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Description of two new species of *Myxobolus* Bütschli, 1892, *M. peleci* n. sp. and *M. cultrati* n. sp., detected during an intensive mortality of the sichel, *Pelecus cultratus* (L.) (Cyprinidae), in Lake Balaton, Hungary

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Abstract In the summer of 2014, mass mortality of the sichel, *Pelecus cultratus* (L.), was observed in Lake Balaton, Hungary. Parasitological examination conducted in the framework of a complete diagnostic survey revealed myxozoan infections. Two species of *Myxobolus* Bütschli, 1892 were found, one in the gill lamellae and another in the eyes. Following this mass mortality, 113 sichel specimens were examined during a 14-month period. Gill infection with scattered spores in the lamellae was found in 51 fish, while infection in the eyes was recorded in three specimens only. Based upon the morphological and molecular biological data the species from the gills is described here as *Myxobolus peleci* n. sp. and the species from the eye as *M. cultrati* n. sp. The 18S rDNA sequences of the two species proved that they differ from all known *Myxobolus* spp. with sequence data available in the GenBank database. Histological examinations revealed that the spores found in the gill lamellae were derived from plasmodia developing in and around the afferent branchial arteries of the gill arches. No mortality of sichel was recorded in 2015. Infection with these two *Myxobolus* spp. does not seem to play a role in the mortality of the host fish.

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Introduction

The sichel *Pelecus cultratus* (L.) is a common cyprinid fish in Lake Balaton. Earlier this fish species had been seined by commercial fishermen in quantities of several hundred tons in the late autumn months when the fish gathered in large shoals. Since 2014 commercial fishing in the lake has been stopped. In the first half of June 2014 about 40,000 specimens of sichel died and were washed to the shore of Lake Balaton. So far, similar mortality occurring in that season year by year could be observed only among common bream *Abramis brama* (L.) due to intensive infection with the copepod parasite *Tracheliastes maculatus* Kollar, 1836 and a concomitant *Aeromonas* spp. infection (Molnár et al., 2001, 2002; Székely et al., 2010). No similar massive mortality of sichel had been recorded up to that time in Lake Balaton. The detailed complex parasitological examination of moribund sichel specimens did not demonstrate a remarkable parasite burden, but in some of the fish heavy infection of the gills and eyes with two unknown species of *Myxobolus* Bütschli, 1892 was found. Myxozoan infection is less studied in the sichel than in other cyprinid species (Molnár & Székely, 1995). Only a single *Myxobolus*

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61 spp., *Myxobolus ladogensis* Rumyantsev and Shul-
62 man, 1997 has been described from the sichel so far
63 (Rumyantsev & Shulman, 1997).
64   In this paper we describe two new species of
65 *Myxobolus* from the sichel but we cannot state that
66 these myxozoans played an important role in the
67 massive mortality of the host.

Materials and methods

Collection of fish

70 In June 2014, 15 moribund sichels were freshly-
71 collected in the deep water region, close to the centre
72 of Lake Balaton. They were immediately placed on ice
73 and sent to the fish disease laboratory of the National
74 Food Chain Safety Office, Veterinary Diagnostic
75 Directorate. Complete pathological examination was
76 carried out, including a general survey as well as
77 parasitological, bacteriological, virological and histo-
78 logical examinations. After finding myxozoan infec-
79 tion on the gills and the eyes, samples were submitted
80 for further investigation to the laboratory of the Fish
81 Pathology and Parasitology Team of the Institute for
82 Veterinary Medical Research, Centre for Agricultural
83 Research, Hungarian Academy of Sciences.
84   In order to monitor the prevalence of these newly
85 detected myxozoans, further sichel samples were
86 collected from Lake Balaton (Table 1). Between
87 September and December 2014, 34 sichel specimens
88 were collected. Of them, 20 specimens were dissected
89 in fresh condition, while 14 specimens were deep-
90 frozen and dissected only a year later. The sichels were
91 mostly 28 to 33 cm long, three to five year-old
92 specimens. In 2015, 47 sichel specimens were

93 examined from March to May and 17 specimens from
94 July to September.

Morphological methods

96 The majority of fish were carefully collected with a
97 dragnet, placed into a plastic bag filled with oxygen, and
98 carried to the laboratory alive. A smaller part of the fish
99 were deep-frozen and dissected later after thawing. A
100 complete parasitological examination was also per-
101 formed on a few samples, but this study was mainly
102 focused on the gills and eyes. The hemibranchia were
103 cut out, the gill filaments were removed from the
104 cartilaginous gill arches, and examined for myxozoan
105 infection first under stereomicroscope and subsequently
106 under a compound microscope at different magnifica-
107 tions. After finding plasmodia in the arteries within the
108 cartilaginous gill arch, these arteries were also carefully
109 separated and examined. The eyes were removed from
110 the orbit *in toto*. After making an incision on the cornea
111 under a stereomicroscope, the lens and the vitreous
112 humour were separated and checked for the presence of
113 *Myxobolus* spp. spores. A piece of the vitreous humour
114 was checked under a coverslip at 400× magnification.
115 In a similar way, pieces of the choroid and the retina
116 were studied under high magnification.
117   *Myxobolus* spp. spores were separated from the
118 tissues. Supposing that scattered spores in the gills had
119 been carried to the lamellae from other organs, squash
120 preparations from the muscles, kidneys and liver were
121 also made and checked for the presence of myxozoan
122 plasmodia. Unfixed spores found in both the gill
123 lamellae and the retina were studied using Nomarski
124 differential interference contrast with an Olympus
125 BH2 microscope. The spores and histological sections
126 were photographed with an Olympus DP20 digital

Table 1 Summary of sichel specimens examined in this study

Year	Month	Examined sichel specimens	Infected with *M. peleci* n. sp.	Plasmodia of *M. peleci* n. sp.	Spore residues in gill arch	Infected with *M. cultrati* n. sp. spores
2014	June	15	4	2		2
	September–December	20	10			
	September–December (refrigerated)	14	8		8	1
2015	March	28	2			
	May	19	15	6		
	July–September	17	12		2	
Total		113	51	8	10	3

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127 camera. Measurements of fresh spores were taken with
128 a calibrated eyepiece micrometer according to the
129 guidelines of Lom & Arthur (1989), or the spores were
130 measured on the basis of digital images. All measure-
131 ments are given in micrometres and are given in the
132 text and tables as the range followed by the mean ± s-
133 tandard deviation and the number of measurements in
134 parentheses. Tissue samples from infected organs
135 containing developing and mature plasmodia were
136 fixed in Bouin's solution, embedded in paraffin wax,
137 cut to 4–5 μm sections, and stained with haematoxylin
138 and eosin. After finding the first plasmodia inside the
139 cartilaginous gill arch, the afferent and efferent
140 arteries in some fish were removed and dissected from
141 the gill arch and sectioned lengthways. In a similar
142 way, some gill arches were cut in the plane of the gill
143 filaments. Part of the spores was collected in Eppen-
144 dorf tubes and stored in 80% ethanol until further use
145 for molecular analysis, while the rest of the spores
146 were preserved in glycerine-gelatine as slide
147 preparations.

148 *Molecular methods*
149 In order to determine the 18S rDNA sequences of the
150 myxozoan samples, DNA was extracted from the
151 spores preserved in ethanol using the DNeasy® Blood
152 & Tissue Kit (Qiagen, Hilden, Germany). The samples
153 were centrifuged at 10,000 rpm for 10 min and the
154 supernatant was removed. Spore pellets were treated
155 according to the manufacturer's instructions, and
156 100 μl DNA was extracted at the final elution step.
157 The 18S rDNA gene was amplified using a nested PCR
158 described in detail by Cech et al. (2015). Universal
159 eukaryotic primers ERIB1 and ERIB10 (Barta et al.,
160 1997) were used in the first round PCR. Myxozoan-
161 specific primers Myx1F and SphR (Hallett & Diamant,
162 2001; Eszterbauer & Székely, 2004) were used in the
163 second round PCR. The primer sequences are listed in
164 Table 2.

165 After the nested PCR the amplicons were analysed
166 by electrophoresis in 1% agarose gel. All the appro-
167 priate PCR products were excised from the gel,
168 purified with the Gel/PCR DNA Fragments Extraction
169 Kit (Geneaid, New Taipei City, Taiwan) and
170 sequenced directly using the BigDye Terminator
171 v3.1 Cycle Sequencing Kit (Life Technologies) with
172 an ABI PRISM® 3100 Genetic Analyser (Life Tech-
173 nologies). The sequencing primers are also listed in
174 Table 2.

175 The sequence fragments were assembled using
176 MEGA 5.2 (Tamura et al., 2011) and ambiguous bases
177 were clarified using corresponding ABI chro-
178 matograms. Nucleotide sequences and reference
179 sequences from the GenBank database based on
180 BLAST matches were aligned with the software
181 CLUSTAL W (Thompson et al., 1994). DNA pairwise
182 distances were calculated with the Mega 5.2 software
183 using the Maximum Composite Likelihood model.
184 Phylogenetic analysis was performed *via* Maximum
185 Likelihood (ML) and Bayesian Inference (BI), and
186 *Ceratonova shasta* (Noble, 1950) was chosen as the
187 outgroup. The dataset was tested using MEGA 5.2 for
188 the nucleotide substitution model of best fit and the
189 model shown by the Akaike Information Criterion
190 (AIC) as the best-fitting one was chosen (GTR +
191 G + I model). Bootstrap values based on 1,000
192 resampled datasets were generated. BI was computed
193 by Topali 2.5 (Milne et al., 2004). The likelihood
194 parameters for BI were based on the GTR + G model.
195 Posterior probabilities were estimated over 1,000,000
196 generations *via* two independent runs of four simul-
197 taneous MCMCMC chains with every 100th tree
198 saved. The first 25% of the sampled trees were
199 discarded as 'burn-in'.

Findings

200

201 The main external signs seen on fresh carcasses and
202 moribund specimens of sichel were exophthalmia and
203 haemorrhage of the eyes. At the laboratory inspection,
204 swelling and hyperaemia of the gills were observed,
205 and the gills were covered by abundant slime. Mucosal
206 hyperaemia of the empty intestine was also recorded.
207 The iris of the eye became larger and free erythrocytes
208 were found in the vitreous humour.

209 Two unknown *Myxobolus* spp. were found in some
210 of the 15 specimens examined for parasitic infections.
211 The gill lamellae of four fish were infected by one of
212 the species, having spherical spores and described
213 below as *Myxobolus peleci* n. sp. Two fish out of the
214 four were heavily infected with this myxozoan, while
215 only a few spores were found in the sinusoids of the
216 lamellae in the other two specimens (Table 1). In the
217 heavily infected specimens, free spores of *M. peleci* n.
218 sp. filled out the sinusoids of the gill lamellae
219 (Figs. 1A, B, 2A, B), and almost every secondary
220 lamella of the gill filaments was jammed with spores.

Table 2 Primers used in the PCRs and in the sequencing reactions

Primer name	Sequence (5′–3′)	Application	Reference
ERIB 1	ACC TGG TTG ATC CTG CCA	1st round PCR	Barta et al. (1997)
ERIB 10	CTT CCG CAG GTT CAC CTA CGG	1st round PCR	Barta et al. (1997)
Myx1F	GTG AGA CTG CGG ACG GCT CAG	2nd round PCR	Hallett & Diamant (2001)
SphR	GTT ACC ATT GTA GCG CGC GT	2nd round PCR & sequencing	Eszterbauer & Székely (2004)
ACT1FR	TTG GGT AAT TTG CGC GCC TGC	sequencing	Hallett & Diamant (2001)
CR1 R	GAT YAG ATA CCG TCS TAG T	sequencing	Székely et al. (2015)
CR1 F	CGA AGA CGA TCA GAT ACC GTC CTA	sequencing	Székely et al. (2015)
NSF573/19	CGC GGT AAT TCC AGC TCC A	sequencing	Li et al. (2013)
1700 Rv	GGC ATC ACW GAC CTG YTA T	sequencing	Based on Dyková et al. (2008)
MB3 F	GAT GAT TAA CAG GAG CGG TTG	sequencing	Eszterbauer (2004)

221 In two of the fishes infected with *M. peleci* n. sp.,
222 *Myxobolus* spp. spores of another type were found in
223 the eyes. These elongated elliptical spores, which are
224 described below as *Myxobolus cultrati* n. sp., were
225 located in large batches among the pigmented cells of
226 the retina layer (Figs. 1C, 2C). No myxozoan infection
227 was found in other organs.

228 Out of the 20 sichel specimens examined in the
229 autumn of 2014, scattered spores of *M. peleci* n. sp.
230 were found in the gill lamellae in ten specimens, while
231 no spores of *M. cultrati* n. sp. were detected in the eye.
232 Among the 28 sichel specimens examined in March
233 2015, only two fish showed infection with scattered
234 spores in the lamellae; however, in May 15 of the 19
235 examined sichel specimens showed *M. peleci* n. sp.
236 infection in the lamellae and inside the lumen of the
237 afferent arteries of the cartilaginous gill arch. Plas-
238 modia with developing and mature spores of *M. peleci*
239 n. sp. were first found in three of the latter specimens.
240 These plasmodia of oval or elongated shape were
241 located in the wall of the afferent artery and in the
242 loose connective tissue close to the base of the gill
243 filaments (Fig. 3A–C). After rechecking histological
244 slides made during the fish mortality in 2014, similar
245 plasmodia were found in two preparations.

246 In the late summer months of 2015 *M. peleci* n. sp.
247 spores were found in the gill lamellae and in the
248 arteries running inside the cartilaginous gill arch in
249 12 of the 17 sichel specimens examined. In these fishes
250 no plasmodia were detected but elongated conglom-
251 erations of aged spores (named pseudocysts) were
252 found around the gill arteries in two fish.

253 In order to obtain more data on infection and the
254 location of spores at the site of plasmodial develop-
255 ment, the gills of 14 sichel specimens deep frozen in
256 autumn 2014 were examined histologically. Eight of
257 these fishes had dispersed spores in the gill lamellae
258 and each of them had free spores in the afferent artery.
259 In addition, aged spores of *M. peleci* were found in
260 pseudocysts in the gill arch (Fig. 4A, B). Spores of *M.*
261 *cultrati* were recorded only in one sichel.

262 No *Myxobolus* spores were found in the muscles
263 and the liver; however, some damaged spores were
264 detected in the melano-macrophage centres of the
265 kidney, corresponding in shape and size to the *M.*
266 *peleci* n. sp. spores found in the gills.

Molecular data and phylogenetic analyses 268

269 PCR amplification of the 18S rDNA produced ampli-
270 cons 1,567 nt long for the samples of *M. peleci* n. sp.
271 (isolated from the gill lamellae) and 1,672 nt for *M.*
272 *cultrati* n. sp. (isolated from the eye). The alignment of
273 the two samples and reference sequences (overall 20
274 sequences) was 1,607 nt long, of which 722 positions
275 were variable and 498 parsimony informative. ML and
276 BI analyses of the sequences generated highly similar
277 topologies, except for a few species, e.g. *Hungactino-*
278 *myxon* type 1 of Rácz et al. (2005), *Myxobolus dispar*
279 Thélohan, 1895, *Myxobolus wootteni* Molnár, Marton,
280 Székely & Eszterbauer, 2010 and *Myxobolus divers-*
281 *icapsularis* Slukhai, 1966, but the phylogenetic posi-
282 tions of the two new species were identical in both
283 phylograms (Fig. 5A, B). The two analysed samples
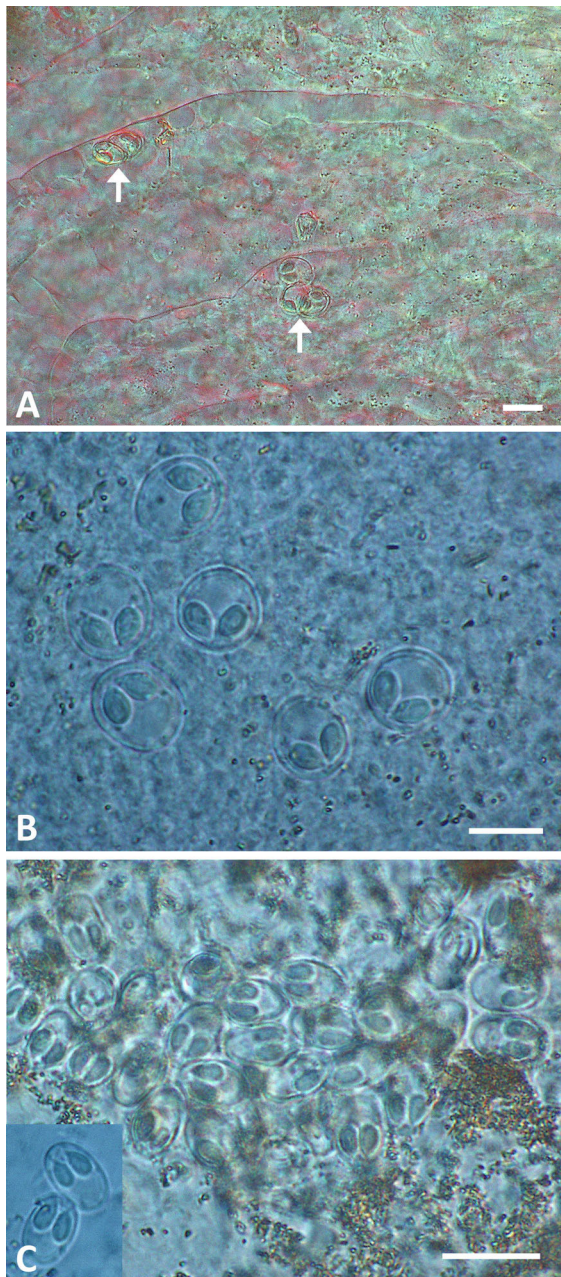284 differed from all of the available reference sequences

Fig. 1 A, Spores of *Myxobolus peleci* n. sp. (*arrows*) in the gill lamellae of *Pelecus cultratus* Fresh preparation. B, Round spores of *M. peleci* n. sp. in the gill lamellae of *Pelecus cultratus* (fresh preparation); C, Elongated ellipsoidal spores of *Myxobolus cultrati* n. sp. in the retina layer of the eye in *Pelecus cultratus* (fresh preparation). Inset: spores with triangular intercapsular appendix. *Scale-bars*: 10 μm

in the GenBank database. *Myxobolus peleci* n. sp. (KU170934) showed the closest similarity (96.5%) to sequences of *Myxobolus alburni* Donec, 1984 (EU567313), a parasite of the bleak *Alburnus alburnus* (L.). *Myxobolus cultrati* n. sp. (KU170935) collected from the retina layer of the eyeball of a sichel specimen did not show close similarity to any other *Myxobolus* spp. from Hungarian cyprinids (Fig. 5A, B). This species showed, however, a relatively close relationship to the actinosporean stage of the Far-Eastern common carp parasite *Myxobolus cultus* Yokoyama, Ogawa & Wakabayashi, 1995 (AB121146) (97.4%) and also exhibited a close similarity (96.1%) to sequences of *Myxobolus lentisuturalis* Dyková, Fiala & Nie, 2002 (AY278563, AY119688) from the gibel carp *Carassius auratus gibelio* (L.) and the goldfish *Carassius auratus auratus* (L.).

Based on the morphology and the 18S rDNA sequences of the spores, the two *Myxobolus* spp. found in the gills and in the eyes proved to be undescribed new species, and are described as follows.

Myxobolus peleci n. sp.

Type-host: *Pelecus cultratus* (L.) (Cyprinidae), sichel.
Type-locality: Lake Balaton, Hungary.
Type-material: Spores in glycerine-gelatine and phototypes were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-19508.
Prevalence: 45% (51 out of 113 fish examined).
Site of tissue development: Histological examination proved that plasmodial development takes place in the afferent artery inside the cartilaginous gill arch, from which solitary spores are carried to the gill filaments and to other parts of the fish body.
Representative DNA sequence: 18S rDNA sequence for *M. peleci* n. sp. collected from the gill filaments of one sichel specimen was deposited in the GenBank database under accession number KU170934.
Etymology: The species is named after its host generic name.

Description (Figs. 1A, B, 2A, B, 3, 4)

Vegetative stages. Ellipsoidal plasmodia, 600–800 × 300–400, filled with spores in and around the afferent artery of the cartilaginous gill arch (Fig. 4).

Spores. Spores round or roundish in frontal view (Figs. 1B, 2A), in most cases with length somewhat greater than width, less frequently with equal length and width. Spores lemon-shaped in sutural view (Fig. 2B). Spore length 11.6–13.2 (12.1 ± 0.55)
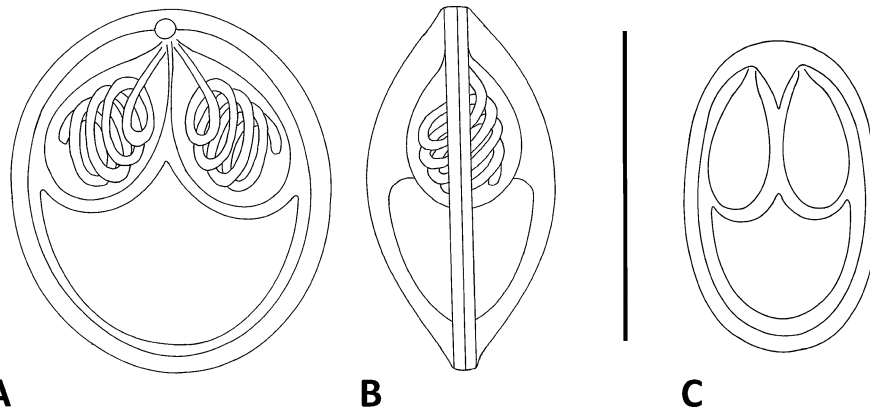
Fig. 2 Schematic drawings of spores A, *Myxobolus peleci* n. sp. in frontal view; B, *Myxobolus peleci* n. sp. in sutural view; C, *Myxobolus cultrati* n. sp., frontal view; *Scale-bar*: 10 μm

334 (n = 50), width 10.8–12.5 (11.4 ± 1) (n = 50),
335 thickness 5.6–6.0 (5.8 ± 0.2) (n = 11). Polar cap-
336 sules drop-like, equal in size, slightly converging
337 anteriorly, 5.2–6.0 (5.5 ± 0.28) long (n = 50),
338 2.8–3.4 (3.2 ± 0.21) wide (n = 50). Four to 5
339 filament coils arranged obliquely to capsule length,
340 wounding loosely in polar capsule. Intercapsular
341 appendix not found, but a small knob-like structure
342 measuring 0.6–0.8 (0.7 ± 0.2) at spore anterior end
343 observed. Sutural protrusion in frontal view with thick
344 0.8–1.0 (0.9 ± 0.1) circular rim around spore but in
345 sutural view emerging only slightly over surface of
346 spore at anterior and posterior extremities. Sutural
347 edge markings not seen. Sporoplasm with small
348 iodinophilous vacuole. Mucous envelope not found.

349 *Histology*: Ellipsoidal cysts of 600–800 × 300–400 in
350 the thick-walled afferent artery of the gill arch
351 (Fig. 3A). Plasmodia embedded into the wall of the
352 artery having no blood cells in the lumen (Fig. 3B).
353 Some other plasmodia located under the base of the
354 cartilaginous gill rays in close proximity to branches
355 of the afferent artery entering the gill filaments
356 (Fig. 3C). In gill arch preparations fixated in the late
357 summer and autumn period, conglomerations of
358 spores called pseudocysts in the wall of the afferent
359 branchial artery and in the loose connective tissue
360 close to the base of the gill lamellae (Fig. 4A).
361 Pseudocysts formed by the fusion of ageing plas-
362 modia. The pseudocysts by some live spores staining
363 red with haematoxillin & eosin and by poorly staining
364 old spores. Some pseudocysts damaged and therefore
365 spores found in the cell debris of the loose connective

366 tissue (Fig. 4B). Unfortunately the structure of this
367 tissue could not be studied because of cell degener-
368 ation of the deep-frozen material.

369 Remarks

370 The intralamellar location of free *M. peleci* n. sp.
371 spores suggested that the plasmodial development
372 took place in another part of the gills or the fish body.
373 Finding free spores in the afferent artery of the
374 cartilaginous gill arch supported this view. Histolog-
375 ical sections made of the branchial arteries of the gill
376 arch definitely demonstrated that spores disseminated
377 in the gill lamellae were formed in plasmodia inside
378 the afferent artery of the gill arch and in subfilamental
379 position close to arteries entering the gill filaments.
380 After disruption of the cysts, the spores scattered in the
381 blood and got stuck in the capillary network of the gill
382 lamellae. Up to this time, only a single species (*M.*
383 *ladogensis*) is known from the sichel as type-host. This
384 species was described by Rumyantsev & Shulman
385 (1997) from the muscles of the sichel. The spores of *M.*
386 *ladogensis* are larger than those of *M. peleci* n. sp.,
387 they have an oval shape with different-sized polar
388 capsules and with a large intercapsular appendix. By
389 its spherical shape and by lacking an intercapsular
390 appendix, *M. peleci* n. sp. closely resembles *M.*
391 *alburni*, a fin parasite of the bleak. The shape of *M.*
392 *peleci* n. sp. shows some resemblance to that of *M.*
393 *ridouti* Easy & Cone, 2009, a parasite infecting the
394 muscles of an American cyprinid fish, *Pimephalus*
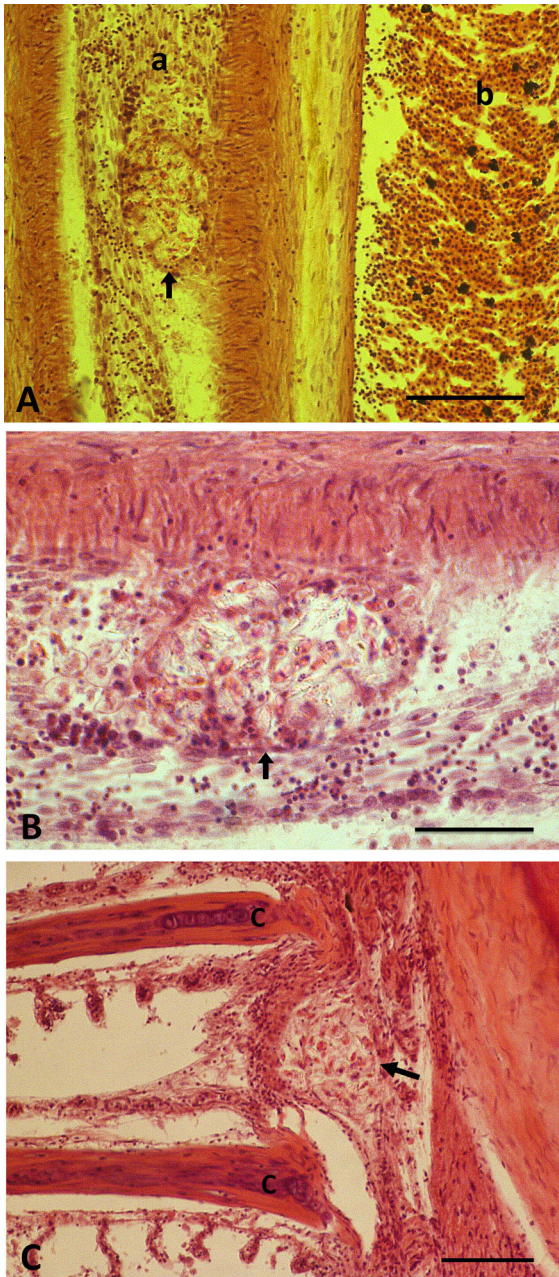395 *notatus* Rafinesque (see Easy & Cone, 2009), which

Fig. 3 A, *Myxobolus peleci* n. sp. plasmodium (*arrow*) in the thick wall of the afferent branchial artery (a) inside the cartilaginous gill arch. The thin-walled efferent branchial artery (b) is filled with blood cells. Histological section, haematoxillin & eosin staining; B, Enlarged picture of the *Myxobolus peleci* n. sp. plasmodium (*arrow*) with spores and developing stages inside. Histological section, haematoxillin & eosin staining; C, *Myxobolus peleci* n. sp. plasmodium (*arrow*) at the base of the gill filaments (c, cartilaginous gill rays of filaments). Histological section, haematoxillin & eosin staining. *Scale-bars*: A, C, 100 μm; B, 50 μm

also has round spores, a knob-like structure at the opening of the spores and loose turns of polar filaments in the capsule. However, the genetic and geographical differences between the two hosts seem to exclude a close relationship between these two parasites.

Myxobolus cultrati n. sp.

Type-host: Sichel *Pelecus cultratus* (L.) (Cyprinidae).
Type-locality: Lake Balaton, Hungary.
Type-material: Phototypes of spores were deposited in the parasitological collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. HNHM-19509.
Prevalence: 2.7% (3 out of 113 fish examined).
Site of tissue development: Plasmodia were not found, batches of spores were located in the retina layer of the eyes.
Representative DNA sequences: 18S rDNA sequence for *M. cultrati* n. sp. was deposited in the GenBank database under accession number KU170935.
Etymology: The species is named after the specific name of its host.

Description (Figs. 1C, 2C)

Vegetative stages. Not found.

Spores. Spores elongate-ellipsoidal in frontal view (Figs. 1C, 2C); no spores recorded in sutural view. Spore length 9.2–10.4 (9.8 ± 0.18) (n = 50), width 6.0–6.8 (6.4 ± 0.51) (n = 50). Polar capsules elongate, equal in size, slightly running parallel to each other, 4.4–4.8 (4.5 ± 0.35) long (n = 50), 2.1–2.4 (2.3 ± 0.18) wide (n = 50). Filament coils not detected. Strong, triangular intercapsular appendix measuring 1.6–2.1 (1.9 ± 0.3) present. Sporoplasm with small iodinophilous vacuole. Mucous envelope not visible.

Although plasmodial stages were not found, the batches of spores detected in the retina layer of the eyes suggest that plasmodial development took place in this specific site of the fish body. Spores of *M. cultrati* n. sp. found in the eye seemed to be aged, but their elongate ellipsoidal shape resembled spores of *Myxobolus muelleri* Bütschli, 1882 and *M. donecae* Kashkovsky, 1969. Its spores differ, however, from the spores of the latter species by their large intercapsular appendix and by their different location in the
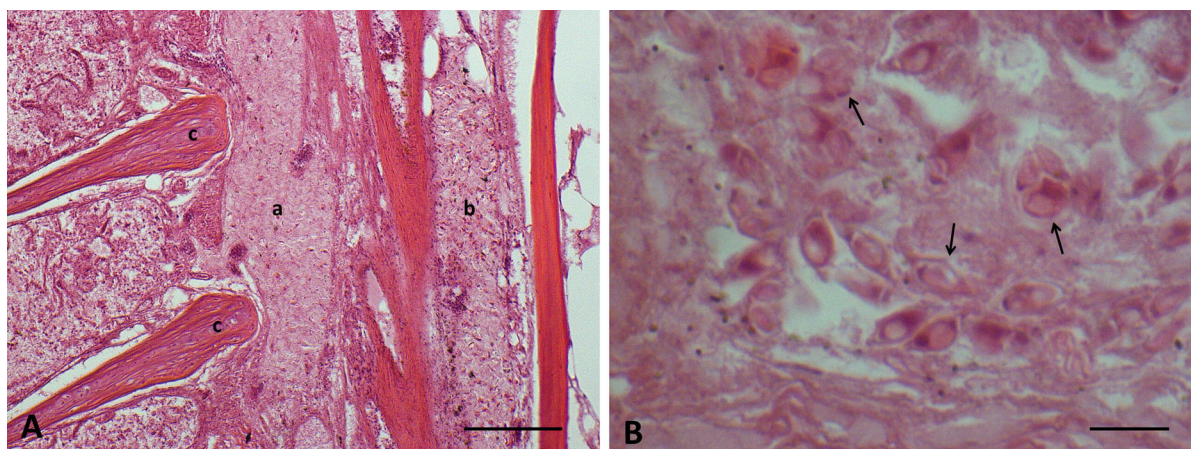
Fig. 4 A, *Myxobolus peleci* n. sp. pseudocysts in the cartilaginous gill arch (a, agglomeration of ageing spores at the base of the gill filaments; b, agglomeration of spores in the wall of the afferent artery; c, cartilaginous gill rays of the gill filaments). Histological section, haematoxillin & eosin staining made from deep-frozen gills; B, Aged *Myxobolus peleci* n. sp. spores (*arrows*) in the cell debris of a pseudocyst. Histological section, haematoxillin & eosin staining made from deep-frozen gills. *Scale-bars*: A, 100 μm; B, 10 μm
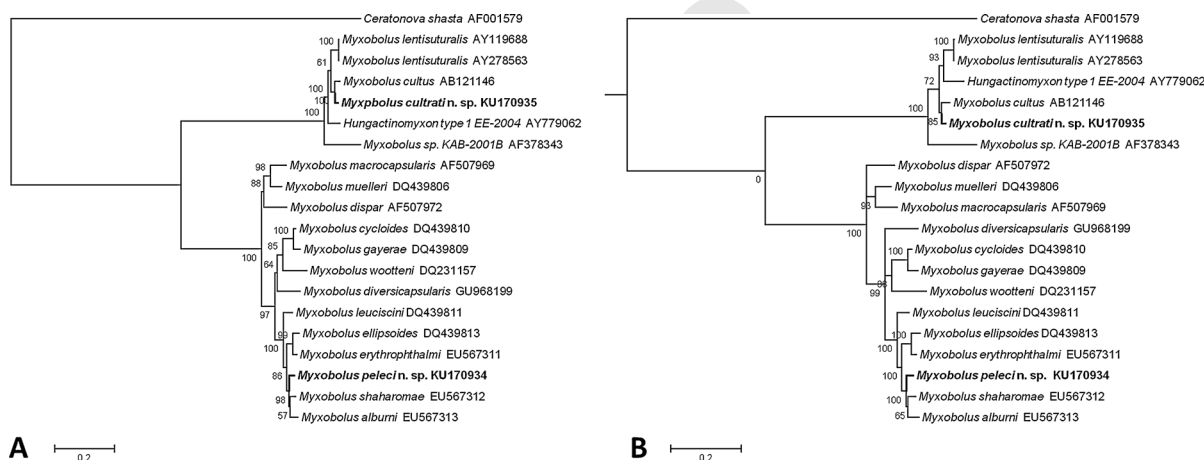

Fig. 5 A, Phylogenetic position of *Myxobolus peleci* n. sp. ex *Pelecus cultratus* based on 18S rDNA analysis by the Maximum Likelihood algorithm (A) and Bayesian Inference algorithm (B). *Ceratonova shasta* was used as the outgroup. Bootstrap values and posterior probabilities are given at the nodes. The scale-bar indicates the number of expected substitutions per site

441 fish body. Differences found in 18S rDNA sequences
442 between *M. cultrati* and *M. muelleri* and all other
443 *Myxobolus* spp. parasitising Hungarian cyprinids also
444 suggest that *M. cultrati* is a specific species of the
445 sichel. Although a completely reliable description of a
446 new species usually requires more detailed data on
447 spore morphology and site selection of the species,
448 differences from other species in the partial 18S rRNA
449 gene (see above) and the specific location of spores in
450 the fish body support the description of the species as a
451 new one. The number of myxozoan species infecting
452 the eyes is relatively low. Most of them infect the

453 cornea of the eye, e.g. *Myxobolus magnus* Awerinzev,
454 1913 in *Gymnocephalus cernua* (L.), *Myxobolus*
455 *heterolepis* Lee & Desser, 1985 in *Notropis hetero-*
456 *lepis* Eigemann & Eigemann, *Myxobolus corneus*
457 Cone, Horner & Hoffman, 1990 in *Lepomis macro-*
458 *chirus* Rafinesque, *Myxobolus scleroperca* Muzzal,
459 1995 in *Perca flavescens* Mitchill and *Myxobolus*
460 *cordeiroi* Adriano, Arana, Alves, Silva, Ceccarelli,
461 Henrique-Silva & Maia, 2009 in *Zungaro yahu* Ihering
462 (see Awerinzew, 1913; Li & Desser, 1985; Cone et al.,
463 1990; Muzzal, 1995; Adriano et al., 2009); however,
464 *Thelohanellus oculileucisci* (Trojan, 1909) of the

roach infects the vitreous humour of the eye (Lom, 1961).

Discussion

Although the new species of *Myxobolus* were found in connection with a fish mortality, our studies proved that neither of the two species played a role in fish mortality. On the other hand, these studies called attention to the fact that the sichel, a little-studied cyprinid fish, might be infected with some specific unknown *Myxobolus* spp., which might cause heavy infection. Only a single species, *Myxobolus ladogensis*, had been described from the sichel as type-host previously (Rumyantsev & Shulman, 1997). In their work on the myxozoan fauna of the former Soviet Union, Donec & Shulman (1984) listed the occurrence of 12 other *Myxobolus* spp. (*M. carassii* Klokaceva, 1914; *M. chondrostomi* Donec, 1962; *M. dispar* Thélohan, 1895; *M. dogieli* Bykhovskaya-Pavlovskaya & Bykhovsky, 1940; *M. dujardini* (Thélohan, 1892); *M. exiguus* Thélohan, 1895; *M. gigas* Auerbach, 1906; *M. kuleminae* Donec, 1984; *M. macrocapsularis* Reuss, 1906; *M. muelleri*; *M. musculi* Keysselitz, 1908; and *M. rotundus* Nemeczek, 1911), known from other type-hosts. It is supposed that these data cover false identifications and some of the listed cases represent new undescribed species specific to the sichel. A proper description of a new species requires a detailed study of the myxospores, finding plasmodia with correct identification of their histotropism and location, and a molecular study of the spores. In the case of *M. peleci* n. sp. these criteria have been fulfilled; however, in the case of *M. cultrati* n. sp. the plasmodia were not found and the tissue tropism was not determined; these represent shortcomings in this respect. Nevertheless, we are convinced that the intraocular location of the spores, the morphological data and the DNA sequences provide a solid basis for the distinct status of the species and will enhance a subsequent diagnosis. Finding the exact site of plasmodial development is a task for the future in the case of several myxozoan species. In the case of *M. peleci* n. sp., clarification of the location of plasmodial development took us a whole year. The fact that only solitary spores were found in the gill lamellae and this infection persisted practically during a whole year,

rendered unlikely the location of plasmodia in the gill filaments, and suggested that plasmodial development of this species takes place in another organ or in some other part of the gills. Molnár & Kovács-Gayer (1985) proved that the spores of *Myxobolus cyprini* Doflein, 1898, formed in the muscle cells, were found practically in all organs. In *M. peleci* n. sp. infection, however, the muscles proved to be free of infection, and in the kidney only a relatively moderate infection was found. This suggested that the source of lamellar infection should be located between the heart and the gills. Histological findings demonstrated that the plasmodia developed either in the wall of the afferent branchial artery or in a basifilamental location inside the gill arch. In the case of intra-arterial development, spores from bursting plasmodia could get directly into the blood, to be carried to the filaments and lamellae of the gills. Although plasmodial stages were found only in the spring, histological preparations made in the summer and autumn showed that thousands of spores were preserved in ageing pseudocysts or dispersed inside the loose connective tissue. It seems to be obvious that spores reserved in pseudocysts are the source of the permanent infection of gill lamellae with spores, which was found throughout the year. The rare occurrence of spores in the melano-macrophage centres of the kidney proves that some of the spores could leave the gills and were carried away by the blood stream.

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. Fishing was carried out with fishing permit for research purposes: HHgF/110-2/2016, Ministry of Agriculture, Hungary.

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