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FISH PARASITOLOGY - SHORT COMMUNICATION

Myxobolus pelecicola Voronin et Dudin 2015 is a junior synonym of *Myxobolus ladogensis* Rumyantsev et Schulman 1997 (Myxosporea: Myxobolidae) infecting the skeletal muscle of sichel *Pelecus cultratus* (Actinopterygii: Cyprinidae) in Russia

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Abstract
Myxobolus pelecicola Voronin et Dudin, 2015 was recently described from the skeletal musculature of sichel *Pelecus cultratus*. However, another species, *Myxobolus ladogensis* Rumyantsev et Schulman, 1997, was described previously from the same host, displaying identical tissue localization and spore morphology as in *M. pelecicola*. Unfortunately, *M. ladogensis* was overlooked when *M. pelecicola* was described, resulting in the superfluous description of the latter species, which, according to the International Code of Zoological Nomenclature, is a junior synonym of *M. ladogensis*. The description of *M. ladogensis* is supplemented with SSU rDNA sequence analysis supporting the conspecificity with *M. pelecicola.* The closest relatives of *Myxobolus ladogensis* (syn. *M. pelecicola*) include several muscle-infecting *Myxobolus* spp. with sequence similarity below 97%.

Keywords Myxozoa · Morphology · 18S rDNA · Molecular phylogenetics · Taxonomy

Introduction

Myxobolus Bütschli 1882 is the most species-abundant genus among the myxozoans with more than 800 species described worldwide (Eiras et al. 2005, 2014). The traditional classification of myxosporeans is based on spore morphology; however, this has been drastically challenged by molecular phylogenetics based on small subunit (SSU) ribosomal DNA (rDNA) sequence analysis (Fiala et al. 2015). Molecular data are of great importance nowadays for the description of new species and revision of previously described taxa, especially for those described using a limited set of morphological data primarily using light microscopy, which is the case for most *Myxobolus* species and myxozoans in general. Being able to discriminate similar species is of critical importance. For example, SSU rDNA sequencing allowed discrimination of a new species, *Myxobolus turpisrotundus* (Zhang et al. 2010), initially described as *Myxobolus rotundus* from several hosts in China (Chen and Ma 1998), although both species are morphologically very similar. Conversely, a myxosporean species identified as *Myxobolus dispar* from the gill filaments of the common carp *Cyprinus carpio* in China was later diagnosed as *Myxobolus musseliusae* based on the 100% sequence identity (Liu et al. 2013). Two isolates of *Myxobolus arcticus* from Japan and Canada showing distinct morphological features and different invertebrate hosts were found to be conspecific as evidenced by 99.9% 18S rDNA sequence similarity (Urawa et al. 2011). Moreover, the diagnosis of the genus *Sphaerospora* (Myxosporea: Sphaerosporidae) was emended with molecular genetic characteristics (Jirků et al. 2007).

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Availability of rDNA sequences is essential for examination of myxosporean life cycles and parasite detection at early phases of development (Eszterbauer et al. 2015).

High diversity of fish myxosporeans has been reported in Russia (Donec and Shulman 1984), though morphological data available for some species are scarce, sometimes including line drawings only. *Myxobolus pelecicola* Voronin et Dudin, 2015 was recently reported from the skeletal musculature of sichel collected from Finnish Bay of Baltic Sea (Voronin and Dudin 2015). In the present paper, we demonstrate this taxon name to be a junior synonym of *Myxobolus ladogensis* Rumyantsev et Schulman, 1997 (which was overlooked when *M. pelecicola* was described) and provide molecular phylogenetic data for this species.

Materials and methods

The material analyzed in the present work originates from the previously published description of *M. pelecicola* (Voronin and Dudin 2015). Here, we briefly describe the material collection and morphological data analysis because the original paper is of limited availability being written in Russian.

Adult fishes *Pelecus cultratus* (L.) of varied size were collected from Finnish Bay (60° 05′ N, 29° 55′ E) and Ladoga Lake (60° 07′ N, 32° 19′ E) during 2010–2014 and transported to the laboratory within cooled plastic bags. Fishes were dissected and a standard examination of myxosporean infection was performed based on Lom and Arthur (1989). Pseudocysts were used to make smear preparations for identification using light microscopy. Infected host tissues were fixed with 95% ethanol for genomic extraction. Prevalence of myxosporean infection was 86.3% ($N = 22$) and 77.7% ($N = 18$) for sichel from Finnish Bay and Ladoga Lake, respectively. Examination of the parasite morphology using light microscopy confirmed its attribution to the species *M. pelecicola* from skeletal musculature of *P. cultratus* (Voronin and Dudin 2015). The spores were elongated oval or oval. The polar capsules were pyriform, unequal in size. Each capsule contained 5–6 distinct filament coils oriented obliquely to the longer axis of the capsule. The mucous envelope was absent. There were no differences in spore shape and size between the two sampling points.

For molecular phylogenetic studies, the fixed samples were centrifuged at 8000*g* for 10 min to pellet the myxospores and remove the ethanol remnants. Genomic DNA was extracted using the Qiagen DNeasy Blood &Tissue Kit (Qiagen, Germany) and eluted in 100 μl of AE buffer. A primer pair of MyxospecF 5′-TTCTGCCCTATCAACTWGTTG-3′ (Fiala 2006) and 18R 5’-CTACGGAAACCTTGTTACG-3' (Whipps et al. 2003) was used to amply the partial SSU rDNA sequence. The reaction system (50 μl) contained 200 ng template DNA, 1× PCR mixture (CWbiotech, China), and 20 pmol of each primer. The PCR program was as follows: 94 °C for 30 s, 46 °C for 30 s, and 65 °C for 70 s for 35 cycles after initial denaturation at 94 °C for 5 min and terminating by an extension period at 65 °C for 10 min. The PCR products were separated by electrophoresis in 1% agarose gel in TBE buffer stained with 1% ethidium bromide and then purified with a PCR purification kit (CWbiotech, China). The purified products were then cloned into PMD-18T vector system (Takara, Japan) and then sequenced in both directions using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyzer automated DNA sequencer.

Two replicates of myxosporean samples collected in different year (2013 and 2014) were sequenced (one clone per sample) to compare the possible sequence variation. The resulting sequences were homology searched by BLAST to find the related species with high sequence similarity and submitted to GenBank with accession numbers KP241961 and KU160629. Sequences of *Myxobolus* spp. and *Dicauda atherinoi* (outgroup) were retrieved from GenBank and aligned using the CLUSTAL W algorithm in BioEdit (Hall 1999). Ends were trimmed and ambiguous sites were removed manually. An optimal evolutionary model for sequence change was determined in jModelTest (Posada 2008) using Akaike information criteria to be the general time reversible model with a proportion of invariable sites and gamma distributed rate variation among sites (GTR+I+G). A maximum likelihood approach using this model was applied with 100 bootstrap replicates in raxmlGUI v. 1.5 (Silvestro and Michalak 2012).

Results

Partial sequences of SSU rDNA were obtained for the specimens collected in 2013 (1714 bp) and 2014 (1714 bp) and showed 99.4% similarity to each other. The sequence similarity and genetic distance between the isolates genotyped in the present study and isolates of *Myxobolus pseudodispar* ranged from 0.031 to 0.054 and from 94.6 to 97.9%. The closest relatives were the *M. pseudodispar* isolates from roach, *Rutilus rutilus* (AF380145) and rudd, *Scardinius erythrophthalmus* (AF380142). Additionally, 99.8% sequence similarity was found between *M. ladogensis* and a triactinomyxon (DQ231145) from *Tubifex tubifex* (Oligochaeta: Naididae) in Hungary (Eszterbauer et al. 2006). Bayesian inference showed a monophyletic position with 100% branch support of all muscle-infecting *Myxobolus* species with *Myxobolus stanlii* infecting the connective tissue and muscle of largescale stoneroller, *Campostoma oligolepis* in a basal position (Iwanowicz et al. 2013). *Myxobolus ladogensis* formed a sister relationship with *M. pseudodispar* within the clade of the muscle-infecting species (Fig. 1).

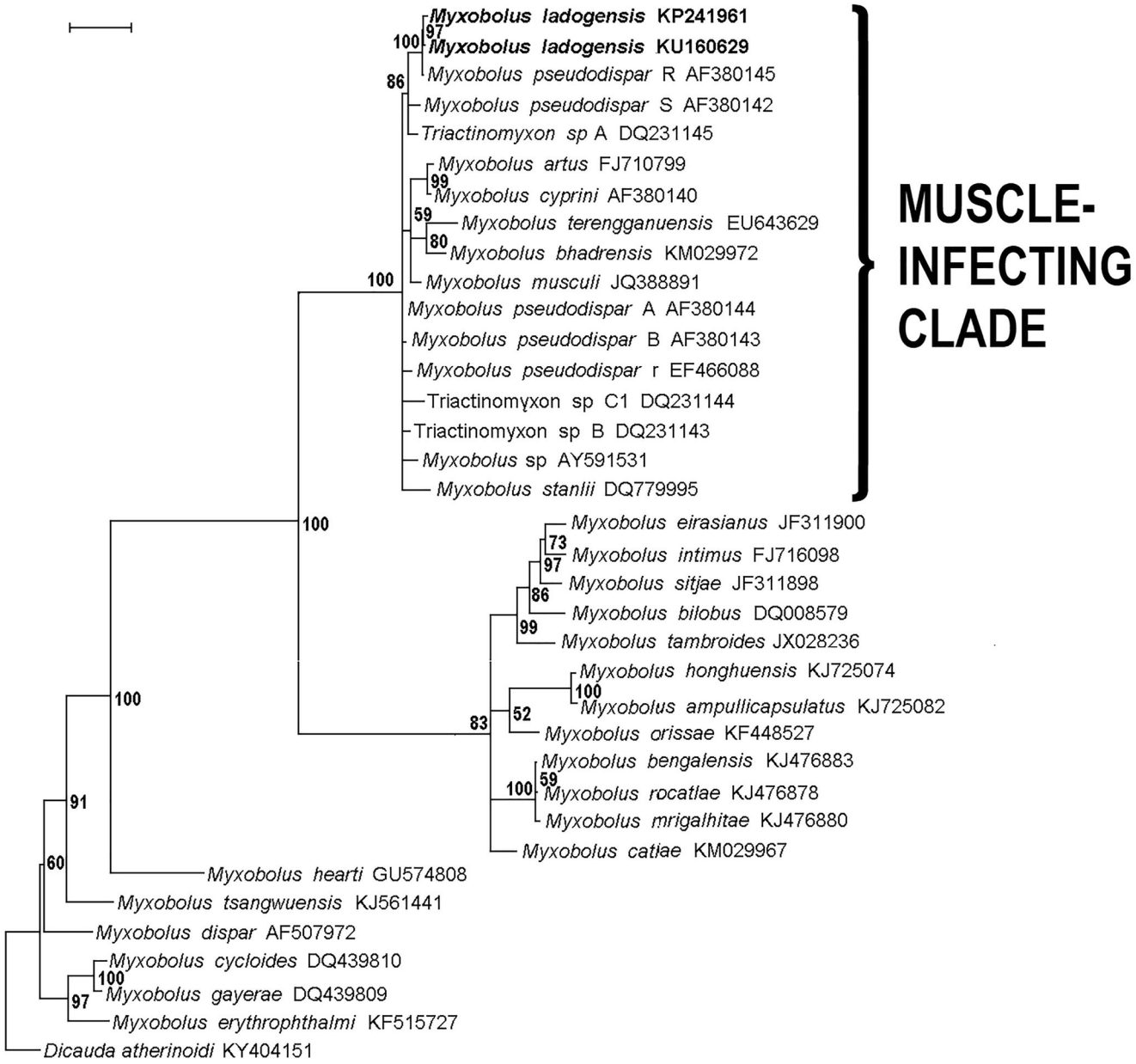

Fig. 1 Phylogenetic tree based on the partial SSU rDNA sequences, generated by Maximum Likelihood approach using GTR+I+G model (see "Materials and methods"), showing the phylogenetic position of *Myxobolus ladogensis (*syn. *Myxobolus pelecicola)* within the muscle-infecting *Myxobolus* clade from fish of Cypriniformes. Genbank accession numbers are given after species names. Numbers next to the nodes indicate bootstrap support. Scale bar is 0.1 expected nucleotide changes per site

Discussion

Myxobolus is one of the most extensively studied myxozoan genera. These parasites can be found in every organ of a fish and some of them have been known to cause serious disease in both wild and cultured species, including *Myxobolus cerebralis* in salmonids (Halliday 1976) and *Myxobolus ampullicapsulatus* in allogynogenetic gibel carp (Xi et al. 2011). Simplicity of morphological features of myxospores as the most important taxonomic criteria makes the classification and identification of highly diversified myxosporeans challenging (Fiala et al. 2015). Variation of morphological characteristics of a given myxozoan due to difference of host and infection sites, as well as environmental factors, has frequently resulted in incorrect identification for some morphologically similar species (Urawa et al. 2011). Furthermore, *Myxobolus* species with *Henneguya*-like spores have been increasingly reported which also challenge the validity of discrimination of these two species-abundant genera among myxozoans (Liu et al. 2010). So, the role of spore morphology in the classification of myxozoa at both species and genus level warrants cautious treatment.

Table 1 Spore measurements of *Myxobolus ladogensis* and *Myxobolus pelecicola* from *Pelecus cultratus*

Parasite species	Spore length (μm)	Spore width (μm)	Spore thickness (μm)	Polar capsule length (μm)		Polar capsule width (μm)		Reference
				Large	Small	Large	Small	
M. ladogensis	14.5–18.0 (16.0)[a]	11–14	7.2-8.4	7.2–9.5	6.0–8.5	4.0–5.3	3.6–4.5	Rumyantsev and Schulman (1997)
M. pelecicola	14.9–18.0 (16.5)	11.6–13.3 (12.1)	7.4–8.3 (7.8)	7.5–8.8 (8.0)	7.2–7.8 (7.6)	4.2–4.7 (4.5)	3.8–4.3 (4.0)	Voronin and Dudin (2015)

[a] Average meaning is given in parentheses (where available)

Moreover, erroneous descriptions may result from incomplete information in taxonomic summaries. Unfortunately, two editions of the synopsis of the species of *Myxobolus* Bütschli, 1882 were missing a species of *Myxobolus* infecting musculature of sichel, *M. ladogensis* (Eiras et al. 2005, 2014). Voronin and Dudin (2015) also missed this species and superfluously described *M. pelecicola* which we contend is conspecific with *M. ladogensis*. A comparison of morphological and morphometric data (Table 1) of *M. pelecicola* to *M. ladogensis* described from the same host with identical tissue localization shows notable overlap in spore dimensions. We therefore suggest they are the same species. According to the International Code of Zoological Nomenclature, *M. pelecicola* is a junior synonym of *M. ladogensis*.

Both *M. ladogensis* and *M. pelecicola* were reported with complete morphological and morphometric data, but DNA sequence information was unavailable at the time of both original descriptions. In the present work, the partial sequence of SSU rDNA of *M. ladogensis* (syn. *M. pelecicola*) was obtained and proved to be dissimilar from any available myxosporean species in Genbank. Phylogenetic analysis for muscle-infecting species from Cypriniformes produced results congruent with previous reports (Iwanowicz et al. 2013), showing a monophyly of all muscle-infecting species collected from different subfamily of Cypriniformes. This observation corresponds to the conclusion of relative importance of tissue specificity as an evolutionary feature at family level (Molnár et al. 2002). Two isolates of *M. ladogensis* formed an independent evolutionary branch with high support value confirming it as a valid species within the muscle-infecting clade.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The biological material examined in the present study was obtained in accordance with Guidelines for the Use of Fishes in Research (AFS 2014).

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