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Two new *Myxobolus* spp. (Myxozoa: Myxobolidae) from white bream, *Blicca bjoerkna* (Linnaeus, 1758) developing in basifilamental location of gills

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Abstract. Two new *Myxobolus* species, *M. bjoerknae* sp. n. and *M. lamellobasis* sp. n. have been described from the gills of white bream, *Blicca bjoerkna*. Plasmodia of *M. bjoerknae* sp. n. developed in the connective tissue inside the cartilaginous gill arch, while plasmodia of *M. lamellobasis* sp. n. seem to start their development in the multilayered epithelium between two lamellae close to the base of gill filaments. Then they may bulge out of the interlamellar space fused to a large bulk locating at the base of filaments. The large, ellipsoidal spores of *M. bjoerknae* sp. n. 17.4 × 13.1 μm in size, resembled the spores of other species developing in the gill arch (e.g. *M. fundamentalis*, *M. gayerae*, and *M. pfeifferi*), but differed from them in its 18S rDNA sequence. Roundish spores of *M. lamellobasis* sp. n. with a size of 11.1 × 8.6 μm resembled the spores of *M. impressus* developing interlamellarly and the spores of *M. rotundus*, *M. parviformis*, and *M. muellericus* having intralamellar localization. However, the detected genetic difference clearly distinguished it from the other species developing in similar tissue location. The phylogenetic location of the two newly described species seems to correlate both with spore shape and fish host species.

Key words: Myxozoa, site selection, tissue tropism, occurrence, histology, 18S rDNA, phylogeny.

INTRODUCTION

Myxobolus spp. of cyprinid fishes show high specific tissue tropism and in most cases they select a specific site for their development in the fish body. First Lom and Arthur (1989) drew attention to the importance of host, organ and tissue specificity for the identification

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of myxosporean spp. Later several authors provided further details concerning this issue (e.g. Molnár 1994, Cone and Overstreet 1998, Dyková and Lom 2007, Ferguson *et al.* 2008). Most of the known species infect either the gill filaments or gill lamellae (Eiras *et al.* 2005, Kallert *et al.* 2005), but some species such as *M. basilamellaris* Lom and Molnár, 1983, develop at the basal part of gill filaments or inside the cartilaginous gill arch (Lom and Molnár 1983). Of the non-cyprinid fishes, Bahri *et al.* (1996, 2003) reported the basifilamental location of myxobolids. They found large plasmodia of *Myxobolus ichkeulensis* Bahri and Marques, 1996,

developing in the gill arch of the marine fish *Mugil cephalus* Linnaeus, 1758. A similar location close to the base of gill filaments was observed for *Henneguya basifilamentalis* Molnár *et al.*, 2006, by Molnár *et al.* (2006) in the Asian redtail catfish *Hemibagrus nemurus* (Valenciennes, 1840).

In the present paper two new *Myxobolus* species, *M. bjoerknae* sp. n. and *M. lamellobasis* sp. n. are described from the gills of white bream developing inside the cartilaginous gill arch and at the basal part of gill filaments. A detailed morphological description of the spores is completed with histology and phylogenetic analysis of the new species. Furthermore, *Myxobolus* spp. found in the same location in other cyprinid fish species are reported.

MATERIALS AND METHODS

The fish material was obtained from natural waters in Hungary between 2005 and 2008, as a part of a general survey on the parasite fauna of fishes from Hungarian natural waters. In the course of the project, the parasitic infections of economically important and some commonly occurring fish species have been studied. Most of the examined fish originated from Lake Balaton and from reaches of the Danube River north to Budapest, less frequently from the Tisza River. In most cases, a complete parasitological examination was performed, however special attention was made on the infections affecting gills.

During the survey, 79 specimens of white bream *Blicca bjoerkna* (Linnaeus, 1758) was studied for basifilamental *Myxobolus* infections and compared with similar infections found in other cyprinid fishes, such as in common carp *Cyprinus carpio carpio* Linnaeus, 1758, roach *Rutilus rutilus* (Linnaeus, 1758) and chub *Squalius cephalus* (Linnaeus, 1758). Small-sized white bream specimens (up to 14 cm in length) were seined by a small, closed-meshed net, while large-sized specimens were caught with gill nets. All fish were carried to the laboratory alive, and kept in aquaria. Fish were anaesthetized with clove oil in 100 ppm concentration, and killed and immobilized by a cervical cut before extermination. They were examined within 4 days of collection. In the course of the complete parasitological examination, the hemibranchia of the gills were examined under a dissecting microscope for the presence of *Myxobolus* plasmodia. *Myxobolus* spores from the isolated and ruptured plasmodia were first studied in a wet mount, and then some of the spores were placed in glycerine-jelly onto a slide under a coverslip and preserved as reference slide. Another part of the spores from the same plasmodia were collected in 1.5 ml Eppendorf tubes and stored at –20°C for later molecular examination. Spores were stained with Lugol's iodine as described by Lom and Dyková (1992) to detect the presence or absence of iodinophilic vacuole. The presence of mucus envelop was studied by using Nomarski differential interference contrast.

Tissue samples from infected organs containing developing and mature plasmodia were fixed in Bouin's solution, embedded in par-affin wax, cut to 4–5 μm sections, and stained with haematoxylin and eosin. The maturity of spores was checked by immersing them in a 0.4% solution of urea. Spores of a given plasmodium were regarded mature when at least 90% of the spores extruded polar filaments in the solution. Unfixed spores were studied with Nomarski differential interference contrast of an Olympus BH2 microscope. Fresh spores were photographed with an Olympus DP20 digital camera. Measurements of fresh spores were taken with a calibrated eyepiece micrometer according to the guidelines of Lom and Arthur (1989), or they were measured on the basis of digital images.

The thawed spore samples preserved for molecular examination were first centrifuged at $7000 \times g$ for 5 min. After the removal of supernatant, 470 μl lysis buffer (100 mM NaCl, 10 mM Tris, 10 mM EDTA, 0.2% SDS, and 0.4 mg/ml Proteinase K) was added to the pellet, and samples were incubated at 55°C for 3 h. Genomic DNA was then purified using the Miniprep Express Matrix (BIO101, Qbiogene) solution as per Eszterbauer (2004), and DNA was eluted in 50 μl ultrapure water. 18S rDNA of the parasite was amplified using a semi-nested PCR assay. In the first round, parasite DNA was amplified with the primer pair EriB1 (5'-ACCTGGTTGATCCTGC-CAG-3') and EriB10 (5'-CTTCCGCAG GTTCACCTACGG-3') (Barta *et al.* 1997). The total volume of PCR reactions was 25 μl, which contained approx. 10 ng DNA (0.5 μl), 1 × *Taq* PCR reaction buffer (MBI Fermentas), 1.5 mM MgCl$_2$, 0.2 mM dNTP mix (Sigma), 1 μM of each primer and 1.25 units of recombinant *Taq* DNA Polymerase (MBI Fermentas). Amplification conditions were: 95°C for 50 s, 52°C for 50 s and 72°C for 120 s for 35 cycles, with a terminal extension at 72°C for 5 min. In the second round of the semi-nested PCR, the primer combinations of EriB1 – Myx4r (5'-CT-GACAGATCACTCCACGAAC-3') (Hallett and Diamant 2001) and MB5 (5'-GGTGATGATTAACAGGAGCGGT-3') (Eszterbauer 2004) – EriB10 were used with the same conditions as above. PCR products of the second round amplification were purified with MEGAquick-spin PCR & Agarose Gel DNA Extraction System (Intron).

The purified PCR products were subjected to direct sequencing with ABI BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3100 Genetic Analyzer automated DNA sequencer (Applied Biosystems) using the amplification primers. 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 BIOEDIT software (Hall 1999).

18S rDNA 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 megaBLAST searches and morphological findings. Bayesian Inference analysis was performed using MrBayes (Ronquist and Huelsenbeck 2003) integrated in the software package TOPALi v2.5 (Milne *et al.* 2009). A general time reversible model with gamma-shaped rate variations across sites (Invgamma) (GTR+I+Γ) was found to be the best for the analysis, as determined by the modeltest algorithm of the software. Two independent runs were conducted with four chains for 1 million generations. Phylogenetic trees were sampled every 100 generations. The first 25% of the samples were discarded from the cold chain (burninfrac = 0.25), and a 50% majority-rule consensus tree was created, which was visualised by Treeview. *Ceratomyxa shasta* was chosen as outgroup.

RESULTS

In the course of the study, two *Myxobolus* spp. were found at basifilamental location of gills of white bream. One of the *Myxobolus* species to be described as *M. bjoerknae* sp. n. formed plasmodia in the cartilaginous gill arch, while plasmodia of the other species, *M. lamellobasis* sp. n. developed at the basal part of gill lamellae. In some cases, the two infections occurred simultaneously. *Myxobolus* spp. located at certain basifilamental position on gills inside the cartilaginous gill arch was found in four other cyprinid species as well. *M. basilamellaris* found in common carp is a well known parasite of this fish species (Lom and Molnár 1983), just like *M. fundamentalis* Molnár *et al*., 2010, that often occurs in roach (Molnár *et al.* 2010). *M. gayerae* Molnár *et al*., 2007 and *M. pfeifferi* Thélohan, 1893 were known up to this time as infecting the intestine wall of chub and the musculature of barbel, respectively (Molnár *et al.* 2007, 2012), however our findings suggest that their plasmodia may develop in gill arch as well.

Descriptions of the novel myxozoan species found in white bream are as follows. Measurements are given in μm.

Myxobolus bjoerknae sp. n. (Figs 1a, 2a, b, 3)

Type host: White bream *Blicca bjoerkna* (Linnaeus, 1758) (Cyprinidae).

Type locality: Lake Balaton, Hungary.

Site of tissue development: Connective tissue in the cartilaginous gill arch close to the base of gill filaments.

Type material: Syntype spores in glycerine-jelly were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-18453. The 18S rDNA sequence of *M. bjoerknae* sp. n. was deposited in the GenBank under the accession number KF314823.

Prevalence of infection: 3.8% (3 infected specimens).

Etymology: The species is named after its host.

Description of the species: Three specimens of 4 to 5 year-old white bream were found to be infected with this *Myxobolus* species. The ellipsoidal or amorphous plasmodia, 240 to 300 in length and 80 to 130 in width, filled by large spores were located inside the cartilaginous gill arch close to the base of gill filaments. Spores in frontal view (Figs 1a and 2a) were symmetrically ellipsoidal. In sutural view, spores showed a citric shape with a small tapered extension at the distal end and a thickening at the anterior end (Figs 1a inset and 2b). Length of spores was 17.4 ± 0.86 (16.2–18.4) (N = 50), width 13.1 ± 0.63 (11.9–13.9) (N = 50), thickness 10.5 ± 0.55 (10–11) (N = 11). Polar capsules were drop-like, equal in size, slightly converging anteriorly, 6.7 ± 0.33 (6.3–7.4) in length (N = 50) and 4.1 ± 0.24 (3.6–4.5) in width (N = 50) (Table 1). Six filament coils arranged obliquely to the capsule length and wound up less densely in the polar capsule. A stout, triangular intercapsular appendix measuring 2.1 ± 0.25 (1.8–2.5) was found in the spores. The emerging suture evenly surrounding the spores (described frequently as thickness of shell valve) formed a 0.8 ± 0.15 (0.7–0.11) rim in frontal view. Sutural edge markings were not seen. The sporoplasm had a large, iodinophilous vacuole. Around spores a distinct, usually round mucous envelope was found.

Molecular data: The obtained two 18S rDNA consensus sequences of *M. bjoerknae* sp. n. isolates collected from the gill arch of two white bream specimen, differed in 1 nucleotide at the position nr 660 of the 1957 bp-long DNA sequence (the G/A alteration was labelled with "R" in the deposited DNA sequence, accession nr KF314823). The closest genetic similarity was found to *M. gayerae* by having 98.5% similarity in their 18S rDNA sequences. The DNA sequence of *M. fundamentalis* locating also at the base of gill filaments, was 93.3% similar to the examined species. *M. bjoerknae* sp. n. differed significantly from *M. pfeifferi* the parasite of barbel, a fish belonging to another subfamily of cyprinids, as their genetic similarity was 89.1% only. On the other hand, *M. bjoerknae* sp. n. genetically also resembled *M. cycloides* Gurley, 1893 (96.9% similarity) and *M. wootteni* Molnár *et al*., 2010 (91.1%), which possess very similar spore morphology, but develop in the connective tissue of other organs, in the wall of swim bladder of chub, and in the fins of common roach, respectively.

Histology: In the gills of the examined white bream specimen mostly ellipsoidal or amorphous plasmodia (Fig. 3) were found inside the gill arch or at the outer surface of the cartilaginous gill arch at the base of gill filaments. Plasmodia were surrounded by two or three dens layers of connective tissue. Under the layers, a thin collagenous capsule was formed around plasmodia.

Remarks: The 'intra-archal' (i.e inside the cartilaginous gill arch) location of plasmodia resembled that of *M. basilamellaris*, the parasite of common carp, but the spores of *M. bjoerknae* sp. n. are much larger
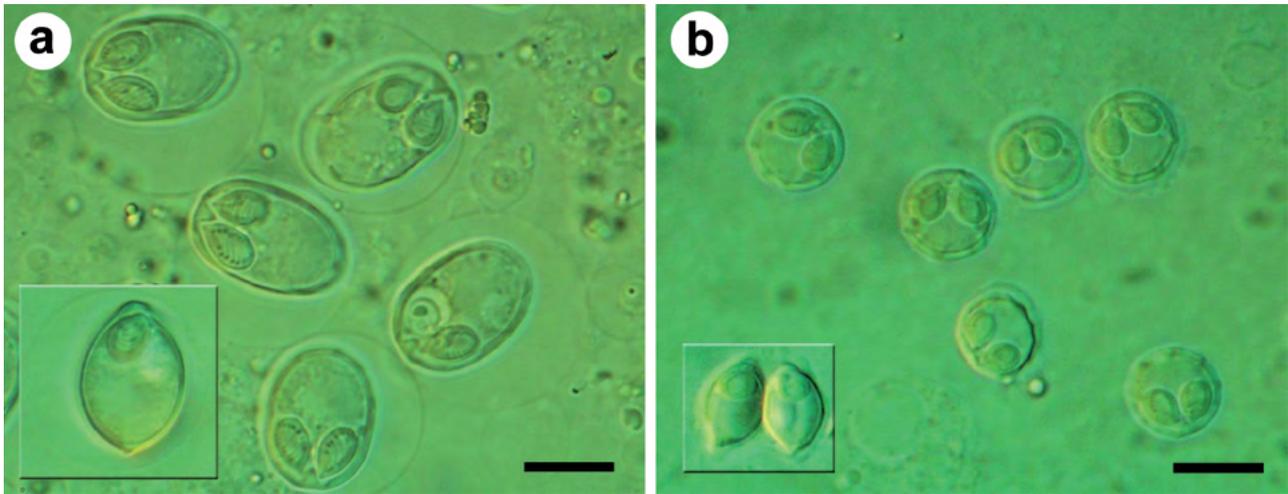
Fig. 1. a – spores of *Myxobolus bjoerknae* sp. n. infecting the cartilaginous gill arch in frontal view; inset: spores in sutural view; **b** – spores of *M. lamellobasis* sp. n. from the base of the gill filaments in frontal view; inset: spores in sutural view. Scale bars: 10 μm.
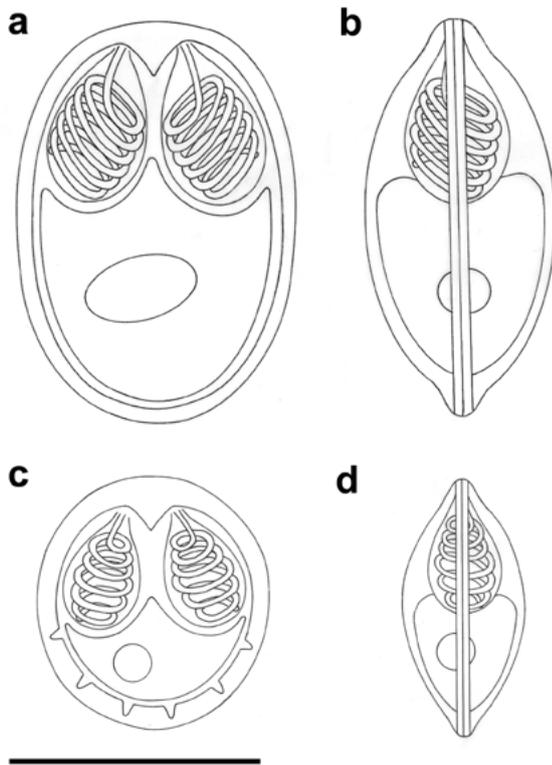

Fig. 2. Schematic illustration of *Myxobolus* spp. myxospores. **a** – *M. bjoerknae* sp. n. in frontal view, **b** – in sutural view; **c** – *M. lamellobasis* sp. n. in frontal view, **d** – in sutural view. Scale bar: 10 μm.

in size than those of *M. basilamellaris*. The spores of *M. pfeifferi, M. fundamentalis* and *M. gayerae* developing in "intra-archal" plasmodia (in barbel, roach and chub, respectively) resembled the spores of *M. bjoerknae* sp. n. due to their shape and large size, however, the 18S rDNA sequence of *M bjoerknae* sp. n. significantly differs from that of the other species.

Myxobolus lamellobasis sp. n. (Figs 1b, 2c, d, 4)

Type host: White bream *Blicca bjoerkna* (Linnaeus, 1758) (Cyprinidae).

Type locality: Lake Balaton, Hungary.

Site of tissue development: Multilayered epithelium of the gill lamellae close to the base of filaments.

Type material: Syntype spores in glycerine-jelly were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-18454. The 1947 bp-long, consensus 18S rDNA sequence of *M. lamellobasis* sp. n. was deposited in the GenBank under the accession number KF314824.

Prevalence of infection: 6.3% (5 infected specimens).

Etymology: The species is named after its specific location close to the base of gill filaments.

Description of the species: Five specimens of 4 to 5 year-old white bream were infected with this *Myxobolus* species. Plasmodia filled with round-shaped spores were located interlamellarly at the basal part of

Table 1. Comparison of spore measurements of *Myxobolus* spp. forming plasmodia in the cartilaginous gill arch and at the base of gill filaments of European cyprinid fishes. Mean ± SD and range in parentheses are in µm. PC – polar capsule; n.d. – no data.

	M. lamellobasis **sp**. n. present study	*M. bjoerknae* sp. n. present study	*M. fundamentalis* Molnár *et al.* 2010	*M. gayerae* Molnár *et al.* 2007	*M. pfeifferi* Molnár *et al.* 2012	*M. basilamellaris* Lom and Molnár 1983
Host	*Blicca bjoerkna*	*Blicca bjoerkna*	*Rutilus rutilus*	*Squalius cephalus*	*Barbus bocagei*	*Cyprinus carpio carpio*
Location in fish	basal part of filaments	gill arch	gill arch	gill arch	gill arch and muscles	gill arch and base of filaments
Spore length	10.1 ± 0.5 (9.1–10.8)	17.4 ± 0.86 (16.2–18.4)	15.5 ± 0.81 (14.4–17)	16.6 ± 0.57 (15.6–17.4)	15 ± 0.51 (14.0–15.7)	7.7–12.2
Spore width	9.7 ± 0.58 (8.6–10.5)	13.1 ± 0.63 (11.9–13.9)	11.8 ± 0.58 (11.2–13.2)	13 ± 0.19 (12.9–13.5)	11.2 ± 0.37 (10.5–11.9)	7.3–9.9
Spore thickness	5.5 ± 0.38 (5.2–6.1)	10.5 ± 0.55 (10.0–11.0)	9.2 ± 0.3 (9.0–9.6)	9.4 ± 0.53 (9.0–10.2)	6.8 ± 0.4 (6.2–7.4)	4.5 (4.2–5.0)
PC length	4.7 ± 0.26 (4.4–5.0)	6.7 ± 0.33 (6.3–7.4)	6.7 ± 0.24 (6.5–7.2)	7.5 ± 0.25 (7.2–7.8)	7 ± 0.85 (6.0–9.5)	3.2–5.4
PC width	3.3 ± 0.54 (2.7–4.8)	4.1 ± 0.24 (3.6–4.5)	4 ± 0.26 (3.7–4.3)	4.3 ± 0.22 (3.9–4.5)	3.6 ± 0.23 (3.2–3.9)	2.2–3.3
Intercaps. appendix	1.2 ± 0.2 (1.0–1.6)	2.1 ± 0.25 (1.8–2.5)	1,7 ± 0.1 (1.6–1.8)	2.8 ± 0.24 (2.4–3.0)	1.3 ± 0.28 (1.0–1.7)	n.d.
No. coils in PC	6	6	6	6	6	5–6
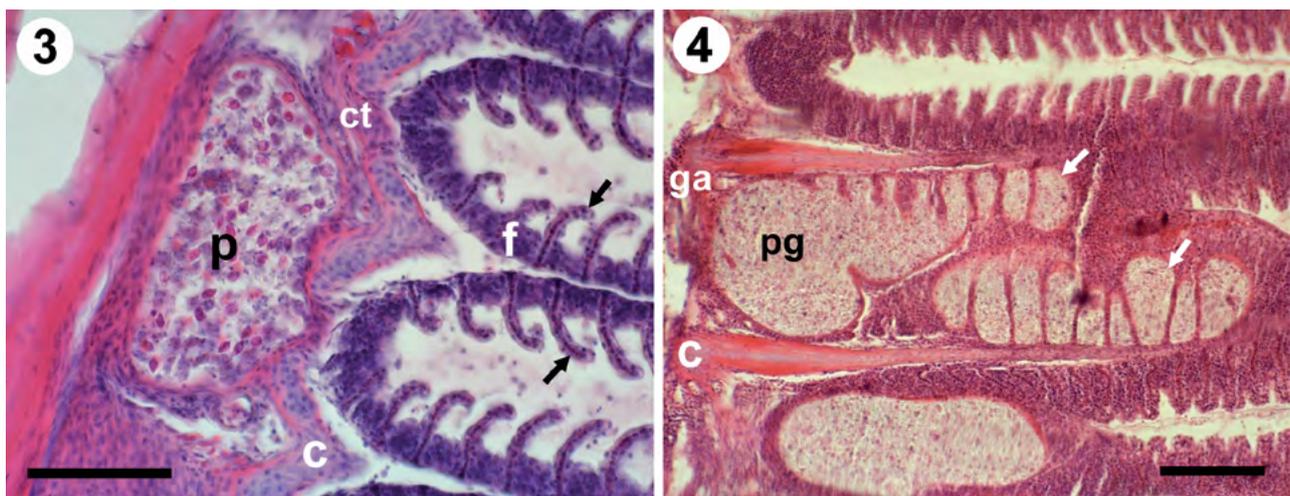
Fig. 3. Histological section of the cartilaginous gill arch of white bream. **p** – plasmodium of *Myxobolus bjoerknae* sp. n., **c** – cartilaginous base of the filaments, **f** – gill filament, **ct** – connective tissue; arrows indicate gill lamellae. Haematoxylin and eosin staining (H & E). Scale bar: 100 µm.

Fig. 4. Histological section of the basal part of gill filaments in white bream. Young interlamellarly developing plasmodia of *Myxobolus lamellobasis* sp. n. (arrows) and a large plasmodium conglomeratum (pg) are located close to the cartilaginous base of filaments (c) and gill arch (ga). H & E. Scale bar: 100 µm.

gill filaments. A part of plasmodia was found as large amorphous bodies 600 to 900 in length and 100 to 140 in width between two gill filaments close to the gill arch, while for other cases, only the interlamellar epithelium of the filaments close to the gill arch were affected by roundish or elliptical plasmodia of 120 × 80

in average. The gill lamellae at the central and apical parts of the filament were not affected by the infection in any cases. For one case, plasmodia of *M. bjoerknae* sp. n. in the gill arch and plasmodia of *M. lamellobasis* sp. n. at basally locating lamellae occurred simultaneously close to each other. Spores in frontal view (Figs 1b and 2c) were round or roundish, their length was somewhat longer than their width, but less frequently spores wider than their length were also found. In sutural view, spores showed a citric shape (Figs 1b inset and 2d). Length of the spores was 10.1 ± 0.5 (9.1–10.8) (N = 50), width 9.7 ± 0.58 (8.6–10.5) (N = 50), thickness 5.5 ± 0.38 (5.2–6.1) (N = 11). Polar capsules were drop-like, equal in size, slightly converging anteriorly, 4.7 ± 0.26 (4.4–5.0) in length (N = 50) and 3.3 ± 0.54 (2.7–4.8) in width (N = 50) (Table 1). Six filament coils arranged perpendicular or slightly obliquely to the capsule length and wound up densely in the polar capsule. The spores showed a stout, triangular intercapsular appendix measuring 1.2 ± 0.2 (1.0–1.6). Sutural protrusion in frontal view formed a thick 1.0 ± 0.07 (0.9–1.1) circular rim around the spore, and in sutural view, it emerged 1.0 ± 0.13 (0.8–1.1) in size, both at the anterior and posterior end over the surface of the spore. Sutural edge markings (6 to 8) were clearly seen in the sutural rim. The sporoplasm had a small iodinophilous vacuole. Mucous envelope apparently absent.

Molecular data: The 18S rDNA sequences of three *M. lamellobasis* sp. n. isolates collected from the gill filaments of different white bream specimens were 100% identical. They show high genetic similarity to *M. rotundus* Nemeczek, 1911 (93.5%), to *M. impressus* Miroshnichenko, 1980 (93.4%), to *M. parviformis* Kallert *et al.*, 2005 (92.5%), and to *M. muellericus* Molnár *et al.*, 2006 (91.3%), which develop in the gill filaments of leuciscine fishes intralamellarly or interlamellarly, and form morphologically similar, rounded spores, which are relative small in size.

Histology: In the gills of the examined white bream specimen, the infection was observed close to the base of gill filaments. In this location, solitary plasmodia developed between two neighbouring lamellae (Fig. 4). Plasmodia overgrowing the lamellae and bulging into the interfilamental space fused and formed a large plasmodial conglomeration at the base of two neighbouring filaments (Fig. 4).

Remarks: The interlamellar-epithelial location of *M. lamellobasis* sp. n. plasmodia as well as the shape and size of spores most closely resemble *M. impressus* infecting the gills of common bream. However, intralamellar plasmodia of the latter species never merged into a large conglomeration, and the genetic similarity of the two species based on their 18S rDNA is 93.4% only. The round spores of *M. lamellobasis* sp. n. also resemble *M. rotundus* and *M. parviformis*, but 18S rDNA sequences of *M. lamellobasis* sp. n. differ from the above species (by having 93.5 and 92.5% similarity, respectively).

Phylogeny

Phylogenetic analysis revealed that *M. bjoerknae* sp. n. clustered with *M. gayerae*, *M. cycloides*, *M. fundamentalis* and *M. wootteni*, which all possess large, ellipsoidal spores. Compare to this, the small-sized, round-spored *M. lamellobasis* sp. n. formed a group with the morphologically similar *M. rotundus*, *M. parviformis* and *M. impressus*. *M. pavlovskii* (Akhmerov, 1954) developing plasmodia between gill lamellae and *M. pfeifferi* that may develop also in gill arch, located distantly from both novel species on the phylogenetic tree (Fig. 5).

DISCUSSION

Our findings indicate that more attention has to be taken to the cartilaginous gill arch and the basal parts of gill filaments, where plasmodia of different myxosporean species may occur. Infections at these sites may easily be overlooked.

The site selection of *Myxobolus* spp. infecting the cartilaginous gill arch or at the base of lamellae is apparently not absolute in all cases. For some species, like *M. bjoerknae* sp. n. and *M. fundamentalis*, the development in connective tissue inside the gill arch seems to be the only site, while for some other species, like *M. gayerae* and *M. pfeifferi* the plasmodial development might take place in other organs as well. The plasmodia of *M. gayerae* occur in the connective tissue of both the gill arch and the intestine (unpublished data), while plasmodia of *M. pfeifferi* occur in both the intermuscular connective tissue and in the cartilaginous gill arch (Molnár *et al.* 2012). On the other hand, plasmodia of *M. basilamellaris* can only be found in close contact with the cartilaginous gill arch, forming plasmodia inside the arch. However, these plasmodia often bulge out of the arch and hence may be located under the base of gill filaments (Kovács-Gayer and Molnár 1983).
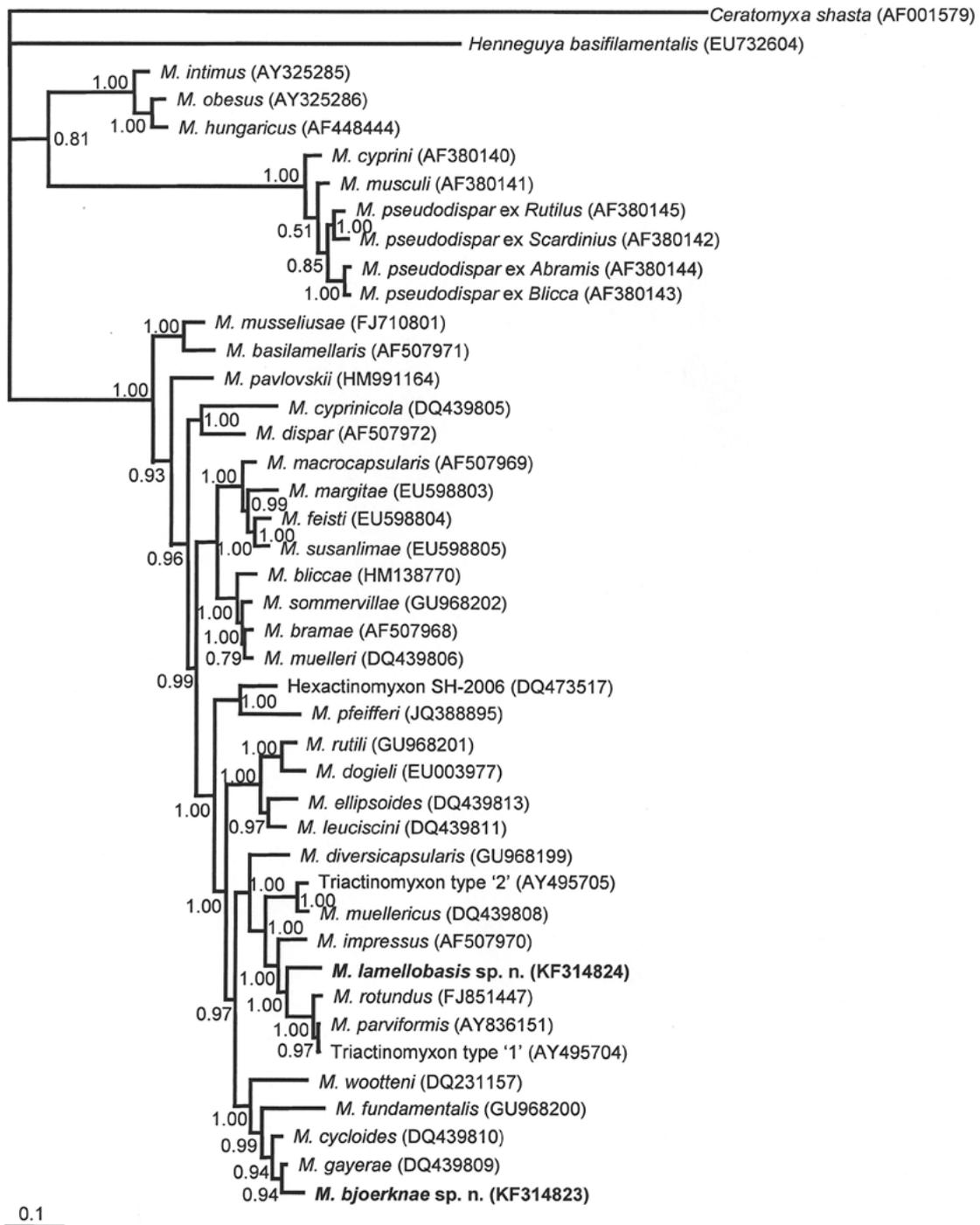
Ceratomyxa shasta (AF001579)

Henneguya basifilamentalis (EU732604)

1.00 ┌ M. intimus (AY325285)
     ├ M. obesus (AY325286)
0.81 1.00 └ M. hungaricus (AF448444)

1.00 ┌ M. cyprini (AF380140)
     ├ M. musculi (AF380141)
0.51 ┌ M. pseudodispar ex Rutilus (AF380145)
1.00 └ M. pseudodispar ex Scardinius (AF380142)
0.85 ┌ M. pseudodispar ex Abramis (AF380144)
1.00 └ M. pseudodispar ex Blicca (AF380143)

1.00 ┌ M. musseliusae (FJ710801)
     └ M. basilamellaris (AF507971)

1.00 ─ M. pavlovskii (HM991164)

1.00 ┌ M. cyprinicola (DQ439805)
     └ M. dispar (AF507972)

0.93

1.00 ┌ M. macrocapsularis (AF507969)
0.99 ┌ M. margitae (EU598803)
1.00 ├ M. feisti (EU598804)
1.00 └ M. susanlimae (EU598805)

0.96

1.00 ┌ M. bliccae (HM138770)
1.00 ├ M. sommervillae (GU968202)
0.79 ├ M. bramae (AF507968)
     └ M. muelleri (DQ439806)

0.99

1.00 ┌ Hexactinomyxon SH-2006 (DQ473517)
     └ M. pfeifferi (JQ388895)

1.00 ┌ M. rutili (GU968201)
1.00 └ M. dogieli (EU003977)

1.00 ┌ M. ellipsoides (DQ439813)
0.97 └ M. leuciscini (DQ439811)

1.00 ┌ M. diversicapsularis (GU968199)
1.00 ┌ Triactinomyxon type '2' (AY495705)
1.00 ├ M. muellericus (DQ439808)
     └ M. impressus (AF507970)

1.00 ┌ **M. lamellobasis sp. n. (KF314824)**
1.00 ├ M. rotundus (FJ851447)
0.97 1.00 ┌ M. parviformis (AY836151)
0.97 └ Triactinomyxon type '1' (AY495704)

1.00 ┌ M. wootteni (DQ231157)
     ├ M. fundamentalis (GU968200)
0.99 ├ M. cycloides (DQ439810)
0.94 ┌ M. gayerae (DQ439809)
0.94 └ **M. bjoerknae sp. n. (KF314823)**

0.1

Fig. 5. Phylogenetic tree generated by Bayesian inference (GTR + I + Γ model) based on a 1613 bp-long alignment of 18S rDNA sequences of 43 myxosporean taxa. New *Myxobolus* spp. sequences are in bold. *Ceratomyxa shasta* was chosen as outgroup. Posterior probabilities are shown at nodes. NCBI accession numbers are in brackets.

Except *M. basilamellaris*, most of the *Myxobolus* spp. developing in the gill arch show relatively uniform spore morphology, with relatively large-sized spores having symmetric ellipsoid shapes and possessing a well-developed intercapsular appendix. The 18S rDNA data and the phylogenetic analyses of these morphologically similar species showed that they are genetically close to each other, but differences in their DNA sequences indicate their validity as independent species. Of the species forming plasmodia in the cartilaginous gill arch, *M. bjoerknae* sp. n. showed close similarity to *M. gayerae* and *M. fundamentalis*, the parasites of cyprinid fishes of the Leuciscinae subfamily, but differed significantly from *M. pfeifferi*, a parasite of *Barbus* spp. members of subfamily Barbinae, thereby being genetically distant from leuciscine fishes. On the other hand, *M. bjoerknae* sp. n. is phylogenetically related to *M. cycloides* and *M. wootteni* as well, both having very similar spore morphology, but they develop plasmodia in the connective tissue of other organs.

Myxobolus lamellobasis sp. n. differs from the above species both in its morphology and location. This myxosporean seems to start its development in the interlamellar epithelium at the basal parts of filaments and in a late stage of plasmodial development, individual plasmodia may merge together. This development resulted in formation of large plasmodia at the basal part of gill filaments, presumably by a similar way as it happens for *Henneguya basifilamentalis* (Molnár *et al.* 2006). Of the well-known *Myxobolus* species, *M. impressus* and *M. pavlovskii* have a similar interlamellar development, but the plasmodia of the latter species are found in different parts of the gill filaments and form spores in separate small plasmodia without evident fusion (Molnár 1979, Molnár and Székely 1999, Marton and Eszterbauer 2011).

The findings of the phylogenetic analysis showed that *M. lamellobasis* sp. n. is in close relation to *M. rotundus*, *M. parviformis*, *M. impressus* and *M. muellericus*, which all develop in the gill lamellae of leuciscine fishes and form morphologically similar, relatively small-sized spores. It is remarkable that genetic differences between *M. lamellobasis* sp. n. and *M. pavlovskii* proved to be very high despite their similar location in gills. These differences might relate to the high genetic difference between the host fishes. Previous studies have already proved that tissue tropism represents a strongly determining factor in myxozoan phylogeny (Eszterbauer 2004, Holzer *et al.* 2004, Fiala 2006). The present phylogenetic results suggest that for *Myxobolus* spp., also host relatedness and spore morphology correlate with genetic relationship among species, thus they are relevant features in the taxonomy of genus *Myxobolus* similarly to tissue tropism.

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