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

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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.

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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* Klokacewa, 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. exiguus* 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

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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), Sződliget (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 Sződliget 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

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158 directly with the primers listed in Table 1 using the
159 BigDye Terminator v3.1 Cycle Sequencing Kit (Life
160 Technologies) with an ABI PRISM® 3100 Genetic
161 Analyser (Life Technologies).
162 Sequence fragments were assembled using MEGA
163 V6.06 (Tamura et al., 2013) and ambiguous bases
164 clarified using corresponding ABI chromatograms.
165 Nucleotide sequences and reference sequences from
166 GenBank based on BLAST matches were aligned with
167 the software CLUSTAL W (Thompson et al., 1994).
168 DNA pairwise distances were calculated with MEGA
169 V6.06 software using the Maximum Composite Like-
170 lihood model. Phylogenetic analysis was performed
171 *via* Maximum Likelihood (ML) and Bayesian Infer-
172 ence (BI); *Ceratonova shasta* (Noble, 1950) was
173 chosen as the outgroup. The dataset was tested using
174 MEGA V6.06 for the nucleotide substitution model of
175 best-fit and the model, shown by the Akaike Informa-
176 tion Criterion (AIC) as the best-fitting one, was chosen
177 (GTR + G + I model). Bootstrap values were gener-
178 ated based on 1,000 resampled datasets. BI was
179 computed using Topali 2.5 (Milne et al., 2004). The
180 likelihood parameters for BI were based on the
181 GTR + G + I model. Posterior probabilities (pp)
182 were estimated over 1,000,000 generations *via* two
183 independent runs of four simultaneous MCMCMC
184 chains with every 100th tree saved. The first 25% of
185 the sampled trees were discarded as 'burn in'.

186 Results

187 Fourteen out of the 27 specimens of two- to four-year-
188 old *C. nasus* were infected with *Myxobolus* spp.
189 Altogether eight spore types located in different
190 organs and in different types of plasmodia were found
191 in the fish studied, but only three spore types were
192 sequenced and studied by 18S rDNA sequence
193 analysis. No infections with *Myxobolus* spp. were
194 found in 121 fingerlings of common nase collected at
195 Szentendre. PCR amplification of the 18S rDNA
196 produced amplicons ranging between 1,600 and 1,700
197 bp in size. The ten samples analysed belonged to three
198 different species of *Myxobolus* and the alignment of
199 the different samples and the reference sequences
200 (overall 40 sequences) was 1,731 bp long, of which
201 692 positions were variable and 479 parsimony
202 informative. ML and BI analyses of the sequences
203 generated highly similar topologies, except for some
204 branches with low support and the location of *M. sitjae*
205 Cech, Molnár & Székely, 2012 (see Figs. 1, 2), but the
206 phylogenetic positions of the three new species were
207 identical on both phylograms. All three spore types
208 differed from similar spores from genetically closely
209 related fish hosts and proved to be undescribed species
210 specific to *C. nasus*. There are no available sequence
211 data of *Myxobolus chondrostomi* for comparison.
212 Some of the other species (*M. bliccae*, *M. bramae*,
213 *M. dispar* and *M. macrocapsularis*) reported for this
214 host by Donec & Shulman (1984) are presented on the
215 phylogenetic tree, but their sequences were not
216 identical with any of our samples found in the present
217 study.
218 *Myxobolus arrabonensis* n. sp. possessed small
219 short elliptical spores and formed large plasmodia in
220 the arteries of the gill filaments. *Myxobolus szenten-*
221 *drensis* n. sp. developed in small plasmodia inside the
222 gill lamellae and had spores resembling those of *M.*
223 *intimus*, a parasite of the roach. *Myxobolus paksensis*

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Table 1 Primers used for PCR or sequencing

Primer	Sequence	Application	Source
ERIB1	5′-ACCTGGTTGATCCTGCCAG-3′	1st round PCR	Barta et al. (1997)
ERIB10	5′-CTTCCGCAGGTTCACCTACGG-3′	1st round PCR	Barta et al. (1997)
Myx1F	5'-GTGAGACTGCGGACGGCTCAG-3'	2nd round PCR	Hallett & Diamant (2001)
SphR	5′-GTTACCATTGTAGCGCGCGT-3′	2nd round PCR and sequencing	Eszterbauer & Székely (2004)
ACT1fr	5′-TTGGGTAATTTGCGCGCCTGCTGCC-3′	sequencing	Hallett & Diamant (2001)
MC5	5′-CCTGAGAAACGGCTACCACATCCA-3′	sequencing	Molnár et al. (2002)
MC3	5′-GATTAGCCTGACAGATCACTCCACA-3'	sequencing	Molnár et al. (2002)
MB5r	5'-ACCGCTCCTGTTAATCATCACC-3′	sequencing	Eszterbauer (2004)
MB5f	5′-GATGATTAACAGGAGCGGTTGG-3′	sequencing	Eszterbauer (2004)

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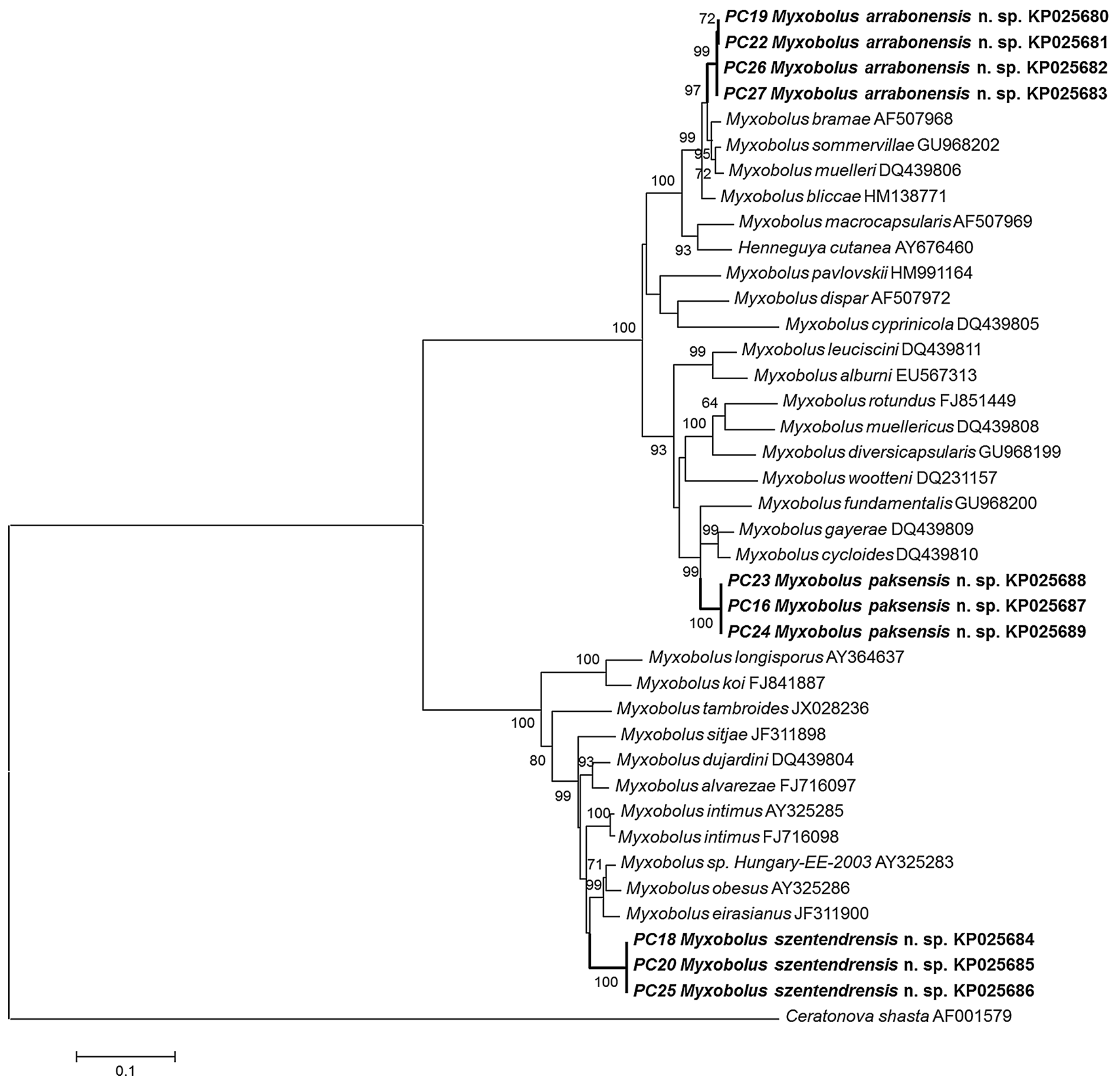

Fig. 1 Phylogenetic position of the new species of *Myxobolus* ex *Chondrostoma nasus* based on the 18S rDNA by Maximum Likelihood algorithm. *Ceratonova shasta* was used as the outgroup. Bootstrap values are given at the nodes. Bootstrap values under 70 were omitted. Scale-bar indicates the number of expected substitutions

n. sp. had large ellipsoidal spores formed in round plasmodia on the surface of the swim bladder.

Myxobolus arrabonensis n. sp.

Type-host: Common nase, *Chondrostoma nasus* (L.) (Cyprinidae).
Type-locality: River Danube at Győr (47°46′06.2″N, 17°41′34.8″E) Hungary.
Other localities: River Danube close to Szentendre, Sződliget and Surány, cities located north of Budapest, 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

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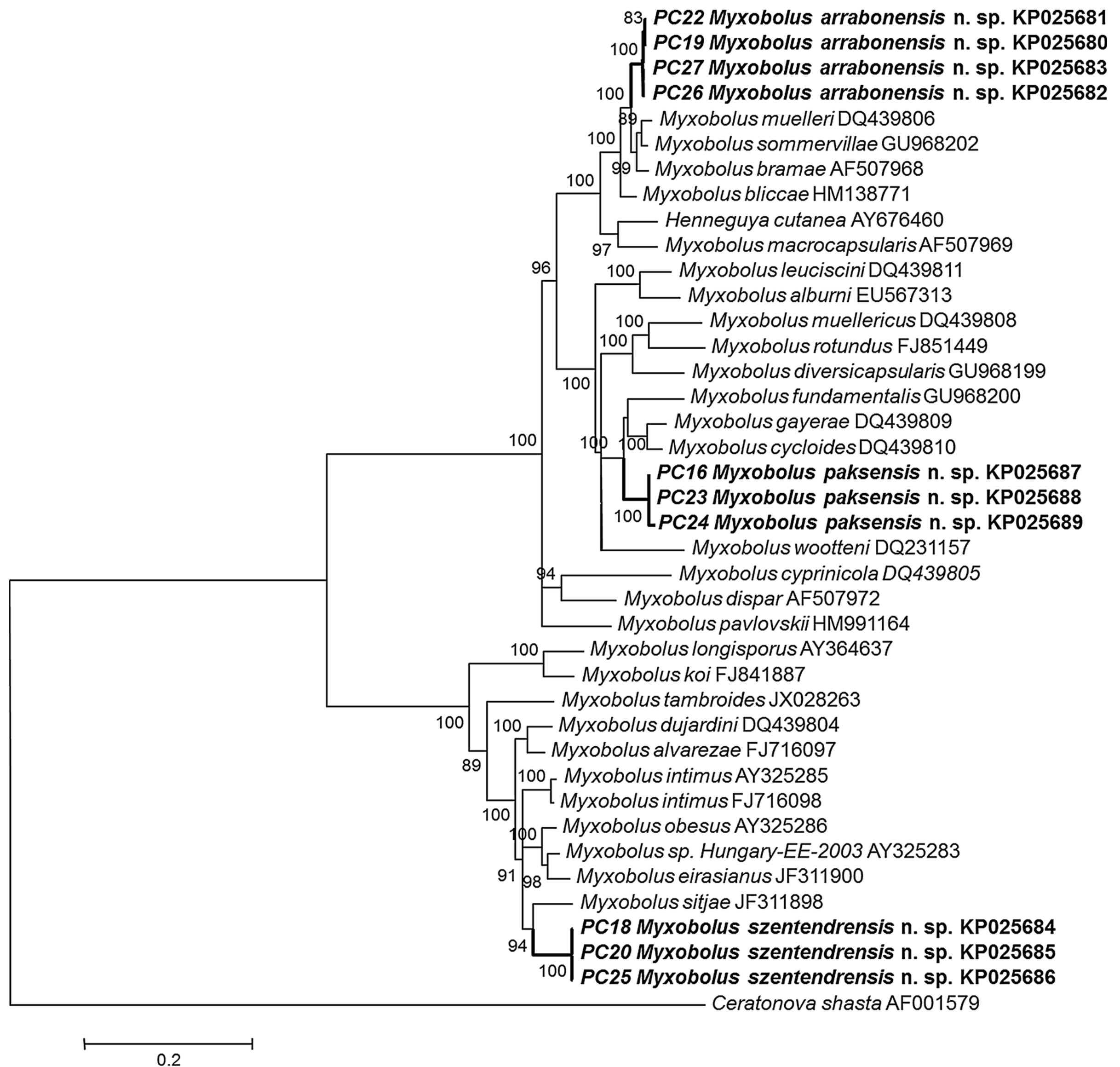

Fig. 2 Phylogenetic position of the new species of *Myxobolus* ex *Chondrostoma nasus* based on the 18S rDNA by Bayesian Inference algorithm. *Ceratonova shasta* was used as the outgroup. Posterior probabilities (only > 0.7 shown) are given at the nodes. Scale-bar indicates the number of expected substitutions per site

239 Zoological Department, Hungarian Natural History
240 Museum, Budapest (Coll. No. HNHM–18910).
241 *Representative sequences*: GenBank accession num-
242 bers KP025680–KP05683 (18S rDNA).
243 *Etymology:* The name of the species refers to the Latin
244 name (Arrabona) of the city of Győr, close to the type-
245 locality.

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

247 *Vegetative stages*

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

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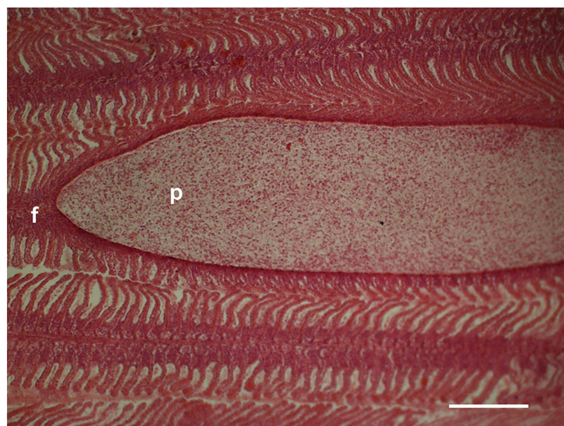

Fig. 3 A large plasmodium (p) of *Myxobolus arrabonensis* n. sp. in the aorta of the gill filament (f). Histological section (haematoxylin and eosin staining). *Scale-bar*: 100 µm

Fig. 4 Spores of the new species of *Myxobolus* ex *Chondrostoma nasus*. A, *M. arrabonensis* n. sp., frontal view (inset: sutural view); B, *M. szentendrensis* n. sp., frontal view (inset: sutural view); C, *M. paksensis* n. sp., frontal view (inset: sutural view). *Scale-bars*: 10 µm

251 *Spores*

252 Spores small, ellipsoidal or short ellipsoidal in frontal
253 view (Figs. 4A, 5A), lemon-shaped in sutural view
254 (Figs. 4A inset, 5B). Length of spores 8.4–10
255 (8.7 ± 0.61) (n = 50), width 7.6–8 (7.8 ± 0.34)
256 (n = 50), thickness 5.4–5.6 (5.5) (n = 12). Polar
257 capsules 2, pyriform, subequal in size, 4.5–5.9
258 (4.8 ± 0.44) long (n = 50), 2.7–3.1 (2.9 ± 0.12)
259 wide (n = 50), tapering toward discharging canals of
260 polar filaments. Polar filaments coiled with 6 turns in
261 polar capsule, situated perpendicularly to its longitu-
262 dinal axis. Spore intercapsular appendix at anterior
263 end relatively large, triangular, 1.3–1.9 (1.5) long
264 (n = 10). Sutural line indistinct; sutural edge moder-
265 ately protruding. Valves thin, symmetrical, smooth
266 with indistinct 4–6 edge markings. Sutural extensions
267 present, *c.*0.6 at anterior and 1 at posterior pole of
268 spores. Sporoplasm nuclei indiscernible; small iodi-
269 nophilous vacuole found in sporoplasm; mucous
270 envelope not observed.

271 Molecular data

272 The 18S rDNA sequences of four isolates of *Myxobo-*
273 *lus arrabonensis* n. sp. (KP025680– KP025683)
274 collected from the gill filaments of four fish specimens
275 showed 100% similarity. The highest similarity
276 (98.5%) to other sequenced myxosporeans was that
277 to *M. sommervillae* Molnár, Marton, Székely &
278 Eszterbauer, 2010 (GU968202). The new species
279 was also similar (98.4%) to *M. bramae* (AF507968)
285 and *M. muelleri* Bütschli, 1882 (DQ439806), and to *M.*
286 *bliccae* (HM138771) (98.1%).

287 Remarks

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

299 ***Myxobolus szentendrensis* n. sp.**

300 *Type-host:* Common nase, *Chondrostoma nasus* (L.)
301 (Cyprinidae).
302 *Type locality*: River Danube at Szentendre
303 (47°39′51.1″N, 19°04′51.9″E), Hungary.
304 *Other localities*: River Danube, close to Surány and
305 Győr, Hungary.
306 *Site of tissue development*: Gill lamellae.
307 *Prevalence*: 18% (5/27 specimens; TL = 18–42 cm).
308 *Type-material*: Photo-types and histological sections
309 were deposited in the parasitological collection of the
310 Zoological Department, Hungarian Natural History
311 Museum, Budapest (Coll. No. HNHM–18911.
312 *Representative sequences*: GenBank accession num-
313 bers KP025684–KP025686 (18S rDNA).
314 *Etymology:* The name of the species is after the name
315 of the type-locality.

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

317 *Vegetative stages*

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

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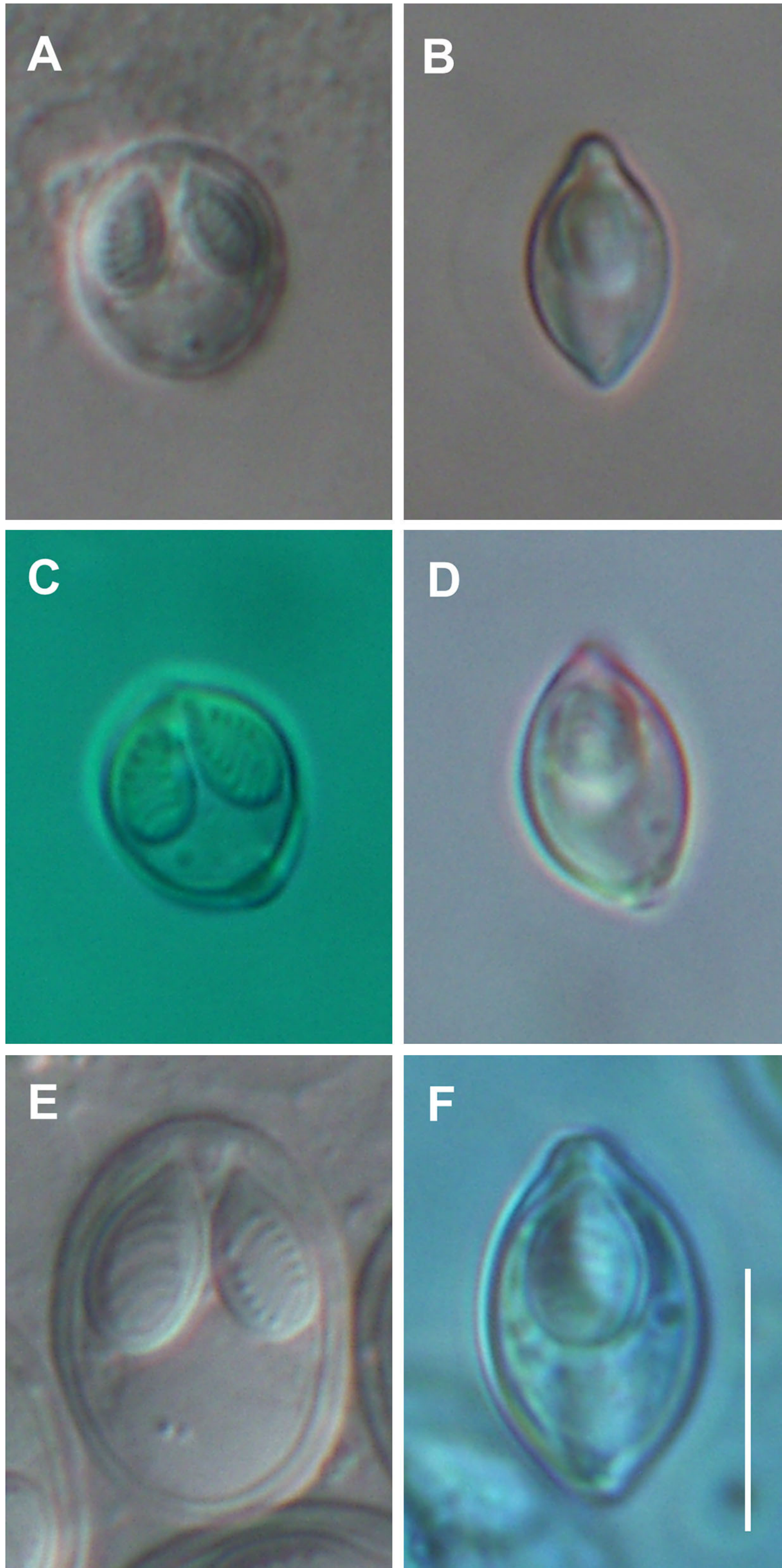

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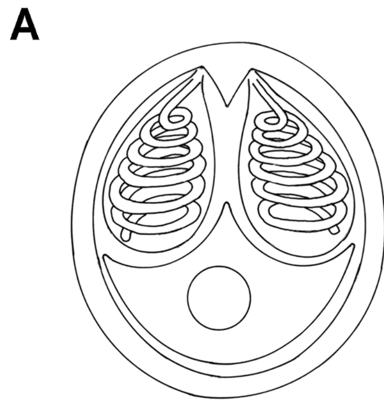

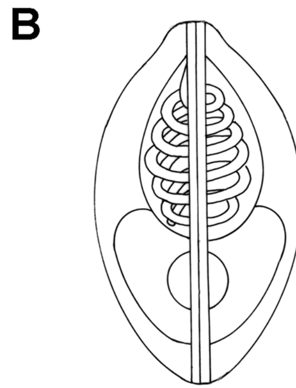

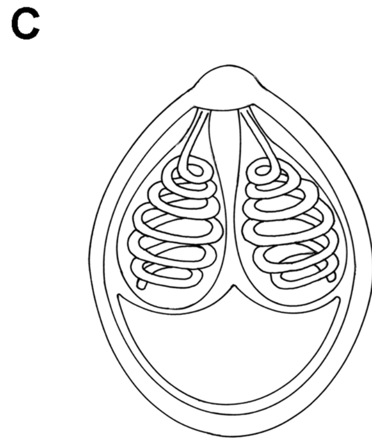

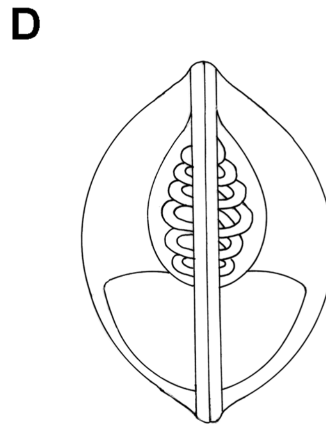

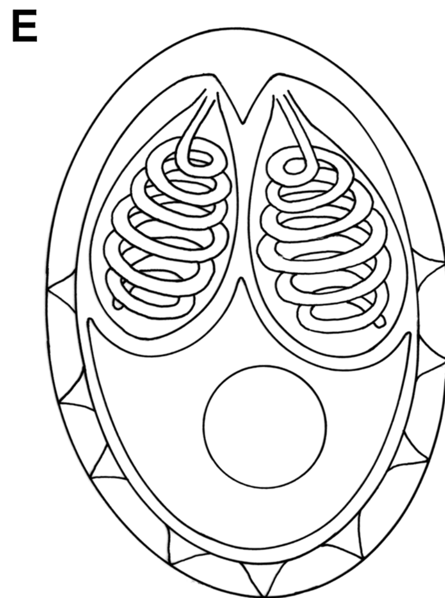

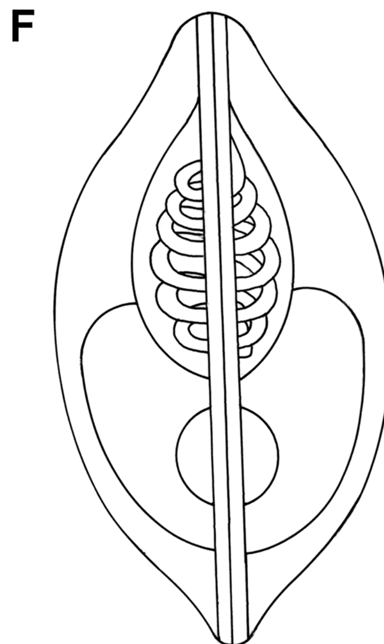

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◀ **Fig. 5** Schematic drawings of spores of the new species of *Myxobolus* ex *Chondrostoma nasus*. A, *M. arrabonensis* n. sp., frontal view; B, *M. arrabonensis* n. sp., sutural view; C, *M. szentendrensis*. n. sp., frontal view; D, *M. szentendrensis* n. sp., sutural view; E, *M. paksensis* n. sp., frontal view; F, *M. paksensis* n. sp., sutural view. *Scale-bar*: 10 µm

321 *Spores*

322 Spores pyriform in frontal view (Figs. 4B, 5C) with
323 well-defined extrusion at anterior end and lemon
324 shaped in sutural view (Figs. 4B inset, 5D). Length of
325 spores 8.8–9.6 (9.2 ± 0.34) (n = 50), width 7.6–8
326 (7.9 ± 0.74) (n = 50), thickness 6.4–7.1 (6.7)
327 (n = 11). Polar capsules 2, pyriform, equal in size,
328 slightly converging anteriorly, 4.8–5.6 (5.3 ± 0.29)
329 long (n = 50), 2.8–3.2 (3.0 ± 0.17) wide (n = 50).
330 Polar filament coils 6, arranged perpendicular or
331 oblique to capsule longitudinal axis, coiled densely in
332 polar capsule. No intercapsular appendix observed.
333 Sutural protrusion with relatively thick circular rim
334 around spore, emerging *c.*0.5 over spore surface; in
335 sutural view rim of suture emerging from spore
336 surface *c.*0.5 at both anterior and posterior poles.
337 Sutural edge markings not seen. No iodinophilous
338 vacuole in single binucleated sporoplasm.

339 Molecular data

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

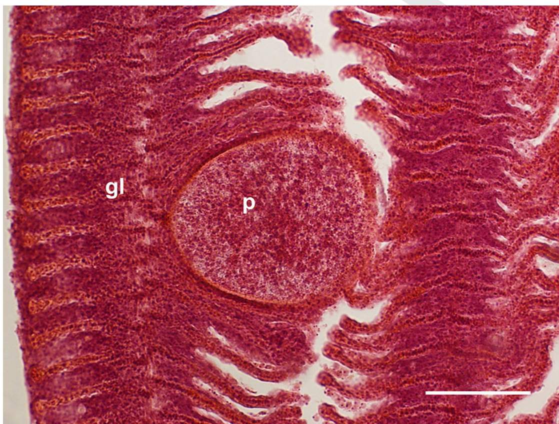

Fig. 6 Plasmodium (p) of *M. szentendrensis* n. sp. in a gill lamella (gl) of *Chondrostoma nasus*. Histological section (haematoxylin and eosin staining). *Scale-bar*: 60 µm

348 Remarks

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

359 ***Myxobolus paksensis* n. sp.**

360 *Type-host*: Common nase, *Chondrostoma nasus* (L.)
361 (Cyprinidae).
362 *Type-locality*: River Danube at Paks (46°37′11.8″N,
363 18°51′42.0″E), Hungary.
364 *Other localities*: River Danube close to Győr and
365 Szentendre, Hungary.
366 *Site of tissue development*: Swim bladder.
367 *Prevalence:* 11% (3/27 specimens; TL = 18–42 cm).
368 *Intensity:* 1 to 8 plasmodia per swim bladder.
369 *Type-material*: Photo-types were deposited in the
370 parasitological collection of the Zoological Depart-
371 ment, Hungarian Natural History Museum, Budapest
372 (Coll. No. HNHM–18912).
373 *Representative sequences*: GenBank accession num-
374 bers KP025687–KP025689 (18S rDNA).
375 *Etymology:* The name of the species is after the name
376 of the type-locality.

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

378 *Vegetative stages*

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

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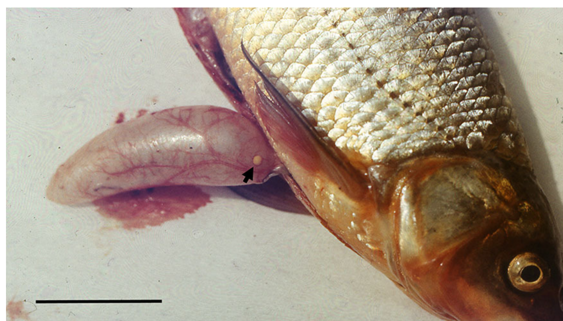

Fig. 7 Plasmodium (*arrowed*) of *M. paksensis* n. sp. in the swim bladder of *Chondrostoma nasus*. *Scale-bar*: 5 cm

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383 *Spores*

384 Spores relatively large, ellipsoidal in frontal view 385 (Figs. 4C, 5E) and lemon shaped in sutural view 386 (Figs. 4C inset, 5F). Spores 14.4–15.2 (14.8 ± 0.62) 387 long (n = 50), 10.4–12 (11 ± 0.68) wide (n = 50), 388 8.4–9.2 (8.7) thick (n = 14). Polar capsules 2, pyri- 389 form, equal in size, slightly converging anteriorly, 390 6.8–7.6 (7.0 ± 0.39) long (n = 50), 4–4.6 391 (4.3 ± 0.21) wide (n = 50). Polar filament coils 6, 392 arranged obliquely to capsule longitudinal axis. Inter- 393 capsular appendix relatively small, 1.6–2.3 (2) 394 (n = 16), triangular, located anteriorly between cap- 395 sules. Sutural protrusion with circular rim around 396 spore emerging *c.*0.9–1.2 over spore surface 397 (Figs. 4C, 5E). Rim 1 thick in sutural view, forming 398 sutural protrusions 1 to 1.3 at anterior pole and 0.5–0.8 399 at posterior pole. Sutural edge markings rarely seen in 400 fresh spores. Single binucleated sporoplasm with 401 large, round iodinophilous vacuole present; mucous 402 envelope not observed.

403 Molecular data

404 The three identical 18S rDNA sequences of isolates of 405 *Myxobolus paksensis* n. sp. (KP025687– KP025689) 406 collected from the swim bladder of three fish specimens 407 showed the highest similarity (96.8%) to *M. cycloides* 408 (DQ439810). The sequence for the new species was also 409 similar to *M. gayerae* Molnár, Marton, Eszterbauer & 410 Székely, 2007 (96.7%; DQ439809) and *M. fundamen-* 411 *talis* Molnár, Marton, Székely & Eszterbauer, 2010 412 (95.5%; GU968200).

Remarks 413

The new species develops typically in the multi- 414 layered, dense connective tissue of the swim bladder 415 wall. The location of plasmodia was the same as in *M.* 416 *cycloides*, but the cysts of *M. paksensis* n. sp. differ 417 from those of *M. cycloides* in the round shape and 418 typically yellow colour of the plasmodia on the swim 419 bladder (*vs* less regular shape and white colour, 420 respectively, in *M. cycloides*). Similarly, the spores 421 of the two species are similar in size and shape but the 422 18S rDNA sequences of *M. paksensis* n. sp. differed 423 from those of *M. cycloides* by 3.2%. The sequences for 424 *M. paksensis* n. sp. also resemble those for *M.* 425 *fundamentalis*, but differed by 4.5%. Differences in 426 the size and shape of plasmodia and spores indicate 427 that *M. paksensis* should be regarded as a new species. 428

Discussion 429

Since Andree et al. (1999) first compared the 18S 430 rDNA sequences of some *Myxobolus* spp., researchers 431 have been provided with an excellent tool for differ- 432 entiating new, host-specific species from known 433 species infecting closely related fishes, which have 434 in most cases morphologically similar spores (Eszter- 435 bauer, 2002). Using this method and comparing the 436 sequence differences, there is no need anymore to 437 perform tiresome and long-lasting cross-infection 438 experiments for identifying new species. Studies on 439 salmonid and cyprinid fishes showed the existence of 440 relatively loose or strict host-specificity in species of 441 *Myxobolus*. Some species, such as *M. cerebralis* 442 Hofer, 1903, are able to infect salmonids belonging 443 to different genera, e.g. *Salmo* (L.), *Salvelinus* 444 Richardson and *Oncorhynchus* Suckley (see El-Mat- 445 bouli et al., 1999; Hedrick et al., 2001; Ferguson et al., 446 2008). In a similar way, *M. pseudodispar* Gorbunova, 447 1936 might occur in cyprinid fishes from different 448 subfamilies (Molnár et al., 2002). Other *Myxobolus* 449 spp. show a relatively strict host range and infect only 450 a single host or some closely related fish species 451 (Marton & Eszterbauer, 2011; Cech et al., 2012). In a 452 study on the host-specificity of some *Myxobolus* spp. 453 in closely related cyprinids of the subfamilies Leu- 454 ciscinae and Abraminae, Cech et al. (2012) found that 455 morphologically similar spores of *Myxobolus* spp. 456 infecting hosts of the leuciscine genera *Rutilus* 457

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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 the subfamily Leuciscinae (see Briolay et al., 1998; Zardoya & Doadrio, 1999) but differs from other members of this subfamily by its mouth structure and feeding habits. In spore morphology, in the location of plasmodia and in the close relationship of the 18S rDNA sequences the species found in *C. nasus* in the present study show very close relationships with some 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 posrterior probabilities under 70). The three new species clustered together with morphologically similar species which also exhibit the same tissue specificity. The only major difference is the position of *M. sitjae*, which was associated with *M. szentendrensis* n. sp. by BI analysis, but into the same group (containing *M. intimus*, *M. alvarezae* Cech, Molnár & Székely, 2012, *M. dujardini* 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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