Vol. 109: 213–222, 2014
doi: 10.3354/dao02745

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published July 3

Myxobolus anatolicus sp. nov. (Myxozoa) infecting the gill of Anatolian khramulya *Capoeta tinca* (Cyprinidae) in Turkey

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ABSTRACT: This work is part of an ongoing investigation into the characteristics of myxozoan parasites of freshwater fish in Turkey and was carried out using morphology, histopathology and molecular analysis. A new species of the genus *Myxobolus* (*M. anatolicus* sp. nov.) was found infecting the gills of 3 of 34 specimens (8.8%) of Anatolian khramulya *Capoeta tinca* from the Samsun Province, Northern Turkey. Both morphology and 18S rDNA sequence data revealed that *M. anatolicus* sp. nov. was distinct from other *Myxobolus* species found in the gills of cyprinid fishes. The small, white and round-shaped plasmodia, measuring 0.2 to 1.4 mm in diameter, were observed macroscopically in the gills. Histological analysis revealed that the cyst-like plasmodia have an intralamellar-vascular type development. Mature spores of *M. anatolicus* sp. nov. were oval in both frontal and sutural views, and tapered at the anterior poles. The spores were 10.1 ± 0.41 (9.4 to 10.7) µm long, 6.9 ± 0.28 (6.6 to 7.2) µm wide, and 4.5 ± 0.36 (4.4 to 4.6) µm thick. The 2 polar capsules were pyriform, equal in size, 4.6 ± 0.45 (4.4 to 4.8) µm long and 2.1 ± 0.12 (2 to 2.3) µm wide. Polar filaments within the polar capsules were coiled with 5 or 6 turns. Phylogenetic analysis placed *M. anatolicus* sp. nov. in a clade of gill-infecting myxobolids. This is the first record of a *Myxobolus* species infecting Anatolian khramulya *Capoeta tinca*, and the first record of this species from Eurasia.

KEY WORDS: *Myxobolus anatolicus* sp. nov. · Myxozoan · Anatolian khramulya · Parasite · Histology · 18S rDNA · Phylogeny · Turkey

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INTRODUCTION

Anatolian khramulya *Capoeta tinca* (Heckel, 1843) is a species of the family Cyprinidae and has a wide distribution in Western Asia. Its original distribution areas in Turkey are the rivers and streams of the northern and northwestern Black Sea regions. *C. tinca* is an herbivorous fish that can easily adapt to aquatic environment changes, and occurs both in lotic and lentic habitats. Furthermore, the species has economic value as a commercial fish in natural and man-made lakes due to its delicious flesh (Ekmekci 2002, Geldiay & Balik 2007).

Myxosporean parasites have a significant role as pathogens of fish in wild and cultured stocks throughout the world (Lom & Dyková 2006). Among the myxosporeans, species of the genus *Myxobolus* are, so far, the most commonly found in fish, with more than 700 known species throughout the world (Eiras et al. 2005). Using spore morphology alone, it is often difficult to determine the validity of morphologically similar myxosporean species, particularly those with identical tissue affinity and that develop in taxonomically closely related host species (Molnár et al. 2002, 2009). The combination of morphology and molecular-based classification, with the considera-

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tion of host range and tissue specificity, provides a precise approach to distinguish valid species from identified taxa (Molnár et al. 2009).

To date, only 4 *Myxobolus* species have been found in wild fish species in Turkey. *M. exiguus*, *M. muelleri*, *M. episquamalis* and *M. ichkeulensis* were morphologically identified in *Mugil cephalus* (Altunel 1983, Umur et al. 2010, Ozak et al. 2012). Up to now, most of the *Myxobolus* species that have been described were obtained from cyprinid fishes (Eiras et al. 2005, Lom & Dyková 2006), although no myxosporeans from cyprinid fishes in Turkish waters have yet been described. This is the first record of a *Myxobolus* species infecting *C. tinca*, and the first record of this species from Eurasia.

The present study is part of an ongoing investigation into the characteristics of myxosporean parasites of freshwater fish in Northeast Turkey. Morphological, histopathological and molecular data were used to describe a new *Myxobolus* species found infecting the gills of *C. tinca* from Turkey.

MATERIALS AND METHODS

Sampling

A total of 34 specimens of *Capoeta tinca* were collected from the Ondokuz Mayıs University I. Pond (wild populations), Samsun, Turkey in February 2013 and September 2013 (41° 20' 55'' N, 36° 10' 15'' E). Immediately after collection, fish were transported alive to the laboratory and kept in aerated aquaria.

Fish necropsy and morphological methods

Standard procedures were used for myxosporean examination within 1 to 2 d after transportation (Lom & Dyková 1992). Examinations for the presence of myxosporeans were carried out using a stereo microscope. Gills, skin, kidney, gall bladder, intestine and other organs were also examined under a stereo microscope. When cyst-like plasmodia were found in the gills, plasmodia were carefully removed from the tissues and dissected with a fine needle on a slide. The locations of the plasmodia in filaments, lamellae or in the gill arches were differentiated according to Molnár (2002) into epithelial, vascular, muscular or chondroid. Some of the spores were studied in fresh preparations, others were fixed in absolute ethanol, collected into 1.5 ml tubes and stored at −20°C until required for further morphological and molecular examinations. The rest of the spores found were preserved in glycerine jelly as reference slide preparations in the laboratory. Morphological and morphometric features of spores were characterised according to Lom & Arthur (1989) by measuring 25 freshly isolated mature spores in wet preparations. All measurements are given in micrometres (µm). Line drawings were made based on the fresh wet mounts with the aid of a drawing tube (Y-IDT, Nikon). The spores were photographed and measured with a microscope (Eclipse 80i, Nikon) connected to a digital camera with a liquid crystal display and measurement-specific software (Digital Sight DS-L1, Nikon).

Histological methods

Tissue samples from infected gills were fixed in 10% neutral formaldehyde solution for pathologic examination. Tissue samples were routinely processed and embedded in paraffin. Tissue sections, 4 to 6 µm in width, were stained with haematoxylin and eosin (H&E) and examined under a microscope (Eclipse 80i, Nikon).

Molecular methods

For genomic DNA (gDNA) extractions, samples preserved in ethanol were centrifuged at 5000 × *g* for 5 min to pellet the myxospores, and then the ethanol was removed. The gDNA was extracted using the Qiagen DNeasy Tissue Kit (Qiagen) and eluted in 50 µl AE buffer (Qiagen). The gDNA content was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific) at 260 nm. The 18S rDNA was amplified using the primers 18e and 18g' (Table 1) in a 25 µl reaction mixture comprising 10 to 50 ng DNA, 1× *Taq* PCR buffer (Thermo Scientific), 1.5 mM $MgCl_2$ (Thermo Scientific), 1 mM dNTPs (Thermo Scientific), 25 µM of each primer and 2 U *Taq* DNA polymerase (Thermo Scientific) in diethyl pyrocarbonate (DEPC)-treated water. The PCR cycle consisted of an initial denaturation step of 94°C for 4 min, followed by 35 cycles of 94°C for 50 s, 56°C for 50 s, and 72°C for 80 s, and a terminal extension at 72°C for 7 min. The reaction was then rested at 4°C.

This was followed by a second round of PCR with the primer pair MX5 and MX3 (Table 1). The total volume of the nested PCR reactions was 50 µl, which contained 1 µl amplified DNA, 1× *Taq* PCR buffer

Table 1. Primers used for PCR and/or sequencing

Primer	Sequence (5′–3′)	Application	Source
18e	CTG GTT GAT TCT GCC AGT	1st round PCR	Hillis & Dixon (1991)
18g′	CGG TAC TAG CGA CGG GCG GTG TG	1st round PCR	Hillis & Dixon (1991)
MX5	CTG CGG ACG GCT CAG TAA ATC AGT	2nd round PCR and sequencing	Andree et al. (1999)
MX3	CCA GGA CAT CTT AGG GCA TCA CAG A	2nd round PCR and sequencing	Andree et al. (1999)
MC5	CCT GAG AAA CGG CTA CCA CAT CCA	Sequencing	Molnár et al. (2002)
MC3	GAT TAG CCT GAC AGA TCA CTC CAC A	Sequencing	Molnár et al. (2002)
MB5f	GAT GAT TAA CAG GAG CGG TTG G	Sequencing	Eszterbauer (2004)
MB5r	ACC GCT CCT GTT AAT CAT CAC C	Sequencing	Eszterbauer (2004)

(Thermo Scientific), 1.5 mM $MgCl_2$ (Thermo Scientific), 1 mM dNTPs (Thermo Scientific), 25 µM of each primer and 2 U *Taq* DNA polymerase (Thermo Scientific) in DEPC-treated water. Amplification conditions in the second round were 35 cycles of 94°C for 50 s, 56°C for 50 s, and 72°C for 60 s, and a terminal extension period at 72°C for 10 min. The reaction was then rested at 4°C. Both PCR cycles were performed in a Thermo PxE 0.2 thermal cycler (Thermo Scientific). The PCR products were electrophoresed in 1.0% agarose gels in Tris-borate-EDTA buffer gel stained with 1% ethidium bromide and then purified with the QIAquick Gel Extraction Kit (Qiagen). Purified PCR products were sequenced in both directions with the primers listed in Table 1 using an ABI PRISM 3130xl automatic sequencer (Applied Biosystems).

Phylogenetic analyses

The various forward and reverse sequence segments were aligned in BioEdit (Hall 1999) and ambiguous bases clarified using corresponding ABI chromatograms. The obtained sequences were verified by forward and reverse comparisons, assembled and edited using Contig Express in Vector NTI Advance 11.5 (Invitrogen). Nucleotide sequences were aligned with the software CLUSTAL W (Thompson et al. 1994). The alignment was corrected manually using the alignment editor of the software MEGA 5.0 (Tamura et al. 2011). Genetic distances were calculated using the Kimura 2-parameter model (K2P) with pairwise deletion in MEGA 5.0 (Tamura et al. 2011). Phylogenetic analysis with other known gill *Myxobolus* species was conducted using neighbour-joining (NJ) and maximum likelihood (ML) analyses in MEGA 5.0 (Tamura et al. 2011). Sequences of *Myxobolus* species were downloaded from GenBank for the phylogenetic analysis and *Ceratomyxa shasta* (AF001579) was used as an out-group species. Taxa were selected on the basis of Basic Local Alignment Search Tool (BLAST) matches. Most were gill-infecting species; however, species from other organs and tissues were also represented. The K2P evolution sequence model was used in the NJ analysis, with gaps treated with complete deletion. The aligned sequences were tested with the MEGA 5.0 model test to find the best DNA model to infer the phylogenetic trees (Tamura et al. 2011). The General Time Reversible model (GTR) with gamma distribution (+G) and a proportion of invariable sites (+I) was selected using Akaike's Information Criterion (AIC). The evolutionary history was inferred using the ML method based on the GTR+G+I model for 18S rDNA sequences. Bootstrap confidence values were calculated with 1000 and 100 repetitions for NJ and ML, respectively (Felsenstein 1985) using the MEGA 5.0 program. Bootstrap values exceeding 70 were considered well supported (Hillis & Bull 1993).

RESULTS

Myxozoan infection was found in 3 (8.8%) of 34 *Capoeta tinca*. Small, white, spherical polysporic plasmodia measuring 0.2 to 1.4 mm in diameter were located in the gills. The plasmodia on the gill filaments of *C. tinca* were easily detected under a stereomicroscope (Fig. 1a). Plasmodia with mature *Myxobolus* spores were found in intralamellar-vascular locations in the gills (Fig. 1b). Spores obtained from mature plasmodia showed the typical characteristics of *Myxobolus* spp. This species differs from the known *Myxobolus* spp. morphologically and in its DNA sequence, and is described as new below. Pathological changes or deformities were not observed, and no other myxozoans were found in other organs of the *C. tinca*.

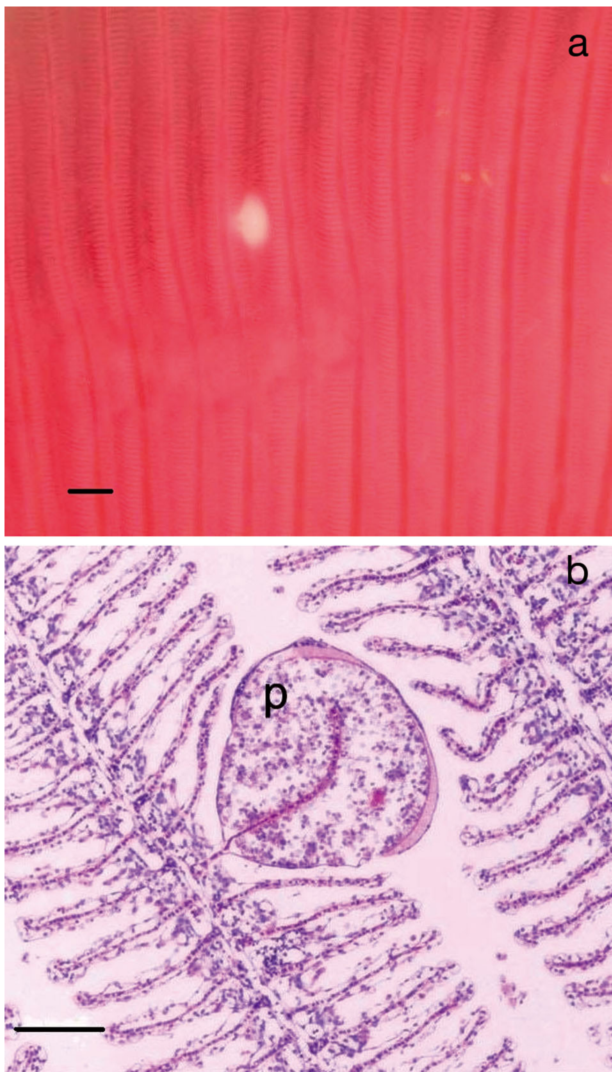

Fig. 1. (a) *Myxobolus anatolicus* sp. nov. infection in the gills of *Capoeta tinca*. Fresh preparation, Scale bar = 1 mm. (b) *M. anatolicus* sp. nov. plasmodium (p) located inside a gill lamella. Around the plasmodium, serum fills the space inside the lamella. Histological section with haematoxylin and eosin (H&E) staining. Scale bar = 100 µm

Myxobolus anatolicus sp. nov.

Type host. Anatolian khramulya *C. tinca* (Heckel, 1843) (Cyprinidae). Local name: Siraz.

Type locality. Ondokuz Mayıs University I. Pond, Samsun, Turkey. Position: 41° 20′ 55″ N, 36° 10′ 15″ E.

Site of tissue development. The plasmodia were found in the capillary network of the secondary lamellae in a vascular intralamellar position (large-cyst type, intralamellar type).

Type material. Syntype spores in glycerine jelly and histological sections were deposited in the parasitological collection of the Department of Aquatic Animal Diseases, Veterinary Faculty, Ondokuz Mayıs University, Samsun, Turkey, coll. no. OMU DAA 2013.12. The 18S rDNA sequence of *M. anatolicus* sp. nov. was deposited in GenBank under accession no. KF537629.

Prevalence of infection. A prevalence of 8.8% (3 out of 34) in 10 to 18 cm sized fish.

Intensity of infection. An intensity of 1 to 3 'cysts' per fish.

Etymology. The species was named in honour of the Anatolian region and the fish host Anatolian khramulya *C. tinca*.

Trophozoites. Mature plasmodia, spherical or ellipsoidal, containing spores were found in intralamellar locations in the gills.

Spores. Spores with typical characteristics of the genus *Myxobolus* were observed (Figs. 2a & 3a). The myxospores were oval in both frontal and sutural views, and tapered at the anterior poles (Figs. 2b,c & 3). The spores were 10.1 ± 0.41 (9.4 to 10.7) µm long (n = 25), 6.9 ± 0.28 (6.6 to 7.2) µm wide (n = 25), and 4.5 ± 0.36 (4.4 to 4.6) µm thick (n = 10). The two polar capsules were pyriform, equal in size, 4.6 ± 0.45 (4.4 to 4.8) µm long (n = 25) and 2.1 ± 0.12 (2 to 2.3) µm wide (n = 25). Polar filaments within the polar capsules were coiled with 5 or 6 turns and situated along the longitudinal axis of the capsule. Polar filaments extruding from mature spores of fresh material were estimated to be ~60 µm in length (Fig. 2d). No triangular intercapsular appendix was found in the spores. Sutural edge markings were not seen. Sporoplasm nuclei were indiscernible, and no iodinophilous vacuole or mucous envelope were found.

Histology. Plasmodia with a diameter of 200 to 1400 µm (deformed frequently in histological sections) were found in intralamellar locations in the gills. The plasmodia detached the epithelium from the endothelium of the capillary network, such that they were located between the epithelium and the endothelium of the infected gill lamellae (Fig 1b).

Molecular data. The 18S rDNA sequences of 4 replicates of *M. anatolicus* sp. nov. (KF537629) collected from the gills of 3 *C. tinca* showed 100% identity over a 1503 bp long alignment. The 18S rDNA sequence of *M. anatolicus* sp. nov. (1503 bp) did not show a close relationship with any other *Myxobolus* represented in GenBank. *M. anatolicus* sp. nov. was most similar to *M. tambroides* (JX028236) (94.9%), and was also similar to *M. dykovae* (EU643627), *M. sitjae* (FJ311898), *M. hungaricus* (AF448444), *M. branchialis* (JQ388887), *M. bilobus* (DQ008579), *Myxobolus* sp. Hungary-EE-2003 (AY325283), *M. intimus* (FJ716098), *M. dujardini* (DQ439804), *M. obe-*

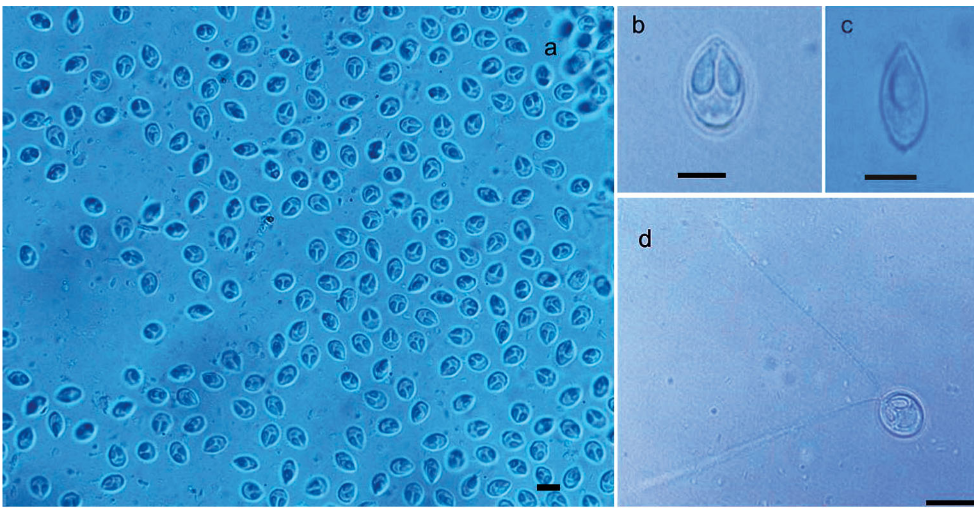

Fig. 2. Fresh spores of (a) *Myxobolus anatolicus* sp. nov. Scale bar = 10 µm. (b) Frontal view. Scale bar = 5 µm. (c) Sutural view. Scale bar = 5 µm. (d) Polar filaments extruding from fresh spore. Scale bar = 10 µm

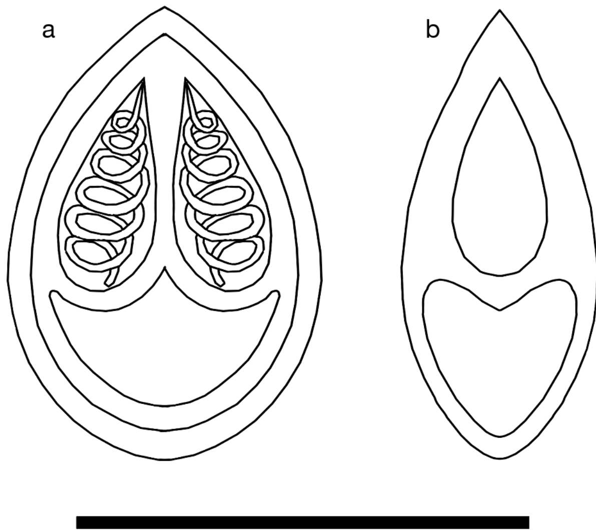

Fig. 3. Line drawings of the spore of *Myxobolus anatolicus* sp. nov., (a) frontal and (b) sutural view. Scale bar = 10 µm

sus (AY325286), and *M. alvarezae* (FJ716097) (88.3 to 91.3%).

Molecular phylogeny. Both ML and NJ analyses gave highly similar tree topologies for *M. anatolicus* sp. nov.; therefore, only the ML tree is presented here. *M. anatolicus* sp. nov. occurred, with high bootstrap support, within a clade that included 11 other species of *Myxobolus* obtained from the gills of various cyprinid fishes from North America or Eurasia (Fig. 4). Phylogenetic analysis placed *M. anatolicus* sp. nov. as a sister to *M. tambroides* in a clade of gill-infecting myxobolids (Fig. 4).

Remarks. The type host *C. tinca* is an entirely freshwater fish species, and its original distribution areas in Turkey are the rivers and streams of the northern and northwestern Black Sea regions. No *Myxobolus* species or morphologically related myxozoans have been recorded in this host before. The parasite isolated in the present study, therefore, appears to be a previously undescribed species, which we have named *M. anatolicus* sp. nov. However, *Myxobolus* species from *Capoeta* (*Varicorhinus*) spp. have been morphologically described (Shulman 1966, Baska & Masoumian 1996, Eiras et al. 2005). A comparison with other *Myxobolus* species infecting *Capoeta* (*Varicorhinus*) spp. reveals that the spore dimensions of *M. anatolicus* sp. nov. are smaller (length: 9.4 to 10.7 µm; width: 6.6 to 7.2 µm) than those of *M. musajevi* Kardilov, 1963, *M. molnari* Baska & Masoumian, 1996, *M. mokhayeri* Baska & Masoumian, 1996, *M. bottliformis* Chen & Ma, 1998, *M. capoeta* Chen & Ma, 1998 (infecting gills), *M. nanhaiensis* Chen & Ma, 1998, and *M. sikiangensis* Chen & Ma, 1998 (Shulman 1966, Baska & Masoumian 1996, Eiras et al. 2005) (Table 2). In addition, spores of *M. kovali* Allamuratov, 1967, *M. capoeta* Chen & Ma, 1998 (infecting kidneys), and *M. hochingensis* Chen & Ma, 1998 are smaller than those of *M. anatolicus* sp. nov. (Table 2). This new species has 2 equal pyriform polar capsules and lacks an intercapsular appendix, allowing it to be easily differentiated from *M. cristatus* Shulman, 1962, *M. musajevi* Kardilov, 1963, *M. samgoricus* Gobebashvili, 1966, *M. kovali* Allamuratov, 1967, *M. tadzhikistanicus* Daniyarov, 1975, *M. varicorhini* Dzhalilov & Daniyarov, 1975, *M. mokhayeri* Baska & Masoumian, 1996, *M. molnari* Baska & Masoumian, 1996, *M. bottliformis* Chen & Ma, 1998, *M. capoeta* Chen & Ma, 1998 (infecting gills and kidneys), *M. hainanensis* Chen & Ma, 1998, *M. hochingensis*

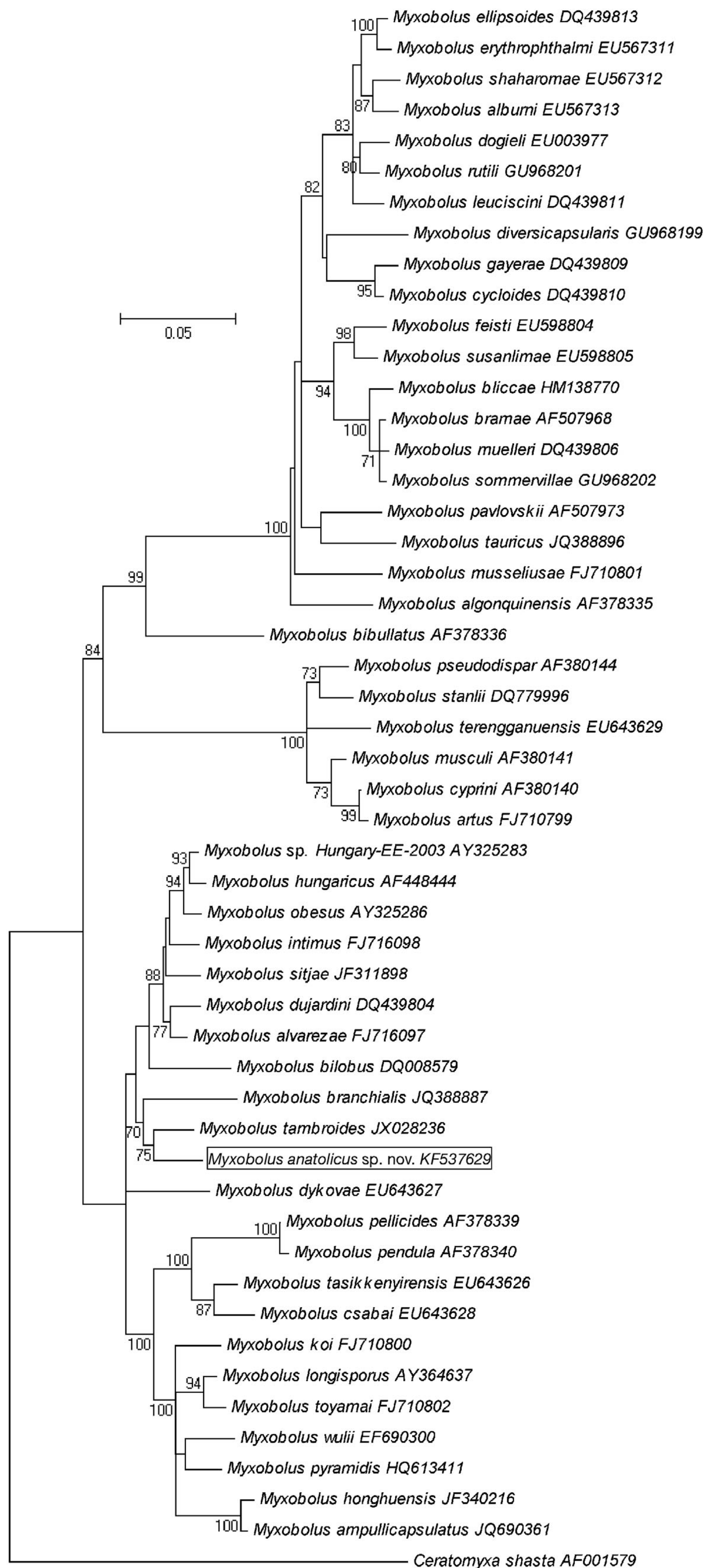

Fig. 4. Phylogenetic tree generated by maximum likelihood analyses of the 18S rDNA sequences of myxosporeans, rooted at *Ceratomyxa shasta*. *Myxobolus anatolicus* sp. nov. is boxed. Numbers above/below the branches are bootstrap confidence levels based on 100 replicates. Branches with a bootstrap value under 70 were cut off. GenBank accession numbers are given after the species name. The distance scale is shown beside the tree

Chen & Ma, 1998, *M. liaohoensis* Chen & Ma, 1998, *M. nanhaiensis* Chen & Ma, 1998, and *M. sikiangensis* Chen & Ma, 1998 (Shulman 1966, Baska & Masoumian 1996, Eiras et al. 2005). Although *M. karelicus* Petrushevski, 1940 and *M. kovali* Allamuratov, 1967 have been found in the gills of *V. capoeta heratensis* in Russia and Central Asia, these species can be discriminated from *M. anatolicus* sp. nov. by spore thickness (Table 2). *M. cristatus* Shulman, 1962, *M. samgoricus* Gobebashvili, 1966, *M. capoeta* Chen & Ma, 1998, *M. musajevi* Kardilov, 1963, *M. molnari* Baska & Masoumian, 1996 have also been observed in the gills of *Capoeta* (*Varicorhinus*) spp. (Shulman 1966, Baska & Masoumian 1996, Eiras et al. 2005). The present species, *M. anatolicus* sp. nov., can be distinguished from *M. samgoricus* Gobebashvili, 1966, *M. capoeta* Chen & Ma, 1998, *M. musajevi* Kardilov, 1963, and *M. molnari* Baska & Masoumian, 1996 by the lack of an intercapsular appendix (Baska & Masoumian 1996, Eiras et al. 2005), while *M. cristatus* Shulman, 1962 and *M. kovali* Allamuratov, 1967 have unequal polar capsules, unlike *M. anatolicus* sp. nov. Therefore, *M. anatolicus* sp. nov. can be distinguished from each of these species infecting *Capoeta* (*Varicorhinus*) spp. Unfortunately, no molecular studies have yet been conducted on these *Myxobolus* species. Moreover, *M. anatolicus* sp. nov. spores also morphologically resemble those of some species (*M. tambroides*, *M. alvarezae*, *M. sitjae*, *M. dujardini, M. dykovae* and *M. branchialis*) infecting the gill lamella of cyprinid fishes, and of them, *M. tambroides* is the most similar to *M. anatolicus* sp. nov. in its DNA sequence.

Table 2. Comparison of spore morphometric data (dimensions in µm) of *Myxobolus anatolicus* sp. nov. with those of other selected *Myxobolus* species infecting *Capoeta* (*Varicorhinus*) spp. All measurements are means with range in parentheses

Myxobolus species	Spore Length	Spore Width	Spore Thickness	Polar capsule Length	Polar capsule Width	No. of polar filament coils	Infection site	Original host	Locality	Reference
M. anatolicus sp. nov.	10.1 (9.4–10.7)	6.9 (6.6–7.2)	4.5 (4.4–4.6)	4.6 (4.4–4.8)	2.1 (2.0–2.3)	5–6	Gills	*Capoeta tinca*	Turkey	Present study
M. molnari	14.2 (13.3–14.6)	10.7 (10.4–11.6)	7.4 (6.9–7.8)	6.8 (6.7–7.2)	3.9 (3.5–4.2)	6–7	Gills	*Capoeta trutta*	Iran	Baska & Masoumian (1996)
M. capoeta	14.4 (14.2–14.8)	8.4 (8.2–9.6)	6.2 (6.0–6.4)	5.1 (4.8–5.4)	2.8 (2.6–3.0)	6–7	Gills	*Capoeta semifasciolata*	China	Eiras et al. (2005)
M. kovali	7.5–9.0	6.0–10.0	7.0	3.7–4.5, 3.2–3.6[a]	2.4–3.9, 2.1–2.7[a]	–	Gills	*Varicorhinus capoeta heratensis*	Central Asia	Eiras et al. (2005)
M. musajevi	11.5–14	10–11	–	6–7	3.3–5.0	–	Gills, kidneys, gall bladder	*Varicorhinus capoeta*	Caucasus	Shulman (1966)
M. samgoricus	9.1–11.0	7.8–9.2	4.9–5.2	3.1–3.9	1.8–3.0	–	Gills, fins, kidneys	*Varicorhinus capoeta*	Caucasus	Eiras et al. (2005)
M. karelicus	7–13	6–10	7	4.0–6.3	2–4	–	Gills, gut, eyes	*Varicorhinus capoeta heratensis*	Russia	Eiras et al. (2005)
M. cristatus	9.0–10.5	6–8	6–8	5–6, 3–5[a]	4–6, 3–5[a]	–	Gills, skin, muscles	*Varicorhinus heratensis steindachneri*	Central Asia	Shulman (1966)
M. tadzhikistanicus	9–12	5–8	–	5.0–8.3, 1.2–1.3[a]	2–3, 1.2–1.8[a]	–	Kidney, spleen	*Varicorhinus heratensis steindachneri*	Central Asia	Eiras et al. (2005)
M. mokhayeri	15.8 (14.4–16.6)	12.5 (11.6–13.3)	8.6 (8.3–9.1)	7.5, 6.6[a]	4.6, 3.6[a]	7–9, 7–8[a]	Fin rays	*Capoeta trutta*	Iran	Baska & Masoumian (1996)
M. capoeta	8.2 (7.9–8.4)	6.6 (6.0–7.2)	5.3 (5.0–5.8)	3.5 (3.0–3.8)	2.4 (2.2–2.5)	–	Kidneys	*Capoeta semifasciolata*	China	Eiras et al. (2005)
M. bottliformis	12.2 (12–13.2)	9.0 (8.6–9.6)	5.5 (5.0–6.0)	4.9 (4.8–5.4)	2.6 (2.4–2.8)	–	Skin	*Capoeta semifasciolata*	China	Eiras et al. (2005)
M. hochingensis	8.6 (8.4–9.2)	7.4 (7.2–8.4)	6.0 (5.7–6.3)	3.5, 3.3[a]	2.2, 3.1[a]	5–6	Skin, kidneys	*Capoeta semifasciolata*	China	Eiras et al. (2005)
M. liaohoensis	9.9 (9.4–10.8)	7.9 (6.2–8.4)	5.8 (5.5–6.0)	5.1 (4.6–6.0)	3.0 (2.6–3.4)	6–7	Skin	*Capoeta semifasciolata*	China	Eiras et al. (2005)
M. sikiangensis	12.1 (12.0–12.4)	8.4 (7.9–8.0)	7.2	6.0 (5.7–6.2)	2.6 (2.4–2.7)	5–6	Skin	*Capoeta semifasciolata*	China	Eiras et al. (2005)
M. hainanensis	10.4 (10.2–10.8)	7.9 (7.4–8.1)	5.2 (5.0–5.4)	6.0 (5.8–6.2)	2.5 (2.4–2.8)	5–6	Skin	*Capoeta semifasciolata*	China	Eiras et al. (2005)
M. nanhaiensis	13.1 (12.0–13.8)	8.4 (8.0–8.6)	6.0 (5.4–7.0)	4.8 (4.6–5.2)	2.7 (2.4–3.2)	–	Kidneys	*Capoeta semifasciolata*	China	Eiras et al. (2005)

[a]Species has asymmetric polar capsules

DISCUSSION

We have described here a new myxobolid species based on spore morphology and molecular analysis. The classification detailed by Lom & Dyková (1992, 2006) was used as the spores appear to belong to the genus *Myxobolus* (family Myxobolidae). More than 700 species within the genus *Myxobolus* have been described to date (Eiras et al. 2005, Molnár et al. 2011) and a number of these show spore morphology matching that of the *Capoeta tinca* myxozoan of the present study. The synopsis of *Myxobolus* spp. by Eiras et al. (2005) lists different species found in the various organs of *Capoeta* (*Varicorhinus*) spp. in the Caucasus, Russia, Central Asia and China. Moreover, *M. cristatus* Shulman, 1962, *M. suturalis* Shulman, 1962, *M. musajevi* Kardilov, 1963, *M. saidovi* Gasimagomedov, 1970, *M. varicorhini* Dzhalilov & Daniyarov, 1975, *M. molnari* Baska & Masoumian, 1996, *M. mokhayeri* Baska & Masoumian, 1996, and *M. buckei* Longshaw et al. 2003 have been found in *Capoeta* (*Varicorhinus*) spp. in Iran and Central Asia (Shulman 1966, Baska & Masoumian 1996, Eiras et al. 2005, Chamak et al. 2009, Pazooki & Masoumian 2012). No morphologically related myxozoans have previously been reported from Anatolian khramulya. However, measurements of the new *Myxobolus* species spores were compared to data from other gill-infecting *Myxobolus* spp. found in cyprinid hosts (Table 3) as well as from species that were congeners to *M. anatolicus* sp. nov. on the phylogenetic tree.

Due to the great similarity of *Myxobolus* spp. spores, the identification of different species on an exclusively morphological basis is rather difficult. Considering host specificity and location of vegetative developmental stages in organs and tissues is a great help in differentiating morphologically identical or similar spores, but a final identification can only be obtained by studying spores with molecular biological methods (Székely et al. 2009). The importance of the infection site for the phylogenetic cluster-

Table 3. Comparison of spore morphometric data (dimensions in µm) of *Myxobolus anatolicus* sp. nov. with those of other selected *Myxobolus* species infecting cyprinid host gills. Gill-parasitic *Myxobolus* species from cyprinid hosts are close phylogenetic relatives to *M. anatolicus* sp. nov. All measurements are means with range in parentheses

Myxobolus species	Spore			Polar capsule		No. of polar filament coils	Infection site in gill	Original host	Locality	GenBank acc. no.	Reference
	Length	Width	Thickness	Length	Width						
M. anatolicus sp. nov.	10.1 (9.4–10.7)	6.9 (6.6–7.2)	4.5 (4.4–4.6)	4.6 (4.4–4.8)	2.1 (2.0–2.3)	5–6	Lamellae	*Capoeta tinca*	Turkey	KF537629	Present study
M. tambroides	9.9 (8.8–10.6)	7.4 (6.8–7.9)	7.2 (7.0–7.9)	5.7 (5.0–7.0)	2.6 (2.4–2.9)	5–6	Lamellae	*Tor tambroides*	Malaysia	JX028236	Székely et al. (2012)
M. alvarezae	13.6 (12.6–14.7)	6.7 (5.6–8.4)	6.5 (5.9–8.4)	6.8 (6.3–7.7)	2.9 (2.1–4.2)	6	Filaments	*Aspius aspius*	Hungary	FJ716097	Cech et al. (2012)
M. dujardini	11.5 (11.0–12.0)	7.4 (6.5–8.0)	7.1 (6.5–7.3)	5.5 (5.0–6.0)	2.3 (2.0–3.0)	6	Filaments	*Leuciscus cephalus*	Hungary	DQ439804	Molnár et al. (2006)
M. intimus	11.5 (10.8–12.6)	8.4 (7.2–9.0)	7.0 (6.5–7.6)	6.1 (5.8–6.8)	3.3 (3.0–3.6)	6	Lamellae	*Leuciscus idus*	Hungary	FJ716098	Cech et al. (2012)
M. bilobus	21.0 (20.0–22.1)	8.4 (7.5–9.3)	6.0	10.8, 10.1[a]	2.8 (2.4–3.2)	7–9	Filaments	*Notemigonus crysoleucas*	Canada	DQ008579	Cone et al. (2005)
M. branchialis	9.7 (9.0–10.4)	7.6 (7.0–8.6)	6.6 (6.3–7.1)	5.3 (4.9–6.0)	3.0 (2.2–4.1)	6	Lamellae	*Barbus barbus*	Hungary	JQ388887	Molnár et al. (2012)
M. sitjae	12.0 (11.0–13.0)	7.5 (6.8–8.1)	6.7 (6.3–6.9)	6.6 (6.0–7.2)	2.9 (2.3–3.2)	6	Filaments	*Blicca bjoerkna*	Hungary	JF311898	Cech et al. (2012)
M. dykovae	12.0 (11.0–12.7)	6.2 (5.6–6.7)	5.8 (5.7–6.0)	6.0 (5.9–6.1)	2.1 (2.0–2.3)	6–7	Lamellae	*Barbonymus schwanenfeldii*	Malaysia	EU643627	Székely et al. (2009)

[a]Species has asymmetric polar capsules

ing of myxozoans has been recently demonstrated by a number of studies that found stronger linkages between Myxosporea infecting the same target organs as opposed to common host groups or species with similar spore morphology (Eszterbauer 2004, Holzer et al. 2004, Fiala 2006). In the present study, *M. anatolicus* sp. nov. fell within a well-supported clade of *Myxobolus* spp. that infects the gills of various cyprinid fishes from North America or Eurasia (Fig. 4).

Molnár (2002) described the site preference of myxosporeans in the gills as lamellar- or filamental-type infections. In the present study, *M. anatolicus* sp. nov. was found to develop inside the intralamellar-vascular location.

Although *M. exiguus*, *M. muelleri*, *M. episquamalis* and *M. ichkeulensis* have been found in wild mullet in Turkey, these 4 species have only been morphologically identified in *Mugil cephalus* (Altunel 1983, Umur et al. 2010, Ozak et al. 2012). Therefore, this is the first molecular study of a myxosporean parasite in a Turkish freshwater fish, which renders the comparison of molecular results with *Myxobolus* spp. from this region impossible. However, the BLAST search using the partial 18S rDNA sequence of the *Myxobolus* species parasitic in *C. tinca* did not match any of the myxozoans available in GenBank. Thus, the *Myxobolus* species studied here differs in morphological aspects from other gill-parasitic *Myxobolus* species and differs on the molecular level from *Myxobolus* species reported in GenBank. It can therefore be considered a new species, for which the name *M. anatolicus* sp. nov. is proposed.

The distribution and prevalence of this myxobolid species around Turkey and in other regions where the host is found are as yet unknown. Further studies are needed to determine the current distribution and population status, and the ecological interactions that *Myxobolus* species may have with native hosts of Turkish rivers and streams.

Acknowledgements. The authors thank Dr. Didem Pekmezci for her valuable help in preparing the manuscript. We also thank Dr. Didem Pekmezci and Dr. Cenk Bolukbas for preparing drawings.

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Editorial responsibility: Dieter Steinhagen, Hannover, Germany

Submitted: November 20, 2013; Accepted: March 17, 2014
Proofs received from author(s): June 11, 2014