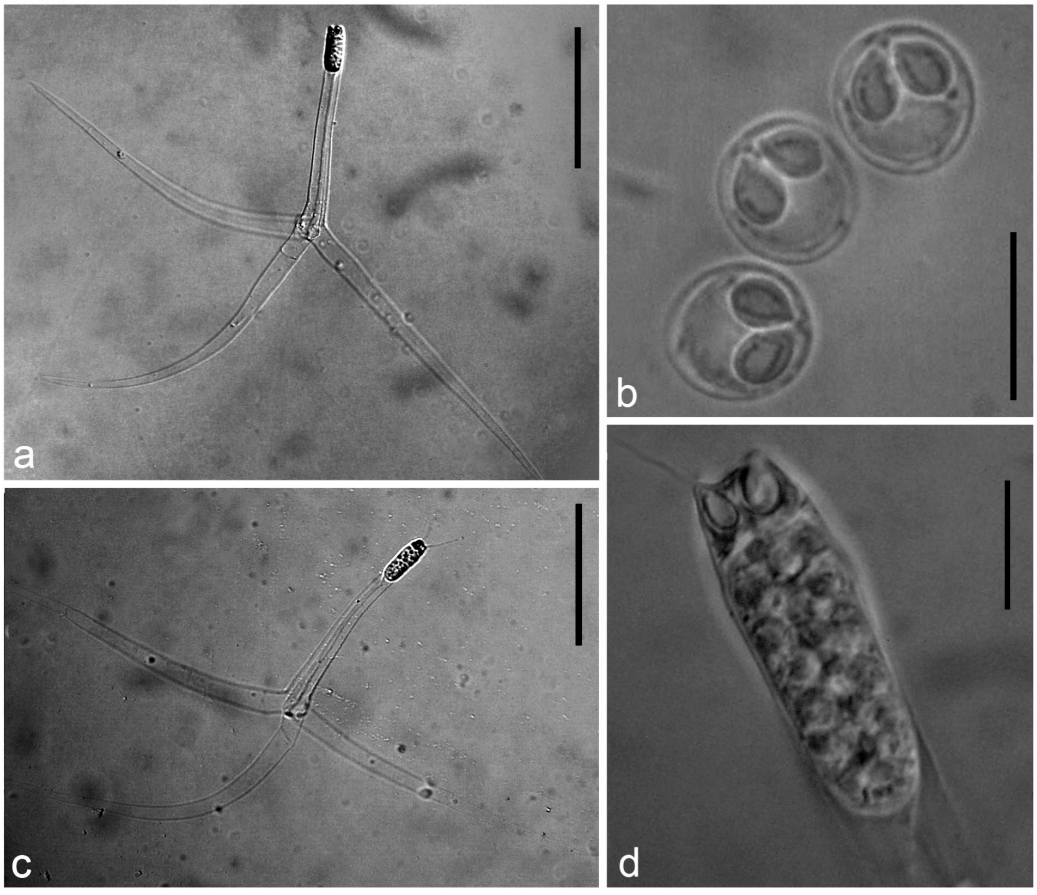

Fig. 2. *Myxobolus rotundus.* (a) First-generation triactinomyxon actinospore, scale bar = 100 µm; (b) *Myxobolus* myxospore from gill plasmodia from experimentally infected bream, scale bar = 10 µm; (c) second-generation triactinomyxon, scale bar = 100 µm; (d) spore body of triactinomyxon showing germ cells, scale bar = 10 µm

(125 to 146.5 µm) long and 15.1 µm (13.2 to 17.6 µm) wide at base. Polar capsules were pyriform, 5.3 µm long and 3.4 µm wide (Fig. 3d). Spore body was 31.4 µm (27.9 to 34.9 µm) long and 12.1 µm (10.6 to 12.9 µm) wide, and the sporoplasm contained 32 spherical germ cells (Figs. 1c & 2d). Caudal processes were 228.8 µm (205 to 279 µm) long and 13.8 µm (12.8 to 15 µm) wide at the base of the spore axis. The processes curved slightly downwards or upwards and tapered to terminate in a 'pencil point.' The valve cell nuclei in processes were irregularly positioned.

Type host: *Tubifex tubifex*

Site in host: Intestinal epithelium

Type locality: Experimental flow-through system of VMRI Fish Pathological Laboratory.

Type specimens: Stored in 70% ethanol deposited in the collection of the first author.

Phototypes: Phototype deposited in the Parasitological Collection of the Hungarian Natural History Museum, accession numbers HNHM 17833 and 17834.

Remarks: More than 60 triactinomyxon types are described in the literature, of which 4 are *Myxobolus* spp. parasitising the gill of common bream. Except for *M. hungaricus*, these 4 have 32 germ cells in their sporoplasm (Table 1). Our type is morphologically very similar to Triactinomyxon type 4 (Hallett et al. 2005) and also the *M. bramae* triactinomyxon (Eszterbauer et al. 2000).

Myxobolus rotundus development in fish

When bream fry were added into the system, the estimated number of waterborne triactinomyxons was 80 000 to 90 000, a rather intense infection. Infection in bream was first recorded on Day 17, when large numbers of small intralamellar plasmodia were seen in the gill filaments in squashed preparations (Fig. 3a). Histological sections showed that plasmodia (Fig. 3b) were located inside the lamellae of the gill filaments. They deformed the lamellae so that the plasmodium was

Table 1. *Myxobolus* spp. Measurements (in µm) of actinospore stages parasitising the gill of common bream *Abramis brama*. ND: not determined

Species	Spore axis		Polar capsule		Spore body		Process		No. of germ cells	GenBank acc. no.	Source
	Length	Width	Length	Width	Length	Width	Length	Width	Termination			
M. rotundus	136.4 (125–146.5)	15.1 (13.2–17.6)	5.3	3.4	31.4 (27.9–34.9)	12.1 (10.6–12.9)	228.8 (205–279)	13.8 (12.8–15)	Pencil point	32	FJ851447	Present study
M. parviformis	127 (111–142.5)	10.3 (6.3–12.1)	7.3 (5.8–9.5)	3.89 (3.7–4.7)	33.9 (28.9–42.1)	11.8 (9.5–14.7)	101.7 (86.2–118.3)	9.3 (6.3–12.1)	Long fine taper (myxospore)	32	AY 836151	Kallert et al. (2005a)
M. hungaricus	Style length: 60.2 (53–69)	6 (anterior part)	7.7 (7–8)	3 (2.5–3.5)	38.9 (30–45)	9.5 (9–10)	196.7 (160–240)	ND	Sharp point	18?	—	El-Mansy & Molnár (1997b)
M. bramae	139 (128–152)	11.6 (10–12)	4.8 (4–6)	3.2 (3–4)	34.1 (28–40)	11.6 (10–12)	246.8 (224–260)	11 (10–12)	Sharp point	32	—	Eszterbauer et al. (2000)
M. macrocapsularis	Style length: 80.8 (80–84)	9.5 (9–10)	5.14	3.8	27.2 (23–31)	12 (10–14)	116.6 (94–141)	8.5 (8–10)	Sharp point	32	—	Székely et al. (2002)
Triactinomyxon type 4	133.6 (123–145)	17.1 (13–20.7)	5.4	3.5	31.6 (28.5–36.3)	13.3 (12.3–15.5)	253 (220–290)	14.9 (11.7–19.4)	Pencil point	32	AY495707	Hallett et al. (2005)
Triactinomyxon type 1a	82.9 (71.9–89.9)	10.6 (5.7–12.8)	5.2	3.3	32.7 (28.2–38.5)	13.2 (10.3–15.4)	109.5 (84.7–123.2)	10.1 (9.2–10.3)	Long fine taper	32	AY495704	Hallett et al. (2005)

mostly located on one side of the lamella closely attached to the neighbouring lamella or plasmodium. Inside these plasmodia, only a few generative cells could be seen. At Day 24 the plasmodia were larger and the number of vegetative nuclei and cells of the pansporoplasm development were more numerous (Fig. 3c,d). The plasmodium could be located in the centre of the lamella surrounded by sera and blood cells (Fig. 3c), or in an eccentric position (Fig. 3d). Although the plasmodium was bordered on both sides by the epithelial and endothelial layer of the infected lamella, at the less extended side this layer was thicker (Fig. 3d) and within it red blood cells could circulate relatively freely (arrow). In more advanced cases (Day 45; Fig. 4a,b) plasmodia were filled by young spores and sporogonic stages. At Day 58, plasmodia packed with spores were still located inside the lamellae of the gill filaments (Fig. 4c,d), but where multiple plasmodia developed in close proximity, the original structure of the gill filaments was hard to observe. For samples taken on Days 17, 24, 45 and 58, prevalence of small intralamellar plasmodia in fresh mounts and histological preparations was 12 to 33%.

Myxospores corresponded in size and structure to those collected from wild fish and were morphologically identical with those of *Myxobolus rotundus* (Molnár et al. 2009). Phototypes of myxospores were deposited in the Parasitological Collection of the Hungarian Natural History Museum, accession number HNHM 17832.

DNA sequence data

Partial SSU rRNA genes from 4 samples were amplified and sequenced (isolate number, read length and GenBank accession numbers in parentheses): first generation triactinomyxon spores (Sample 1; 971 nt; FJ851446), myxobolus plasmodia (Sample 4; 1986 nt; FJ851448) and myxospores (Sample 6; 1977 nt; FJ851449) from exposed fingerlings and second-generation triactinomyxon spores (Sample 11; 1985 nt; FJ851447). These were identical to each other and to the type sequence of *Myxobolus rotundus* (GenBank accession number EU710583). Characteristic polymorphic loci were apparent in all reads, consistent with those in the type sequence (Molnár et al. 2009). The sequences were 99.9% (1439/1440 nt) similar to Triactinomyxon type 4 of Hallett et al. (2005).

DISCUSSION

Contrary to the large number of myxospores described for different myxosporean species, the num-

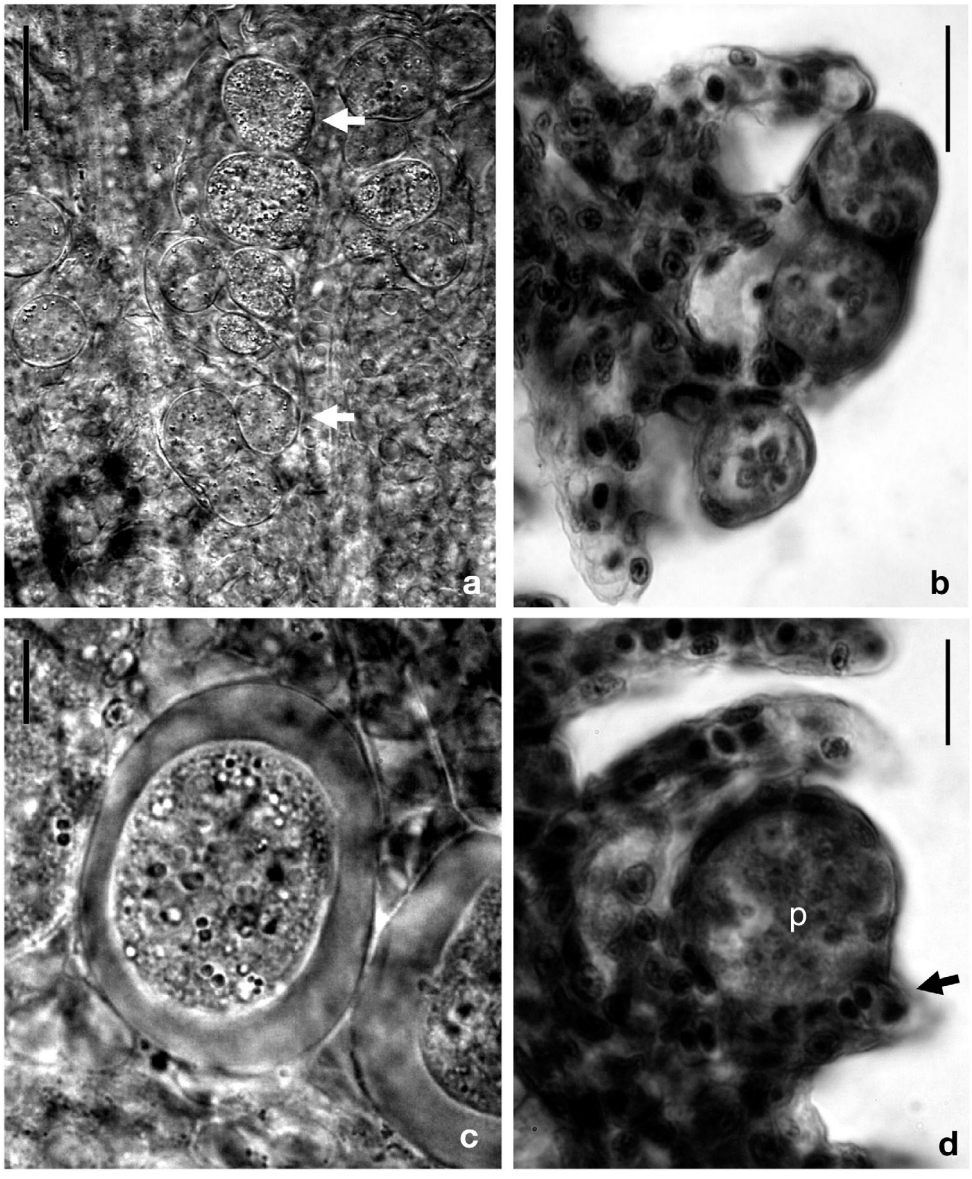

Fig. 3. *Myxobolus rotundus.* Developing plasmodia from experimentally infected bream fry: (a) squash preparation of gill 17 d post-exposure (Day 17), with numerous developing plasmodia inside the lamellae (arrows), scale bar = 50 µm; (b) histological section of the same gill filament with plasmodia in lamellae, H&E, scale bar = 50 µm; (c) developing plasmodia Day 24, fresh mount, scale bar = 100 µm; (d) histological section of the same gill filament with a plasmodium (p) bulging out laterally from the lamella (arrow) H&E, scale bar = 25 µm

ber of known actinospores is rather low. In their review on Myxozoa, Kent et al. (2001) reported on 25 known myxospore–actinospore pairs. The majority of researchers produced actinospores by infecting oligochaetes with myxospores of well-identified myxozoan species. Yokoyama et al. (1995) and Kallert et al. (2005a), however, obtained data on the life cycle of *Myxobolus cultus* and *M. parviformis*, respectively, by infecting fish with actinospores released from oligochaetes and describing myxosporean developmental stages in the fish. In other cases, actinospores and myxospores have been linked by homology of their SSU rRNA gene sequences (Holzer et al. 2004, Eszterbauer et al. 2006) or in combination with exposure experiments (Bartholomew et al. 2006, Atkinson et al. 2007, Atkinson & Bartholomew 2009). Understandably, there are few studies that undertake the time-consuming and laborious task of experimentally following a complete myxozoan life cycle (Székely et al. 1999, 2001) in a way similar to the classical work on the life cycle of *M. cerebralis* by Wolf & Markiw (1984).

By infecting naïve oligochaetes with well-identified

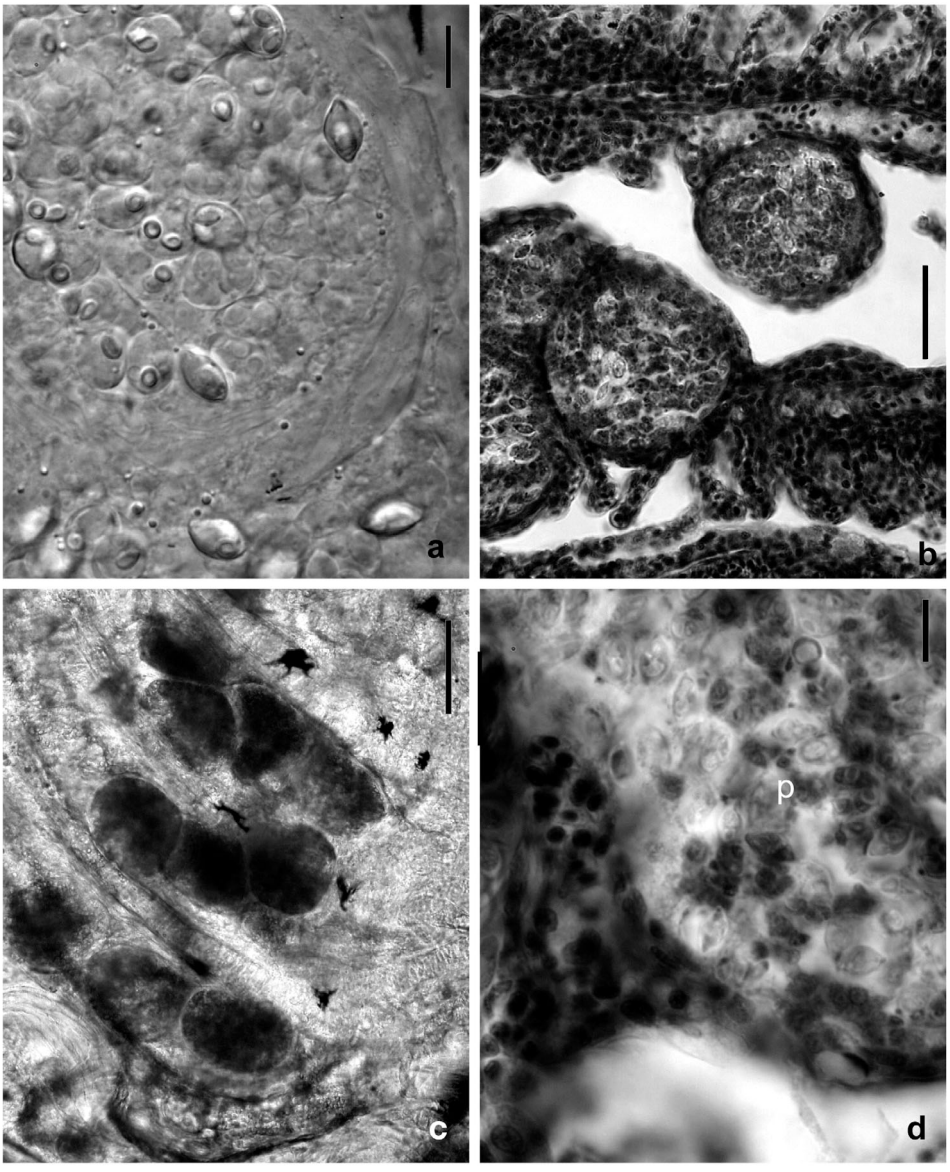

Fig. 4. *Myxobolus rotundus.* Developing plasmodia from experimentally infected bream fry: (a) fresh mount of a semi-mature plasmodium in a gill lamella 45 d post-exposure (Day 45)—young spores and sporogonic stages of *M. rotundus* are visible in squashed plasmodium, scale bar = 10 µm; (b) semi-mature plasmodia inside lamellae of 2 gill filaments, H&E, scale bar = 50 µm; (c) fresh mount of gill Day 58, (note dark plasmodia filled with myxospores), scale bar = 100 µm; (d) histological section of a plasmodium (p) filled by matured spores in the same gill, H&E, scale bar = 10 µm

myxospores, our Hungarian research team successfully determined the actinospore stages of 11 myxosporean species (El-Mansy & Molnár 1997a,b, El-Mansy et al. 1998a,b,c, Molnár et al. 1999a,b, Székely et al. 1999, 2001, 2002, Eszterbauer et al. 2000, Rácz et al 2004). Attempts to reinfect naïve fish with the actinospores were only successful for *Myxobolus pseudodispar* (Székely et al. 1999, 2001).

The experimental flow-through system described herein was devised to clarify the developmental cycle of *Myxobolus bramae*. Serendipitously, it resulted in elucidation of the complete cycle of a related species, *M. rotundus* (Nemeczek 1911). Though this parasite was described almost 100 yr ago, relatively little is known about it. Molnár & Székely (1999) thought that *M. bramae* sometimes forms large plasmodia in the gill filaments of bream, while other times its spores develop in small plasmodia inside the secondary lamellae. From the combined data of the present life cycle experiments and molecular data (Molnár et al. 2009) we concluded that spores developing in small plasmodia and earlier regarded as being *M. bramae*

actually correspond to spores of *M. rotundus*, and only spores developing in large filament cysts represent *M. bramae*.

Masoumian et al. (1996) found that a large, seemingly single cyst of *Myxobolus nodulointestinalis* was the amalgamation of several small plasmodia. Moreover, Molnár & Székely (1999) identified spores of *M. macrocapsularis* in plasmodia of *M. bramae*. In our experiment, we used spores from large filament cysts (i.e. *M. bramae*), however the oligochaetes produced actinospores of *M. rotundus*. We therefore suppose that the large plasmodia of *M. bramae* probably included plasmodia or disseminated spores of *M. rotundus* and it was the small number of these rather than the abundant *M. bramae* that initiated infection in the oligochaetes. The species of oligochaetes present in the experimental stock were compatible hosts only for 1 of the 2 myxozoan species under the experimental conditions.

Our large number of unsuccessful experiments with other *Myxobolus* spp. further emphasises the difficulties of selecting suitable invertebrate host species and providing appropriate experimental conditions. It is apparent that the requirements for studying and successfully repeating the life cycles of *M. cerebralis, M. pseudodispar* and *M. rotundus* apply to only a fraction of the known myxozoans. Clearly, an understanding of environmental and host factors is required to successfully reproduce a complete myxosporean life cycle and the repeated difficulties encountered with experimental approaches highlight the importance and expediency of alternate methods (i.e. molecular).

A BLAST search allowed Molnár et al. (2009) to connect the myxospore of *Myxobolus rotundus* and the actinospore Triactinomyxon type 4 of Hallett et al. (2005). We have now supported this link with life cycle experiments and further molecular analyses described herein. Although Triactinomyxon type 4 was observed from oligochaetes obtained from a petshop in Germany, Hungary was a supply source of the worms. *M. rotundus* is genetically very similar to *M. parviformis* (Kallert et al. 2005a; 99%), another lamellar species from common bream. The myxospores and actinospores of *M. parviformis* differed morphologically from those of *M. rotundus.*

Hallett et al. (2005) noted phenotypic similarity between the purported *M. bramae* triactinomyxon (Eszterbauer et al. 2000) and their Triactinomyxon type 4. We now understand that the '*M. bramae*' triactinomyxon type of Eszterbauer et al. (2000) was misidentified and was actually *M. rotundus*. Thus, the infection experiments and DNA data from the current study support the conclusion that the myxospores of *M. rotundus* (Molnár et al. 2009), the experimentally derived myxospores and actinspores detailed herein, the triactinomyxon type of Eszterbauer et al. (2000) and Triactinomyxon type 4 of Hallett et al. (2005) are conspecific.

Myxobolus and triactinomyxon are the most common morphotypes of freshwater myxospores and actinospores, respectively, and the 2 are common life cycle counterparts. Thus the release of triactinomyxons from oligochaetes following exposure to *M. rotundus* myxospores was not unexpected. However, this morphological pairing is not ubiquitous, as *Myxobolus* has been linked with raabeia and hexactinomyxon actinospores (Kent et al. 2001), and triactinomyxon with *Henneguya* (Kallert et al. 2005b) and *Myxobilatus* (Atkinson & Bartholomew 2009). Myxozoan relationships continue to be discerned.

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