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ORIGINAL PAPER

Life cycles of three *Myxobolus* spp. from cyprinid fishes of Lake Balaton, Hungary involve triactinomyxon-type actinospores

Csaba Székely · Muhammad Hafiz Borkhanuddin · Gábor Cech · Olga Kelemen · Kálmán Molnár

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Abstract A study on the actinosporean fauna of oligochaetes of Lake Balaton was carried out from 2009 to 2011. The morphology of actinosporean stages of myxosporeans obtained from oligochaetes was studied, and their 18S rDNA structure was analyzed by molecular biological methods. Three triactinomyxon types were released from the oligochaete *Isochaetides michaelseni* (Tubificidae). The sequences of Triactinomyxon type 1 proved to be identical with those of *Myxobolus fundamentalis*. The sequences of Triactinomyxon type 2 showed 99.9 % similarity to *Myxobolus eryhtrophthalmi*, while the sequences of Triactinomyxon type 3 showed a 99.9 % similarity to those of *Myxobolus shaharomae*. The life cycles of the above species, just like those of other species with a known life cycle, suggest that most *Myxobolus* spp. develop through triactinomyxon-type actinosporean stages.

Keywords Myxozoa · Actinospores · Developing stages · Life cycle · Lake Balaton · Hungary

Introduction

Myxobolus Bütschli, 1882 is the most speciose genus within the phylum Myxozoa comprising around 800 species to date (Eiras et al. 2005; Lom and Dyková 2006; Zhang et al. 2010). The complex myxozoan life cycle involving a vertebrate and an invertebrate host was first described in 1984 for *Myxobolus cerebralis* (Wolf and Markiw 1984). Since this pioneering discovery, about 50 myxozoan species have been found to follow this life cycle pattern that involves the alternation of oligochaete and vertebrate hosts (Lom and Dyková 2006; Yokoyama et al. 2012). Most of the life cycle studies (45 %) based on experimental infections were performed on *Myxobolus* spp. Such experimental studies have been carried out on *Myxobolus cotti* (El-Matbouli and Hoffmann 1989), *Myxobolus carassii* (El-Matbouli and Hoffmann 1993), *Myxobolus drjagini* (El-Mansy and Molnár 1997a), *Myxobolus hungaricus* (El-Mansy and Molnár 1997b), *Myxobolus portucalensis* (El-Mansy et al. 1998a), *Myxobolus bramae* (Eszterbauer et al. 2000), *Myxobolus pseudodispar* (Székely et al. 1999 and 2001), *Myxobolus macrocapsularis* (Székely et al. 2002), *Myxobolus intimus* (Rácz et al. 2004), and *Myxobolus rotundus* (Székely et al. 2009). These experimental transmission studies were very laborious, time consuming, and very often produced questionable results (Rácz et al. 2004). More recently, it has been assumed that these cumbersome and complicated experimental studies could be substituted by identifying conspecific actinosporean-myxosporean developmental stage pairs through DNA analysis (Atkinson and Bartholomew 2009; Székely et al. 2009; Kallert et al. 2005). Comparison of the 18S rDNA sequences proved to be more accurate and reliable in matching or confirming the life cycle stages of myxozoan species. DNA studies on the genus *Myxobolus* provided useful data on the life cycle of several *Myxobolus* species, such as *M. rotundus* (Hallett et al. 2005; Székely et al. 2009), *Myxobolus diversicapsularis* (Hallett et al. 2005; Molnár et al. 2010), *Myxobolus wootteni* (Eszterbauer et al. 2006; Molnár et al. 2010), *Myxobolus parviformis* (Kallert et al. 2005), and *Myxobolus arcticus* (Kent et al. 1993; Urawa 1994; Urawa et al. 2011); in the last two cases, molecular identity was confirmed also by the results of experimental transmission studies.

C. Székely (✉) · M. H. Borkhanuddin · G. Cech · O. Kelemen · K. Molnár
Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, POB 18, H-1581 Budapest, Hungary
e-mail: szekely.csaba@agrar.mta.hu

M. H. Borkhanuddin
School of Marine Science and Environment, Universiti Malaysia Terengganu, Terengganu, Malaysia

Up to this time, about 60 *Myxobolus* spp. have been described from fishes in Hungary. In most cases, these descriptions were restricted to spore morphology (Lom and Molnár 1983; Molnár and Székely 1995; Székely and Molnár 1997; Molnár 2000). In recent years, however, the morphological description of several species has been supplemented by molecular data, and 18S rDNA sequences of some *Myxobolus* spp. from Hungarian cyprinid fishes have also been determined (Molnár et al. 2007, 2008, 2009, 2010). Among these latter species, *Myxobolus erytrophthalmi* and *Myxobolus shaharomae* were described from the internal organs of *Scardinius erythrophthalmus* (rudd) and *Alburnus alburnus* (bleak), respectively, (Molnár et al. 2009) and *Myxobolus fundamentalis*, *Myxobolus sommervillae*, and *M. wootteni* from the gill arch of fishes collected in Lake Balaton and the Kis-Balaton water reservoir (Molnár et al. 2010). The extrapiscine development of the above species was not studied.

Since Wolf and Markiw (1984) proved that actinosporeans were alternative stages of myxosporeans, several authors have studied the morphological variations of these stages in oligochaete and polychaete hosts. By their morphology, these myxosporean stages have been classified into collective groups. A total of 18 collective groups have been described so far, with around 136 types identified in recent years (Özer et al. 2002; Canning and Okamura 2004; Lom and Dyková 2006). The most common types described belong to Triactinomyxon (53), Aurantiactinomyxon (34), Raabeia (25), Echinactinomyxon (19), and Neoactinomyxum (18) collective groups (Lom and Dyková 2006; Rangel et al. 2011; Zhai et al. 2012). Although actinospores are mostly reported from freshwater annelid hosts such as *Branchiura sowerbyi*, *Tubifex tubifex*, *Limnodrilus hoffmeisteri*, *Isochaetides michaelseni*, *Nais* spp., *Psammoryctides albicola*, and *Dero digitata*, they were also associated with marine worms such as *Nereis diversicolor*, *Nereis succinae*, *Spirorbis spirorbis*, *Hydroides norvegica*, and *Chone infundibuliformis* (El-Mansy et al. 1998b, c; Køie 2000, 2002; Özer et al. 2002; Canning and Okamura 2004; Eszterbauer et al. 2006; Székely et al. 2007; Yokoyama et al. 2012).

In Hungary, studies on the actinosporean fauna associated with oligochaetes have been focused on Lake Balaton, the Kis-Balaton Reservoir, the river Tisza, and the Temperate Water Fish Farm located at Százhalombatta near Budapest (El-Mansy et al. 1998b, c; Eszterbauer et al. 2006). Thirty-eight isolates of actinospore were determined in a morphological study by El-Mansy et al. (1998b, c), and the identity of 14 actinosporeans was determined by morphological and genetic studies by Eszterbauer et al. (2006). In another study, Rácz et al. (2005) described a novel actinosporean designated as hungactinomyxon, which differs in spore morphology from the actinospore types previously described in Hungary.

Most of the already known myxospore-actinospore pairs have been revealed by experimental infections but in some instances, e.g., in the case of *M. rotundus*, *M. diversicapsularis*, *M. wootteni*, *M. parviformis*, and *M. arcticus*, the correspondence of molecular sequences between myxospores and actinospores helped the correct identification of the two stages (Kallert et al. 2005; Székely et al. 2009; Molnár et al. 2010; Urawa et al. 2011).

In the present study, we examined the morphology and 18S rDNA sequences of triactinomyxon actinospore stages collected from the oligochaete *I. michaelseni*. The aim of the study was to compare DNA sequences of some triactinospores with the data of known *Myxobolus* spp. deposited in the GenBank and to identify actinospore and myxospore pairs. The sequences of three triactinomyxon spore types corresponded to those of the species *M. fundamentalis*, *M. erythrophthalmi*, and *M. shaharomae*.

Materials and methods

Source of oligochaetes

Oligochaetes were collected two to three times each month at different sites of Lake Balaton (Keszthely, Tihany, Balatonszemes, Balatonvilágos, and Siófok) and the Kis-Balaton water reservoir system, where most *Myxobolus* spp. known from Hungary were found. A 1,000 μm mesh size net was used to collect mud-dwelling oligochaetes near the water vegetation at about 0.5 to 1 m depth. Oligochaetes trapped within debris particles were transported to the laboratory and placed into a tray. Isolated oligochaetes were then kept individually in "cell-well" plates with clean dechlorinated tap water (Yokoyama et al. 1991). Each cell-well plate was scanned using a Zeiss Treval 3 inverted microscope for released actinosporeans. Detected floating actinospores were removed from the wells with a plastic pipette, mounted under a coverslip, and 30 spores were imaged digitally. Photomicrographs were taken from the fresh material of actinospores under both bright and phase-contrast field, using a DP-20 digital camera mounted on an Olympus BH-2 microscope. The remaining spores were preserved in 80 % ethanol in 2.0-mL microcentrifuge tubes for DNA analyses. Line drawings of actinospores were prepared based on the photos. Spore measurements of actinosporean types were taken according to the guidelines of Lom et al. (1997). All measurements are given in micrometers (μm) unless stated otherwise. Oligochaetes were identified according to the key of Timm (1999). Photo samples and some of the less easily identifiable oligochaete specimens preserved in 70 % ethanol were sent to Dr. Tarmo Timm for definitive identification (T. Timm, pers. comm.; vouchers in the personal collection of T. Timm,

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia).

Genetic analyses

The ethanol-fixed actinospore samples were spun for 10 min at 8,000 rpm to pellet the spores, the ethanol was removed and the retained spores were vacuumed to dry. Total DNA was extracted using a DNeasy™ tissue kit (animal tissue protocol, QIAGEN, Germany) according to the manufacturer's instructions. The 18S rDNA was amplified with a set of universal eukaryotic primers ERIB1 and ERIB10 (Barta et al. 1997) in a 25-μL reaction mixture comprising 2 μL of extracted genomic DNA, 5 μL of 1 mM deoxyribonucleotide triphosphates (dNTPs, MBI Fermentas), 0.25 μL of each primer, 2.5 μL of 10× Taq buffer (MBI Fermentas), 0.1 μL of Taq polymerase (2 U; MBI Fermentas), and 15 μL of water. The following profile was used to amplify the 18S rDNA region: an initial denaturation step of 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, and was completed with terminal extension at 72 °C for 7 min, then stored at 4 °C.

This was followed by a second round of PCR with the Myx1F (Hallett and Diamant 2001) and SphR (Eszterbauer and Székely 2004) primer pair. To amplify products for sequencing, 50-μL reaction volumes were used with 1.0 μL of template DNA and the following cycle profile: 95 °C for 3 min, and then 35 cycles of 95 °C for 50 s, 50 °C for 50 s, 72 °C for 1 min 40 s, and the cycle was terminated with an extension period at 72 °C for 7 min, and then rested at 4 °C. PCR cycles were run in a PTC-200 thermocycler (MJ Research, Waltham, MA, USA).

PCR products were electrophoresed in 1 % agarose gels in Tris-acetate-EDTA buffer gel stained with 1 % ethidium bromide. Amplified DNA was purified with EZ-10 Spin Column PCR Purification Kit (Bio Basic Inc., Amherst, NY, USA). Purified PCR products were sequenced with the primers listed in Table 1, using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyser. DNA sequences assembling and similarities (pairwise-distance) were calculated with MEGA 5.10 software package using the Tamura-Nei substitution model (Tamura et al. 2011). Consensus sequences were submitted and close relatives of the sequences were determined with standard nucleotide-nucleotide BLAST search. Oligochaete hosts were not identified to DNA level.

Results

A total of 7,818 samples of tubificid oligochaetes from Lake Balaton and the Kis-Balaton Reservoir were screened in this study. Altogether, four triactinomyxon, five aurantiactinomyxon, two raabeia, one synactinomyxon, and one neoactinomyxum morphotypes were identified (to be described elsewhere). Of them, sequences of three triactinomyxon morphotypes, released from the oligochaete *I. michaelseni* Lastockin, 1936 (Annelida: Oligochaeta: Tubificidae) corresponded to known *Myxobolus* spp. deposited in the GenBank database. The 18S rDNA sequences of the isolated triactinomyxon spores gave 99.9–100 % identity to myxospores that infect cyprinids collected in the same locality, which indicates their conspecificity. The morphology and molecular characteristics of the above three triactinomyxon spp., named as Triactinomyxon type 1, Triactinomyxon type 2, and Triactinomyxon type 3, are presented below.

Systematics and morphology of actinospores studied

Phylum Myxozoa Grasse, 1970

Class Myxosporea, Bütschli, 1881

Actinosporean forms of Kent, Margolis & Corliss (1994)

Collective group *Triactinomyxon*, Štolc, 1899

The morphology and morphometry of the actinospores collected are consistent with triactinomyxon spores. The spores had the typical triactinomyxon "anchor" morphology with no extravalvular protrusions or augmentation, slightly elongated spore body in spherical shape, with three relatively

Table 1 Primers used for PCR and sequencing

Primer	Sequence	Application	Source
ERIB1	5' -ACC TGG TTG ATC CTG CCA G-3'	1st round PCR	Barta et al. (1997)
ERIB10	5'-CTT CCG CAG GTT CAC CTA CGG-3'	1st round PCR	Barta et al. (1997)
Myx1F	5'-GTG AGA CTG CGG ACG GCT CAG-3'	2nd round PCR	Hallett and Diamant (2001)
SphR	5'-GTT ACC ATT GTA GCG CGC GT-3'	2nd round PCR and sequencing	Eszterbauer and Székely (2004)
MC5	5'-CCT GAG AAA CGG CTA CCA CAT CCA-3'	Sequencing	Molnár et al. (2002)
MC3	5′-GAT TAG CCT GAC AGA TCA CTC CAC A-3′	Sequencing	Molnár et al. (2002)
MB5r	5'-ACC GCT CCT GTT AAT CAT CAC C-3'	Sequencing	Eszterbauer and Székely (2004)
MB5f	5'-GAT GAT TAA CAG GAG CGG TTG G-3'	Sequencing	Eszterbauer and Székely (2004)
ACT1fr	5'- TTG GGT AAT TTG CGC GCC TGC TGC C -3'	Sequencing	Hallett and Diamant (2001)

long caudal processes equal in length and with three pear-shaped polar capsules situated at the top of the spore body.

Triactinomyxon type 1 nov. (Fig. 1a–c)

Epispore Cylindrical, elongated in side view. Length 41.6 (39.0–44.2). Width 14.04 (13.0–15.6). *Spore axis*: length 152.3 (145.2–161.2). Width 19.2 (18.2–20.8). *Caudal processes*: slightly curved, tapering toward the end, terminating in a sharp point. Length 296.2 (280.8–325). Width 19.5 (18.2–20.8). Largest span 537.3 (533–546). *Polar capsule*: pyriform in side view, positioned at the epispore apex. L: 6.2× W: 4.2. The coils of the polar filament had 5 turns. *Germ cells*: 16.

Triactinomyxon type 2 nov. (Fig. 2a–c)

Epispore Cylindrical, elongated in side view. Length 44.98 (39–49.94). Width 12.61 (10.4–15.6). *Spore axis*: length 148.9 (124.8–171.6). Width 17.9 (15.6–20.8). *Caudal processes*: curved upwards, tapering toward the end, terminating in a sharp point. Length 255.9 (176.8–286). Width 13.4 (10.4–16.9). Largest span 455.5 (395.2–522.6). *Polar capsule*: pyriform in side view, positioned at the epispore apex. L: 5.6×W: 4.0. Polar filament turns were 4. *Germ cells*: >26 (probably 32).

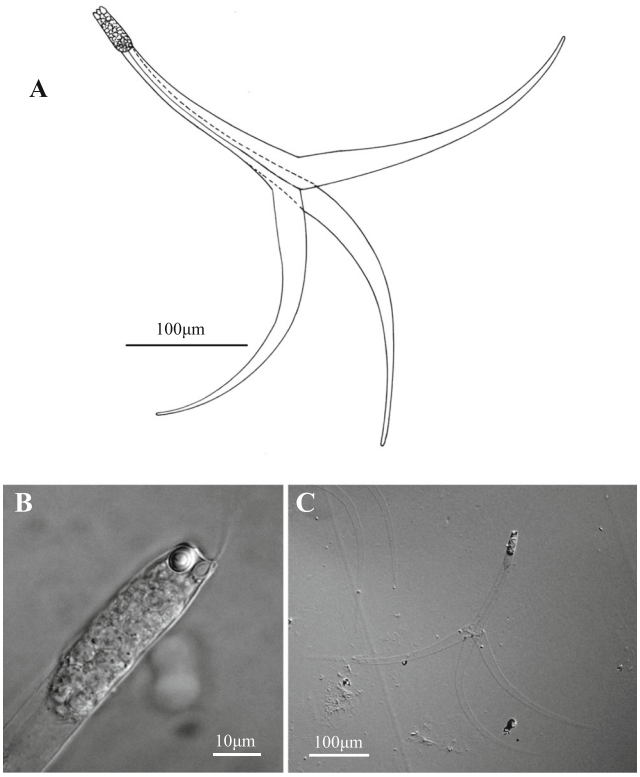

Fig. 1 Triactinomyxon type 1 nov. **a** Line drawing of a mature spore. **b** Higher magnification of spore body showing germinal cells. **c** Waterborne spore

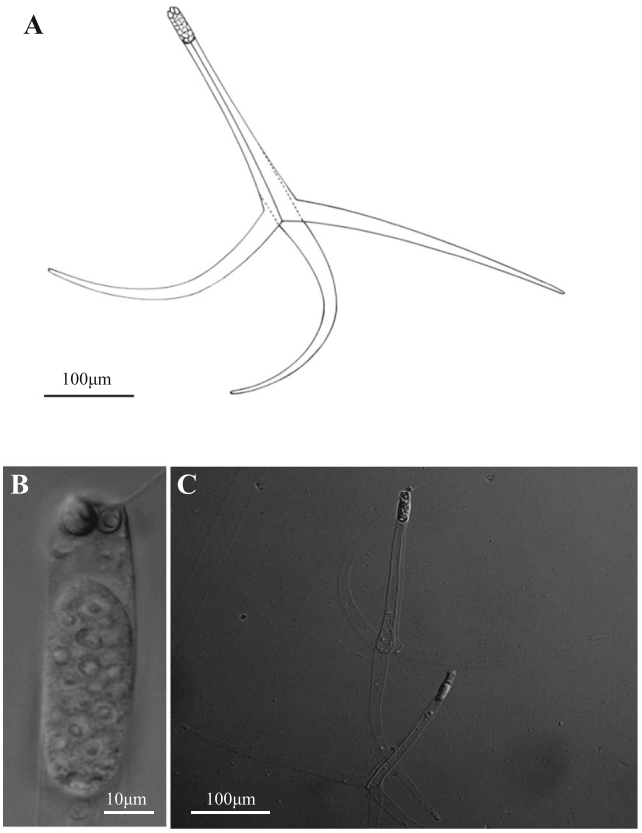

Fig. 2 Triactinomyxon type 2 nov. **a** Line drawing of a mature spore. **b** Higher magnification of spore body showing germinal cells. **c** Waterborne spore

Triactinomyxon type 3 nov. (Fig. 3a–c)

Epispore Barrel shaped in side view. Length 46.15 (36.4–59.8). Width 18.36 (13.0–23.4). *Spore axis*: length 260.81 (221.0–296.4). Width 23.56 (20.8–26.0). *Caudal processes*: minimal or without curves and tapers to blunt or almost rounded terminus. Length 252.5 (182–283.4). Width 24.8 (20.8–26.0). Largest span 426.08 (358.8–566.8). *Polar capsule*: pyriform in side view, positioned apically at the epispore. L: 5.2×W: 3.5. Polar filament turns were 4–5. *Germ cells*: >30.

These triactinomyxons were distinct from those recorded in the literature. The three types appear to differ in the annelid host type, *I. michaelseni*, while several dimensions of these spores overlap and all three have a sporoplasm with a different germ cell count. The detailed measurements are presented in Table 2.

18S rDNA analysis of actinospores

[Performed on three triactinomyxon spore morphotypes designated Triactinomyxon type 1 (1 isolate), Triactinomyxon type 2 (2 isolates), and Triactinomyxon type 3 (3 isolates)].

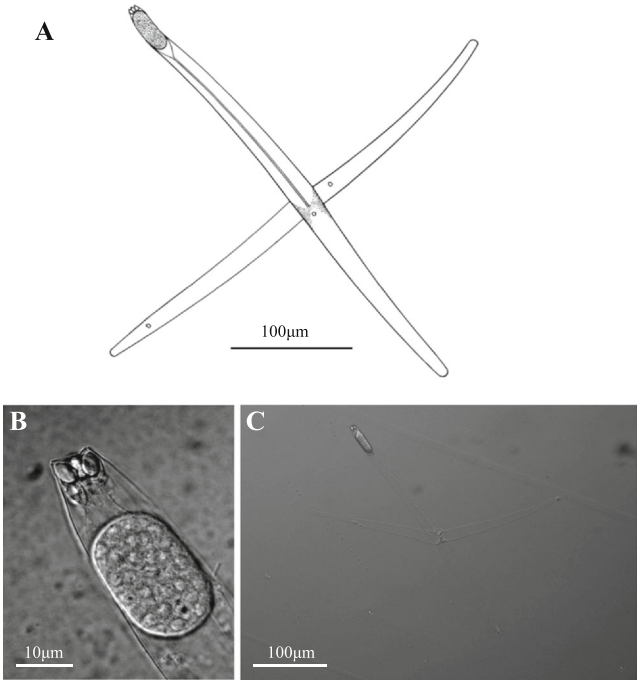

Fig. 3 Triactinomyxon type 3 nov. **a** Line drawing of a mature spore. **b** Higher magnification of spore body showing germinal cells. **c** Waterborne spore

The complete 18S rDNA sequence fragments of the triactinomyxon isolates varied as shown in Table 3. The amplified fragment of the Triactinomyxon type 1 (1625 bp), Triactinomyxon type 2 (1606, 1158 bp), and Triactinomyxon type 3 (1618, 1243, 1499 bp) were obtained and deposited in the GenBank with the accession numbers indicated in Table 3.

Based on their 18S rDNA sequence, these triactinomyxon isolates were found to be genetically highly similar to myxospores investigated from the same locality. The consensus sequence fragment analyzed by BLAST search of Triactinomyxon type 1 (KF515725) gave 100 % identity with *M. fundamentalis* (GU968200), a gill-infecting parasite of the roach *Rutilus rutilus*. Furthermore, the sequence of Triactinomyxon type 2 (KF515727, KF515728) showed 99.9 % identity to that of *M. erythrophthalmi* (EU567311) found to inhabit the blood vessels of the renal insterstitium of the rudd (*S. erythrophthalmus*). The 18S rDNA sequence similarity between Triactinomyxon type 3 (KF515726, KF515729, KF515730) and *M. shaharomae* (EU567312) collected from the blood vessels of the kidney, liver, testes, and the lamina propria of the intestinal folds of bleak *A. alburnus* was 99.9 % (Table 3). Based on the complete identity of *M. fundamentalis*, *M. erythrophthalmi*, and *M. shaharomae* with the triactinomyxon types isolated from *I. michaelseni* in this study, it is apparent that these shall be regarded as myxospore-actinospore conspecific matches.

Discussion

During the present study conducted on actinospores released from the oligochaete *I. michaelseni*, we identified three triactinomyxon spore types which were proved to be the developing stages of *M. fundamentalis*, *M. erythrophthalmi*, and *M. shaharomae*, respectively. The spores show minimal morphometric differences from other triactinomyxon types described in the literature, with overlapping measurements in

Table 2 Morphometrics of the three novel triactinomyxons identified in the present study. Measurements (µm) are of freshly released waterborne spores under a coverslip

Parameter	Triactinomyxon type 1	Triactinomyxon type 2	Triactinomyxon type 3
Locality	Tihany, Lake Balaton, Hungary	Keszthely, Lake Balaton, Kis-Balaton Reservoir, Hungary	Keszthely, Balatonszemes, Tihany, Siófok, Hungary
Oligochaete host	*Isochaetides michaelseni*	*Isochaetides michaelseni*	*Isochaetides michaelseni*
Length of SB	41.6 (39.0–44.2)	44.98 (39–49.94)	46.15 (36.4–59.8)
Width of SB	14.04 (13.0–15.6)	12.61 (10.4–15.6)	18.36 (13.0–23.4)
Length of SA	152.3 (145.2–161.2)	148.9 (124.8–171.6)	260.81 (221.0–296.4)
Width of SA (near base)	19.2 (18.2–20.8)	17.9 (15.6–20.8)	23.56 (20.8–26.0)
Length of CP	296.2 (280.8–325)	255.9 (176.8–286)	252.5 (182–283.4)
Width of CP	19.5 (18.2–20.8)	13.4 (10.4–16.9)	24.8 (20.8–29.9)
CP largest span	537.3 (533–546)	455.5 (395.2–522.6)	426.08 (358.8–566.8)
Dimension of PC	L: 6.2×W: 4.2	L: 5.6×W: 4.0	L: 5.2×W: 3.5
No. of turns of PF	5	4	4–5
No. of GCs	16	>26	>30

Ranges are given in parentheses

SB spore body, *SA* spore axis, *CP* caudal processes, *PC* polar capsule, *PF* polar filaments, *GCs* germ cells

Table 3 Genetic similarity based on the 18S rDNA sequence of triactinomyxon types from Lake Balaton and the Kis-Balaton Reservoir to the myxospore species found from the same localities

Triactinomyxon isolates	GenBank accession number (length of 18S rDNA fragment, bp)	Genetic similarities	References
Triactinomyxon type 1	KF515725 (1625)	100 %, myxosporean stage of *M. fundamentalis* (GU968200)	Molnár et al. (2010)
Triactinomyxon type 2	KF515727 (1606)	99.9 %, myxosporean stage of *M. erythrophthalmi* (EU567311)	Molnár et al. (2009)
	KF515728 (1158)	99.9 %, myxosporean stage of *M. erythrophthalmi* (EU567311)	Molnár et al. (2009)
Triactinomyxon type 3	KF515726 (1618)	99.9 %, myxosporean stage of *M. shaharomae* (EU567312)	Molnár et al. (2009)
	KF515729 (1243)	99.9 %, myxosporean stage of *M. shaharomae* (EU567312)	Molnár et al. (2009)
	KF515730 (1499)	99.9 %, myxosporean stage of *M. shaharomae* (EU567312)	Molnár et al. (2009)

only some of the features. These triactinomyxon spores were phenotypically compatible with triactinomyxon type spores, but possessed at least one different characteristic. The morphometry of Triactinomyxon type 1 is consistent with the Triactinomyxon 'E' described by Xiao and Desser (1998) except that the former has a longer spore body (47–53 *vs*. 41.6). The morphometry of our Triactinomyxon type 2 is similar to that of Triactinomyxon type 4 reported by Hallett et al. (2005), except for its shorter spore body (31.6 *vs*. 44.9) and spore axis (133.6 *vs*. 148.9). However, not all dimensions of our Triactinomyxon type 3 are consistent with the morphotypes of the triactinomyxon collective group. With respect to the length of caudal processes (253.4 *vs*. 252.5), it resembles the Triactinomyxon type 4 of Hallett et al. (2005). Hallett et al. (2002, 2004), Eszterbauer et al. (2006) and Atkinson and Bartholomew (2009) stressed that the identification of myxosporeans (myxospores, actinospores) based solely on morphological features may be false since the parasites are small and have a paucity of consistent measurable characters. The analysis of the 18S rDNA sequences of myxospores and triactinomyxon morphotypes gives more reliable results.

The 18S rDNA sequences of our three triactinomyxon spore types were at least 99.9 % similar to their myxospore counterpart records in the GenBank. Our study provides data on the actinospore stages of myxozoan spp. (*M. fundamentalis*, *M. erythrophthalmi* and *M. shaharomae*) whose myxospore had been described morphologically and genetically (Molnár et al. 2009, 2010), but for which there was no known actinospore counterpart. This study identified *I. michaelseni* as the oligochaete host, which is also a new record. While the role of tubificid oligochaetes (*Tubifex* and *Limnodrilus* spp.) in the life cycle of several *Myxobolus* species such as *M. macrocapsularis* (Székely et al. 2002), *M. parviformis* (Kallert et al. 2005), *M. wootteni* (Eszterbauer et al. 2006; Molnár et al. 2010), and *M. rotundus* (Székely et al. 2009) has been clarified; *I. michaelseni* as their alternative annelid host has never been recorded. The fact that triactinospores had earlier been detected mainly from *Limnodrilus* and *Tubifex* species while in the current study they were isolated from the species *I. michaelseni*, might suggest a new alternative host record for some *Myxobolus* species. However, actually the differences between the present findings and the previous data can be explained by the new systematics of oligochaetes, i.e., by the fact that numerous species formerly belonging to the *Limnodrilus* genus has been reassigned to different genuses including *Isochaetides*. Synonymisation of the genera *Isochaetides* and *Limnodrilus* might explain differences in the identification of the worms. The genus *Isochaetides* now contains more than 15 species, among them are two that were earlier classified as *Limnodrilus* (Ferraguti et al. 2002).

Triactinomyxon-type spores are the most common forms of the actinosporean stages of *Myxobolus* species (Wolf and Markiw 1984; Kent et al. 1993; Urawa 1994; El-Matbouli and Hoffmann 1989; Kallert et al. 2005; Hallett et al. 2005). However, some species, e.g., *Myxobolus cultus* (Yokoyama et al. 1995), *Myxobolus dispar* (Molnár et al. 1999; Holzer et al. 2004), and *Myxobolus lentisuturalis* (Caffara et al. 2009), have been recorded as having raabeia-type spore developing stages. Our findings seem to be consistent with the general trend in correlating the actinosporean and myxosporean counterparts for *Myxobolus* species. By forming triactinomyxon-type actinospores, the life cycles of *M. fundamentalis*, *M. eryhtrophthalmi*, and *M. shaharomae* followed the most common routes of myxozoan infection in vertebrate and invertebrate hosts. In Hungary, triactinomyxon-type actinospores have been described for *M. drjagini* (El-Mansy and Molnár 1997a), *M. hungaricus* (El-Mansy and Molnár 1997b), *M. portucalensis* (El-Mansy et al. 1998a), *M. bramae* (Eszterbauer et al. 2000), *M. pseudodispar* (Székely et al. 1999 and 2001), *M. macrocapsularis* (Székely et al. 2002), *M. intimus* (Rácz et al. 2004), *M. rotundus* (Székely et al. 2009), *M. wootteni*, and *M. diversicapsularis* (Molnár et al. 2010) developmental cycles (Table 4).

Eight *Myxobolus* species have been described from roach (*R. rutilus*) as a type host, i.e., *M. diversicapsularis*, *M. pseudodispar*, *M. sommervillae*, *M. rutili*, *M. intimus*,

Table 4 List of *Myxobolus* species and the corresponding actinosporean types found in Hungary

No.	*Myxobolus* species	Host	Type of actinospore	References
1	*M. dispar*	*Cyprinus carpio* common carp	Raabeia	Molnár et al. (1999)
2	*M. hungaricus*	*Abramis brama* common bream	Triactinomyxon	El-Mansy and Molnár (1997b)
3	*M. drjagini*	*Hypophthalmichthys molitrix* silver carp	Triactinomyxon	El-Mansy and Molnár (1997a)
4	*M. portucalensis*	*Anguilla anguilla* European eel	Triactinomyxon	El-Mansy et al. (1998a)
5	*M. bramae*	*A. brama*	Triactinomyxon	Eszterbauer et al. (2000)
6	*M. pseudodispar*	*Rutilus. rutilus* Roach	Triactinomyxon	Székely et al. (1999 and 2001)
7	*M. macrocapsularis*	*A. brama*	Triactinomyxon	Székely et al. (2002)
8	*M. intimus*	*R. rutilus*	Triactinomyxon	Rácz et al. (2004)
9	*M. rotundus*	*A. brama*	Triactinomyxon	Székely et al. (2009)
10	*M. diversicapsularis*	*R. rutilus*	Triactinomyxon	Molnár et al. (2010)
11	*M. wootteni*	*R. rutilus*	Triactinomyxon	Molnár et al. (2010)
12	*M. fundamentalis*	*R. rutilus Roach*	Triactinomyxon	Present study
13	*M. erythrophthalmi*	*Scardinius erythrophthalmus* Rudd	Triactinomyxon	Present study
14	*M. shaharomae*	*Alburnus alburnus* Bleak	Triactinomyxon	Present study

Myxobolus feisti, *M. wootteni*, and *M. fundamentalis* (Eiras et al. 2005; Eszterbauer et al. 2006; Molnár et al. 2008, 2010; Rácz et al. 2004). In addition to finding the triactinomyxon stages of *M. fundamentalis*, we also have data on the actinosporean stages of *M. diversicapsularis*, *M. pseudodispar*, *M wootteni*, and *M. fundamentalis* spp. A triactinomyxon was assumed to be the developing stage of *M. intimus* by Rácz et al. (2004), but Hallett et al. (2005) and Molnár et al. (2010) stressed that these stages should be identified as stages of *M. diversicapsularis*. Nine *Myxobolus* species have been described from rudd (*S. erythrophthalmus*) and bleak (*A. alburnus*) as typical hosts in Hungary: *Myxobolus scardinii*, *M. eryhtrophthalmi*, *Myxobolus alburni*, *Myxobolus margitae*, *Myxobolus obesus*, *M. pseudodispar*, *M. shaharomae*, *Myxobolus susanlimae*, and *Myxobolus ergensi* (Molnár 2000; Molnár et al. 2008; Molnár et al. 2009). However, the developmental cycles of the above-mentioned *Myxobolus* species of rudd and bleak have not yet been elucidated. The actinospore stages of *M. erythrophthalmi* and *M. shaharomae* presented in this study expand our knowledge in this field.

Up to now, actinospore stages have been identified for about twenty *Myxobolus* species (Yokoyama et al. 2012). Lom and Dyková (2006) reported fourteen completed developmental cycles of the genus, while in recent reviews of the group Yokoyama et al. (2012) have added three more cycles. These data should be complemented with the results obtained on *M. diversicapsularis* (Hallett et al. 2005; Molnár et al. 2010) and *M. wootteni* (Eszterbauer et al. 2006; Molnár et al. 2010). The identification of the 18S rDNA in triactinomyxon actinospores of *M. fundamentalis*, *M. erythrophthalmi*, and *M. shaharomae* released from *I. michaelseni* (Fig. 4) expands the number of *Myxobolus* species with known developmental stages, provides evidence of their presence in Hungarian biotopes and enhances our knowledge on the life cycle of *Myxobolus* species from cyprinid fishes.

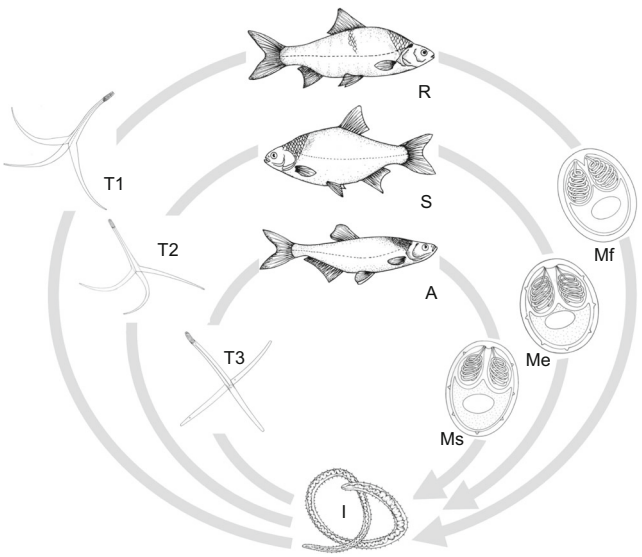

Fig. 4 Schematic illustration of *M. fundamentalis*, *M. erythrophthalmi*, and *M. shaharomae* life cycles: Triactinomyxon type 1 (T1), Triactinomyxon type 2 (T2), and Triactinomyxon type 3 (T3) actinospores infect the vertebrate hosts roach, *R. rutilus* (*R*), rudd, *S. erythrophthalmus*, (*S*), and bleak, *A. alburnus* (*A*) in which then myxospores (Mf, Me, and Ms) infecting the invertebrate host *I. michaelseni* (*I*) develop

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