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ORIGINAL ARTICLE

Common dace (*Leuciscus leuciscus*) – A new host of the myxozoan fish parasite, *Myxobolus elegans* (Cnidaria: Myxozoa) – Short communication

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ABSTRACT

This paper reports the detection of the myxozoan species *Myxobolus elegans* Kashkovsky 1966 in common dace (*Leuciscus leuciscus*) that has not been previously listed as its host. The problem of differentiation of phenotypically similar *Myxobolus* species is addressed. During parasitological survey of common dace from the desalinated part of the Gulf of Finland at the city of Sestroretsk, Russia, numerous oval-shaped plasmodia, 0.2–0.4 mm in size, filled with *Myxobolus* spores were found on the gills. Pear-shaped myxospores were 15.4 (14.8–16.0) × 10.2 (9.6–10.9) μm in size with a rib on each valve. On the basis of spore morphology, the species appeared to be similar to *M. elegans* and *Myxobolus hungaricus* Jaczó, 1940. In order to identify the species, molecular genetic analysis was performed, and the species was identified on the basis of morphological characteristics and 18S rDNA data. The results obtained indicate that the *Myxobolus* species observed on the gills of dace is *M. elegans*. Thus, common dace is another valid host of *M. elegans* besides the type host, ide (*Leuciscus idus*).

KEYWORDS

Myxobolus elegans, *Leuciscus leuciscus*, Baltic Sea, morphotype, 18S rDNA

Members of the genus *Myxobolus* (Cnidaria: Myxozoa) are among the most widespread endoparasites of fish. Over 800 valid species of this genus are known, and the number of new species is increasing constantly (Eiras et al., 2005, 2014). The differentiation of *Myxobolus* species is based on spore morphology, host specificity and tissue tropism. Due to the high degree of morphological similarity, species identification is completed by the use of molecular methods – revealing differences in nucleotide sequences such as 18S rRNA genes (Molnár, 1994; Eszterbauer, 2002, 2004; Kallert et al., 2005; Molnár et al., 2010; Borzák et al., 2018; Sokolov and Lebedeva, 2018). We performed an ichthyoparasitological study of common dace (*Leuciscus leuciscus*) caught in the freshened part of the Gulf of Finland near the city of Sestroretsk (60°05′11.7″N 29°55′31.9″E) in April 2015. On the gills of one fish specimen, multiple oval plasmodia 0.2–0.4 mm in size, containing spores of *Myxobolus*-like shape, were found. Temporary and glycerol-gelatin preparations for microscopy were made from the detected myxospores. Plasmodia were fixed in 96% ethanol for subsequent molecular analysis. Genomic DNA (gDNA) was extracted using a 100x DNA extraction kit (Biosilika LLC, Russia) following the manufacturer's manual.

Nested PCR assay was used to amplify the 18S rDNA of the parasite, with the pair of universal primers 18e (5′ CTG GTT GAT TCT GCC AGT 3′) and 18g (5′ CGG TAC TAG CGA CGG GCG GTG TG 3′ (Hillis and Dixon, 1991) in the first PCR round, and the primer pair of Myx1F (5′ GTG AGA CTG CGG ACG GCT CAG 3′) and Myx4R

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(5′ CTG ACA GAT CAC TCC ACG AAC 3′) in the second round (Hallett and Diamant, 2001).

The PCR reaction mixture of 20 μL volume contained (final concentrations are given): 1 × Taq buffer (SibEnzyme Ltd, Russia), 1.5 mM $MgCl_2$, 250 μM of each deoxyribonucleotide, 200 nM of forward and reverse primers, 1 unit of Taq DNA-polymerase (SibEnzyme Ltd, Russia) and 100 ng of genomic DNA. The amplification program consisted of the following stages: DNA denaturation at 94 °C for 2 min, 36 cycles including denaturation at 94 °C for 30 sec, primer annealing at 54 °C for 20 sec and elongation at 72 °C for 30 sec.

Agarose gel electrophoresis was performed in 1% agarose gel containing 0.1% ethidium bromide in Tris-acetate buffer. Before DNA sequencing, the PCR products were purified on spin columns using Sephadex G-50 Superfine sorbent (GE Healthcare, USA).

Sanger sequencing was performed using Big Dye terminator cycle sequencing kit v.3.1 (Applied Biosystems, Thermo Fisher Scientific, USA) using the primers 18e, 18g, Myx1F and Myx4R. Sanger reaction products were analysed using ABI 3130XL Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA).

Nucleotide sequence polymorphism was analysed using MEGA6 software (Tamura et al., 2013). The degree of nucleotide sequence differences was evaluated by calculating the p-distance; a JC model of nucleotide substitutes selected during the model testing was used (Jukes and Cantor, 1969). The partial 18S rDNA sequence was deposited to GenBank (NCBI) under the accession number MH069207.

The myxospores found were pyriform, 15.4 (14.8–16.0) × 10.2 (9.6–10.9) μm in size (Fig. 1), and detailed inspection showed that each valve possessed a ridge (Fig. 1, inset). On the basis of spore morphology the species at hand was identified as *Myxobolus elegans* Kashkovsky 1966.

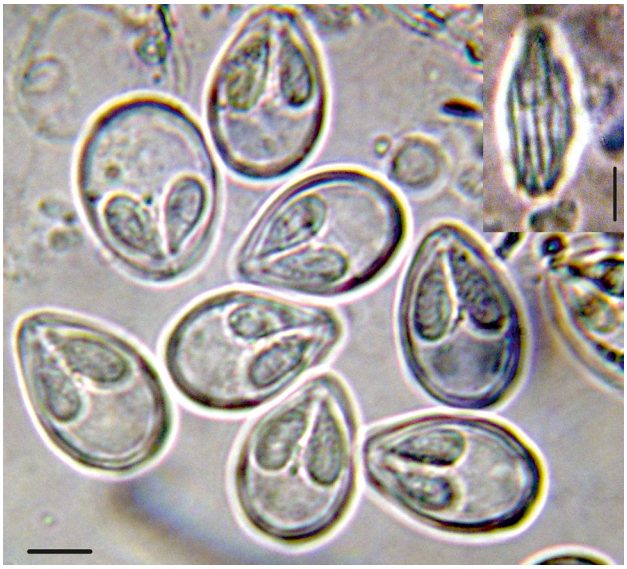

Figure 1. Myxospores of *Myxobolus elegans* from common dace (*Leuciscus leuciscus*), in frontal view. Native preparation. Scale bar: 5 μm. Inset: myxospore in side view. Scale bar: 5 μm

According to the original description (by Kashkovsky, referred in Shulman, 1966), *M. elegans* was found on the gill arches, gills and fins of two fish species: ide, *Leuciscus idus* (26.2% occurrence) and roach, *Rutilus rutilus* (1.7% occurrence) from the Iriklinskoe Reservoir (the Ural River) and the Chusovaya River (the Volga River basin). They found that ide was the most intensively infested species, with an average of 14 plasmodia per fish. The plasmodia of *M. elegans* were rounded or oval in shape, 0.17–0.34 mm long by 0.23–0.55 mm wide, and encircled by a thin connective tissue envelope. The myxospores were ovoid in shape, narrowing towards the anterior end. The sutural edge was slightly protruded above the spore surface. Each valve possessed one ridge located near the sutural edge, almost parallel to it. The myxospores were 13.5–17 μm long, 7.4–10 μm wide and 7.4–8 thick. Polar capsules were pyriform and uniform in size. They were 6.8–8 μm long and 2.5–3.5 μm wide. The shape and morphometrics of *M. elegans* detected in the present study were similar to those in the original description.

The spore morphology of *M. elegans* is similar to that of *Myxobolus hungaricus* Jaczó, 1940. Re-description of *M. hungaricus* found in common bream *Abramis brama* (Hungary, Lake Balaton and the River Danube) showed that mature plasmodia were rounded or elliptical in shape, reaching 0.35–0.45 × 0.17–0.35 mm in size (Baska and Molnár, 1999). The myxospores were symmetric with a distinct ridge parallel to the valve, averaging 11.9 × 7.0 μm. The polar capsules were similar in size and shape. Differentiation of *M. hungaricus* and *M. elegans* spores was found to be difficult as they were morphologically similar although slightly different in size (Baska and Molnár, 1999). Currently, the identification of morphologically indistinguishable *Myxobolus* species is based on the 18S rDNA (Molnár et al., 2010; Cech et al., 2012). The two *Myxobolus* species, *M. hungaricus* and *M. elegans*, could be easily distinguished using the PCR-related restriction fragment length polymorphism (RFLP) technique (Eszterbauer et al., 2001; Eszterbauer, 2002).

We have performed DNA sequencing of the myxozoan found in common dace in order to confirm the identification of *M. elegans*. A 522-bp-long 18S rDNA fragment of *M. elegans* was analysed. The sequenced fragment was located in the region between nucleotide positions 40 and 561 of *M. elegans* sequences available in GenBank (AF448445, JN252485). BLAST search revealed the highest similarity to *M. elegans* sequences (99.43–99.81%). When comparing the obtained *M. elegans* DNA sequence with the ones in GenBank (AF448445, JN252485), the p-distance varies from 0.2 to 0.6%. Comparison of the obtained *M. elegans* DNA sequence to the 18S rDNA of *M. hungaricus* (AF448444) showed a p-distance of 60.9%. The p-distance between *M. elegans* (AF448445) and *M. hungaricus* (AF448444) was 61.3%, while that between the studied *M. elegans* (JN252485) and *M. hungaricus* (AF448444) was 61.7%.

Therefore, the obtained results proved that the myxozoan found in common dace was indeed *M. elegans*. Furthermore, we confirmed that common dace is another

valid host of *M. elegans* besides the type host, ide. According to Shulman (1966), *M. elegans* has been found in the following hosts: roach (*Rutilus rutilus*), ide (*Leuciscus idus*), common bream (*Abramis brama*), sichel (*Pelecus cultratus*), asp (*Leuciscus aspius*) and lake minnow (*Rhynchocypris percnurus*) (Bauer, 1984). Ide and common dace represent the same genus, while all other fish belong to other cyprinid genera. It is possible that common bream caught in Russia was in fact infested with *M. hungaricus,* and the parasite was misidentified as *M. elegans*. Thus, we assume that the host range of *M. elegans* described so far might contain invalid hosts, and additional studies are required for clarification.

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