

Morphological and molecular characterizations of *Myxobolus curriculum* sp. nov. (Myxozoa, Myxobolidae) infecting the intestine of Barbel chub *Squaliobarbus curriculum* in China

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Abstract

During a myxosporean biodiversity survey in Hunan province, a novel *Myxobolus* species was found in the intestinal wall of *Squaliobarbus curriculum*. Infection was evident as numerous whitish plasmodia distributed throughout the intestinal wall, with a prevalence of 32.35% (11/34). Mature myxospores were round in the valvular view, and lenticular in the sutural view, measuring 8.8 ± 0.2 (7.8–9.4) μm long, 8.3 ± 0.3 (7.5–9.3) μm wide, and 3.8 ± 0.3 (3.5–4.2) μm thick. Two pyriform polar capsules of equal size located at the anterior end, and measured 3.8 ± 0.2 (3.5–4.3) μm long and 2.4 ± 0.3 (2.2–3.0) μm wide. The polar tubules were coiled with four to five turns. A mucous envelope and intercapsular appendix were absent, but three to four distinct posterior folds were present. Histopathological examination indicated that plasmodia developed within the circular muscle layer of the host intestine, surrounded by a thin connective tissue capsule, and elicited no observable inflammatory response. BLASTn analysis revealed that the SSU rDNA sequence was unique, showing the highest similarity (93.02%) to *Myxobolus algonquinensis* Xiao & Dessler, 1997 (AF378335), a parasite of the ovary of *Notropis cornutus*. Phylogenetic construction yielded two primary clades (A and B). Within Clade A, the novel species formed a distinct sister lineage with *M. dabryi*, *M. abitus*, and *M. tauricus* in the subclade III, rather than clustering with other *Myxobolus* species known to infect intestine.

Key Words

China, intestine, Myxozoa, *Myxobolus curriculum* sp. nov., *Squaliobarbus curriculum*

Introduction

The genus *Myxobolus* Büschusi, 1882 represents the most speciose lineage within the Myxozoa (Cnidaria), with more than 1000 species reported globally (Eiras et al. 2005, 2014, 2021; Whipps et al. 2026; Vieira et al. 2024). It primarily infects fish, with target tissues and organs including gill, intestine, skin, and liver (Chen and Ma 1998; Fiala et al. 2015). The pathogenicity of *Myxobolus* species varies greatly; many are considered non-pathogenic. How-

ever, several notable exceptions cause severe diseases with significant economic impacts, such as *Myxobolus cerebralis* Hofer, 1903, the causative agent of whirling disease in rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 (Bartholomew et al. 2022), *Myxobolus honghuensis* Liu et al., 2012 causing pharyngeal myxosporidiosis in *Carassius auratus gibelio* Bloch, 1782 (Liu et al. 2012), *Myxobolus wulii* Lansberg & Lom, 1991 (the etiology of liver myxosporidiosis) from the *C. auratus gibelio* Bloch (Zhang et al. 2010), and *Myxobolus drjagini* Akherov, 1954 (the etiology of

ology of twist disease) infecting the cranial cavity of silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) can cause large-scale fish mortality (Xi et al. 2019).

In China, more than 400 *Myxobolus* species have been reported (Chen and Ma 1998; Zhang 2023). Many of these species were documented in the last century primarily based on morphological measurements and schematic drawings (Chen and Ma 1998), which may have led to reports of the same nominal species from multiple hosts and tissues. Although host specificity and tissue tropism are valuable for distinguishing species with different hosts or parasitic sites, they are often insufficient for delineating morphologically similar species infecting the same host and site (e.g., *M. musseliousae* and *M. dispar* in the gills of common carp) (Liu et al. 2013). The integration of molecular data has proven crucial in resolving such complexes, enabling the reclassification of cryptic species (Zhang et al. 2010; Liu et al. 2013). Therefore, the current classification of Myxosporea adopts an integrative identification approach combining morphology, parasitic characteristics, and molecular data, which can efficiently discover new species and clarify cryptic species.

Hunan Province, with its abundant aquatic resources and habitats, includes major river systems (the Xiang, Zi, Yuan, and Li Rivers) and Dongting Lake and hosts a rich ichthyofauna of 218 documented species (Wu et al. 2021). To our knowledge, despite this diversity, surveys of fish-infecting myxosporeans in this region remain limited and outdated, with only two studies conducted to date (Jin et al. 1993; Liu 2015). These efforts are either temporally obsolete (carried out in the last century) or taxonomically narrow (covering twelve fish species), failing to accurately reflect the true diversity of this parasite group in Hunan.

The barbel chub *Squaliobarbus curriculum* Richardson, 1846 (Cyprinidae), is a widely distributed species of growing aquacultural interest due to its disease resistance, including potential resistance to grass carp hemorrhage disease (Jin et al. 2018; Zheng et al. 2024). Despite its ecological and economic relevance, myxosporean infections in this host are poorly documented, with only approximately 11 species reported globally including 2 *Myxidium* spp., 7 *Myxobolus* spp., and 2 *Thelohanellus* spp. (Chen and Ma 1998). As part of an ongoing survey of myxosporean diversity in Hunan Province, a *Myxobolus* infection was detected in the intestinal wall of *S. curriculum*. By synthesizing evidence from morphology, histopathology, site specificity, and molecular phylogeny, we identify this isolate as a novel species, *Myxobolus curriculum* sp. nov. This discovery not only enriches the known myxosporean fauna of Hunan Province but also establishes the first record of any myxosporean parasite in *S. curriculum* from this region.

Material and methods

Fish sampling and morphological observation

A parasitological survey of natural fish was carried out from June to December 2024 in Zi River (27°43'46.8"N,

111°19'18.9"E), Xinhua County, Loudi City, Hunan Province, China. Thirty-four specimens of Barbel chub *S. curriculum*, with body weights ranging from 316.5 g to 498.5 g, were collected by gill-nets. Fish were isolated from the nets and conducted immediate examination upon arrival at the local laboratory. Each specimen was subjected to a comprehensive examination. First, external surfaces (skin, fins, gills) were visually inspected, and scrutinized under a Motic SMZ-161 stereo microscope (Motic, China) for the presence of suspected plasmodia. Subsequently, internal organs (liver, intestine, spleen, kidney) were inspected macroscopically and microscopically during dissection. Suspected plasmodia were excised, and wet mounts were prepared for confirmation of myxosporean infection with a microscope of Olympus BX53 (Olympus, Japan). For preservation, infected tissues and isolated plasmodia were fixed in 70% ethanol and 10% neutral buffered formalin. An imaging system consisting of an Olympus BX53 microscope and an MSX 11 digital camera (Mingmei, China) was employed to capture micrographs of fresh spores. Morphometric data for plasmodia and spores were obtained from these digital images following Lom and Arthur (1989). Thirty mature spores were randomly measured. Schematic drawings were prepared from micrographs using CorelDRAW 2024 software (Corel, Canada).

Histopathological examination

Formalin-fixed intestinal tissues containing plasmodia were processed using standard histological techniques: dehydration through a graded ethanol series, clearing in xylene, and embedding in paraffin wax. Serial sections (3–5 µm in thickness) were sectioned, adhered to glass slides, and subjected to routine hematoxylin and eosin (H&E) staining. Sections were examined under the Olympus BX53 microscope to characterize the histopathology of the infection.

Molecular analyses and phylogeny

Using the SteadyPure Universal Genomic DNA Extraction Kit (SteadyPure, China), genomic DNA was extracted from ethanol-preserved plasmodia according to the manufacturer's guidelines. PCR amplification of the partial SSU rDNA was carried out with the myxosporean universal primers MyxospecF (5'-TTCTGCCCTATCAACTWGTG-3') and 18R (5'-CTACGGAAACCTTGTTACG-3') (Fiala 2006; Whipps et al. 2003). The PCR was conducted in a 25 µL reaction volume containing 1 µL of template DNA, 1 µL of each primer (10 µM), 12.5 µL of 2× Rapid Taq MasterMix, and 9.5 µL of ddH₂O. The cycling protocol consisted of an initial 4 min denaturation at 94 °C; 35 cycles of 94 °C for 1 min, 46 °C for 50 s, and 65 °C for 90 s; and a final 10 min extension at 65 °C. Following visualization on a 1% agarose gel, PCR products of the target size were

gel-extracted and purified with a Trelief DNA Gel Extraction Kit (Trelief, China). Subsequently, the amplicons were cloned into the pMD18-T vector (Takara, Japan) and introduced into *E. coli* DH5 α competent cells via transformation. For sequencing, recombinant plasmids from positive clones were purified and sent to Tsingke Biotechnology Co., Ltd. for bidirectional sequencing.

The newly generated SSU rDNA sequence was deposited in GenBank. Sequence similarity was assessed using the BLASTn algorithm on the NCBI platform (Altschul et al. 1990). For phylogenetic reconstruction, a dataset of 43 SSU rDNA sequences, including close matches (BLAST identity >80%) and taxa of interest selected based on sequence similarity and species with more rounded spore morphology, was compiled. *Zschokkella nova* (DQ377690) served as the outgroup. Sequences were aligned using the MAFFT algorithm in PhyloSuite v.1.2.2 with default parameters (Zhang et al. 2020). The best-fit nucleotide substitution model (GTR+I+G) was selected under the Bayesian Information Criterion (BIC) using ModelFinder (Kalyaanamoorthy et al. 2017). Phylogenetic trees were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML analysis was performed with IQ-TREE, employing 1000 ultrafast bootstrap replicates (Nguyen et al. 2015). Bayesian analysis (MrBayes v.3.2.7) involved two independent MCMC runs, each lasting 2 million generations with a sampling frequency of every 100 generations (Ronquist et al. 2012). The first 25% of sampled trees were discarded as burn-in. Convergence was assessed using the average standard deviation of split frequencies (0.010322) and PSRF values (average = 1.000, maximum = 1.007), indicating that the MCMC runs reached convergence. Consensus trees were visualized and edited using FigTree v.1.4.3 (Rambaut et al. 2016) and Adobe Illustrator CC 2023 (Adobe Systems Inc., CA). To quantify genetic divergence, the Kimura 2-parameter (K2P) model in MEGA 11 was employed to calculate pairwise distances and similarities between the new species and six closely related taxa (Tamura et al. 2021).

Results

During the survey, 34 individuals of *Squaliobarbus curriculus* were examined for myxosporean infection. After dissection, numerous whitish plasmodia could be easily observed throughout the intestinal wall in 11 of the 34 hosts (prevalence: 32.35%) (Fig. 1). The plasmodia were ellipsoidal, measuring up to 2 mm long and 1.5 mm wide (Fig. 1B). No infections were found in other examined organs, such as the kidney, liver, or gills. Despite the presence of extensive plasmodia, infected fish exhibited no abnormal behavior compared to that of healthy individuals.

Myxobolus curriculus sp. nov.

<https://zoobank.org/7F2199D5-F3AA-4921-8D5F-5ED521492E5A>

Figs 1–4

Morphological description. Upon rupturing the plasmodia, numerous myxospores were released and observed (Fig. 1C). Mature myxospores were round in the valvular view, and lenticular in the sutural view (Fig. 1C, Fig. 2), measuring 8.8 ± 0.2 (7.8–9.4) μm long, 8.3 ± 0.3 (7.5–9.3) μm wide, and 3.8 ± 0.3 (3.5–4.2) μm thick. Two equal, pyriform polar capsules were located anteriorly, with dimensions of 3.8 ± 0.2 (3.5–4.3) μm in length and 2.4 ± 0.3 (2.2–3.0) μm in width. Polar tubes formed 4–5 coils. Spores lacked a mucous envelope and intercapsular appendix but possessed 3–4 distinct posterior folds.

Species. *Myxobolus curriculus* sp. nov.

Host. Barbel chub *Squaliobarbus curriculus* (Cyprinidae).

Site of infection. Intestinal wall.

Plasmodia size. Ellipsoidal, approximately 2×1.5 mm in size.

Prevalence. 32.35% (11/34).

Locality. Zi River, Xinhua County, Loudi City, Hunan Province, China (27°43'46.8"N, 111°19'18.9"E).



Figure 1. Barbel chub *Squaliobarbus curriculus* infected with *Myxobolus curriculus* sp. nov. **A.** Barbel chub with myxosporean infection; **B.** The symptom with numerous plasmodia in the intestinal wall of the host; **C.** Myxospore in the valvular view; **D.** Myxospore in the sutural view (arrow). Scale bars: 5 cm (**A**, **B**); 10 μm (**C**, **D**).

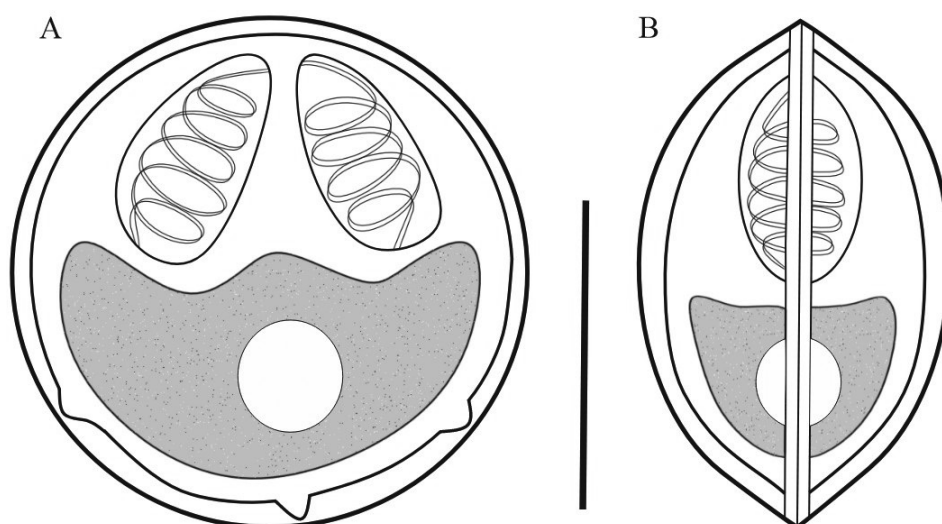


Figure 2. Schematic drawings of *Myxobolus curriculus* sp. nov. from barbel chub, myxospore in the valvular view (A) and in the sutural view (B). Scale bar: 5 μ m.

Type material. Specimens (fixed in 10% formalin and 95% ethanol), and digitized images of H&E-stained tissue sections are deposited in the Museum of Hydrobiological Sciences, Hunan Agricultural University, China (accession numbers HNAU-CF-PAR-24061).

Gene sequence. A Partial SSU rDNA gene sequence (1731 bp) is deposited in GenBank under accession number **PV785691**.

Etymology. The species epithet derives from the species name of the host.

Remarks. To date, seven *Myxobolus* species have been reported in barbel chub *S. curriculus* in China, including *M. squamae* Keysseltz, 1908, infecting the intestine, *M. rotundatus* Achmerov, 1956, infecting the intestinal wall, *M. vastus* Kudo, 1934, infecting the kidney and liver, *M. squamosus* Kudo, 1934, infecting the intestine, liver, and intestinal wall, *M. pfeifferi* Thelohan, 1890, infecting the gills, *M. permagnus* Wegener, 1910, infecting the gills, *M. weishanensis* Ma & Chen, 1998 infecting the gills (Chen and Ma 1998). The new species is morphologically compared to these and distinguished by a combination of characters summarized in Table 1. Among them, *M. squamae* and *M. vastus* possessed ellipsoidal spores, while *M. rotundatus* and *M. squamosus* possessed round myxospores, as in the case of the new species. Furthermore, *M. squamae* could be easily differentiated from *M. curriculus* sp. nov. by bigger myxospore thickness and more folds. *M. vastus* possessed a bigger myxospore length and thickness, slightly more coils of polar tubes (5–6 vs. 4–5), an intercapsular appendix, and the absence of folds in the posterior. The plasmodia of *M. rotundatus* were smaller (2×1.5 mm vs. 0.193×0.181 mm) than those of the new species, but the size of the myxospore length and thickness was bigger. *M. squamosus* was recorded to be capable of infecting three different sites, while the present species was only found in the intestine of the host. In addition, *M. squamosus* differed from the novel species by more folds and bigger myxospore thickness (5.8–6.0 μ m vs. 3.5–4.2 μ m). The infection sites for *M. pfeifferi*, *M. permagnus*, and *M. weishanensis*

were gills, different from intestinal wall for *M. curriculus* sp. nov. *M. pfeifferi* was distinguished from the concerned species by bigger myxospores (10.8–14.4 $\times$ 9.2–9.7 $\times$ 6.6–7.4 μ m vs. 7.8–9.4 $\times$ 7.5–9.3 $\times$ 3.5–4.2 μ m) and polar capsules (6.0–7.2 $\times$ 3.3–3.6 μ m vs. 3.5–4.3 $\times$ 2.0–3.0 μ m), more turns of polar tubes and less folds in the posterior. *M. permagnus* and *M. weishanensis* could be easily differentiated from *M. curriculus* sp. nov. by different myxospore shapes (pyriform vs. round), bigger myxospore, and more turns of polar tubes (>7 vs. 4–5) (Table 1).

Histopathological examination

Histopathological analysis indicated that the new species developed within the circular muscle layer of intestine (Fig. 3). A development gradient was evident, with mature spores concentrated centrally and immature stages peripherally (Fig. 3C). Expanding plasmodia compressed adjacent muscle fibers and elicited the formation of a thin outer connective tissue capsule (Fig. 3D, E). No evident muscle fiber atrophy, necrosis, mucosal damage, epithelial desquamation, or obstruction of the intestinal lumen was observed, and no significant inflammatory response was detected.

Molecular characterization

The SSU rRNA sequence obtained is 1,731 base pairs in length and has been deposited in GenBank (**PV785691**). BLAST analysis did not match any available species sequence in GenBank, but exhibited high similarity with *Myxobolus algonquinensis* Xiao & Desser, 1997 (**AF378335**, 93.02%) infecting the ovary of *Notropis cornutus*, *Myxobolus muellericus* Molnár, Marton, Eszterbauer & Székely, 2006 (**DQ439808**, 92.86%) infecting the gills of *Leuciscus cephalus* L., *Myxobolus diversicapularis* Slukhai, 1966 infecting the gills of *Rutilus rutilus* (**GU968199**, 92.83%), *Myxobolus feisti* Molnár et al., 2008

Table 1. Comparison of *Myxobolus curriculus* sp. nov. with morphologically similar species reported previously.

	Site of infection	Host	Shape	LS (µm)	WS (µm)	TS (µm)	LPC (µm)	WPC (µm)	PFC	Folds	Refs
<i>M. curriculus</i> sp. nov.	intestinal wall	<i>S. curriculus</i>	Round	8.8 ± 0.2 (7.8–9.4)	8.3 ± 0.3 (7.5–9.3)	3.8 ± 0.3 (3.5–4.2)	3.8 ± 0.2 (3.5–4.3)	2.4 ± 0.3 (2.2–3.0)	4–5	3–4	Present study
<i>M. squamae</i>	intestine	<i>S. curriculus</i>	Ellipsoidal	9.4 (8.4–10.4)	7.5 (7.2–8.4)	5.9 (5.8–6.0)	3.6 (3.4–3.8)	2.4 (2.4–2.6)	4–5	4–7	Chen and Ma 1998
<i>M. rotundatus</i>	intestinal wall	<i>S. curriculus</i>	Round	10.1 (9.2–10.8)	9.2 (8.4–9.6)	7.2 (7.0–7.4)	4.6 (4.2–4.8)	2.7 (2.1–2.9)	4–5	4–5	Chen and Ma 1998
<i>M. vastus</i>	kidney and liver	<i>S. curriculus</i>	Ellipsoidal	10.9 (9.6–12.0)	7.5 (7.2–8.4)	6.0 (5.8–6.2)	4.9 (4.8–5.4)	2.8 (2.4–3.0)	5–6	–	Chen and Ma 1998
<i>M. squamosus</i>	liver, intestine and intestinal wall	<i>S. curriculus</i>	Round	9.4 (8.4–10.4)	7.5 (7.2–8.4)	5.9 (5.8–6.0)	3.6 (3.4–3.8)	2.4 (2.4–2.6)	4–5	4–7	Chen and Ma 1998
<i>M. pfeifferi</i>	gills	<i>S. curriculus</i>	Ellipsoidal	12.6 (10.8–14.4)	9.7 (9.2–9.7)	7.1 (6.6–7.4)	6.1 (6.0–7.2)	3.3 (3.3–3.6)	5–6	2–3	Chen and Ma 1998
<i>M. permagnus</i>	gills	<i>S. curriculus</i>	Pyriform	18.1 (18.0–19.2)	10.2 (9.2–12.0)	8.4	9.7 (9.6–10.8)	3.5–4.0	9–10	–	Chen and Ma 1998
<i>M. weishanensis</i>	gills	<i>S. curriculus</i>	Pyriform	12.28 (10.0–12.5)	8.26 (7.5–10.0)	6.07 (5.0–6.25)	6.12 (5.62–6.25)	2.65 (2.5–3.12)	> 7	4–6	Chen and Ma 1998
<i>M. squamae</i>	scales	<i>Barbus barbus</i>	Ellipsoidal	11 ± 0.32 (10–11.4)	9.1 ± 0.45 (8.4–9.7)	–	5.3 ± 0.3 (4.9–5.6)	3 ± 0.15 (2.7–3.2)	6	–	Molnár et al. 2012
<i>M. pfeifferi</i>	gill arch	<i>Luciobarbus bocagei</i>	Ellipsoidal	15 ± 0.51 (14–15.7)	11.2 ± 0.37 (11–12.5)	–	7 ± 1.35 (6–9.5)	3.6 ± 0.29 (3.2–3.9)	6	–	Molnár et al. 2012

LS: Length of spores; WS: Width of the spores; TS: Thickness of spores; LPC: Length of polar capsule; WPC: Width of polar capsule; PFC: number of polar filament coils; Folds: number of valvular folds; Refs: references; –: absent.

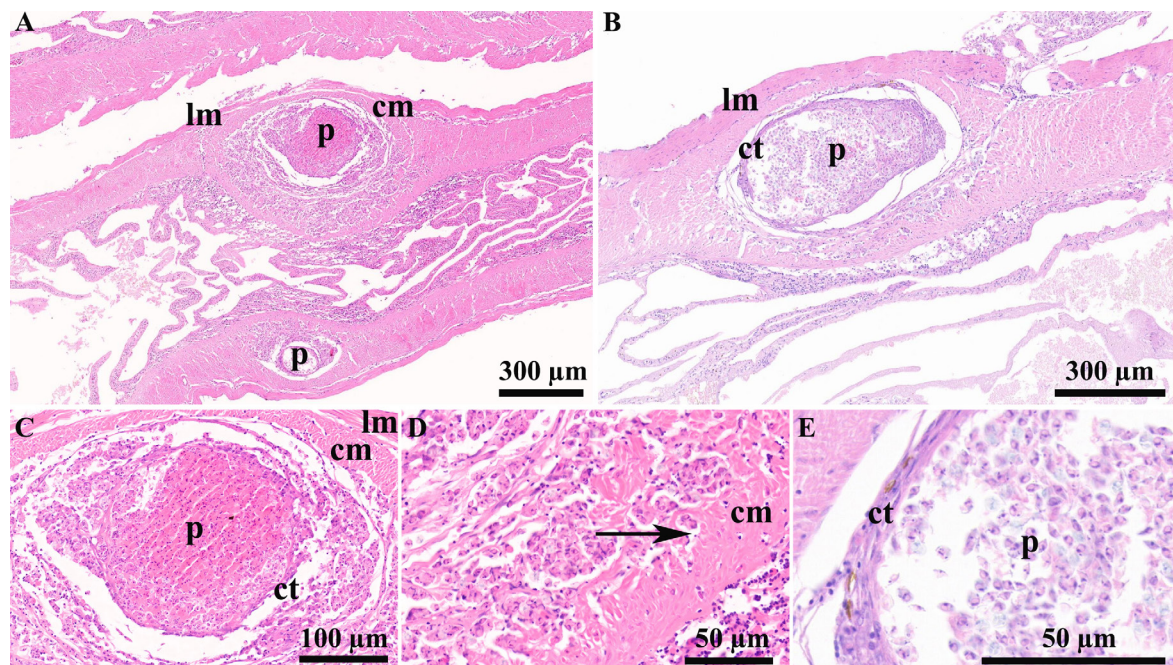


Figure 3. Histopathological analysis of the intestinal wall of barbel chub infected with *Myxobolus curriculus* sp. nov. **A.** Two developing plasmodia (p) located in the circular muscle layer (cm) of the intestine; **B.** A developed plasmodium (p) was surrounded by a thin connective tissue (ct) in the circular muscle layer; **C, D.** The magnification of the plasmodium in **A**, showing the developing plasmodium surrounded by connective tissues (ct) with mature spores located centrally and immature spores peripherally and compressed the adjacent circular muscle layer (cm); **E.** The magnification of the plasmodium in **B** showing the developed plasmodium surrounded by a thin connective tissue (ct). Abbreviations: p, plasmodium; ct, connective tissue; cm, circular muscle layer; lm, longitudinal muscle layer. Scale bars: 300 µm (**A, B**); 100 µm (**C**); 50 µm (**D, E**).

infecting the cartilaginous gill rays of *R. rutilus* (EU598804, 92.66%), *Myxobolus abitus* Li & Nie, 1973 (MG520367, 92.43%) infecting the gills of *H. molitrix*, and *M. pavlovskii* (Akhmerov, 1954) Landsberg & Lom, 1991 infecting the gills of *H. molitrix* and bighead carp *Hypophthalmichthys nobilis* Richardson (MG520368, 92.26%). Genetic distance analysis indicated that the distances between this sequence and those of the most closely related species ranged from 0.0765 to 0.1230 (Table 2).

Both BI and ML analyses yielded phylogenetic trees with highly similar topologies. Minor discrepancies were confined to variations in support values at certain nodes (Fig. 4). The phylogenetic tree is clearly divided into two major clades, Clade A and Clade B. Species within the Clade A were further divided into three subclades. Subclade I mainly comprised species infecting diverse organs of cypriniform hosts in Hungary and Portugal, Subclade II mainly consisted of species infecting gills of cypriniform hosts in several different countries, and Subclade III mainly comprised species infecting different organs of cypriniform hosts in China, India and Hungary. The node separating Subclade I and Subclade II was moderately supported (BI = 0.86, ML = 60%), while the node grouping Subclade III with the other subclades showed low support (BI < 0.50, ML < 50%). The present species formed a sister lineage with *M. dabryi* infecting the gills of *Chanodichthys dabryi*, Bleeker, 1871, with strong support (BI > 0.95, ML > 95%), *M. abitus* infecting the gills of *Hypophthalmichthys molitrix*, and *M. tauricus* infecting the pin bones and fins of *Barbus barbus* Linnaeus, 1758, and *Luciobarbus bocagei* Steindachner, 1864 within the subclade III (Fig. 4).

Discussion

It is important to note that *M. squamae* Keisselitz, 1908, and *M. pfeifferi* Thélohan, 1895, may represent cryptic species complexes. The specimens identified as *M. squamae* and *M. pfeifferi* in Chen and Ma (1998) differ notably from the corresponding species redescribed by Molnár et al. (2012), including distinct host ranges, infection sites, larger spore and polar capsule dimensions, and a higher number of polar tubule coils. This discrepancy underscores the necessity of an integrative taxonomic approach, combining morphology, ecological data (host and site specificity), and molecular phylogenetics for clarifying myxozoan systematics. This is particularly crucial for numerous Chinese species described in the last century, many of which were reported from a wide range of hosts and organs, likely reflecting the reliance on morphology-based identification in the absence of molecular data at that time (e.g., *M. dispar* was recorded from 14 organs across 15 host species) (Chen and Ma 1998). Furthermore, the *M. squamae* and *M. pfeifferi* characterized by Molnár et al. (2012) are readily distinguished from the novel species described herein by their different hosts and distinct morphometric profiles (Table 1). Finally, the low genetic similarity (<90%) and their separate phylogenetic placements (*M. squamae* and *M. pfeifferi* in subclade I vs. *Myxobolus curriculus* sp. nov. in subclade III) provide conclusive molecular evidence that these are distinct species.

The fish intestine is a complex, multifunctional organ central to digestion, nutrient absorption, and immune function, making it a critical site for host-parasite interactions (Bieczynski et al. 2021; Corrêa et al. 2025).

Table 2. Comparison of sequence similarities and genetic distances of *Myxobolus curriculus* sp. nov. with genetically related species.

Species (GenBank accession number)	1	2	3	4	5	6	7	8	9	10	11	12
1. <i>M. curriculus</i> sp. nov. (PV785691)		93.03%	92.86%	92.83%	92.66%	92.43%	92.26%	92.18%	92.14%	92.01%	91.76%	90.18%
2. <i>M. algonquinensis</i> (AF378335)	0.1145		91.67%	92.95%	94.24%	93.66%	90.60%	91.85%	92.98%	92.64%	88.70%	93.09%
3. <i>M. muellericus</i> (DQ439808)	0.1134	0.1220		92.36%	89.11%	91.50%	88.26%	87.57%	88.38%	87.92%	88.79%	88.83%
4. <i>M. diversicapsularis</i> (GU968199)	0.1171	0.0965	0.0769		92.52%	93.02%	92.94%	90.62%	92.80%	91.63%	92.18%	89.20%
5. <i>M. feisti</i> (EU598804)	0.1209	0.1070	0.1143	0.1233		90.39%	89.78%	91.61%	89.70%	95.51%	88.91%	89.68%
6. <i>M. abitus</i> (MG520367)	0.0775	0.1104	0.1186	0.1009	0.1057		95.26%	91.80%	95.14%	88.94%	92.17%	92.62%
7. <i>M. pavlovskii</i> (MG520368)	0.0786	0.1092	0.1207	0.1036	0.1056	0.0451		91.32%	97.24%	89.20%	91.12%	92.69%
8. <i>Myxobolus</i> sp. (PP859466)	0.1164	0.1231	0.1333	0.1043	0.1364	0.0935	0.0859		93.66%	90.96%	92.10%	89.10%
9. <i>M. kiuchowensis</i> (MG520366)	0.0765	0.1157	0.1194	0.1052	0.1078	0.0483	0.0262	0.0889		88.64%	91.13%	92.11%
10. <i>M. margitae</i> (EU598803)	0.1230	0.1162	0.1244	0.1258	0.0413	0.1148	0.1121	0.1449	0.1207		87.85%	88.12%
11. <i>M. dabryi</i> (OP359407)	0.0800	0.1186	0.1135	0.1227	0.1150	0.0805	0.0914	0.1194	0.0893	0.1245		91.36%
12. <i>M. tauricus</i> (JQ388896)	0.0940	0.1210	0.1105	0.1108	0.1166	0.0733	0.0719	0.1053	0.0755	0.1280	0.0892	

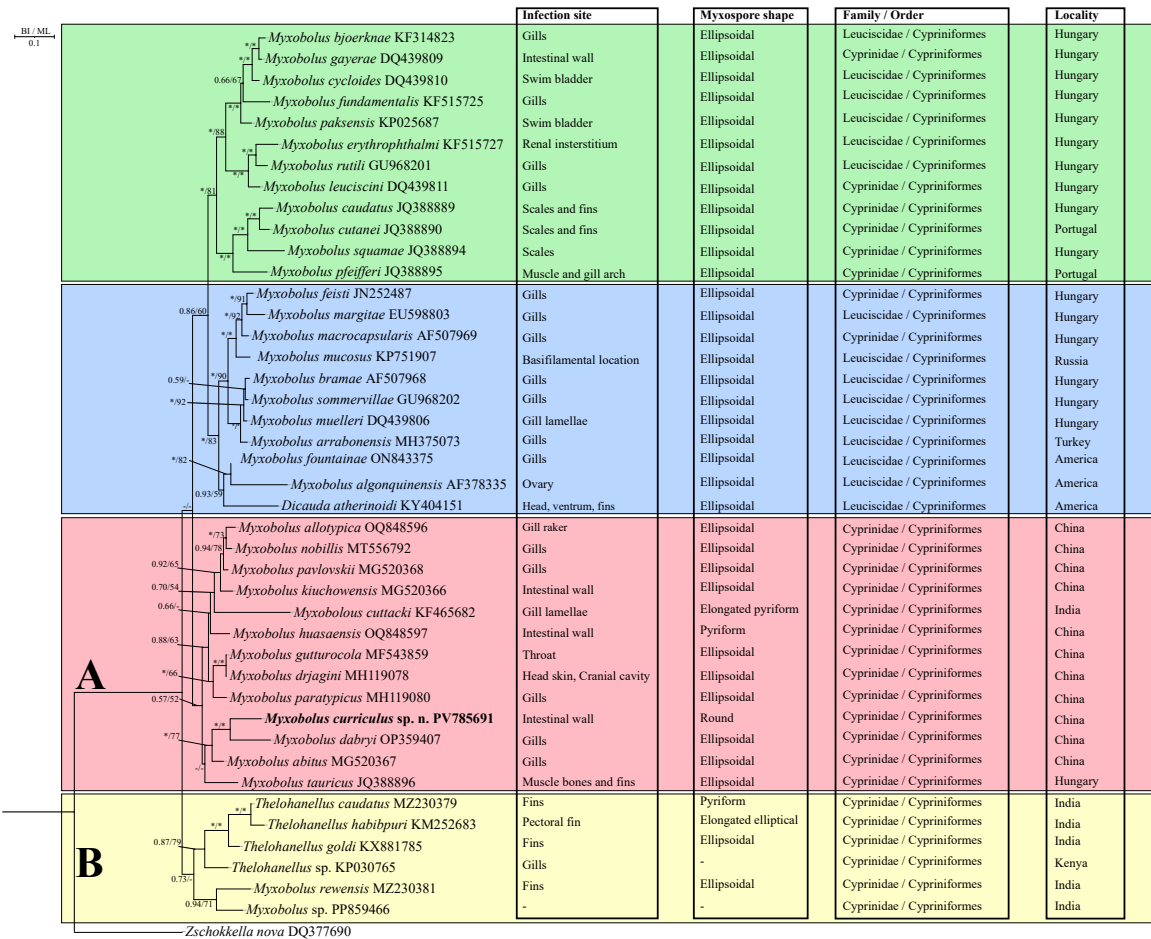


Figure 4. Phylogenetic tree of *Myxobolus curriculus* sp. nov. and its relatives based on the SSU rDNA sequences, rooted at *Zschokella nova* (DQ377690). Support values on the branch nodes are indicated as BI posterior probabilities/ML support values; Asterisks represent support values > 0.95 or 95%, and dashes demonstrate support values < 0.50 or 50%.

It is also a common target for parasitic infections, including those caused by myxozoans (Liu et al. 2016, 2017; Lablack et al. 2022; Corrêa et al. 2025). Myxosporean infections localize to several distinct intestinal tissues: the muscular layers (longitudinal and circular), the epithelium, the lamina propria, the mucosa/submucosa, and the enteric cavity (Molnár et al. 2007; Ye 2012; Liu 2014; Bartholomew et al. 2022). Among them, the muscle layer is the most frequently reported site. The present species, like *M. taibaiensis* (Liu et al. 2017), developed within the circular muscle layer and elicited no obvious inflammatory response. This pronounced tropism for muscular tissue, while not fully understood, may be associated with the relatively high availability of nutrients and metabolic resources in this tissue, which could be advantageous for parasite proliferation.

Infection site specificity is recognized as a significant taxonomic character in myxozoans, a view supported by phylogenetic studies showing that species infecting similar tissues often cluster together. However, our analysis revealed that the present species grouped with *M. dabryi* and *M. abitus* (both possessing round myxospores), rather than with other intestinal parasites such as *M. huasaensis*

infecting the intestinal wall of grass carp *Ctenopharyngodon idella* Valenciennes, 1844, *M. gayerae* infecting the intestinal wall of *Leuciscus cephalus* Linnaeus, 1758, and *M. kiuchowensis* infecting the intestine of *H. nobilis*. This pattern mirrors the finding of Liu et al. (2017) for *M. taibaiensis*, suggesting that intestinal tropism may not provide a strong phylogenetic signal in this group. This observation aligns with the proposed categorization of site tropism into specific (e.g., gills, certain muscles) and non-specific (e.g., intestine, skin, fins) types, as suggested by the present study and previous reports on intestinal *Myxobolus* species.

Beyond host infection dynamics, host phylogenetic affinity, geographic distribution, and myxospore morphology have all been shown to provide substantial phylogenetic signal in myxozoan evolution (Yuan et al. 2024; Zhao et al. 2024). Our analysis corroborates this multi-factor influence. Specifically, Subclade I predominantly comprises species with ellipsoidal myxospores infecting Cyprinidae and Leuciscidae hosts in Europe (Hungary and Portugal). Subclade II also consists of species with ellipsoidal spores infecting these host families but across a broader Holarctic range (Europe and North America). In contrast, Subclade III is primarily composed of species

from China and India infecting Cyprinidae hosts, exhibiting a mix of spore shapes (predominantly ellipsoidal, with some pyriform and round). These structured patterns further support the conclusion that myxozoan evolution is shaped by a combination of ecological, morphological, and biogeographic factors, consistent with prior studies (Yuan et al. 2024).

Myxospore shape is a fundamental morphological trait in myxozoan taxonomy, routinely employed for species comparison and identification (Yuan et al. 2024). Recent phylogenetic studies have reinforced its diagnostic value, demonstrating that species often cluster according to shared myxospore shape (Liu et al. 2016; Yuan et al. 2024; Zhao et al. 2024). For example, Yuan et al. (2024) showed that *Myxobolus* species with round, pyriform, and ellipsoidal spores formed distinct, morphology-correlated clades. In line with this pattern, species within Subclades I and II of our analysis formed cohesive groups characterized exclusively by ellipsoidal spores. In contrast, Subclade III comprised species with ellipsoidal, pyriform, and round spores, suggesting that spore morphology within this lineage is evolutionarily labile and may have undergone multiple transitions.

Conclusions

In summary, the new species *Myxobolus curriculus* sp. nov., which infects the intestinal wall of barbel chub *Squaliobarbus curriculus*, is comprehensively described with morphological, ecological, and molecular data.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Conceptualization, X.C.G. and X.H.L.; methodology, W.J.D. and W.L.; validation, M.Y.H. and D.L.L.; formal analysis, J.G.X. and S.Y.; investigation, S.Y.; writing – original draft preparation, S.Y.; writing – review and editing, X.C.G. and W.J.D.; funding acquisition, X.H.L. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

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