Springer

Dear Author

Here are the proofs of your article.

- You can submit your corrections **online,** via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For **fax** submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
- Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL:

`http://dx.doi.org/10.1007/s00436-010-1982-z`

If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information, go to: http://www.springerlink.com.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us, if you would like to have these documents returned.

Springer

To: **Springer Customer Support 1**
E-mail: CorrAdmin1@spi-bpo.com
Fax: +1-703-5621873
SPi
Re: **SPi Building, Sacsac Bacong**
Oriental Negros 6216
Philippines

Parasitology Research DOI 10.1007/s00436-010-1982-z
Differentiation of Myxobolus spp. (Myxozoa: Myxobolidae) infecting roach (Rutilus rutilus) in Hungary
Molnar · Marton · Szekely · Eszterbauer

Permission to publish

I have checked the proofs of my article and

❑ I have **no corrections.** The article is ready to be published without changes.

❑ I have **a few corrections.** I am enclosing the following pages:

❑ I have made **many corrections.** Enclosed is the **complete article.**

Date / signature: _____

Metadata of the article that will be visualized in OnlineFirst

1	Article Title	**Differentiation of *Myxobolus* spp. (Myxozoa: Myxobolidae) infecting roach (*Rutilus rutilus*) in Hungary**	
2	Article Sub- Title		
3	Article Copyright - Year	**Springer-Verlag 2010 (This will be the copyright line in the final PDF)**	
4	Journal Name	Parasitology Research	
5	Corresponding Author	Family Name	**Molnár**
6		Particle	
7		Given Name	**Kálmán**
8		Suffix	
9		Organization	Hungarian Academy of Sciences
10		Division	Veterinary Medical Research Institute
11		Address	PO Box 18, Budapest 1581, Hungary
12		e-mail	molnar@vmri.hu
13	Author	Family Name	**Marton**
14		Particle	
15		Given Name	**Szilvia**
16		Suffix	
17		Organization	Hungarian Academy of Sciences
18		Division	Veterinary Medical Research Institute
19		Address	PO Box 18, Budapest 1581, Hungary
20		e-mail	
21	Author	Family Name	**Székely**
22		Particle	
23		Given Name	**Csaba**
24		Suffix	
25		Organization	Hungarian Academy of Sciences
26		Division	Veterinary Medical Research Institute
27		Address	PO Box 18, Budapest 1581, Hungary
28		e-mail	
29	Author	Family Name	**Eszterbauer**
30		Particle	
31		Given Name	**Edit**
32		Suffix	
33		Organization	Hungarian Academy of Sciences
34		Division	Veterinary Medical Research Institute
35		Address	PO Box 18, Budapest 1581, Hungary
36			

		e-mail	
37	Schedule	Received	16 March 2010
38		Revised	
39		Accepted	13 July 2010
40	Abstract	During a survey on fishes from Hungarian freshwaters, the occurrence of eight *Myxobolus* species was registered in roach (*Rutilus rutilus* L.). Most species had a specific location within the fish host. Of the known species infecting roach, the intramuscularly developing species *Myxobolus pseudodispar* was found to be the most common. Besides *Myxobolus intimus* and *Myxobolus diversicapsularis* infecting the capillary network of gill lamellae, *Myxobolus feisti* infecting the cartilaginous gill rays were observed. Of the “muelleri-type” *Myxobolus* spp, *Myxobolus rutili* and *Myxobolus sommervillae* sp. n. formed elongated plasmodia in the gill filaments outside and inside the arteria efferens, respectively, while *Myxobolus wootteni* sp. n. developed cysts in fins. Plasmodia and spores of *Myxobolus fundamentalis* sp. n. were detected in the cartilaginous gill arch under the basis of gill filaments. Despite similarities of some species in spore morphology, 18S rDNA sequences and phylogenetic analyses showed clear differences among all species examined. The findings of the study demonstrate that morphologically similar spores could only be correctly identified by considering the location of plasmodia and the genetic characters of the myxozoan species.	
41	Keywords separated by ' - '		
42	Foot note information		

Parasitol Res
DOI 10.1007/s00436-010-1982-z

ORIGINAL PAPER

Differentiation of *Myxobolus* spp. (Myxozoa: Myxobolidae) infecting roach (*Rutilus rutilus*) in Hungary

Kálmán Molnár · Szilvia Marton · Csaba Székely · Edit Eszterbauer

Received: 16 March 2010 / Accepted: 13 July 2010

Abstract During a survey on fishes from Hungarian freshwaters, the occurrence of eight *Myxobolus* species was registered in roach (*Rutilus rutilus* L.). Most species had a specific location within the fish host. Of the known species infecting roach, the intramuscularly developing species *Myxobolus pseudodispar* was found to be the most common. Besides *Myxobolus intimus* and *Myxobolus diversicapsularis* infecting the capillary network of gill lamellae, *Myxobolus feisti* infecting the cartilaginous gill rays were observed. Of the "muelleri-type" *Myxobolus* spp, *Myxobolus rutili* and *Myxobolus sommervillae* sp. n. formed elongated plasmodia in the gill filaments outside and inside the arteria efferens, respectively, while *Myxobolus wootteni* sp. n. developed cysts in fins. Plasmodia and spores of *Myxobolus fundamentalis* sp. n. were detected in the cartilaginous gill arch under the basis of gill filaments. Despite similarities of some species in spore morphology, 18S rDNA sequences and phylogenetic analyses showed clear differences among all species examined. The findings of the study demonstrate that morphologically similar spores could only be correctly identified by considering the location of plasmodia and the genetic characters of the myxozoan species.

Introduction

Roach, *Rutilus rutilus* (L.), is one of the most common fish in Europe. Its parasite fauna, among them *Myxobolus* infections, are also well studied (Donec and Shulman 1984; Landsberg and Lom 1991; Lom and Dyková 1992; Longshaw et al. 2003). Donec and Shulman (1984) recorded more than 20 *Myxobolus* spp. from roach and its subspecies. However, Eiras et al. (2005), in their synopsis on the genus *Myxobolus*, listed only eight species described from roach as a type host (*Myxobolus alievi* Gasimagomedov, 1970; *Myxobolus chernovae* (Chernova, 1970); *Myxobolus diversicapsularis* Slukhai, 1984; *Myxobolus dujardini* Thelohan, 1892; *Myxobolus elegans* Kashkovski, 1966; *Myxobolus marginatus* Kulemina, 1969; *Myxobolus pseudodispar* Gorbunova, 1936; and *Myxobolus rutili* Donec and Tozyyakova, 1984). Besides these species, *Myxobolus buckei* Longshaw et al. (2005) from the vertebral column and *Myxobolus feisti* Molnár et al. (2008) from the gill cartilage were recently described from roach. *M. buckei* was described from chub as original host, but its occurrence in roach was also assumed. Morphological and molecular studies by Molnár et al. (2006) on the *Myxobolus* fauna of chub suggest that *Myxobolus* species are rather host-specific parasites, and seem to infect a single host species or genetically closely related hosts. The above mentioned paper and previous studies (Molnár 1994; Molnár et al. 2002; Eszterbauer 2002, 2004) also suggest that *Myxobolus* species bear strict tissue specificity and moderate organ specificity.

This paper presents data on the spore morphology and phylogeny of five known and three new *Myxobolus* species found in roach. Furthermore, the typical locations of infection and the histological changes in fish caused by these species are also investigated.

Materials and methods

Roach (*Rutilus rutilus*) specimens originated from several Hungarian lakes and rivers. Most specimens were collected

K. Molnár (✉) · S. Marton · C. Székely · E. Eszterbauer
Veterinary Medical Research Institute,
Hungarian Academy of Sciences,
PO Box 18, 1581 Budapest, Hungary
e-mail: molnar@vmri.hu

in the framework of fish parasitological monitoring projects in Lake Balaton (no. of specimens were 339) and the Kis-Balaton water reservoir (217 specimens), but roach were examined also from Danube (12 specimens) and Tisza rivers, and from some small creeks (35 specimens). The majority of roach specimens were 4- to 13-cm long fingerlings of the age group 1+ year, but 14 to 25 cm long, 3- to 6-year-old specimens were also examined (Table 1).

The fishes were subjected to complete parasitological dissection. However, infection of muscle, by compressing pieces between two glass plates, was studied only partially. When mature plasmodia were found, some of the spores were studied in fresh preparations, some of them were collected into Eppendorf tubes and stored at –20°C until further molecular characterization, while the rest of the spores were preserved in glycerine-gelatine as slide preparations. Tissue samples from infected organs containing developing and mature plasmodia were fixed in Bouin's solution, embedded in paraffin wax, cut to 4–5-µm sections, and stained with haematoxylin and eosin. Naïve spores were studied by Nomarski differential interference contrast of an Olympus BH2 microscope. The spores were photographed with an Olympus DP10 digital camera, or recorded on videotapes; and then digitized images were obtained, and measurements were taken. All measurements are given in micrometer.

Molecular methods

For DNA extraction, samples were centrifuged at 5,000×*g* for 5 min. Spore pellets were suspended in 500 µl lysis buffer (100 mM NaCl, 10 mM Tris, 10 mM EDTA, 0.2% SDS, and .4 mg/ml proteinase K) and incubated at 55°C for 3–4 h. DNA was then purified using the Miniprep Express Matrix (BIO 101, USA). Genomic DNA was amplified with the primer pair 18e (5′-CTG GTT GAT TCT GCC AGT-3′) (Hillis and Dixon 1991) and 18r (5′-CTA CGG AAA CCT TGT TAC-3′) (Whipps et al. 2003). The total volume of the PCR reactions was 50 µl, which contained approximately 10 to 50 ng DNA, 1× Taq PCR reaction buffer (MBI Fermentas), 1.5 mM MgCl2, 0.2 mM dNTP mix (Sigma), 25 µM of each primer, and 2 U of Taq DNA polymerase (MBI Fermentas). Amplification conditions were 95°C for 50 s, 58°C for 50 s, and 72°C for 80 s for 35 cycles, with a terminal extension at 72°C for 7 min. When a weak band was detected on 1.0% agarose gel in Tris–acetate–EDTA (TAE) buffer, the amplification was followed as nested PCR assay with inner primer pairs MX5 (5′-CTG CGG ACG GCT CAG TAA ATC AGT-3′) and MX3 (5′-CCA GGA CAT CTT AGG GCA TCA CAG A-3′) (Andree et al. 1999), or SphF (5′-ACT CGT TGG TAA GGT AGT GGC T-3′) and SphR (5′-GTT ACC ATT GTA GCG CGC GT-3′) (Eszterbauer and Székely 2004). Biometra T1 thermocycles were used for amplification. Cycling conditions with primers MX5 and MX3 were 95°C for 30 s, 50°C for 30 s, and 72°C for 60 s for 35 cycles, and were terminated with an extension period at 72°C for 7 min. For PCR using SphF and SphR primers, the same condition was applied for those with the 18e–18r primer pair. The PCR products were electrophoresed in 1.0% agarose gels (Sigma) in TAE buffer, and then purified with QIAquick Gel Extraction Kit (Qiagen).

For samples with insufficient amount of amplified PCR product for direct DNA sequencing, purified DNA was cloned into a pGEM-T Vector System II (Promega) following the manufacturer's manual. Purified PCR products and/or at least two positive clones per sample were sequenced in both directions using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyzer automated DNA sequencer (Applied Biosystems). The following primers were used for sequencing: amplification primers 18e and 18r, MX5 and MX3, SphF and SphR, Myx4r and Act1f by Hallett and Diamant (2001) and MB5, MB3, MC5, and MC3 described by Eszterbauer (2004). For sequence assembling, the STADEN Sequence Analysis Package version 2001.0 (Staden 1996) was used. DNA sequence similarities were calculated with the Sequence Identity Matrix of the software BioEdit (Hall 1999).

Phylogenetic analyses

Nucleotide sequences were aligned with the software Multalin (Corpet 1988) available online. The alignment was corrected manually using the GeneDoc sequence alignment editor program. The dataset for the alignment was chosen on the basis of the results of BLAST searches and morphological findings. Phylogenetic analyses using neighbor-joining (with K2P; NJ) and maximum likelihood (ML) algorithm were conducted in PAUP* version 4.0b10 (Swofford 2001). An optimal evolutionary model (GTR+I+G) for the alignment was determined with AIC in Modeltest 3.06 (Posada and Crandall 1998). Maximum likelihood analysis employed a heuristic search algorithm with random sequence addition (10 replicates) and TBR branch swapping. Bootstrap confidence values were calculated with 1,000 and 100 repetitions for NJ and ML, respectively. Bayesian inference analysis was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). A general time reversible model (GTR) with gamma-shaped rate variations across sites (Invgamma) was chosen for the analysis. Two independent runs were conducted with four chains for 1 million generations. Trees were sampled every 100 generations. The first 25% of the samples were discarded from the cold chain

t1.1 **Table 1** Number of examined roach specimens of two size groups collected in different water basins in the course of the 8-year-long survey (2001–2008

	Lake Balaton		Kis-Balaton		Danube		Other places		All	t1.2
	4–13	14–25	4–13	14–25	4–13	14–25	4–13	14–25		t1.3
2001	20	18	8	4	–	–	12	–	62	t1.4
2002	16	8	3	7	–	2	–	–	36	t1.5
2003	32	14	4	–	–	3	3	–	56	t1.6
2004	18	18	–	10	–	–	–	4	50	t1.7
2005	29	13	12	–	–	–	–	2	56	t1.8
2006	33	24	20	6	4	–	7	5	99	t1.9
2007	8	6	5	–	1	–	–	–	20	t1.10
2008	51	31	98	40	–	2	–	2	224	t1.11
	207	132	150	67	5	7	22	13	603	t1.12
Total	339		217		12		35		603	t1.13

Two size groups (4–13 and 14–25) are given in cm

(burninfrac=0.25), and a 50% majority-rule consensus tree was created, which was visualized by MEGA 4. *Myxobolus cerebralis* was chosen as the outgroup.

Results

Five known species (*Myxobolus intimus* Zaika, 1965; *M. diversicapsularis* Slukhai, 1984; *M. rutili* Donec and Tozyyakova, 1984; *M. feisti* Molnár et al. 2008; and *M. pseudodispar* Gorbunova, 1936) and three new species (*Myxobolus fundamentalis* sp. n, *Myxobolus sommervillae* sp. n. and *Myxobolus wootteni* sp. n.) were identified in roach in the course of the 8-year-long survey. Each species had a specific location within the fish host (Table 2). Measurements of *Myxobolus* spp. examined are listed in Table 3, while their molecular characterization is summarized in Table 4.

M. intimus Zaika, 1984

The species was found in 3- to 6-year-old roach specimens from Lake Balaton and Danube River in March and April of each year. Mature plasmodia filled with spores were found in April. In March, plasmodia harbored sporogonic stages and some young spores. In May, only some aged plasmodia were found. No occurrence of the species was recorded in other seasons of the year. Although in a year's relation, only 6% of the examined roach were infected with this species, in spring, the infection in larger roach specimens was over 40%.

Q2 Round plasmodia of 60 to 220 in diameter were located in the capillary network of gill lamellae. The shape and size of the characteristic spores, with a small extension at the anterior pole (Fig. 1a, Table 3), corresponded to the data given by Rácz et al. (2004); Donec and Shulman (1984) and Lom and Dyková (1992).

Histology Small, round plasmodia developed in the capillary network of gill lamellae. Plasmodia filled most of the capillary network of the lamellae.

Molecular data 18S rDNA sequence of *M. intimus* (AY325285) resembled the best Aurantiactinomyxon "type 1" (AY495708) by Hallett et al. (2006) with 99.9% similarity. Between the two *M. intimus* replicates sequenced, a T/C nucleotide alteration was detected in position no. 52.

M. diversicapsularis Slukhai, 1966

The species was found in 3- to 6-year-old roach specimens from Lake Balaton and Danube River in April and May of each year. Mature plasmodia were first found in April. No occurrence of the species was recorded in any other seasons of the year. Although only 2.6% of the examined roach was found infected in the course of the entire study, in spring 2008, 37% prevalence was detected among roach specimens >3 years of age. Concurrent infections with *M. intimus* were frequently recorded. Ellipsoidal plasmodia of 100–150×175–250 μm in diameter were located in the capillary network of gill lamellae (Fig. 2). Shape and size of the characteristic spores with unequal polar capsules (Figs. 1b and 4a, b) corresponded to the data given by Shulman (1966) and Lom and Dyková (1992).

Type of material Voucher spores in glycerine-gelatine were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Mu-

t2.1 **Table 2** Number of roach specimens found to be infected with *Myxobolus* spp. in different habitats in the course of the 8-year-long survey

t2.2 Name of the species	Location in host	Lake Balaton	Kis-Balaton	Danube	Other localities	In all localities
t2.3 *Myxobolus intimus*	Gill lamellae	35/339 (10.3)	0/217 (0)	1/12 (8.3)	1/35 (2.9)	37/603 (6.1)
t2.4 *M. diversicapsularis*	Gill lamellae	16/339 (4.7)	0/217 (0)	1/12 (8.3)	0/35 (0)	17/603 (2.8)
t2.5 *M. pseudodispar*	Muscles	82/339 (24.2)	130/217 (60)	2/12 (16.7)	30/35 (85.7)	244/603 (40.5)
t2.6 *M. fundamentalis* sp. n.	Gill arch	8/339 (2.4)	0/217 (0)	0/12 (0)	0/35 (0)	8/603 (1.3)
t2.7 *M. wootteni* sp. n.	Fins	24/339 (7.1)	0/217 (0)	1/12 (8.3)	0/35 (0)	25/603 (4.1)
t2.8 *M. feisti*	Gill rays	27/339 (7.9)	52/217 (24)	0/12 (0)	1/35 (2.9)	80/603 (13.3)
t2.9 *Myxobolus rutili*[a] and *M. sommervillae* sp. n.	Gill filaments	18/339 (5.3)	6/217 (2.8)	0/12 (0)	2/35 (5.7)	26/603 (4.3)
t2.10 All *Myxobolus* spp.		210/339	188/217	5/12	34/35	437/603

Prevalence values are in parenthesis (%). Number of fish infected by *Myxobolus* spp/examined fish from a certain water basin

[a] The identification of these species without detailed examination of spores was uncertain

seum, Budapest, Coll. No. HNHM-18215. The 18S rDNA sequence of *M. diversicapsularis* was deposited in Genbank under the accession number GU968199.

Histology Small- to middle-sized ellipsoidal plasmodia (Fig. 2) filled most of the capillary network of the lamellae.

Molecular data 18S rDNA sequences of *M. diversicapsularis* samples resembled the best Triactinomyxon "type 3" (AY495706) with 99.6% similarity (Hallett et al. 2005). Three nucleotide alterations were detected among sample replicates in position no. 1420. (T/C), no. 1429 (T/C), and no. 1508 (T/G).

t3.1 **Table 3** Morphological features and measurements of *Myxobolus* spp. found in roach

t3.2	*M. intimus*	*M. diversicapsularis*	*M. pseudodispar*	*M. fundamentalis* sp. n.	*M. wootteni* sp.n.	*M. rutili*	*M. sommervillae* sp.n.	*M. feisti*
t3.3 Location of plasmodia	Gill lamellae	Gill lamellae	Muscle cells	Gill arch	Fins	Gill filaments	Gill filaments	Cartilaginous gill rays
t3.4 Shape of plasmodia	Small, roundish	Small, roundish	Large, elongated	Large, roundish	Small, ellipsoid	Large, elongated or roundish	Large, elongated	Small, round or ellipsoid
t3.5 Spore shape in FW	Ellipsoidal with extension	Ellipsoidal, often deformed	Ellipsoidal	Ellipsoidal	Ellipsoidal	Ellipsoidal	Ellipsoidal	Ellipsoidal
t3.6 Spore length	13.5±0.96 (12.6–14.7)	10.3±0.51 (9.3–11.0)	12.2±0.79 (11.0–13.6)	15.5±0.81 (14.4–17.0)	13.3±0.96 (11.6–15.0)	13.1±1.26 (11–15.7)	11.8±0.95 (10.5–13.5)	11.7±0.93 (11.5–13.2)
t3.7 Spore width	9.2±0.4 (8.6–9.7)	8.7±0.41 (8.0–9.0)	7.0±0.7 (5.8–8.3)	11.8±0.58 (11.2–13.2)	11.6±0.73 (10.2–13.0)	9.9±0.77 (8–10.8)	9.7±0.76 (8.4–11.2)	10.0±0.77 (9.4–10.8)
t3.8 Spore thickness	8.0±0.4 (7.0–8.5)	6.5±0.15 (6.3–6.8)	5.6±0.3 (5.0–6.0)	9.2±0.3 (9.0–9.6)	5.7±0.37 (5.5–6)	6.8±1.17 (6.0–9.0)	7.2±0.39 (6.6–7.5)	6.7±0.19 (6.6–7.0)
t3.9 Length of (larger) PC	5.5±0.4 (4.8–6.2)	5.4±0.34 (4.8–6.0)	5.9±0.39 (5.6–6.6)	6.7±0.24 (6.5–7.2)	6.2±0.75 (5.0–7.0)	5.9±0.9 (4.0-7.2)	6.0±0.38 (5.4–6.7)	6.2±0.14 (6.0–6.3)
t3.10 Width of (larger) PC	3.4±0.5 (2.9–4.8)	3.5±0.16 (3.3–3.6)	2.9±0.35 (2.1–3.3)	4.0±0.26 (3.7–4.3)	3.9±0.49 (3.0–4.4)	3.4±0.62 (3.0–3.7)	3.3±0.35 (3.0–4.2)	3.7±0.22 (3.3–4.0)
t3.11 Length of smaller PC	–	3.4±0.55 (2.5–4.6)	3.8±0.45 (3.5–5.0)	–	–	–	–	–
t3.12 Width of smaller PC	–	2.6±0.38 (2.2–3.0)	2.8±0.13 (2.7–3.0)	–	–	–	–	–
t3.13 IP length	n.d.	–	–	1.7±0.1 (1.6–1.8)	2.7±0.76 (2.0–3.5)	1.3±0.31 (0.7–1.5)	1.8±1.85 (1.5–2.7)	2.0±0.32 (1.8–2.4)
t3.14 Number of PFC	5–6	4–5/3–4	4–6/3–4	6–7	6–7	6	6	6
t3.15 Reference	Rácz et al. 2004	Present study	Present study	Present study	Present study	Present study	Present study	Molnár et al. 2008

FW frontal view, *PC* polar capsule, *IP* intercapsular process, *PFC* polar filament coils, *n.d.* no data, – absent

t4.1 **Table 4** *Myxobolus* spp. from roach examined at DNA level. The positions of nucleotide alterations are noted in Results

t4.2 Species	No. of replicates sequenced	Genetic similarities (%)	GenBank accession number (length of 18S rDNA fragment)
t4.3 *M. intimus*	2	99.9	AY325285 (1583 nt)
t4.4 *M. diversicapsularis*	3	99.7–99.9	GU968199 (1891 nt)
t4.5 *M. fundamentalis* sp. n.	2	99.9	GU968200 (1357 nt)
t4.6 *M. wootteni* sp. n.	2	99.9	DQ231157 (1599 nt)
t4.7 *M. rutili*	2	100	GU968201 (1326 nt)
t4.8 *M. sommervillae* sp.n.	2	100	GU968202 (1321 nt)
t4.9 *M. feisti*	1	–	EU598804 (1331 nt)

Remarks It is assumed that prior to 2007, immature plasmodia, and less frequently, mature plasmodia of *M. diversicapsularis*, might be erroneously identified in some cases as *M. intimus* occurring concurrently in the same location in fish host.

M. pseudodispar Gorbunova, 1936

Plasmodia or spores of this species were found in each age group and in most of the examined specimens of roach. Plasmodia located in muscle cells showed no seasonality, although developing plasmodia were found mostly in spring, except fingerlings, which got infected in early summer. In autumn and winter, mostly aged plasmodia or disseminated spores in macrophage centers of different organs were detected. The shape and size of the characteristic spores with unequal polar capsules (Fig. 1c, Table 3) corresponded to the data given by Shulman (1966); Lom and Dyková (1992), and Molnár et al. (2002).

M. fundamentalis sp. n.

Plasmodia (Fig. 3), round or ellipsoidal, up to 1.2 mm in length and 0.7 to 0.8 mm in width develop in the cartilaginous gill arch under the gill filaments. Spores develop in disporic pansporoblasts. Spores are relatively large, ellipsoidal in frontal view (Figs. 1d and 4c), and lemon-shaped in sutural view (Figs. 1d (inset) and 4d). Q3 The other characteristics of the spore are the following: spore length, 15.5±0.81 (14.4–17.0; n=50), width, 11.8±0.58 (11.2–13.2; n=50), and thickness, 9.2±0.3 (9.0–9.6; n=15). Polar capsules are equal in size, pyriform, relatively short, slightly converging anteriorly, 6.7±0.24 (6.5–7.2) long (n=50) and 4.0±0.26 (3.7–4.3) wide (n=50). Six to seven polar filament coils are arranged perpendicular to the capsule length. Relatively small triangular intercapsular appendix measuring 1.7±0.1 (1.6–1.8; n=16) are located anteriorly between the capsules. Sutural protrusion forms a circular rim around the spore emerging about 0.8 to 1.0 μm over the surface of the spore. The thickness of the rim in sutural view measures about 0.8 μm. Sutural edge markings (Nos. 8 to 9) are poorly seen in fresh spores. Single binucleated sporoplasm with large, round iodinophilous vacuole is present. Mucous envelope is not found.

Host type: roach, *Rutilus rutilus* (L.) (Cyprinidae). Q4

Localities: Lake Balaton and Kis-Balaton water reservoir

Site of tissue development: connective tissue in the gill arch

Type of material Syntype spores in glycerine-gelatine were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-18211. The 18S rDNA sequence of *M. fundamentalis* sp. n. has been deposited in Genbank under the accession number GU968200.

Etymology The species is named after its specific location in the cartilaginous gill rays at the basis of filaments.

Histology of infection Most plasmodia were located in the gill arch close to the basis of gill filaments, but several plasmodia were found inside the gill arch, attached to the wall of arteries (Fig. 3). Semi-mature plasmodia contained mature spores in the central region, and young sporogonic stages at the periphery.

Molecular data The DNA sequences of two samples of this species were 99.9% similar, as only a single nucleotide difference in position no. 350 (A/T alteration) was detected over a 1,357-bp-long fragment. The most closely related species was *Myxobolus gayerae*, with 93.5% similarity in their 1330-bp-long aligned 18S rDNA sequence.

Remarks M. fundamentalis sp. n. resembled in spore morphology *Myxobolus cyprinicola* Reuss 1906 (DQ439805) from the common carp, and *Myxobolus*

Q5

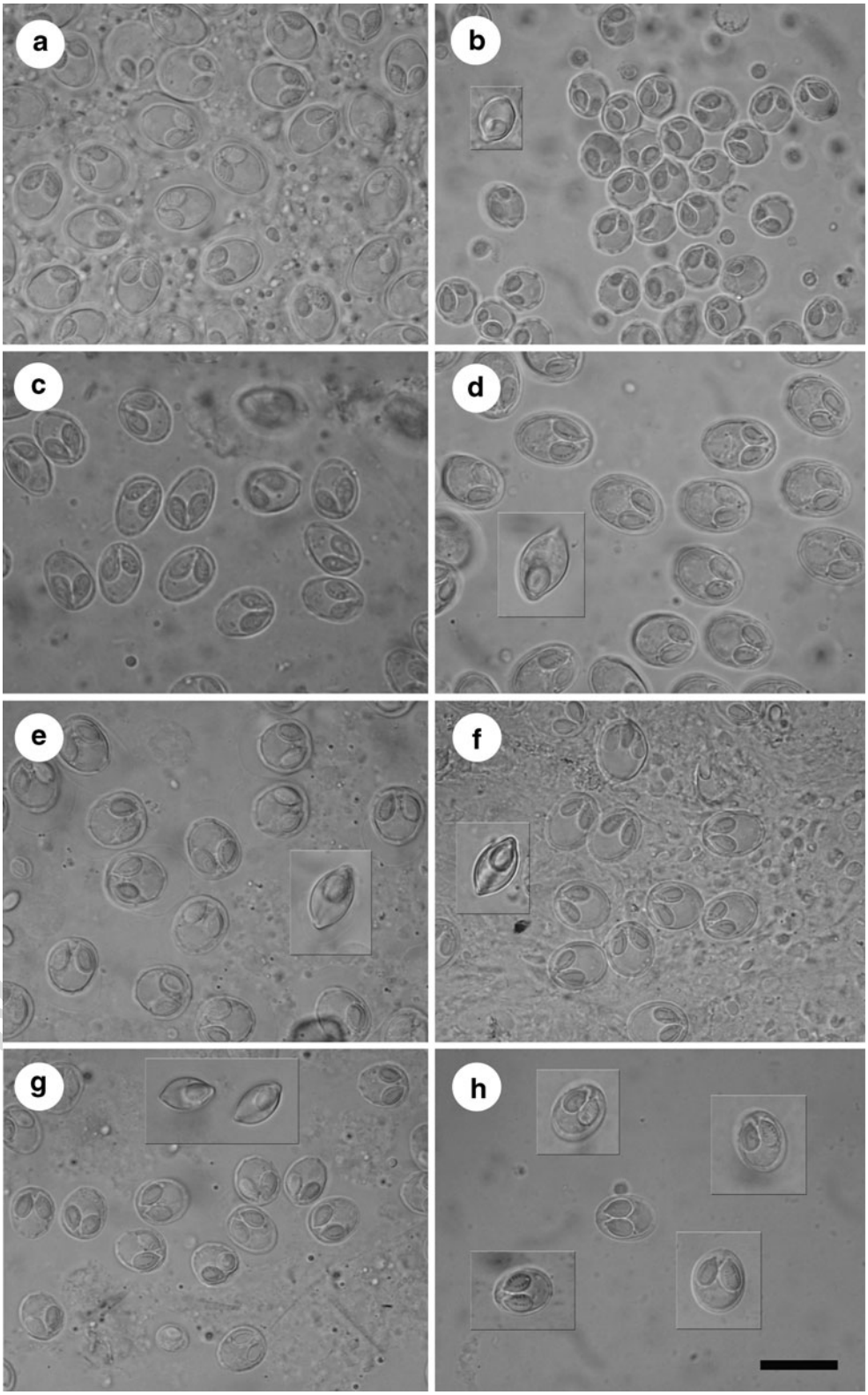
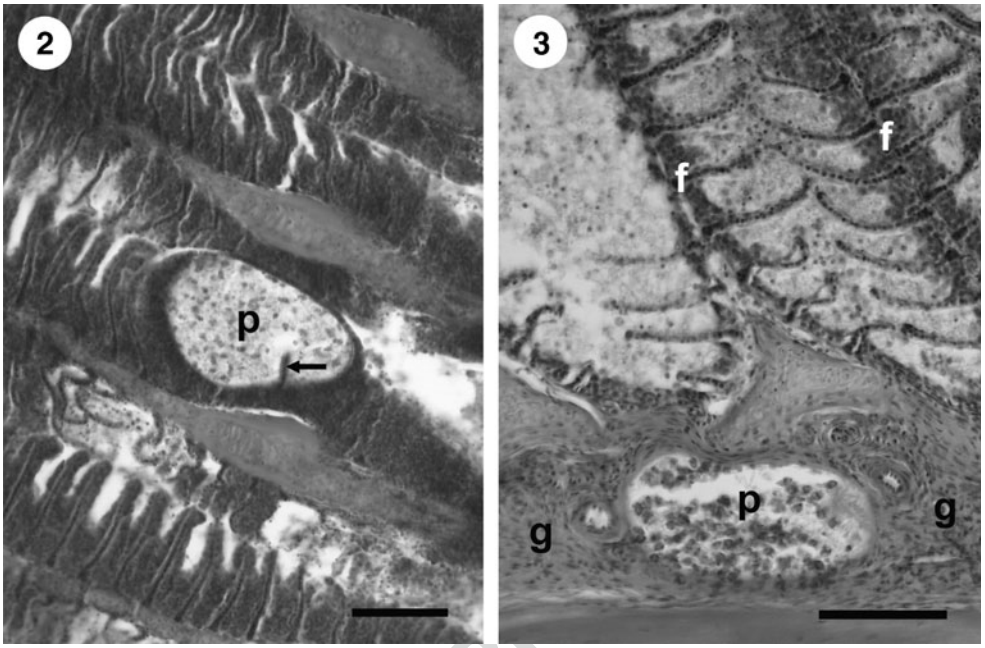


Fig. 1 Spores of *Myxobolus* spp. found in roach. **a** *M. intimus*. **b** *M. diversicapsularis* in frontal view, *inset* in sutural view. **c** *M. pseudodispar*. **d** *M. fundamentalis* sp. n. in frontal view, *inset* in sutural view. **e** *M. wootteni* sp. n. in frontal view, *inset* in sutural view. **f** *M. rutili* in frontal view, *inset* in sutural view. **g** *M. sommervillae* sp. n. in frontal view, *inset* in sutural view and **h** *M. feisti*. Fresh mount. *Bar*=15 μm

cycloides ex *Leuciscus* (DQ439810), a typical parasite of the swimbladder of chub, but it clearly differed from them at the DNA level (86.8% and 93.2% similarity, respectively). By its typical basifilamental location, *M. fundamentalis* sp. n. resembles also *Myxobolus basilamellaris* (AF507971), the parasite of the common carp, but its spores are larger, and their 18S rDNA sequence similarity is only 86.9%.



Figs. 2-3 Plasmodium (*p*) of *M. diversicapsularis* in a gill lamella deforming the neighboring lamellae. The rest of the attacked capillary (*arrow*) can still be seen. Histological section, H&E. *Bar*=200 μm Plasmodium (*p*) of *M. fundamentalis* sp. n. in the lumen of the cartilaginous gill arch (*g*) under the basis of a gill filament (*f*). Histological section, H&E. *Bar*=200 μm

Occurrence of "muelleri-type" spores in different organs of the roach

In the course of the present study, four different "muelleri-type" *Myxobolus* species (*M. wootteni* sp. n., *M. feisti, M. rutili* and *M. sommervillae* sp. n.) were found infecting different organs of roach. Their spores morphologically resembled very much the spores of *Myxobolus muelleri* Buetschli, 1882, a gill-parasite of chub, but differed from it in their site selection and 18S rDNA sequences. The description of these species is as follows:

M. wootteni sp. n.

Large, white, round plasmodia of 300–400×100–180 were found in the fins of roach fingerlings (Figs. 5 and 6). "Muelleri-type" spores were short, ellipsoidal or roundish, somewhat enlarged anteriorly in frontal view (Figs. 1e and 4e), and lemon-shaped in lateral view (Figs. 1e (inset) and 4f). Sutural line is indistinct and the sutural edge is moderately protruding. Valves are thin, symmetrical, and smooth. Spores are 13.3±0.96 (11.6–15) long, 11.6±0.73 (10.2–13) wide, and 5.7±0.37 (5.5–6.0) thick. Two polar capsules pyriform, closely equal in size, 6.2±0.75 (5.0–7.0) long and 3.9±0.49 (3.0–4.4) wide, tapering toward the discharging canals of polar filaments. Polar filaments coiled with six to seven turns in the polar capsule situated perpendicularly to the longitudinal axis of the capsule. The spore has a strong, large, triangular intercapsular appendix of 2.7±0.76 (2.0–3.5) at the anterior end. Sporoplasm nuclei are indiscernible, and a large iodinophilous vacuole is found in the sporoplasm. Four to six sutural edge markings and a thin oval mucous envelope around the posterior end of the spores.

Host type: roach, *Rutilus rutilus* (L.) (Cyprinidae).

Locality: Lake Balaton, Hungary

Site of tissue development: connective tissue between the fin rays

Type of material Voucher spores of *M. wootteni* sp. n. in glycerine-gelatine were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-18212. The 18S rDNA sequence of *M. wootteni* sp. n. has previously been deposited in Genbank by Eszterbauer et al. (2006) under the accession number DQ231157.

Etymology The species is named after the well-known British fish pathologist Dr. Rodney Wootten.

Histology In histological sections, plasmodia were found in the connective tissue under the skin between two cartilaginous fin rays (Fig. 6). The ectoplasm of the plasmodium was bordered by a thin connective tissue layer. No connection was found between plasmodia and cartilaginous elements.

Molecular data DNA sequences of two *M. wootteni* samples from different roach specimens showed 99.9% similarity (G/A nucleotide alteration in position no. 930), and differed from *M. muelleri* ex. chub in 11.2%, from *M. fundamentalis* sp. n. in 10.2%, from *M. gayerae* in 8.9%. Triactinomyxon "type D-1" (DQ231146) characterized by Eszterbauer et al. (2006) showed 99.9% similarity to *M.*

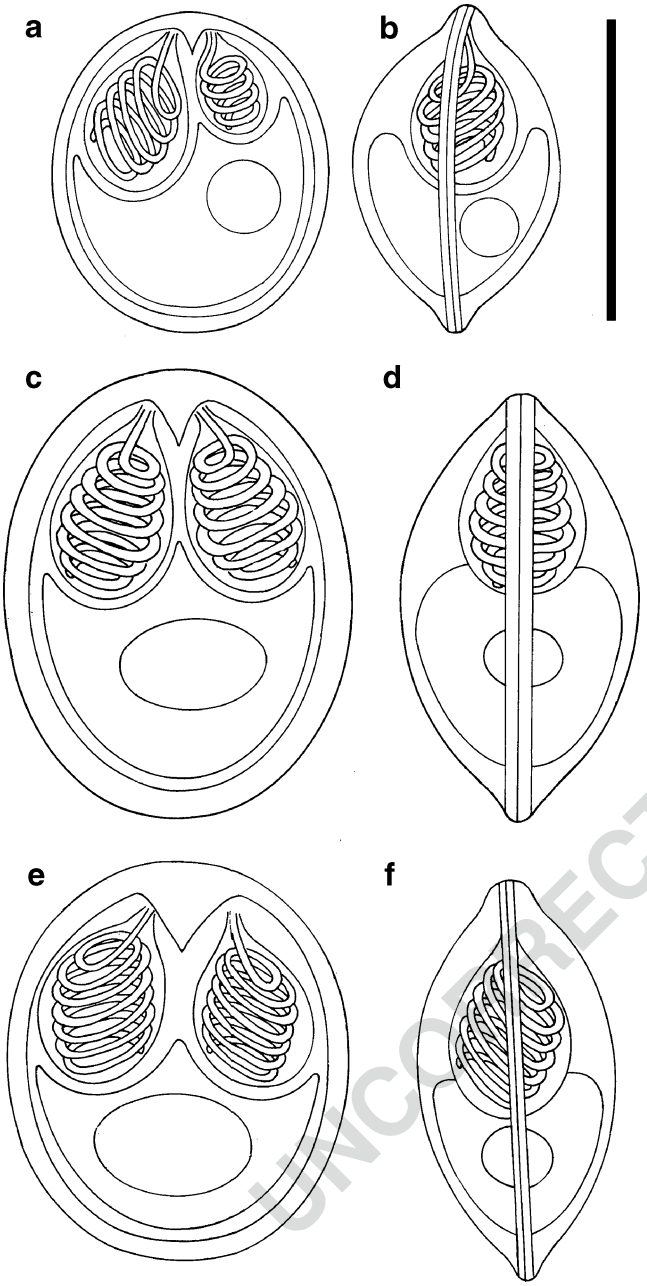


Fig. 4 Schematic drawings of spores of different *Myxobolus* spp. infecting roach. **a** *M. diversicapsularis* in frontal view and **b** in sutural view. **c** *M. fundamentalis* sp. n. in frontal view and **d** in sutural view. **e** *M. wootteni* sp. n. in frontal view and **f** in sutural view. *Bar*=10 μm

wootteni sp. n. (1 nt difference over a 894-bp-long DNA fragment).

Remarks In its spore morphology, *M. wootteni* resembles *Myxobolus bramae* Reuss 1906, *M. muelleri* Buetschli, 1882, and other "muelleri-type" *Myxobolus* spp. (*Myxobolus muellericus, M. rutili, M. sommervillae*, and *M. feisti*), but differs from them in its larger and wider spores and in its well-developed intercapsular appendix. We found *M. wootteni* sp. n. plasmodia that developed exclusively in the fin of roach fingerlings.

M. rutili Donec et Tozyyakova, 1984

Elongated plasmodia of this species, reaching a size of 1–1.5 mm, were located in the central part of gill filaments between the cartilaginous gill ray and the arteria afferens (Fig. 7). Spores were ellipsoidal or short ellipsoidal in frontal view (Figs. 1f and 10a) and lemon-shaped in lateral view (Figs. 1f (inset) and 10b). Spores were 13.1±1.26 (11.0–15.7) long, 9.9±0.77 (8.0–10.8) wide, and 6.8±1.17 (6.0–9.0) thick. Two polar capsules pyriform, closely equal in size, 5.9±0.9 (4.0–7.2) long, and 3.4±0.62 (3.0–3.7) wide, tapering toward discharging canals of polar filaments. Polar filaments are coiled with six turns in polar capsule, situated perpendicularly to the longitudinal axis of the capsule. Spore has a 1.3±0.31 (0.7–1.5) long triangular intercapsular appendix at the anterior end. Sutural line is indistinct. The sutural edge is moderately protruding. Valves are thin, symmetrical, and smooth with indistinct four to six edge markings. Sutural extensions are present, approximately 0.6 at the anterior and 1 at the posterior end of the spores. Sporoplasm nuclei are indiscernible, and a small iodinophilous vacuole is found in the sporoplasm. Mucous envelope is not found.

Host type: roach, *Rutilus rutilus* (L.) (Cyprinidae).

Locality: Kis-Balaton water reservoir, Hungary

Additional localities: Lake Balaton, small creeks inflowing to Lake Balaton

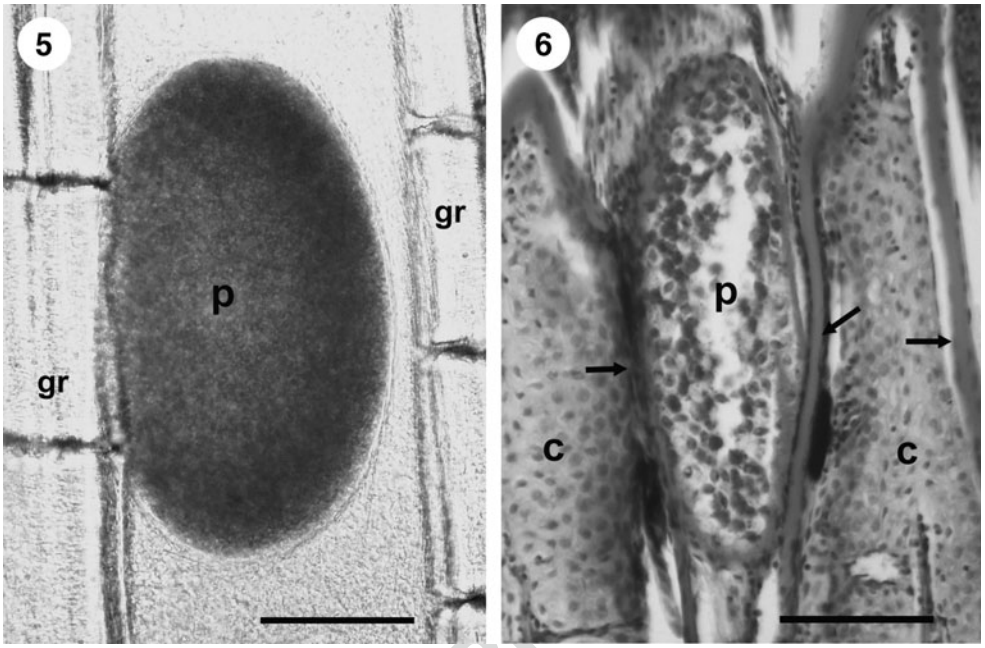
Site of tissue development: central region of the gill filament

Type of material Syntype spores in glycerine-gelatine were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No.HNHM-18214. The 18S rDNA sequence of *M. rutili* was deposited in Genbank under the accession number GU968201.

Histology Large, elongated plasmodia developed at the central part of gill filaments in the connective tissue attaching externally to the arteria afferens (Fig. 7).

Molecular data 18S rDNA sequences of the two *M. rutili* samples were 100% identical. It resembled the best *Myxobolus dogieli* isolate 1 (EU003977) from the heart of *Abramis brama* (95.8%).

Remarks While studying "muelleri-type" *Myxobolus* spp. of roach, Donec and Tozyyakova (1984) (Donec and Shulman 1984) observed that different organs (cornea,



Figs. 5-6 Plasmodium (***p***) of *M. wootteni* sp. n. in the fin between two gill rays (*gr*) attaching to one of the rays. Fresh mount. *Bar*=100 μm. Inside the cartilaginous wall of the fin ray (*arrows*), loose connective tissue can be seen (*c*). Histological section, H&E. *Bar*=100 μm

gills, gill arch, muscles, and fins) were infected with various sizes and shapes, but morphologically with similar spores and described spores with a size of 14–16.5 μm in length as *M. rutili*. In the same study, Donec (Donec and Shulman 1984) depicted some other spores as well, the shape of which better resembles *M. sommervillae* and *M. feisti*. We assume, therefore, that the original description of *M. rutili* covers more than one *Myxobolus* species. Due to our findings, only spores collected from large cysts locating extravasally in the central region of filaments should be designated as *M. rutili*.

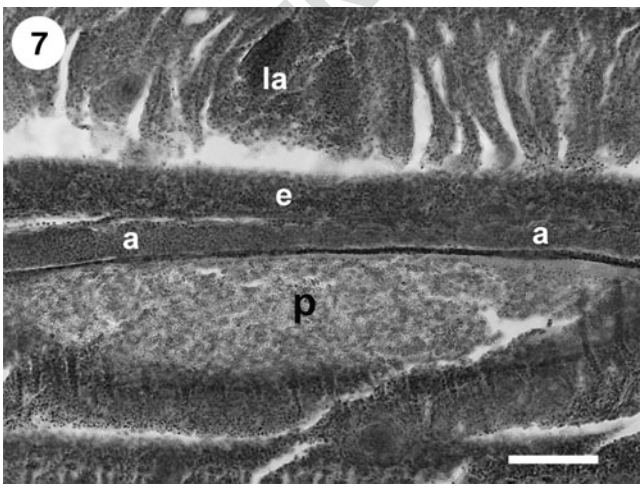


Fig. 7 Plasmodium (*p*) of *M. rutili* lays in the center of a gill filament close to arteria afferens (*a*) covered by multilayered epithelium of the non-lamellated part of the filament. Lamellae (*la*) of the neighboring filament are also seen. Histological section, H&E. *Bar*=50 μm

On the basis of spore morphology, *M. rutili* resembles other "muelleri-like" *Myxobolus* spp., but it differs from them in its 18S rDNA sequence and its specific tissue location. The spores of this species were somewhat more elongated and longer than those of *M. sommervillae* sp. n., but in most cases, no clear morphological difference was observable between the spores of the two species. Both species form elongated plasmodia in the gill filaments, but plasmodia of *M. sommervillae* sp. n. develop inside the lumen of arteria efferens. Plasmodia of *M. rutili*, however, are located outside the lumen of arteries in the central part of gill filaments between the aorta and the cartilaginous gill rays. Two 18S rDNA sequences obtained from samples collected from roach in the Lesence creek entering Lake Balaton and from the Kis-Balaton water reservoir were 100% identical. Their DNA sequences differed from *M. sommervillae* sp. n. in 10.2%. *M. rutili* showed a relatively low similarity to *M. muelleri* (90.5%) and *M. muellericus* (90.4%) as well.

M. sommervillae sp. n.

Elongated plasmodia of the species, reaching a size of 1–1.5 mm, are located in the lumen of the arteria efferens of gill filaments (Fig. 8). Spores are short ellipsoidal in frontal view (Figs. 1g and 10c), and lemon-shaped in lateral view (Figs. 1g (inset) and 10d). Spores are 11.8±0.95 (10.5–13.5) long, 9.7±0.76 (8.4–11.2) wide, and 7.2±0.39 (6.6–7.5) thick. Two polar capsules pyriform, closely equal in size, 6.0±0.38 (5.4–6.7) long, and 3.3±0.35 (3.0–4.2) wide, tapering toward discharging canals of polar filaments. Polar filaments are coiled with six turns in polar capsule, situated perpendicularly to the longitudinal axis of the

capsule. The spore possesses a 1.8±1.85 (1.5–2.7) long triangular intercapsular appendix at the anterior end. Sutural extensions, approximately 0.6 at the anterior and 1 at the posterior end of spores, are seen only in sutural view. Sutural line is indistinct. Sutural edge is moderately protruding. Valves are thin, symmetrical, and smooth with indistinct four to six edge markings. Sporoplasm nuclei are indiscernible, and a small iodinophilous vacuole is found in the sporoplasm. Mucous envelope is not found.

Host type: roach, *Rutilus rutilus* (L.) (Cyprinidae)
Locality: Kis-Balaton water reservoir, Hungary
Additional localities: Tisza River
Site of tissue development: artery of the gill filament

Type of material Syntype spores in glycerine-gelatine were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-18213. The 18S rDNA sequence of *M. sommervillae* was deposited in Genbank under the accession number GU968202

Etymology The species is named after the well-known British fish parasitologist Prof. Christina Sommerville.

Histology Large, elongated plasmodia developed inside the arteria efferens of gill filaments (Fig. 8). Mature spores from ruptured plasmodia were often released into the bloodstream (Fig. 9).

Molecular data 18S rDNA sequences obtained from two roach specimens collected in the Tisza River region and the Kis-Balaton showed 100% identity. The DNA sequence of *M. sommervillae* sp. n. showed high similarity to *M. muelleri* from chub (DQ439806; 98.8% similarity) and *M. bramae* from common bream (AF507968; 98.7%).

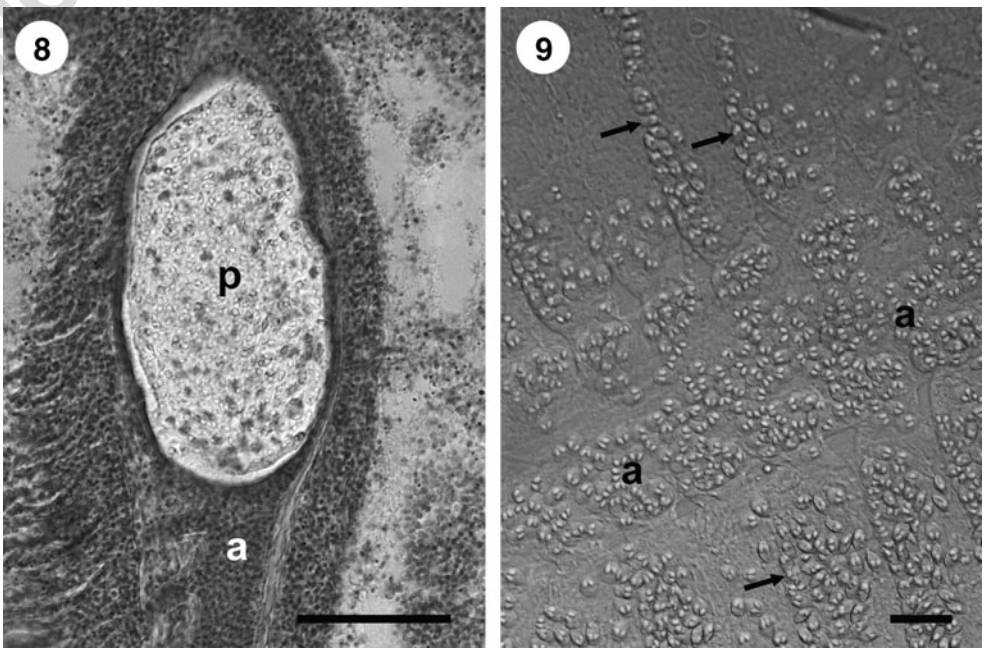
Remarks On the basis of spore morphology, *M. sommervillae* sp. n. resembled other "muelleri-like" *Myxobolus* spp., but it differed from them in its site selection and tissue tropism. Except for minor differences in the size of spores (Table 3) and their site selection, this species resembled the best *M. rutili*. However, the two species clearly differed from each other in their 18S rDNA sequences, as they share only 89.8% identical nucleotides over a 1,323-bp-long DNA fragment.

M. feisti Molnár et al. 2008

The occurrence, histology, and spore morphology (Fig. 1h) of this species was recently described by Molnár et al. (2008). The prevalence of this species might be higher than stated in Table 2, since, in case of low intensity of infection, the detection of small plasmodia in the cartilaginous gill rays is rather difficult.

PCR, DNA sequencing, and phylogenetic analyses

The primer pairs 18e–18r, MX5–MX3, and SphF–SphR successfully amplified approximately 2,000, 1,600, and 1,400 bp fragments of the 18S rDNA from samples



Figs. 8-9 Plasmodium (*p*) of *M. sommervillae* sp. n. located inside the artery (*a*) of a gill filament. Histological section, H&E. *Bar*=100 μm *M. sommervillae* sp. n. spores released from a disrupted plasmodium in the filament artery (*a*) and in the lamellae (*arrows*). Fresh mount. *Bar*=30 μm

examined, respectively. The 14 samples sequenced belonged to eight well-distinguishable *Myxobolus* species, three of which have not been studied at the DNA level previously. 18S rDNA sequences obtained were deposited in GenBank under the accession numbers listed in Table 4. Phylogenetic analyses were performed on the basis of a 1,661-bp-long edited alignment that contained 41 DNA sequences.

Maximum likelihood and Bayesian inference analyses confirmed the topology of the phylogenetic tree generated by neighbor-joining analysis (Figs. 10 and 11). Of the "muelleri-type" species, only *M. sommervillae* sp. n. was located within the cluster of *M. muelleri*. Despite the similar location in gill lamellae, the location of *M. diversicapsularis* sp. n. samples were distant from *M. intimus*, which formed a sister clade of the muscle parasite *M. pseudodispar*. *M. wootteni* sp. n. clustered with the group of *M. fundamentalis* sp. n. and *M. diversicapsularis*. Furthermore, *M. rutili*, another "muelleri-type" species, was located more closely to *M. muellericus* then to *M. muelleri* itself.

Discussion

Most of the known *Myxobolus* species have been described from European freshwater fishes (Eiras et al. 2005). However, the number of papers describing new myxozoan species from North and South America (Cone et al. 2004; Eiras et al. 2010), from Asia (Baska et al. 2009; Kaur and Singh 2010), from Africa (Abdel-Ghaffar et al. 2008), and from marine fishes (Ali et al. 2007) have been constantly increasing in recent years.

Roach *(Rutilus rutilus)*, a common cyprinid fish in Europe, seems to be infected by several, mostly site-specific *Myxobolus* species. Of them, three new species were found in Hungarian lakes and rivers, and the occurrence of five known species was recorded. Of the species studied in this paper, *M. pseudodispar* was detected most frequently. The occurrence, development, electron microscopic, and molecular aspects of the parasite were previously studied in detail (Baska 1986; Székely et al. 1999, 2001; Alvarez Pellitero et al. 2002; Molnár et al. 2002), although several questions concerning its host specificity and genetic variability still need to be answered. In the course of the present study, the plasmodia of this species could easily be detected in the muscle of fish, and its disseminated spores were found in different seasons in the melano-macrophage centers of inner organs like kidney. Similarly, *M. intimus* infection studied by Rácz et al. (2004) in roach from Lake Balaton proved to be rather common in spring months. However, only the present study revealed that *M. diversicapsularis* often caused a concurrent infection with *M. intimus*, and their plasmodia developed in the same location inside the gill lamellae of roach. Due to the DNA sequence analyses of *M. intimus*, developmental stages (both myxospores and actinospores) obtained and preserved from transmission experiments by Rácz et al. (2004), our findings questioned that triactinomyxon-type actinospores observed by Rácz et al. (2004) are the developmental stage of *M. intimus*. Our molecular findings suggested that the triactinomyxons from the transmission experiments of *M. intimus* are the actinospore of *M. diversicapsularis*, as DNA sequences of *M. diversicapsularis* and actinospores from *M. intimus* experiments were 100% identical (data not shown). Besides, a triactinomyxon-type actinospore found by Hallett et al. (2005) showed 99.6% similarity to *M. diversicapsularis*. The DNA sequence analysis of the present study demonstrated that *M. intimus* was 99.9% similar to aurantiactinomyxon "type 1" found by Hallett et al. (2006) in a naturally infected oligochaete stock. These findings suggest that *M. intimus* most likely possesses an aurantiactinomyxon-type actinospore.

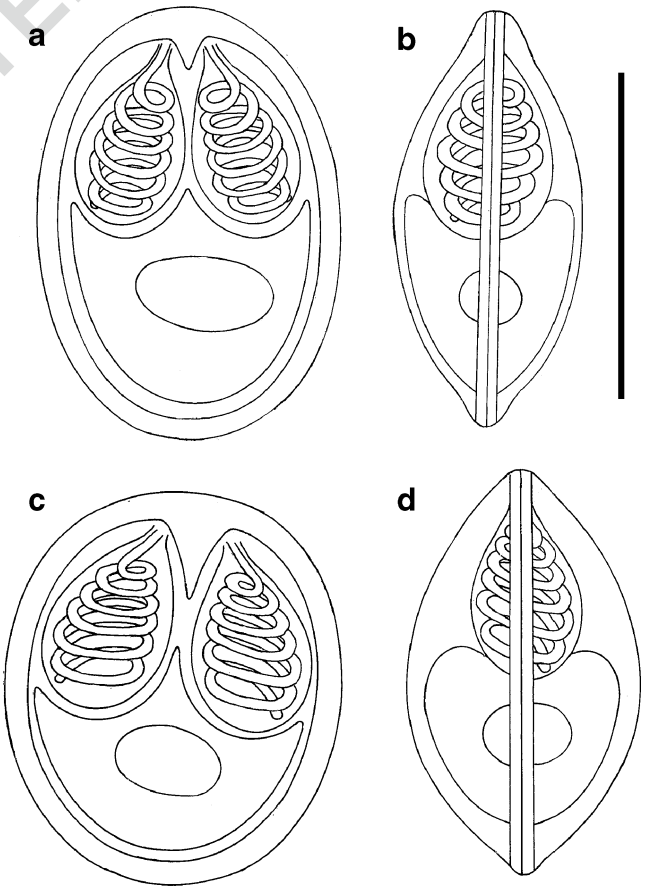


Fig. 10 Schematic drawings of spores of different *Myxobolus* spp. infecting roach. **a** *M. rutili* in frontal view and **b** in sutural view. **c** *M. sommervillae* sp. n. in frontal view and **d** in sutural view. *Bar*=10 μm

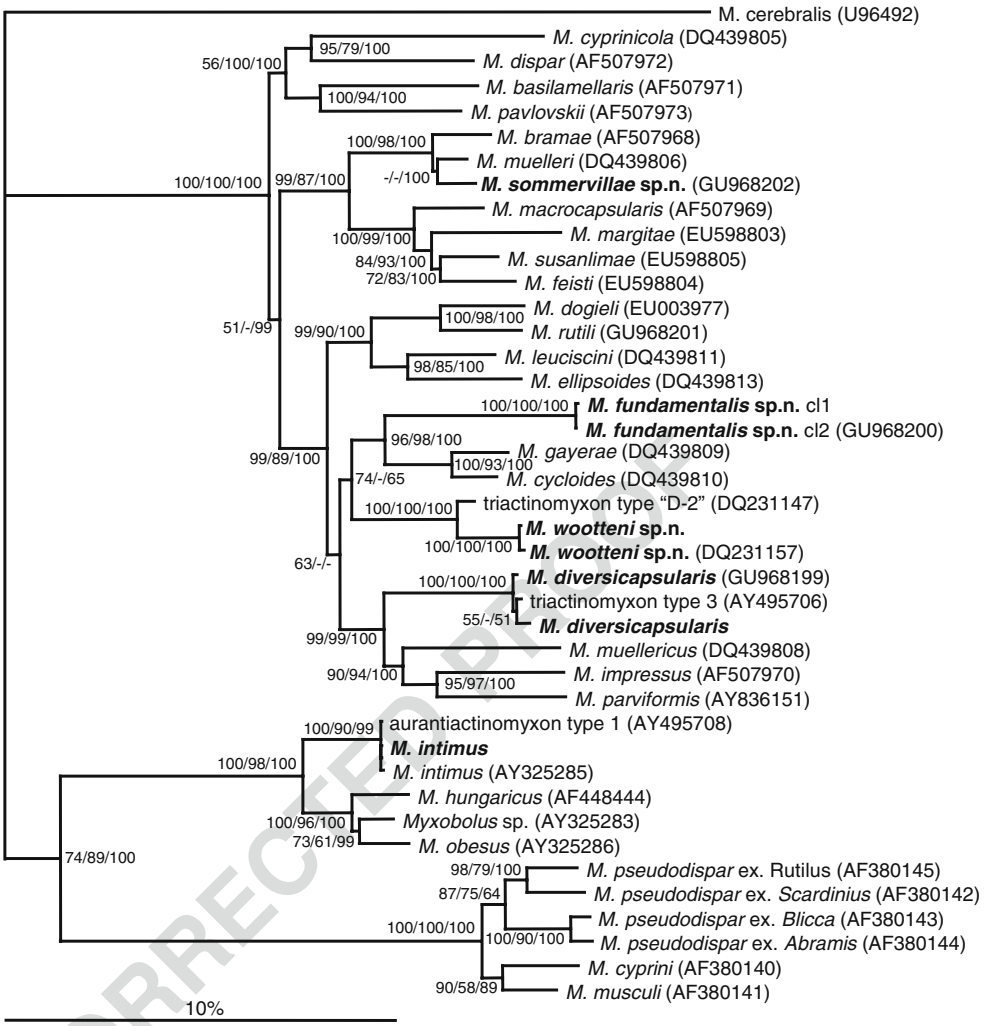


Fig. 11 Phylogenetic tree constructed based on neighbor-joining, maximum likelihood, and Bayesian inference analyses of the 18S rDNA sequences of selected myxozoans. *Numbers at nodes* indicate the respective bootstrap confidence levels in percent. *Myxobolus cerebralis* was used as the outgroup

The above *Myxobolus* spp., as well as *M. fundamentalis* sp. n., have relatively unique spore shapes, and besides their typical locations, they could easily be differentiated even on the basis of morphological features. However, some other *Myxobolus* spp. examined showed rather similar spore morphology, which corresponded to the original description of *M. muelleri* Buetschli, 1882, one of the first described *Myxobolus* species of cyprinid fishes. *M. muelleri* was for a long time thought to be a parasite of several cyprinid fishes (Shulman 1966). However, Reuss (1906) separated "muelleri-type" spores developing in common bream from the above species and described *M. bramae*, while Donec and Tozyyakova (1984) (Donec and Shulman 1984) found slight morphological and size differences in "muelleri-type" spores of roach, and erected *M. rutili* as a new species. Although studies on the fine morphology, site selection, and tissue tropism of morphologically similar spores assist species identification, molecular characterization of spores is required for proper species differentiation. In the course of the present study, four morphologically similar "muelleri-type"species (*M. feisti, M. rutili*, *M. wootteni* sp. n., and *M. sommervillae* sp. n.) were examined. Of them, 18S rDNA sequences of *M. rutili, M. sommervillae* sp. n., and *M. wootteni* sp. n. clearly differed from each other and from the sequences of *M. feisti* described by Molnár et al. (2008). The 89.8% difference between DNA sequences of *M. rutili* and *M. sommervillae* sp. n. was unexpected, as the two species developed in a similar intrafilamental location in roach, and only slight size differences were found in spore measurements. Detailed histological examinations, however, proved that besides differences in 18S rDNA sequences, the two species show different histotropism as well. *M. sommervillae* sp. n. belongs to the filamental vascular group, while *M. rutili* is a filamental but extravascular parasite-forming plasmodia in the non-lamellar part of the central connective tissue, and located between arteria efferens and the cartilaginous gill ray. Molnár et al. (2007) pointed out that in chub, great differences were observed among the 18S rDNA sequences of the filamental species *M. muelleri* and

the DNA sequence of *M. muellericus*, a species developing in gill lamellae. Both in chub and roach, spores, which had a "muelleri-like" morphological character, but were observed in different locations of gills, possessed different 18S rDNA sequences. Of the filamental species with morphologically similar spores, clear difference (89.8%) was detected between *M. rutili* and *M. sommervillae* sp. n., and the chondrophil species *M. feisti* showed only 92.6% similarity to *M. sommervillae* and 87.4% to *M. rutili*. Spores identified as *M. wootteni* sp. n. seem to differ somewhat from the group of *M. muelleri*-like species. By the widened anterior part of its spores, by its roundish polar capsules, and its large intercapsular appendix, this species can even morphologically be distinguished easily from other species examined.

Spores of *M. fundamentalis* sp. n. differ from spores of other species examined in its large, symmetrical spores and in its specific location in the cartilaginous gill arch under the filaments. Its location resembles *M. basilamellaris* from common carp, but the latter species has smaller spores. In its large spores, *M. fundamentalis* sp. n. also resembles morphologically *M. cycloides* Gurley, 1894; *M. cyprinicola* Reuss 1906 and *M. gayerae* Molnár et al. 2007, but it differs from them in its 18S rDNA sequence, and in locating typically in the cartilaginous gill arch.

Although in the course of the survey a great number of fish was dissected, and in most cases complete parasitological examinations were carried out, the prevalence of *Myxobolus* spp. infection in roach (Table 2) has to be considered with caution. In order to be able to detect a given *Myxobolus* infection, fish of various size groups should be collected in large numbers from several habitats and in different seasons. For *M. pseudodispar*, the plasmodia of which could be found both in fingerlings and aged fish and disseminated spores often occur in inner organs, the rate of infection was easily evaluated. However, for species like *M. intimus* and *M. diversicapsularis*, which develop spores in older fish specimens only in spring, prevalence data could have been exact only for a given season and fish age class. The same concerns the occurrence of *M. wootteni* sp. n., which infected only the fins of fingerlings. The observation of small-sized plasmodia in gill cartilage and hidden plasmodia of *M. fundamentalis* sp. n. in the gill arch is rather time consuming and requires a special technique of dissection. Finding large plasmodia of *M. rutili* and *M. sommervillae* sp. n. seemed to be more simple, but in this case, histology and molecular characterization were also required to correctly identify the species.

Three other species (*M. alievi* Gazimagomedov, 1970, *M. chernovae* (Chernova 1970, syn. *M. lomi* Chernova, 1970), *M. marginatus* Kulemina, 1969) have been described from roach. We were unable to identify these poorly described and inadequately illustrated species with those found in the survey.

The primary purpose of the survey was to enlarge our knowledge on the myxosporean fauna of roach. A more general purpose was, however, to obtain further data on the host specificity and site selection of *Myxobolus* spp. Previous studies on chub (Molnár et al. 2007, 2008), a leuciscinid-cyprinid fish with close relation to roach, showed that chub is regularly infected at least by eight different *Myxobolus* species. Though in the present study eight *Myxobolus* species was also found in roach, DNA sequence analysis revealed that none of the morphologically identical or similar species corresponded to those found in chub.

Results obtained in the course of this survey suggest that the number of host-, site- and tissue-specific species is much higher than hitherto expected. A single cyprinid species might be infected by more than a dozen specific *Myxobolus* spp. In the study, our aim was also to highlight the need for the consideration of the host types in the original species description. Our opinion is that in those cases, when the author of the original species description designated more than one fish as host types, the typical host (i.e., in which the parasite is most frequently found) should be considered valid at re-descriptions.

Acknowledgements The study was supported by the Hungarian Scientific Research Fund (OTKA, projects no. T45891, K75873 and K71837). The authors thank Ms. Györgyi Pataki for preparing drawings and histological slides.

References

Abdel-Ghaffar F, Toukhy A, Al-Quarashi S, Al-Rasheid KAS, Abdel-Baki AS, Hegazy A, Bashtar A-R (2008) Five new myxosporean species (Myxozoa: Myxosporea) infecting the Nile Tilapia) *Oreochromis niloticus* in Bahr Shebin, Nile Tributary, Nile Delta, Egypt. Parasitol Res 103:1197–1205

Ali MA, Abdel-Baki AS, Sakran T, Entzeroth R, Abdel-Ghaffar F (2007) *Myxobolus lubati* n. sp. (Myxosporea: Myxobolidae), a new parasite of haffara seabream *Rhabdosargus haffara* (Forsskal, 1775), Red Sea, Egypt: a light and electron transmission microscopy. Parasitol Res 100:819–827

Alvarez-Pellitero P, Molnár K, Sitja-Bobadilla A, Cs S (2002) Comparative ultrastructure of the actinosporean stages of *Myxobolus bramae* and *Myxobolus pseudodispar* (Myxosporea). Parasitol Res 88:198–207

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

Baska F (1986) Histological studies on the development of *Myxobolus pseudodispar* Gorbunova, 1936 in the roach (*Rutilus rutilus*). Acta Vet Hung 35:251–257

Baska F, Voronin VN, Eszterbauer E, Müller L, Sz M, Molnár K (2009) Occurrence of two myxosporean species, *Myxobolus hakyi* sp. n. and *Hoferellus pulvinatus* sp. n., in *Pangasianodon hypophthalmus* fry imported from Thailand to Europe as ornamental fish. Parasitol Res 105:1391–1398

Cone D, Marcogliese DJ, Russel R (2004) The myxozoan fauna of the spottail shiner in the Great Lakes basin: membership, richness, and geographical distribution. J Parasitol 90:921–932

Corpet F (1988) Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res 16:10881–10890

Donec ZS, Shulman SS (1984) Knidosporidii (Cnidosporidia). In: Bauer ON (ed) Key to the determination of parasites of freshwater fishes of the USSR Vol. 1. Nauka, Leningrad, pp 88–251, in Russian

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

Eiras JC, Monteiro CM, Brasil-Sato MC (2010) *Myxobolus franciscoi* (Myxozoa: Myxosporea: Myxobolidae), a parasite of *Prochilodus argenteus* (Actinopterygii: Prochilodontidae) from the Upper Sao Francisco River, Brazil, with a revision of *Myxobolus* spp. from South America. Zoologia 27:131–137

Eszterbauer E (2002) Molecular biology can differentiate morphologically indistinguishable myxosporean species: *Myxobolus elegans* and *M. hungaricus*. Acta Vet Hung 50:59–62

Eszterbauer E (2004) Genetic relationship among gill-infecting *Myxobolus* species (Myxosporea) of cyprinids: molecular evidence of importance of tissue-specificity. Dis Aquat Org 58:35–40

Eszterbauer E, Székely C (2004) Molecular phylogeny of the kidney-parasitic *Sphaerospora renicola* from common carp (*Cyprinus carpio*) and *Sphaerospora* sp. from goldfish (*Carassius auratus auratus*). Acta Vet Hung 52:469–478

Eszterbauer E, Marton S, Rácz OZ, Letenyei M, Molnár K (2006) Morphological and genetic differences among actinosporean stages of fish-parasitic myxosporeans (Myxozoa): difficulties of species identification. Syst Parasitol 65:97–114

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

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

Hallett SL, Atkinson SD, Erséus C, El-Matbouli M (2005) Dissemination of triactinomyxons (Myxozoa) via oligochaetes used as live food for aquarium fishes. Dis Aquat Org 65:137–152

Hallett SL, Atkinson SD, Erséus C, El-Matbouli M (2006) Myxozoan parasites disseminated via oligochaete worms as live food for aquarium fishes: descriptions of aurantiactinomyxon and raabeia actinospore types. Dis Aquat Org 69:213–225

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

Kaur H, Singh R (2010) One new myxosporidian species, *Myxobolus slendrii* sp. nov., and one known species, *M. punjabensis* Gupta and Khera, 1989, infecting freshwater fishes in wetlands of Punjab, India. Parasitol Res 106:1043–1047

Landsberg JJ, Lom J (1991) Taxonomy of the genera of the *Myxobolus/Myxosoma* group (Myxobolidae: Myxosporea), current listing of species and revision of synonyms. Syst Parasitol 18:165–186

Lom J, Dyková I (1992) Protozoan parasites of fishes, volume 26 (Developments in aquaculture and fisheries science). Elsevier, Amsterdam, p 315

Longshaw M, Frear P, Feist SW (2003) *Myxobolus buckei* sp n. (Myxozoa), a new pathogenic parasite from the spinal column of three cyprinid fishes from the United Kingdom. Folia Parasitol 50:251–262

Molnár K (1994) Comments on the host, organ and tissue specificity of fish myxosporeans and on the types of their intrapiscine development. Parasitol Hung 27:5–20

Molnár K, Eszterbauer E, Székely C, Dán Á, Harrach B (2002) Morphological and molecular biological studies on intramuscular *Myxobolus* spp. of cyprinid fish. J Fish Dis 25:643–652

Molnár K, Marton S, Eszterbauer E, Székely C (2006) Comparative morphological and molecular studies on *Myxobolus* spp. infecting chub from the River Danube, Hungary, and description of *M. muellericus* sp. n. Dis Aquat Org 73:49–61

Molnár K, Marton S, Eszterbauer E, Székely C (2007) Description of *Myxobolus gayerae* n. sp. and redescription of *Myxobolus leuciscine* infecting the European chub from the Hungarian stretch of the river Danube. Dis Aquat Org 78:147–1543

Molnár K, Cech G, Székely C (2008) *Myxobolus* species infecting the cartilaginous rays of the gill filaments in cyprinid fishes. Acta Parasitol 53:330–338

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818

ácz OZ, Székely C, Molnár K (2004) Intraoligochaete development of *Myxobolus intimus* (Myxosporea: Myxobolidae), a gill myxosporean of the roach (*Rutilus rutilus*). Folia Parasitol 51:199–207

Reuss H (1906) Neue Myxosporidien von Süsswasserfischen. Bull Acad Imp Sci St-Petersbg Ser 5:199–205

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Shulman SS (1966) Myxosporidia of the fauna of the USSR. Nauka, Moscow, p 504, in Russian

Staden R (1996) The Staden sequence analysis package. Mol Biotechnol 5:233–241

Swofford DL (2001) PAUP*: phylogenetic analysis using parsimony (*and other methods).Version 4.0b8. Sinauer Associates, Sunderland

Székely C, Molnár K, Eszterbauer E, Baska F (1999) Experimental detection of the actinospores of *Myxobolus pseudodispar* (Myxosporea: Myxobolidae) in oligochaete alternate host. Dis Aquat Org 38:219–224

Székely C, Molnár K, Rácz O (2001) Complete developmental cycle of *Myxobolus pseudodispar* (Gorbunova) (Myxosporea: Myxobolidae). J Fish Dis 24:461–468

Whipps CM, Adlard RD, Bryant MS, Lester RJ, Findlay V, Kent ML (2003) First report of three *Kudoa* species from eastern Australia: *Kudoa thyrsites* from Mahi mahi (*Coryphaena hippurus*), *Kudoa amamiensis* and *Kudoa minithyrsites* n. sp. from sweeper (*Pempheris ypsilychnus*). J Eukaryot Microbiol 50:215–219

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

Q1. Please check the modification made in the sentence.
Q2. Please provide the appropriate unit in measuring the diameter of the plasmodia (“60 to 220”)..
Q3. Please check the modification made in the sentence.
Q4. Please check the changes made in this section.
Q5. Please check figure presentations.
Q6. Please check the modifications made in the paragraph.
Q7. Please check the modification made in the paragraph.
Q8. Please provide the unit of measure of the sutural extensions mentioned in this sentence.
Q9. "l" describing "lamellae" was changed to "la". Please check if appropriate. Also, please provide explanation of the letter "e" in the figure.
Q10. Figure 10 was inserted here for proper figure citation sequence. If not appropriate, please indicate where it should be cited.
Q11. Please check the modification made in the sentence.
Q12. Reference title was changed from "Key to the parasites of freshwater fishes of the USSR" to “Key to the determination of parasites of freshwater fishes of the USSR”. Please check if appropriate.
Q13. Please check the change made on the reference title, if appropriate.