Three new species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxobolidae) infecting the common nase *Chondrostoma nasus* (L.) in the River Danube

Gábor Cech · Réka Borzák · Kálmán Molnár · Csaba Székely

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Abstract

The common nase *Chondrostoma nasus* (L.) is a frequent cyprinid fish in the River Danube. In a survey on its infection with myxosporeans, eight different *Myxobolus* spp. spore types were found in the gills, swim bladder, fins and intestinal wall. Of these, spore types representing three species were studied in detail by morphological and molecular methods. Based on the differences in 18S rDNA sequences, two new species of *Myxobolus* Bütschli, 1882 from the gills and one from the swim bladder are described: *M. arrabonensis* n. sp., *M. szentendrensis* n. sp. and *M. paksensis* n. sp. The new species resembled *M. muelleri* Bütschli, 1882, *M. intimus* Zaika, 1965 and *M. cycloides* Gurley, 1893, all parasitic in leuciscine cyprinids, in spore size and location in the host, but exhibited differences in partial 18S rDNA sequences as follows: *M. arrabonensis* - *M. muelleri* (1.4%), *M. szentendrensis* - *M. intimus* (2.8%), *M. paksensis* - *M. cycloides* (2.4%). Based on the significant differences in rDNA sequences, the three forms are considered to represent new, hitherto undescribed species in spite of their morphological similarities to some *Myxobolus* spp. forming spores in identical locations in genetically closely related cyprinids of the subfamily Leuciscinae.

Introduction

The common nase *Chondrostoma nasus* (L.) is one of the most common cyprinid fishes in European rivers. Molecular studies have demonstrated that this fish is closely related to species of the genera *Leuciscus* Cuvier and *Squalius* Bonaparte (see Briolay et al., 1998; Zardoya & Doadrio, 1999). The myxosporean fauna of the common nase is poorly studied, and only a single species of *Myxobolus* Bütschli, 1882, *M. chondrostomi*Donec, 1962, has been described from *C. nasus* as its type-host (Eiras et al., 2005). However, spores of *Myxobolus* spp. of various shapes and sizes found in different organs of this fish were identified as known species, and Donec & Shulman (1984) reported *C. nasus* as a host of 15 *Myxobolus* spp. originally described from other cyprinid fishes: *M. albovae* Krasilnikova, 1966; *M. bliccae* Donec & Toziyakova, 1984; *M. bramae* Reuss, 1906; *M. carassii* Klockewa, 1914; *M. caudatus* Gogebashvili, 1966; *M. chondrostomi* Donec, 1962; *M. circulus* Akhmerov, 1960; *M. cyprini* Doflein, 1898; *M. dispar* Thélohan, 1895; *M. donecae* Kashkovsky 1969; *M. ellipsoides* Thélohan, 1892; *M. exigus* Thélohan, 1895; *M. lobatus* Dogiel & Bychowsky, 1934; *M. macrocapsularis* Reuss, 1906; and *M. musculi* Keysselitz, 1908.

Due to the relatively strict host-, tissue- and organ-specificity of myxosporeans (Molnár, 1994), the majority of spores collected from the common nase but classified to the above *Myxobolus* spp. might represent undescribed species. In contrast to that of the
common nase, the *Myxobolus* fauna of other leuciscine fishes is well studied, and several species have been described and/or recorded (Donec & Shulman, 1984; Lom & Dyková, 1992; Eiras et al., 2005; Molnár et al., 2006, 2010, 2012; Cech et al., 2012).

This paper presents data on the spore morphology and phylogenetic relationships of three new species of *Myxobolus* from *C. nasus* in the River Danube in Hungary. Furthermore, the typical infection sites and the histology of plasmodia are also described.

**Materials and methods**

**Morphological methods**

The myxozoan fauna of the common nase *Chondrostoma nasus* (L.) was studied in fish samples caught by fishermen between 1997 and 2014 at six different sampling sites of the River Danube in Hungary: Győr (47°46′06.2″N, 17°41′34.8″E), Surány (47°42′24.7″N, 19°07′19.2″E), Szöldilit (47°43′52.9″N, 19°07′59.7″E), Szentendre (47°39′51.1″N, 19°04′51.9″E) located north of Budapest, as well as Ercsi (47°14′49.1″N, 18°54′36.2″E) and Paks (46°37′11.8″N, 18°51′42.0″E) located south of Budapest. Altogether 27 specimens of two- to four-year-old fish (total length, TL = 18–42 cm), were purchased (eight from Győr, seven from Szentendre, four each from Szöldilit and Ercsi, and two each from Surány and Paks). Additionally, fingerlings of *C. nasus* were seined or collected by electrofishing. Fish were carried to the laboratory alive, in oxygenated plastic bags, kept in aerated aquaria and subjected to complete parasitological dissection within three days. When mature plasmodia were found, some of the spores were studied as fresh preparations, some were stored in 70% ethanol until further molecular analysis, and the remaining were mounted in glycerine-gelatine 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 thick sections, and stained with haematoxylin and eosin. The vitality of the spores was checked by adding spores into a 0.4% urea solution; spores of a given plasmodium were regarded as mature when at least 90% of the spores extruded polar filaments in this solution. Unfixed spores were studied with an Olympus BH2 microscope equipped with Nomarski differential interference contrast optics. The spores were photographed with an Olympus DP 20 digital camera. All measurements are expressed in micrometres and given as the range followed by the mean ± standard deviation and the number of measurements (n) in parentheses. Descriptions follow the guidelines of Lom & Arthur (1989).

**Molecular data**

DNA was extracted from the spores of single plasmodia (all isolated from different fish specimens) preserved in ethanol using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany). The samples were centrifuged at 10,000 rpm for 10 min and the supernatant was removed. Spore pellets were treated according to the manufacturer’s instructions, and 100 μl DNA was extracted at the final elution step. The 18S rDNA gene was amplified using nested polymerase chain reaction (PCR). The universal eukaryotic primers ERIB1 and ERIB10 (Barta et al., 1997) were used in the first round PCR. The reaction mixture consisted of 14.4 μl nuclease-free water, 2.5 μl of 10× DreamTaq buffer (Thermo Scientific, Vilnius, Lithuania), 0.1 μl of DreamTaq polymerase (1 U; Thermo Scientific), 0.2 mM dNTPs (Thermo Scientific), 0.325 μM of each primer and 2 μl of the extracted DNA in a final volume of 25 μl. The following profile was used for amplification: an initial denaturation step at 95°C for 3 min, followed by 40 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 2 min, and completed with terminal extension step at 72°C for 7 min.

This was followed by a second round PCR using the myxozoan-specific primers Myx1F and SphR (Hallett & Diamant, 2001; Eszterbauer & Székely, 2004). The reaction mixture contained 31.8 μl nuclease-free water, 5 μl of 10× DreamTaq buffer (Thermo Scientific), 0.2 μl of DreamTaq polymerase (2 U; Thermo Scientific), 0.2 mM dNTPs (Thermo Scientific), 0.325 μM of each primer and 1 μl from the first round PCR product in a final volume of 50 μl. The amplification conditions were: 95°C for 3 min, followed by 35 cycles at 95°C for 50 s, 50°C for 50 s, 72°C for 1 min 40 s, and terminated with an extension step at 72°C for 7 min.

The results of the second round PCR were analysed by electrophoresis in 1% agarose gel. PCR products were excised from the gel, purified with the Gel/PCR DNA Fragments Extraction Kit (Geneaid, New Taipei City, Taiwan). The purified products were sequenced...
directly with the primers listed in Table 1 using the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) with an ABI PRISM® 3100 Genetic Analyser (Life Technologies).

Sequence fragments were assembled using MEGA V6.06 (Tamura et al., 2013) and ambiguous bases clarified using corresponding ABI chromatograms.

Nucleotide sequences and reference sequences from GenBank based on BLAST matches were aligned with the software CLUSTAL W (Thompson et al., 1994).

DNA pairwise distances were calculated with MEGA V6.06 software using the Maximum Composite Likelihood model. Phylogenetic analysis was performed via Maximum Likelihood (ML) and Bayesian Inference (BI); Ceratonova shasta (Noble, 1950) was chosen as the outgroup. The dataset was tested using the software CLUSTAL W (Thompson et al., 1994).

MegaTrees were estimated over 1,000,000 generations via 24 independent runs of four simultaneous MCMCMC chains with every 100th tree saved. The first 25% of the sampled trees were discarded as ‘burn in’.

Results

Fourteen out of the 27 specimens of two- to four-year-old C. nasus were infected with Myxobolus spp. Altogether eight spore types located in different organs and in different types of plasmodia were found in the fish studied, but only three spore types were sequenced and studied by 18S rDNA sequence analysis. No infections with Myxobolus spp. were found in 121 fingerlings of common nase collected at Szentendre. PCR amplification of the 18S rDNA produced amplicons ranging between 1,600 and 1,700 bp in size. The ten samples analysed belonged to three different species of Myxobolus and the alignment of the different samples and the reference sequences (overall 40 sequences) was 1,731 bp long, of which 692 positions were variable and 479 parsimony informative. ML and BI analyses of the sequences generated highly similar topologies, except for some branches with low support and the location of M. sitjae Cecc, Molnár & Székely, 2012 (see Figs. 1, 2), but the phylogenetic positions of the three new species were identical on both phylograms. All three spore types differed from similar spores from genetically closely related fish hosts and proved to be undescribed species specific to C. nasus. There are no available sequence data of Myxobolus chondrostomi for comparison. Some of the other species (M. bliccae, M. brazae, M. dispar and M. macrocapsularis) reported for this host by Donec & Shulman (1984) are presented on the phylogenetic tree, but their sequences were not identical with any of our samples found in the present study.

Myxobolus arrabonensis n. sp. possessed small short elliptical spores and formed large plasmidia in the arteries of the gill filaments. Myxobolus szentendresi n. sp. developed in small plasmodia inside the gill lamellae and had spores resembling those of M. intimus, a parasite of the roach. Myxobolus paksensis

Table 1 Primers used for PCR or sequencing

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<th>Primer</th>
<th>Sequence</th>
<th>Application</th>
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<td>ERIB1</td>
<td>5'-ACCTGGTTGATCTGCGCAG-3'</td>
<td>1st round PCR</td>
<td>Barta et al. (1997)</td>
</tr>
<tr>
<td>ERIB10</td>
<td>5'-CTTGCCAGTTCTACCTGCA-3'</td>
<td>1st round PCR</td>
<td>Barta et al. (1997)</td>
</tr>
<tr>
<td>Myx1F</td>
<td>5'-GTGAGCTCGGGACGCTCAG-3'</td>
<td>2nd round PCR</td>
<td>Hallett &amp; Diamant (2001)</td>
</tr>
<tr>
<td>SphR</td>
<td>5'-GTTAATGTTAGCCTGCGC-3'</td>
<td>2nd round PCR and sequencing</td>
<td>Eszterbauer &amp; Székely (2004)</td>
</tr>
<tr>
<td>ACT1fr</td>
<td>5'-TGGGTTATTTCGCCCTGCTGC-3'</td>
<td>sequencing</td>
<td>Hallett &amp; Diamant (2001)</td>
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<tr>
<td>MC5</td>
<td>5'-CCTGAGAAGCGGGCTACCACAT-3'</td>
<td>sequencing</td>
<td>Molnár et al. (2002)</td>
</tr>
<tr>
<td>MC3</td>
<td>5'-GATTAGCTGACAGATCTCCAC-3'</td>
<td>sequencing</td>
<td>Molnár et al. (2002)</td>
</tr>
<tr>
<td>MB5r</td>
<td>5'-ACCGGTCTTGAATCATCACCC-3'</td>
<td>sequencing</td>
<td>Eszterbauer (2004)</td>
</tr>
<tr>
<td>MB5f</td>
<td>5'-GATGATACAGCGGCGTGG-3'</td>
<td>sequencing</td>
<td>Eszterbauer (2004)</td>
</tr>
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</table>

Myxobolus arrabonensis n. sp. possessed small short elliptical spores and formed large plasmidia in the arteries of the gill filaments. Myxobolus szentendresensis n. sp. developed in small plasmodia inside the gill lamellae and had spores resembling those of M. intimus, a parasite of the roach. Myxobolus paksensis
**Myxobolus arrabonensis** n. sp.

*Type-host*: Common nase, *Chondrostoma nasus* (L.)

*Type-locality*: River Danube at Győr (47°46′06.2″N, 17°41′34.8″E) Hungary.

*Site of tissue development*: Gill filaments.

*Prevalence*: 15% (4/27 specimens; TL = 18–42 cm).

*Intensity*: 2 to 18 plasmodia per hemibranch.

*Type-material*: Photo-types and histological sections were deposited in the parasitological collection of the Hungarian University of Agriculture and Life Sciences, Budapest, Hungary.

*Other localities*: River Danube close to Szentendre, Sződliget and Surány, cities located north of Budapest, Hungary.

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**Fig. 1** Phylogenetic position of the new species of *Myxobolus* ex *Chondrostoma nasus* based on the 18S rDNA by Maximum Likelihood algorithm. *Ceratonova shasta* was used as the outgroup. Bootstrap values are given at the nodes. Bootstrap values under 70 were omitted. Scale-bar indicates the number of expected substitutions.
Elongated plasmodia of this species, reaching 1.0–1.5 mm in size, were located inside the afferent artery of the central and distal part of the gill filaments (Fig. 3).

Description (Figs. 3,4A, 5A, B)

Vegetative stages

Elongated plasmodia of this species, reaching 1.0–1.5 mm in size, were located inside the afferent artery of the central and distal part of the gill filaments (Fig. 3).
Spores

Spores small, ellipsoidal or short ellipsoidal in frontal view (Figs. 4A, 5A), lemon-shaped in sutural view (Figs. 4 inset, 5B). Length of spores 8.4–10 (8.7 ± 0.61) (n = 50), width 7.6–8 (7.8 ± 0.34) (n = 50), thickness 5.4–5.6 (5.5) (n = 12). Polar capsules 2, pyriform, subequal in size, 4.5–5.9 (4.8 ± 0.44) long (n = 50), 2.7–3.1 (2.9 ± 0.12) wide (n = 50), tapering toward discharging canals of polar filaments. Polar filaments coiled with 6 turns in polar capsule, situated perpendicularly to its longitudinal axis. Spore intercapsular appendix at anterior end relatively large, triangular, 1.3–1.9 (1.5) long (n = 10). Sutural line indistinct; sutural edge moderately protruding. Valves thin, symmetrical, smooth with indistinct 4–6 edge markings. Sutural extensions present, c.0.6 at anterior and 1 at posterior pole of spores. Sporoplasm nuclei indiscernible; small iodinophilous vacuole found in sporoplasm; mucous envelope not observed.

Molecular data

The 18S rDNA sequences of four isolates of Myxobolus arrabonensis n. sp. (KP025680– KP025683) collected from the gill filaments of four fish specimens showed 100% similarity. The highest similarity (98.5%) to other sequenced myxospores was that to M. sommervillae Molnár, Marton, Székely & Eszterbauer, 2010 (GU968202). The new species was also similar (98.4%) to M. bramae (AF507968) and M. muelleri Bütschli, 1882 (DQ439806), and to M. bliccae (HM138771) (98.1%).

Remarks

The new species seems to be a typical vascular species forming large plasmodia in the gill arteries. In morphology and size, the spores of Myxobolus arrabonensis n. sp. were very similar to those of M. muelleri, M. bramae and M. sommervillae but had a somewhat more roundish form. The 1.5–1.9% differences between the 18S rDNA sequences of M. arrabonensis n. sp. and those of M. bramae (AF507968), M. sommervillae (GU968202), M. muelleri (DQ439806), and M. bliccae (HM138771), indicate that M. arrabonensis n. sp. should be regarded as a new species.

Myxobolus szentendrensis n. sp.

Type-host: Common nase, Chondrostoma nasus (L.) (Cyprinidae).

Type locality: River Danube at Szentendre (47°39'51.1"N, 19°04'51.9"E), Hungary.

Other localities: River Danube, close to Surañy and Győr, Hungary.

Site of tissue development: Gill lamellae.

Prevalence: 18% (5/27 specimens; TL = 18–42 cm).

Type-material: Photo-types and histological sections were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest (Coll. No. HNHM–18911).

Representative sequences: GenBank accession numbers KP025684–KP025686 (18S rDNA).

Etymology: The name of the species is after the name of the type-locality.

Description (Figs. 4B, 5C, D, 6)

Vegetative stages

Small round or roundish plasmodia 60 to 130 in diameter, containing 3,500 to 5,000 spores, developed in the capillary network of the gill lamellae (Fig. 6).
Spores

Spores pyriform in frontal view (Figs. 4B, 5C) with well-defined extrusion at anterior end and lemon shaped in sutural view (Figs. 4B inset, 5D). Length of spores 8.8–9.6 (9.2 ± 0.34) (n = 50), width 7.6–8 (7.9 ± 0.74) (n = 50), thickness 6.4–7.1 (6.7) (n = 11). Polar capsules 2, pyriform, equal in size, slightly converging anteriorly, 4.8–5.6 (5.3 ± 0.29) long (n = 50), 2.8–3.2 (3.0 ± 0.17) wide (n = 50). Polar filament coils 6, arranged perpendicular or oblique to capsule longitudinal axis, coiled densely in polar capsule. No intercapsular appendix observed. Sutural protrusion with relatively thick circular rim around spore, emerging c.0.5 over spore surface; in sutural view rim of suture emerging from spore surface c.0.5 at both anterior and posterior poles. Sutural edge markings not seen. No iodinophilous vacuole in single binucleated sporoplasm.

Molecular data

The 18S rDNA sequences of three isolates of Myxobolus szentendrensis n. sp. were identical. The highest similarity (96.6%) was found to Myxobolus sp. Hungary EE-2003 (AY325283). The new sequences were also similar to M. intimus (96.3%; AY325285 and FJ716098), M. eirasianus Cech, Molnár & Székely, 2012 (96.2%; JF311900) and M. obesus Gurley, 1893 (96.4% AY325286).

Remarks

The new species seems to be a typical vascular species forming small plasmodia in the gill lamellae. The spores of Myxobolus szentendrensis n. sp. were very similar to those of M. intimus and M. eirasianus in morphology and size, but had a somewhat more roundish shape. The c.3% differences between the 18S rDNA sequences of M. szentendrensis n. sp. and those of M. intimus (FJ716098) and M. eirasianus (JF311900) indicate that the present material should be regarded as a new species.

Myxobolus paksensis n. sp.

Type-host: Common nase, Chondrostoma nasus (L.) (Cyprinidae).

Type-locality: River Danube at Paks (46°37′11.8″N, 18°51′42.0″E), Hungary.

Other localities: River Danube close to Győr and Szentendre, Hungary.

Site of tissue development: Swim bladder.

Prevalence: 11% (3/27 specimens; TL = 18–42 cm).

Intensity: 1 to 8 plasmodia per swim bladder.

Type-material: Photo-types were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest (Coll. No. HNHM–18912).

Representative sequences: GenBank accession numbers KP025687–KP025689 (18S rDNA).

Etymology: The name of the species is after the name of the type-locality.

Description (Figs. 4C, 5E, F, 7)

Vegetative stages

Large flat plasmodia 1 to 1.7 mm in size, located in the multilayered connective tissue of the swim bladder, covered by a thin epithelial layer (Fig. 7), containing large-sized ellipsoidal spores.
Spores

Spores relatively large, ellipsoidal in frontal view (Figs. 4C, 5E) and lemon shaped in sutural view (Figs. 4C inset, 5F). Spores 14.4–15.2 (14.8 ± 0.62) long (n = 50), 10.4–12 (11 ± 0.68) wide (n = 50), 8.4–9.2 (8.7) thick (n = 14). Polar capsules 2, pyriform, equal in size, slightly converging anteriorly, 6.8–7.6 (7.0 ± 0.39) long (n = 50), 4–4.6 (4.3 ± 0.21) wide (n = 50). Polar filament coils 6, arranged obliquely to capsule longitudinal axis. Intercapsular appendix relatively small, 1.6–2.3 (2) (n = 16), triangular, located anteriorly between capsules. Sutural protrusion with circular rim around spore emerging c.0.9–1.2 over spore surface (Figs. 4C, 5E). Rim 1 thick in sutural view, forming sutural protrusions 1.3 to 1.3 at anterior pole and 0.5–0.8 at posterior pole. Sutural edge markings rarely seen in fresh spores. Single binucleated sporoplasm with large, round iodinophilous vacuole present; mucous envelope not observed.

Molecular data

The three identical 18S rDNA sequences of isolates of Myxobolus paksensis n. sp. (KP025687– KP025689) collected from the swim bladder of three fish specimens showed the highest similarity (96.8%) to M. cycloides (DQ439810). The sequence for the new species was also similar to M. gayerae Molnár, Marton, Eszterbauer & Székely, 2007 (96.7%; DQ439809) and M. fundamentalis Molnár, Marton, Székely & Eszterbauer, 2010 (95.5%; GU968200).

Remarks

The new species develops typically in the multi-layered, dense connective tissue of the swim bladder wall. The location of plasmodia was the same as in M. cycloides, but the cysts of M. paksensis n. sp. differ from those of M. cycloides in the round shape and typically yellow colour of the plasmodia on the swim bladder (vs less regular shape and white colour, respectively, in M. cycloides). Similarly, the spores of the two species are similar in size and shape but the 18S rDNA sequences of M. paksensis n. sp. differed from those of M. cycloides by 3.2%. The sequences for M. paksensis n. sp. also resemble those for M. fundamentalis, but differed by 4.5%. Differences in the size and shape of plasmodia and spores indicate that M. paksensis should be regarded as a new species.

Discussion

Since Andree et al. (1999) first compared the 18S rDNA sequences of some Myxobolus spp., researchers have been provided with an excellent tool for differentiating new, host-specific species from known species infecting closely related fishes, which have in most cases morphologically similar spores (Eszterbauer, 2002). Using this method and comparing the sequence differences, there is no need anymore to perform tiresome and long-lasting cross-infection experiments for identifying new species. Studies on salmonid and cyprinid fishes showed the existence of relatively loose or strict host-specificity in species of Myxobolus. Some species, such as M. cerebralis Hofer, 1903, are able to infect salmonids belonging to different genera, e.g. Salmo (L.), Salvelinus Richardson and Oncorhynchus Suckley (see El-Matbouli et al., 1999; Hedrick et al., 2001; Ferguson et al., 2008). In a similar way, M. pseudodispar Gorbunova, 1936 might occur in cyprinid fishes from different subfamilies (Molnár et al., 2002). Other Myxobolus spp. show a relatively strict host range and infect only a single host or some closely related fish species (Marton & Eszterbauer, 2011; Cech et al., 2012). In a study on the host-specificity of some Myxobolus spp. in closely related cyprinids of the subfamilies Leuciscinae and Abraminae, Cech et al. (2012) found that morphologically similar spores of Myxobolus spp. infecting hosts of the leuciscine genera Rutilus...
Rafinesque, *Leuciscus* and *Aspius* (Agassiz) were identical with those of *M. intimus*, but similar spores from the abramine *Blicca bjoerkna* (L.) exhibited different sequences and proved to be a new species, *M. eirasianus*. *Chondrostoma nasus* is classified within 478 the subfamily Leuciscinae (see Briolay et al., 1998; Zardoya & Doadrio, 1999) but differs from other species clustered together with morphologically similar species infecting well-studied fish species of the subfamily Leuciscinae (see Briolay et al., 1998). The three new species, *M. intimus*, the chub *Rutilus rutilus* (L.), and the ide *Leuciscus idus* (L.), the chub *Squalius cephalus* (L.), and the roach *Rutilus rutilus* (L.) (Molnár et al., 2005), were classified within the subfamily Leuciscinae (see Briolay et al., 1998).

The two phylogenetic algorithms (ML and BI) yielded very similar topologies; differences are usually at the nodes with low support (bootstrap and posterior probabilities under 70). The three new species infecting well-studied fish species of the subfamily Leuciscinae (Figs. 1, 2), e.g. the ide *Leuciscus idus* (L.), the chub *Squalius cephalus* (L.), and the roach *Rutilus rutilus* (L.) (Molnár et al., 2010; Cech et al., 2012), but the remarkable differences found in the sequences prove that they are closely related but distinct new species.

The two phylogenetic algorithms (ML and BI) yielded very similar topologies; differences are usually at the nodes with low support (bootstrap and posterior probabilities under 70). The three new species, *M. sitjae*, which was associated with *M. szenetderensis* n. sp. by BI analysis, but into the same group (containing *M. intimus*, *M. alvarezae* Cech, Molnár & Székely, 2012, *M. djuardini* Thelohan, 1892, *M. obesus* and *M. eirasianus*), but with a more basal location by ML analysis.

The data obtained in this study resulted in the description of three new species of *Myxobolus*. Although the differences found in the 18S rDNA sequences clearly support the host-specificity of the new species described here, the fact that in genetically closely related fishes morphologically similar spores develop in the same locations, indicates their common phylogenetic origin.

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**Compliance with Ethical Standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable institutional, national and international guidelines for the care and use of animals were followed. Permit for scientific fishing in Hungary (EHVF/121-1/2014) is issued by the Ministry of Agriculture, Hungary.

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