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MOLECULAR BIOLOGY CAN DIFFERENTIATE MORPHOLOGICALLY INDISTINGUISHABLE MYXOSPOREAN SPECIES: *Myxobolus elegans* AND *M. hungaricus* (SHORT COMMUNICATION)

Edit ESZTERBAUER*

Veterinary Medical Research Institute, Hungarian Academy of Sciences,
H-1581 Budapest, P.O. Box 18, Hungary

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Two, morphologically indistinguishable myxosporean species, *Myxobolus elegans* Kashkovsky, 1966 and *M. hungaricus* Jaczó, 1940 were differentiated using molecular biological methods. Polymerase chain reaction (PCR) with primers specific for the family Myxobolidae was used to amplify an approximately 1600 base pairs (bp) long fragment of the 18S ribosomal RNA gene. In restriction fragment length polymorphism (RFLP) study with *Hin*fI, *Msp*I and *Taq*I enzymes, the two parasite species were easily distinguishable. The genetic distinctness was also confirmed by the DNA sequence of their PCR products. Although *M. elegans* and *M. hungaricus* are morphologically very similar, based on the results of the PCR-RFLP and the DNA sequences, we concluded that they are valid species.

Key words: Myxosporeans, *Myxobolus elegans, M. hungaricus,* 18S rRNA gene, PCR, RFLP, *Leuciscus idus, Abramis brama*

The members of genus *Myxobolus* are among the most common endoparasites of fish. To date, approximately 500 valid species are known, of which 444 species were recorded by Landsberg and Lom (1991). The differentiation among *Myxobolus* species is based primarily on phenotypic features including morphological characteristics of the myxospores, host specificity and tissue tropism (Molnár, 1994). In several cases, however, precise identification of the species is rather difficult because of the high degree of similarity in spore morphology and the shape differences existing within a given species. Nowadays molecular biological methods offer a useful opportunity to complete the identification procedure of myxosporeans. Several reports have been published on the molecular characterisation of Myxosporea species using the 18S ribosomal RNA gene sequences (see Kent et al., 2001). Restriction fragment length polymorphism (RFLP) has also been found feasible for identification of myxosporean species (Xiao and Desser, 2000; Eszterbauer et al., 2001).

*E-mail: eedit@vmri.hu; Fax: +36 (1) 252-1069

Myxobolus elegans Kashkovsky, 1966 was originally described from the gills of orfe (*Leuciscus idus*) and roach (*Rutilus rutilus*) from the rivers Volga and Ural and until now, there was no evidence for its occurrence in Hungary. Nonetheless, the phenotypic features of *M. elegans* were very similar to those of *M. hungaricus* Jaczó, 1940 described from common bream (*Abramis brama* L.) (Molnár and Baska, 1999; Molnár and Székely, 1999). Both species have a pair of unique ribs on the surface of the spore valves running parallel to the suture, but the spores of *M. elegans* are slightly larger than those of *M. hungaricus* (Fig. 1). The purpose of this study was to prove the validity of these two, morphologically very similar species, *M. elegans* and *M. hungaricus* with the use of a combined PCR-RFLP method, and by comparing 18S rDNA sequences.

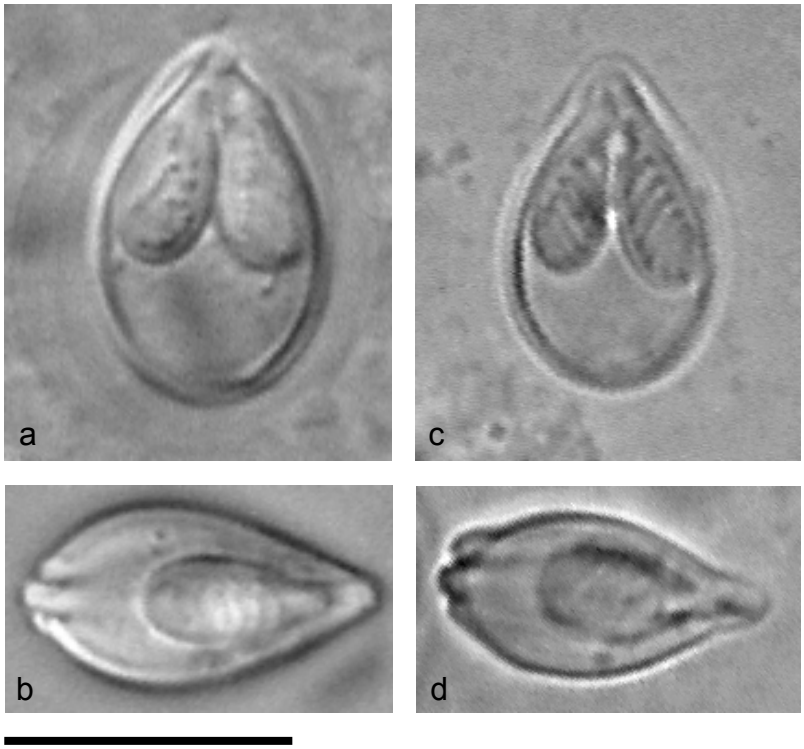

Fig. 1. Myxospores of intracellular *Myxobolus* spp. collected from gills of cyprinid fishes: a-b: *M. elegans* collected from orfe (*Leuciscus idus*), c-d: *M. hungaricus* from common bream (*Abramis brama*). Fresh preparations. Scale bar is equal to 10 µm

Myxospores of *Myxobolus elegans* (Fig. 1 a-b) were collected from orfe in the River Danube, while *M. hungaricus* spores (Fig. 1 c-d) were obtained from common bream in the River Danube and Lake Balaton. Two samples were ex-

amined from each parasite species. The number of spores varied between 10^3 and 10^5. The DNA of spores was extracted as described before by Eszterbauer et al. (2001). Primers (MX5 and MX3) specific for the family Myxobolidae (Andree et al., 1999) were used for PCR amplification of an approx. 1600 bp fragment of the 18S rRNA gene. The conditions of the PCR were published before (Eszterbauer et al., 2001). Three frequent-cutter restriction endonucleases, *Taq*I, *Hin*fI and *Msp*I were applied in the RFLP. Two or three µl of the PCR products were digested in a 20-µl reaction mixture containing 10 U of enzymes (MBI Fermentase, Lithuania). Following 2 h incubation at 37 °C with *Hin*fI and *Msp*I, or at 65 °C in the case of *Taq*I, the digested products were electrophoresed on a 1.5% agarose gel containing 0.1% ethidium bromide.

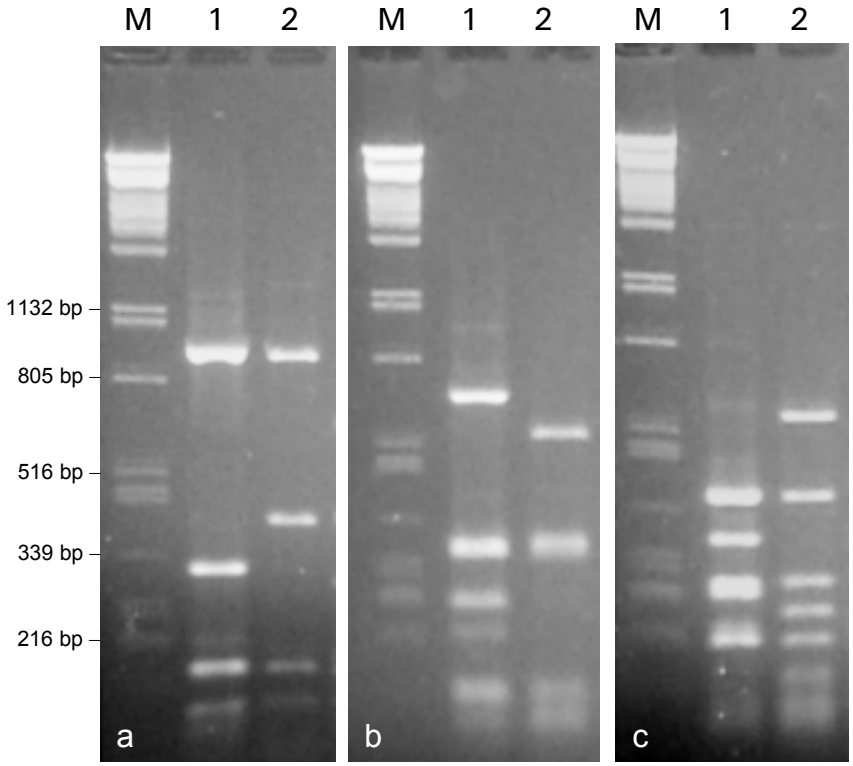

Fig. 2. RFLP patterns of the amplicons digested with (a) *Hin*fI, (b) *Msp*I, and (c) *Taq*I restriction endonucleases. Lane 1: *Myxobolus elegans*; lane 2: *M. hungaricus* and lane M: molecular weight marker

In the PCR, all of the four samples gave an approx. 1600 bp fragment of the 18S rDNA. By direct DNA sequencing, the exact size of the fragments was determined to be 1610 base pairs in *M. elegans* and 1553 bp in the case of *M. hungaricus*. The nucleotide sequence identity was calculated by distance matrix

analysis and was found to be 79.9%. The DNA sequences of the PCR products had been deposited in GenBank. Accession numbers are the following: *Myxobolus elegans* AF448444, *M. hungaricus* AF448445. The restriction fragment patterns of the PCR products generated by *Taq*I, *Hin*fI or *Msp*I enzymes are presented in Fig. 2 a-c. It can be seen that each pattern was characteristic even alone and the patterns were easily distinguishable from each other.

Based on the results of the PCR-RFLP and sequences, *M. elegans* and *M. hungaricus* are proposed to be valid species. As exemplified by the present study, the molecular biological approach may have as great importance in parasitology as in virology, bacteriology or other fields of biology. In several cases the classical taxonomy based on phenotypic features is not able to differentiate parasite species, especially the morphologically very similar Myxosporea species. However, this problem can be solved with the help of molecular biological methods, and thus the phenotypic and the genetic features together can ensure the exact identification of species.

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