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FISH PARASITOLOGY - ORIGINAL PAPER

Morphological and molecular characterization of a new species *Myxobolus gutturocola* n. sp. (Myxozoa: Myxobolidae) from the throat of *Hypophthalmichthys molitrix* in China

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Abstract

Myxobolus gutturocola n. sp. was isolated from the throat of silver carp, *Hypophthalmichthys molitrix*, in Chongqing, China. Myxospore valves are unsymmetrical and smooth. Mature spores are ellipsoidal in frontal view, measuring 12.5 ± 0.2 μm (n = 25) in length, 8.4 ± 0.2 μm (n = 25) in width and 7.1 ± 0.2 μm (n = 25) in thickness. Each spore has two pyriform and unequal sizes polar capsules, the large one with 5.7 ± 0.2 μm in length × 3.6 ± 0.2 μm in width and the small one with 4.6 ± 0.2 μm in length × 2.6 ± 0.1 μm in width. Polar filaments are coiled seven or eight turns in the large polar capsule and four or five turns in the small polar capsule. The coils are arranged almost perpendicularly to the longitudinal axis of the polar capsule. Morphological analysis revealed that *M. gutturocola* n. sp. is distinct from related species of *Myxobolus* Bütschli, 1882. Molecular analysis has demonstrated that its SSU rDNA sequences do not match with any available sequences in GenBank. Phylogenetic analysis of the SSU rDNA sequences indicated this species clustered in a clade composed exclusively of parasites infecting the fishes of the Leucisini lineage and most closely related to *Myxobolus pavlovskii* isolated from the gill filaments of silver carp in Hungary.

Keywords *Myxobolus gutturocola* n. sp. · Myxozoan · *Hypophthalmichthys molitrix* · SSU rDNA · China

Introduction

Myxosporeans are tiny metazoan parasites of fish with high diversity (Bartošová-Sojková et al. 2014; Whipps and Zhao 2015). To date, *Myxobolus* Bütschli, 1882, is the most speciose myxosporean genus with approximately 900 species worldwide (Eiras et al. 2005, 2014; Manrique et al. 2015; Székely et al. 2015; Liu et al. 2016a, b; Abdel-Ghaffar et al. 2015, 2017). Among them, 13 *Myxobolus* species have been reported from silver carp (*Hypophthalmichthys molitrix*), one of the most commonly cultured fish species in China (Eiras et al. 2005, 2014; Zhang et al. 2018). Currently, it is difficult to identify novel species or distinguish myxosporean species only based on spore morphology, especially those with high similarity in morphology, same host, and living environment (Gunter and Adlard 2009; Atkinson et al. 2015; Liu et al. 2016). Molecular data (i.e., DNA sequence similarity, variation sites, genetic distance) have been widely recommended for the identification of novel or obscure myxosporean species along with morphological and ecological as well as phylogenic analysis (Fiala 2006; Molnár et al. 2009; Zhao et al. 2008; Zhang et al. 2010; Liu et al. 2016, 2017). Ran et al. (2014) have shown that based on small subunit (SSU) rRNA gene data, the taxonomic subdivisions of species for myxozoans are significantly correlated with their genetic distance, with the genetic distance of 0–0.007 within species and 0.1352–0.2404 between species. Liu et al. (2016) reported different numbers, based on SSU rRNA gene data, with the genetic distance of 0–0.013 within species and 0.008–0.678 between species. Although these data are not identical, genetic distance is an effective way to identify genetically and morphologically similar species (Andree et al. 1999; Ferguson et al. 2008; Carriero et al. 2013; Zhao et al. 2013; Atkinson et al. 2015; Liu et al. 2016).

In this study, we morphologically and molecularly characterized a new *Myxobolus* species, *Myxobolus gutturocola* n. sp. from the throat of silver carp (*H. molitrix*) in Chongqing, China, and addressed its phylogenetic relationships with other *Myxobolus* species.

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Materials and methods

Sample collection and species identification

During three sampling trips between April and May 2015, a total of 12 host fish, *H. molitrix*, were captured in the Hechuan District, Chongqing, China. These fish were transported alive to the laboratory and necropsied. Some myxosporean plasmodia were excised from the throat of three individual silver carp and their mature spores were smeared on the slides and examined under a Leica DM6000B microscope at × 400. In addition, the released fresh spores were rinsed three times with sterile distilled water, collected by centrifugation at 2000×*g* and used for species identification as reported previously (Lom and Arthur 1989; Zhao et al. 2001, 2013).

Genomic DNA extraction, cloning, and sequencing

The myxosporean samples isolated from the throat of fish were preserved in 95% ethanol. They were collected and washed twice with distilled water by centrifugation at 10,000×*g* for 10 min to remove the ethanol. The washed sample was used to extract genomic DNA using the DNeasy Blood & Tissue Kit (QIAGEN, Düsseldorf, Germany) following the manufacturer's recommended protocol for animal tissue. A portion of the SSU rRNA gene was amplified in a 25 μl polymerase chain reaction (PCR) system containing 0.5 μM of each of the primer pair ERIB1-ERIB10 (5′-ACCT GGTTGATCCTGCCAG-3′ and 5′-CTTCCGCAGGTTCA CCTACGG-3′) (Barta et al. 1997), 3 mM $MgCl_2$, 0.2 mM dNTP, 15 ng genomic DNA, and 1.5 U Taq Ex DNA polymerase (TaKaRa, Otsu, Japan). The PCR cycling conditions used a 5-min initial pre-denaturation at 94 °C followed by 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at 56 °C and 2 min extension at 72 °C, and a final extension at 72 °C for 10 min. The PCR products were separated by electrophoresis on a 1.0% agarose gel. The targeted products were purified with the DNA Agarose Gel Extraction Kit (Omega Bio-Tek, Norcross City, GA) and inserted into pMD18-T vector (TaKaRa, Otsu, Japan), and the recombinant plasmids were purified according to the manual of plasmid extraction kit (OMEGA, GA, USA). Three clones were sequenced on an ABI Prism 377 DNA Sequencer (Applied Biosystems Inc., Foster City, California).

Phylogenetic and sequence analysis

The SSU rRNA gene sequences of the new species were assembled using Seqman v 5.1 (Burland 2000). Our analyses used 25 myxosporean SSU rRNA gene sequences, three that we generated and 22 from the NCBI GenBank database. These sequences include all available SSU rRNA gene sequences of *Myxobolus* species that are genetically and morphologically similar to our species and some representatives of myxosporean species infecting Cyprinidae fishes (Fiala 2006; Zhao et al. 2008; Liu et al. 2010; Chen et al. 2016). *Sphaerospora truttae* was used as outgroup taxon in the present work. The sequences of these SSU rDNA were manually edited using BioEdit (Hall 1999), aligned using Clustal X 1.8, and used to construct phylogenic trees. Maximum likelihood (ML) analysis in RAxML-HPC2 on XSEDE of CIPRES web (Stamatakis 2014) was performed based on 10 starting trees and full gamma estimation. Statistical support was computed using 1000 bootstrap replicates. Bayesian analysis tree was inferred with MrBayes v 3.1.2 using the GTR+I+G substitution model and the Markov chain Monte Carlo algorithm (Ronquist and Huelsenbeck 2003). The program was run for 1000,000 generations with sampling every 100 generations and the first 2500 trees were discarded as burn-in. In addition, the similarity among sequences was calculated using GenBank BLAST (Altschul et al. 1990).

Results

Myxobolus gutturocola n. sp.
Phylum: Cnidaria Hatschek, 1888
Class: Myxosporea Bütschli, 1881
Order: Bivalvulida Shulman, 1959
Family: MyxobolidaeThélohan, 1892
Genus: *Myxobolus* Bütschli, 1882

Diagnosis

The plasmodia are whitish, round, or ellipsoidal and have protrusions of 1.4 × 0.93 mm out of the throat serous surface of each infected host.

The mature spores are ellipsoidal and unsymmetrical in the frontal view, lemon-like in lateral view, measuring 11.6–13.7 (12.5 ± 0.2) μm long, 8.4–10.7 (8.4 ± 0.2) μm wide, and 6.8–7.3 (7.1 ± 0.2) μm thick. They have unsymmetrical and smooth spore valves and two pyriform and unequal polar capsules occupying the anterior half area of spore. The larger polar capsule is 4.9–6.4 (5.7 ± 0.2) μm long and 3.0–4.1 (3.6 ± 0.2) μm wide and the smaller polar capsule is 4.0–5.5 (4.6 ± 0.2) μm long and 2.1–3.5 (2.6 ± 0.1) μm wide. Polar filaments are coiled seven or eight turns in the large polar capsule and four or five turns in the small polar capsule. The coils are arranged almost perpendicular to the longitudinal axis of the polar capsule. A v-shaped intercapsular appendix is observed between the two polar capsules (Fig. 1, Table 1). There are neither evident iodinophilous vacuoles in the sporoplasm nor mucous envelopes around the spore.

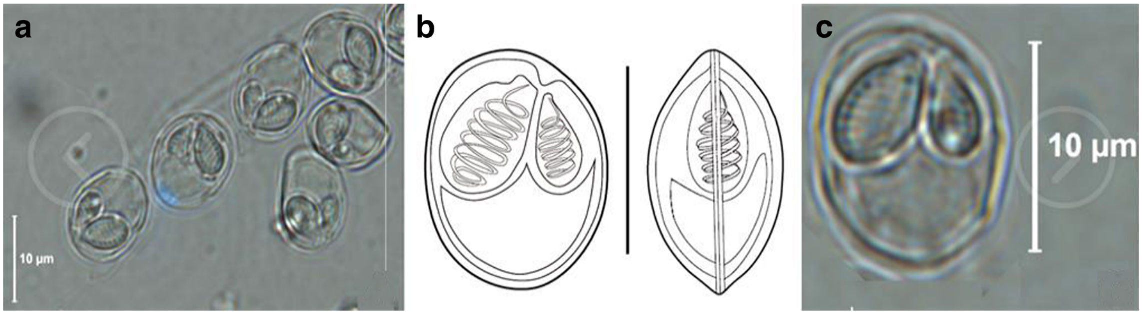

Fig. 1 Spores of *M. gutturocola* n. sp. collected from the throat of silver carp. **a**, **c** Fresh myxospores in frontal view. **b** Line drawing of mature spores of *M. gutturocola* n. sp. in frontal and sutural view. (Scale bars = 10 μm)

Taxonomic summary

Locality: Hechuan District, Chongqing (29° 93′ N, 106° 33′ E), China

Host: *Hypophthalmichthys molitrix* Valenciennes, 1844

Infection site: the throat of infected host fish

Prevalence of infection: 25% (3/12)

Host symptom: slightly swollen eyes

Date of sampling: April 2015

Deposited materials: the holotype (No. CQHC-HM-201504), a fixed plasmodium with the mixture of glycerin/ethyl alcohol 70%/formalin (G./A./F. = 12:108:5), was deposited in the Collection Center of Chongqing Key Laboratory of Animal Biology, Chongqing Normal University.

Etymology: The name *gutturocola* refers to the tissue location of the parasite, and the name *gutturocola* is a combination of *gutturo*- from the Latin *guttur* (= throat) and *cola* from the Latin *colo* (= habit, site).

Molecular characterization

Three SSU rDNA sequences, namely 1929 nt, 1930 nt, and 1929 nt, were obtained from the throat of different silver carp fish and deposited in GenBank under the accession numbers MF543857, MF543858, and MF543859, respectively. Based on GenBank BLAST (Altschul et al. 1990), these three SSU rDNA sequences had 99–100% similarity to each other, and 95%, 92%, 91%, 90%, 92%, and 90% similarity to other morphological similar species *Myxobolus pavlovskii*, *Myxobolus tsangwuensis*, *Myxobolus musseliusae*, *Myxobolus basilamellaris*, *Myxobolus dispar*, and *Myxobolus cyprinicola*, respectively.

Phylogenic analysis

The phylogenetic analyses of SSU rDNA sequences inferred from ML and BI showed highly similar topologies with different support values at some branch nodes. Twenty-five SSU rDNA sequences of *Myxobolus* were placed into two branches. The taxa in one branch bore ellipsoidal mature spores with blunt anterior end (Fig. 2 (a)), while those in the other branch bore elongated pyriform spores with tapering anterior end (Fig. 2 (b)). The branch with *Myxobolus* species bearing blunt anterior end spores also contained two sub-branches, where the three *M. gutturocola* n. sp. SSU rDNA sequences were first clustered together with 100% bootstrap support. This cluster was sister to *M. pavlovskii*, forming a clade of *Myxobolus* species that infect fishes in the subfamily Hypophthaemichthyinae with 100% support. Moreover, *Myxobolus impressus* from the white bream (*Blicca bjoerkna*) and *Myxobolus diversicapsularis* from roach (*Rutilus rutilus*) were clustered together and then grouped with *Myxobolus bjoerknae* parasitized in common bream (*Abramis brama*) to form a subclade of *Myxobolus* species that infect fishes of the subfamily Leuciscinae, and *M. dispar* and *M. cyprinicola* isolated from *Cyprinus carpio* L. were grouped into a subclade of *Myxobolus* species that infect fishes of the subfamily Cyprininae. The two subclades formed a clade of *Myxobolus* species that infect fishes of the subfamily Leuciscinae and Cyprininae. The *Myxoblous* species that infect fishes of the subfamily Hypophthaemichthyinae and those infecting fishes of the subfamily Leuciscinae and Cyprininae were grouped together, forming the first sub-branch. In addition, *M. tsangwuensis*, *M. musseliusae*, and *M. basilamellaris* from common carp, *Cyprinus carpio* also clustered together with high support (98%/1.00), forming the second sub-branch (Fig. 2 (a)). The branch containing species with tapering anterior spore ends also contained two sub-branches. The species (*Myxobolus pseudokoi*, *Myxobolus bilobus*, *Myxobolus intimus*, *Myxobolus hungaricus*, and *Myxobolus obesus*) isolated from Leuciscini fishes clustered together, forming the first sub-branch with strong support (98%/1.00), and the species (*Myxobolus pendula*, *Myxobolus csabai*, *Myxobolus tasikkenyirensis*) isolated from Leuciscini fishes were first grouped into a subclade with 100% support, and other species (*Myxobolus ampullicapsulatus*, *Myxobolus honghuensis*, *Myxobolus wulii*, *Myxobolus longisporus*, and *Myxobolus koi*) isolated from *Carassius auratus* (Cyprininae) were clustered into the other subclade with high support (86%/1.00). These

Table 1 Comparitive description of *M. gutturocola* n. sp with morphologically similar species infecting cyprinid fishes. Mean ± SD and range in parentheses. Measurements ($n = 25$) are in µm

Myxosporean species	Size of spore	Size of large PC	Size of small PC	Host and location	Locality	Source
M. gutturocola n. sp.	L: 12.5 ± 0.2(11.6–13.7), W: 8.4 ± 0.2(8.4–10.7), T: n.d.	L: 5.7 ± 0.2 (4.9–6.4), W: 3.6 ± 0.2 (3.0–4.1), PF: 7–8 coils	L: 4.6 ± 0.3 (4.0–5.5), W: 2.6 ± 0.1 (2.1–3.5), PF: 5–6 coils	*Hypophthalmichthys molitrix* Throat	Chongqing, China	Present study
M. hypophthalmichthydis	L: 11–12, W: 9–10, T: 6	L: 5.5–6, W: 4, PF: 5–6 coils	L: 3, W: 2–2.5 PF: 3–4 coils	*Hypophthalmichthys molitrix* Head, skin	Hungary	Dogiel and Akhmerov (1960)
M. drjagini	L: 13–14, W: 9–10, T: n.d.	L: 5.5–6, W: 3–3.5, PF: 7–8 coils	L: 3.5–4, W: 2–2.5 PF: 5–6 coils	*Hypophthalmichthys molitrix* Skin	Hungary	Akhmerov (1954)
M. nobillis	L: 12.7 (12–14), W: 10.6 (10.2–12), T: 6.6–6.8	L: 7.4 (7.2–7.5), W: 5.4 (4.5–5.8), PF: 9 coils	L: 3.7 (3.2–4.5), W: 2.3 (2.2–2.5), PF: 5 coils	*Hypophthalmichthys molitrix* Almost all organs	Hubei, China	Li and Nie (1973)
M. pavlovskii	L: 9–10, W: 10–11.5, T: n.d.	L: 5–6, W: 3–3.5 PF: n.d.	L: 3–4, W: 2.5–3 PF: n.d.	*Hypophthalmichthys molitrix* Gills	Hungary	Akhmerov (1954)
M. tsangwuensis	L: 11.1 (10.2–12), W: 9.0 (8.4–9.6), T: 6.9 (6.6–7)	L: 4.8 (4.6–5.0), W: 2.9 (2.4–3.), PF: 5–7 coils	L: 3.4 (3.0–3.), W: 2.5 (2.4–2.), PF: 3–4 coils	*Cyprinus carpio* Kidneys, gills	Hubei, China	Chen and Ma (1998)
M. basilamellaris	L: 7.7–12.2, W: 7.3–9.9, T: 4.5 (4.2–5)	L: 3.2–5.4, W: 2.2–3.3 PF: 5–6 coils	L: 2.5–4.4, W: 1.8–3.3, PF: n.d.	*Cyprinus carpio* Gills	Hungary	Lom and Molnár (1983)
M. musseliusae	L: 10.5–11.1, W: 8.8–10, T: 7.2	L: 3.9–4, W: n.d., PF: n.d.	L: 1.7–2.2, W: n.d., PF: n.d.	*Cyprinus carpio* Gills	Russia	Yakovchuk (1979)

L length, *W* width, *T* thickness, *PC* polar capsule, *PF* polar filament, *n.d.* no data

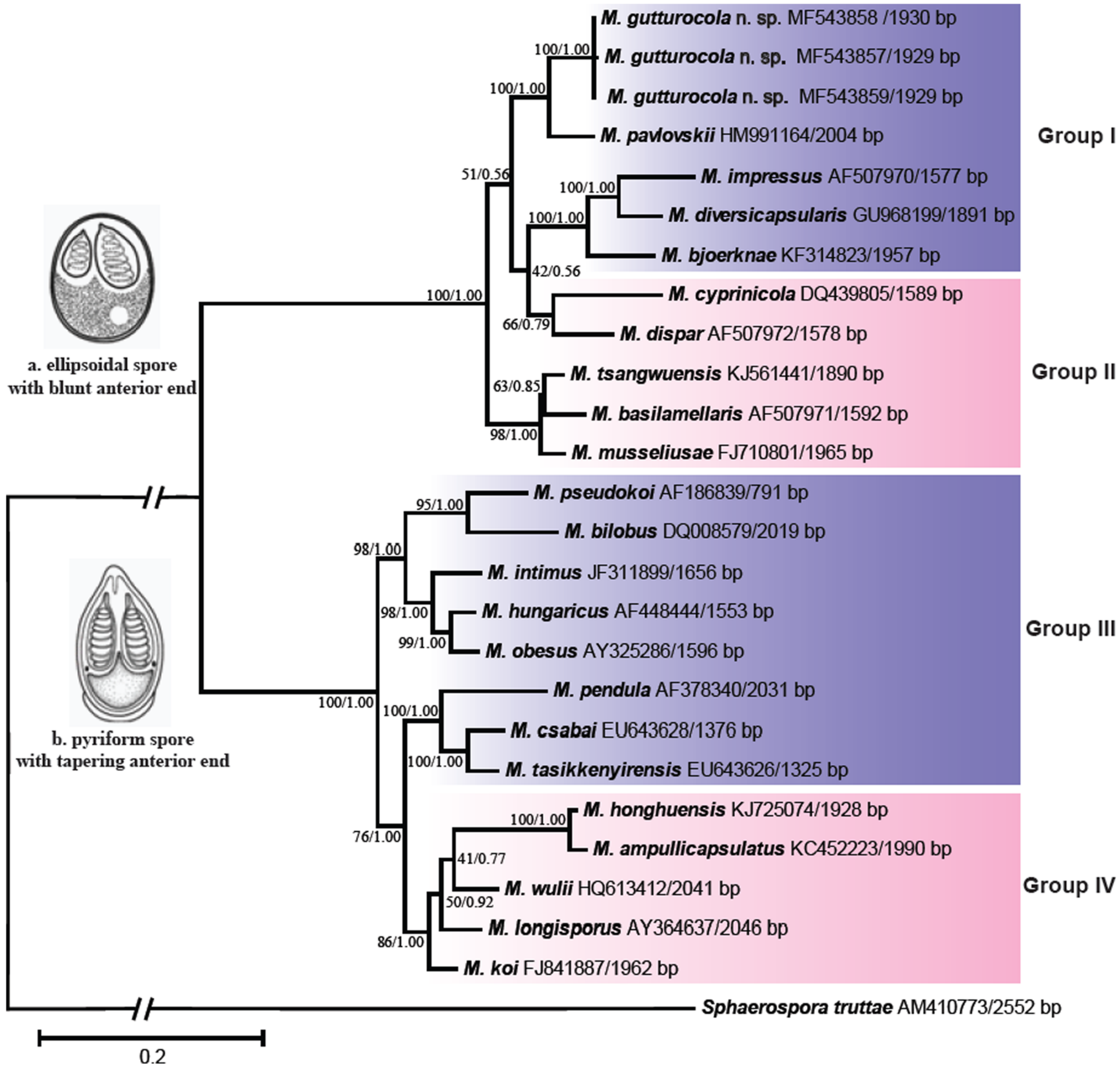

Fig. 2 Phylogenetic tree based on SSU rDNA sequences by maximum likelihood (ML) with the model GTRGAMMA + I implemented in RAxML-HPC2 on XSEDE and Bayesian inference (BI) with the model GTR + I + G applied in MrBayes 3.1.2. Numbers at nodes indicate bootstrap confidence levels (1000 repetitions) and posterior probabilities. (a) *Myxobolus* spp. with ellipsoidal mature spores and blunt anterior end. (b) *Myxobolus* spp. with pyriform mature spores and blunt anterior end. Group I: *Myxobolus* species infecting the fishes of Hypophthaemichthyinae and Leuciscinae. Group II: *Myxobolus* species infecting the fishes of Cyprininae. Group III: *Myxobolus* species infecting the fishes of Leuciscinae. Group IV: *Myxobolus* species infecting the fishes of Cyprininae. The purple rectangle represents Leuciscini lineage, and the pink rectangle represents Barbini lineage. Shortened braches are 50% of their original lengths

two subclades were then grouped together, forming the second sub-branch (Fig. 2 (b)).

Discussion

Morphological remarks

Of the approximately 900 species of *Myxobolus* described (Eiras et al. 2005, 2014; Lom and Dyková 2006), the three isolated samples in the present study have displayed similarity to *Myxobolus hypophthalmichthydis* Dogieland Akhmerov, 1960; *Myxobolus drjagini* Akhmerov, 1954; *Myxobolus nobillis* Li and Nie, 1973; *M. pavlovskii* Akhmerov, 1954; *M. tsangwuensis* Chen, 1998; *M. basilamellaris* Lom and Molnár, 1983; and *M. musseliusae* Yakovchuk, 1979, based on the fact that they all have ellipsoidal spores with two unequal polar capsules (Table 1). In addition, although *M. hypophthalmichthydis*, *M. drjagini*, *M. nobillis*, and *M. pavlovskii* all parasitize on the same host, *H. molitrix*, they all differ from the present species in spore dimension, size of polar capsule, and site of infection.

As for the spore shape, the present organism shows similarity with *M. hypophthalmichthydis*, but it can still be distinguished from it in the following aspects. First, the present organism has remarkably larger polar capsules (4.0–5.5 μm ×

2.1–3.5 μm in length and width) than *M. hypophthalmichthydis* (4 × 3 μm in length and width). Second, the present organism has asymmetrical spores and the polar filament ejection forms some angles to the longitudinal axis of the spore, while the latter has symmetrical spores and the polar filament ejection parallels to the longitudinal axis of spore. Third, the present organism has spores with lower ratio length to width than the *M. hypophthalmichthydis* and does not have the iodinophilous vacuole. Fourth, although the two species are isolated from the same host, their infection locations are different. The present organism infects the throat while *M. hypophthalmichthydis* infects the head or body skin.

The present taxon shows high similarity with *M. drjagini* (Akhmerov, 1954), but can be distinguished from it in the following aspects. Firstly, the present taxon has remarkably broader spores than *M. drjagini* (8.4–10.7 μm vs. 9–10 μm). Secondly, the present taxon has remarkably longer polar capsules than *M. drjagini* (4.0–5.5 μm × 2.1–3.5 μm vs. 2.6–3.6 μm × 2.4–2.6 μm). Thirdly, although the two species were isolated from the same host, their infection locations are different. The present taxon infects the throat while *M. drjagini* infects the skin. Fourthly, the present taxon possesses spores with higher ratio of length to width than *M. drjagini*.

This present species and *M. nobillis* Li and Nie, 1973 also have the following distinct characteristics. (1) The present species has spores with round anterior end while *M. nobillis* has spores with tapered anterior end. (2) The present species has smaller polar capsule than *M. nobillis* (4.9–6.4 μm × 3.0–4.1 μm vs. 7.2–7.5 μm × 4.5–5.8 μm). (3) The present species has less polar filament coils than *M. nobillis* (8 vs. 9). Overall, the present species can be distinguished from *M. nobillis* based on the round anterior end of spores, smaller polar capsules, and fewer polar filament coils.

The present taxon shows higher similarity with *M. pavlovskii* (Akhmerov, 1954), which infects the gills of silver carp, and has also available sequence in NCBI database. The present taxon differs from the latter by having larger spores (11.6–13.7 μm × 8.4–10.7 μm vs. 9–10 μm × 10–11.5 μm), different infection sites (throat vs. gill) and not matching for available sequences with each other.

Moreover, the present congener is also similar to *M. tsangwuensis*, *M. musseliusae*, and *M. basilamellaris*, because they all have ellipsoidal spores with two unequal polar capsules. But it also differs from the three taxa by infecting different hosts (silver carp for the present species vs. common carp for *M. tsangwuensis*, *M. musseliusae*, and *M. basilamellaris*) and different infection sites (throat for the present species vs. gills for *M. tsangwuensis*, *M. musseliusae*, and *M. basilamellaris*), and *M. gutturocola* n. sp. has a larger spore. Overall, the above morphological comparisons support that the three isolated samples belong to a new species.

Molecular identification inferred from SSU rDNA

Molecular characteristics such as similarity, genetic distances, and phylogenic trees have been accepted as important factors for species identification and discrimination of closely related species (Zhang et al. 2010; Zhao et al. 2013; Bartošová-Sojková et al. 2014; Liu et al. 2016; Liu et al. 2017). Previous studies have also indicated that SSU rDNA sequence similarity could be used for identification of *Myxobolus* species (Cone et al. 2005; Easy et al. 2005; Whipps and Kent 2006; Bartošová and Fiala 2011; Ran et al. 2014; Zhao et al. 2013). In this study, the intraspecific differences between the three SSU sequences from *M. gutturocola* n. sp. are 0–1% (99–100% similarity), which are among the reported range for species of myxozoans (≤ 1%) (Zhao et al. 2013; Bartošová-Sojková et al. 2014; Hartikainen et al. 2014). Meanwhile, the present species shows the highest similarity (95%) with *M. pavlovskii*, which is beyond what is expected for intraspecific variation of 2.6% for SSU rDNA sequence difference (Schlegel et al. 1996; Zhao et al. 2013). This supports that the SSU rDNA sequences of the present species are distinctly different from other related species and do not match any other myxosporean sequences in GenBank (Zhao et al. 2013; Atkinson et al. 2015; Liu et al. 2016). Thus, the three isolated samples belong to the same species, distinct from other myxozoans. Phylogenic analysis based on the SSU rDNA sequences from three isolated samples shows that these samples form a distinct lineage within *Myxobolus* clade infecting fishes of the subfamily Hypophthaemichthyinae (Fig. 2). Despite the high bootstrap support (100%) for a sister relationship between the present species and *M. pavlovskii* (Fig. 2), the exact locations of sporulation are different (Atkinson et al. 2015; Huang et al. 2014; Liu et al. 2016b).

Spore morphology dominance and host specificity

Until recently, almost 600 known *Myxobolus* species have been identified using spore morphology alone and the others have corresponding DNA sequence data (Chen and Ma 1998; Landsberg and Lom 1991). Because of the difficulties of experimental studies, little has been known about host specificity. Morphologically similar spores from genetically different hosts are often described as the same species. Shulman (1966) has recorded as many as 40 hosts for certain *Myxobolus* species. Therefore, spore morphology has been often discussed as an important factor in the evolution of myxozoans; for example, spore shape and size are often used to identify and distinguish different species (Shulman 1966; Chen and Ma 1998). With the application of molecular technology, many molecular identifications for species have been carried out based on the comparative analysis of sequence similarity, genetic distance, mutation sites, GC content of SSU rDNA, and phylogenic trees (Easy et al. 2005; Whipps and Kent 2006; Zhao et al. 2008, 2013; Manrique et al. 2015; Székely et al. 2015; Liu et al. 2016a, b; Liu et al. 2016; Abdel-Ghaffar et al. 2015, 2017). In this study, phylogenetic analysis of the SSU rDNA sequences shows that species with similar spore body morphology are clustered into the same branches (Fig. 2). For example, *Myxobolus* species with ellipsoidal spores and round anterior end form a branch, while those with elongated pyriform spores and tapering anterior end form another branch.

In the present study, every member of the two branches in the phylogenic tree is a parasite mainly infecting the gills of Cyprinidae fish. Previous investigations on fish phylogenic analysis have revealed that fish within the family Cyprinidae are mainly placed into two lineages, namely Leuciscini and Barbini lineage. The Leuciscini lineage comprises Hypophthalmichthyinae, Xenocyprinae, Cultrinae, Gobioninae, Acheilognathinae, Leuciscinae, and Danioninae, while the Barbini lineage comprises Schizothoracinae, Barbinae, Cyprininae, and Labeoninae (Kong et al. 2007; Wang et al. 2007, Wang et al. 2012). The present phylogenetic analysis of myxosporean inferred from ML and BI shows that myxosporeans with similar spore shape are clustered together first, and the myxosporeans parasitizing in Leuciscini and Barbini lineage are then placed separately (groups I and III, groups II and IV; Fig. 2).

When compared to GenBank-deposited sequences of the related *Myxobolus* species infecting the fishes of the Cyprinini and Leuciscini lineages, the molecular data obtained from *Myxobolus* species parasitizing silver carp suggest that each phylogenetic branch of *Myxobolus* species isolated from Cyprinini-fishes are clustered differently from the Leuciscini fish parasites. Moreover, the phylogenetic research on both fish and myxozoan show consistency and correlation, which indicate that both tissue specificity and host specificity are important factors in the evolution of myxosporeans (Kent et al. 2001; Molnár et al. 2002, 2006; Zhao et al. 2013; Liu et al. 2016). Host specificity has been perceived as an important factor in the evolution of myxosporeans, especially in some certain myxobolids. Molnár's study on the host range of *M. rotundus* has suggested that type-host should be considered host preference to identify novel or published/known myxosporean species (Molnár et al. 2009). The morphologically branched pattern in the present phylogenetic analysis also shows consistence with our previous research (Zhao et al. 2008). Furthermore, the present study also reveals that these tree branches with similar spore morphology are clustered together first and then split based on host specificity, indicating the importance of spore morphology in the evolution of some lineages of myxosporeans.

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References

Abdel-Ghaffar F, Morsy K, El-Ganainy S, Ahmed M, Gamal S, Bashtar AR, Al-Quraishy S, Mehlhorn H (2015) Twelve myxosporean species of the family Myxobolidae infecting freshwater fishes of the River Nile, Egypt, with the description of four novel species. Parasitol Res 114:2985–2998

Abdel-Ghaffar F, Abdel-Gaber R, Maher S, El-Deeb N, Kamel R, Al-Quraishy S, Mehlhorn H (2017) Morphological and ultrastructural characteristics of *Myxobolus ridibundae* n. sp. (Myxosporea: Bivalvulida) infecting the testicular tissue of the marsh frog *Rana ridibunda* (Amphibia: Ranidae) in Egypt. Parasitol Res 116:133–141

Akhmerov AK (1954) Species complex of a new genus of Myxosporidia. Dok1 Akad.Nauk. SSSR, 97(6):1101–1103 (in Russian)

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Andree KB, Székely C, Molnár K, Gresoviac SJ, Hedrick RP (1999) Relationships among members of the genus *Myxobolus* (Myxozoa: Bilvalvidae) based on small subunit ribosomal DNA sequences. J Parasitol 85: 68–74

Atkinson SD, Bartošová-Sojková P, Whipps CM, Bartholomew JL (2015) Approaches for characterising myxozoan species. In: Myxozoan evolution, ecology and development. Springer International Publishing, pp 111–123

Barta JR, Martin DS, Liberator PA, Dashkevicz M, Anderson JW, Feighner SD, Elbrecht A, Perkins-Barrow A, Jenkins MC, Danforth HD, Ruff MD, Profous-Juchelka H (1997) Phylogenetic relationships among eight *Eimeria* species infecting the domestic fowl inferred using complete small subunit ribosomal DNA sequences. J Parasitol 83:262–271

Bartošová P, Fiala I (2011) Molecular evidence for the existence of cryptic species assemblages of several myxosporeans (myxozoa). Parasitol Res 108:573–583

Bartošová-Sojková P, Hrabcová M, Pecková H, Patra S, Kodádková A, Jurajda P, Tyml T, Holzer AS (2014) Hidden diversity and evolutionary trends in malacosporean parasites (Cnidaria: Myxozoa) identified using molecular phylogenetics. Int J Parasitol 44:565–577

Burland TG (2000) DNASTAR's lasergene sequence analysis software. Methods Mol Biol 132:71–91

Carriero MM, Adriano EA, Silva MR, Ceccarelli PS, Maia AA (2013) Molecular phylogeny of the *Myxobolus* and *henneguya* genera with several new South American species. PLoS One 8:1–12

Chen CL, Ma CL (1998) Fauna Sinica: Myxozoa, Myxosporea. Science Press, Beijing, pp 1–806 (in Chinese)

Chen W, Yang CZ, Zhao YJ (2016) Molecular evolution of *Myxobolus* (Myxosporea) and coevolution with their hosts. Sichuan J Zool 35: 183–189 (In Chinese with English abstract)

Cone DK, Yang J, Sun G, Easy R (2005) Taxonomy and molecular phylogeny of *myxobolus* bilobus n. sp. (myxozoa) parasitizing note migonuscry soleucas (cyprinidae) in Algonquin Park, Ontario, Canada. Dis Aquat Org 66:227–232

Dogiel VA, Akhmerov AK (1960) Myxosporidia of fishes of the Amur River Basin. RybnKhoz Vnutr Vodoemov Latv SSSR 5:239–308 (In Russian)

Easy RH, Johnson SC, Cone DK (2005) Morphological and molecular comparison of *Myxobolus procerus* (kudo, 1934) and *M. intramusculi* n. sp. (myxozoa) parasitising muscles of the trout-perch percopsis omiscomaycus. Syst Parasitol 61:115–122

Eiras JC, Molnár K, Lu YS (2005) Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). Syst Parasitol 61:1–46

Eiras JC, Zhang JY, Molnár K (2014) Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea, Myxobolidae) described between 2005 and 2013. Syst Parasitol 88:11–36

Ferguson JA, Atkinson SD, Whipps CM, Kent ML (2008) Molecular and morphological analysis of *Myxobolus* spp. of salmonid fishes with the description of a new *Myxobolus* species. J Parasitol 94:1322–1334

Fiala I (2006) The phylogeny of Myxosporea (myxozoa) based on small subunit ribosomal RNA gene analysis. Int J Parasitol 36:1521–1534

Gunter NL, Adlard RD (2009) Seven new species of *Ceratomyxa* Thélohan, 1892 (Myxozoa) from the gall-bladders of serranid fishes from the Great Barrier Reef, Australia. Syst Parasitol 73:1–11

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hartikainen H, Gruhl A, Okamura B (2014) Diversification and repeated morphological transitions in endoparasitic cnidarians (Myxozoa: malacosporea). Mol Phylogenet Evol 76:261–269

Huang M, Liu Y, Jia L, Zhai YH, Deng Q, Gu ZM (2014) Supplementary studies on *Myxobolus tsangwuensis* Chen, 1954 (Myxozoa: myxosporea) infecting the gills of common carp *Cyprinus carpio* (L.): molecular and histological data. Acta Parasitol 59:653–660

Kent ML, Andree KB, Bartholomew JL, El-Matbouli M, Desser SS, Devlin RH, Feist SW, Hedrick RP, Hoffmann RW, Khattra J, Hallett SL, Lester RJ, Longshaw M, Palenzeula O, Siddall ME, Xiao C (2001) Recent advances in our knowledge of the Myxozoa. J Eukaryot Microbiol 48:395–413

Kong XH, Wang XZ, Gan XN, Li JB, He SP (2007) Phylogenetic relationships of Cyprinidae (Teleostei: Cypriniformes) inferred from the partial s6k1 gene sequences and implication of indel sites in intron 1. Sci China Life Sci 50:780–788

Landsberg JH, Lom J (1991) Taxonomy of the genera of the *Myxobolus*/*Myxosoma* group (Myxobolidae: Myxosporea), current listing of species and revision of synonyms. Syst Parasitol 18:165–180

Li LX, Nie DS (1973) Myxoporidia. In: Hubei Provincial Research Institute of Aquatic Biology (ed.) The Atlas of Pathogeny of Fish Disease in the Region of Hubei Province. Published by: Science Publishing House, Peking (China). pp 57–93 (In Chinese)

Liu Y, Whipps CM, Gu ZM, Zeng LB (2010) *Myxobolus turpisrotundus* (myxosporea: bivalvulida) spores with caudal appendages: investigating the validity of the genus *Henneguya* with morphological and molecular evidence. Parasitol Res 107:699–706

Liu XC, Yang CZ, Zhao YJ (2016) Redescription of *Myxobolus honghuensis* Liu et al. 2012 and identification on its genetic related species. Acta Hydrobiol Sin 40:352–359

Liu XH, Yuan S, Zhao YL, Fang P, Chen H, Zhang JY (2016a) Morphological and molecular characterization of *Myxobolus sheyangensis* n. sp. (Myxosporea: Myxobolidae) with intralamellar sporulation in allogynogeneticgibel carp, *Carassius auratus gibelio* (Bloch) in China. Parasitol Res 115:3567–3574

Liu XH, Batueva MD, Zhao YL, Zhang JY, Zhang QQ, Li TT, Li AH (2016b) Morphological and molecular characterisation of *Myxobolus pronini* n. sp. (Myxozoa: Myxobolidae) from the abdominal cavity and visceral serous membranes of the gibel carp *Carassius auratus gibelio* (Bloch) in Russia and China. Parasit Vectors 9:562–572

Liu XH, H CJ, Zhang QQ, Zhao YL, Zhang D, Zhang JY (2017) *Myxobolus taibaiensis* sp. n. (Myxozoa: Myxosporea) infecting the intestinal wall of common carp *Cyprinus carpio* Linnaeus in China. Folia Parasitol 64:1–7

Lom J, Arthur JR (1989) A guideline for the preparation of species descriptions in Myxosporea. J Fish Dis 12:151–156

Lom J, Dyková I (2006) Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. Folia Parasitol 53:1–36

Lom J, Molnár K (1983) *Myxobolus basilamellaris* sp. n. (Myxozoa: Myxosporea), a parasite of the gills of common carp (*Cyprinus carpio* L.). Folia Parasitol 30:1–3

Manrique WG, Figueiredo MA, de Andrade Belo MA, Martins ML, de Moraes FR (2015) First report of *Myxobolus* sp. infection in the skeletal muscle of Neotropical freshwater fish *Piaractus mesopotamicus*. Parasitol Res 114:2041–2044

Molnár K, Eszterbauer E, Székely C, Dán Á, Harrach B (2002) Morphological and molecular biological studies on intramuscular *Myxobolus* spp. of cyprinid fish. J Fish Dis 25:643–652

Molnár K, Marton S, Eszterbauer E, Székely C (2006) Comparative morphological and molecular studies on *Myxobolus* spp. infecting chub from the River Danube, Hungary, and description of *M. muellericus* sp. n. Dis Aquat Org 73:49–61

Molnár K, Székely C, Hallett SL, Atkinson SD (2009) Some remarks on the occurrence, host-specificity and validity of *Myxobolus rotundus* Nemeczek, 1911 (Myxozoa: Myxosporea). Syst Parasitol 72:71–79

Ran J, Yang CZ, Zhao YJ (2014) Taxonomic research of the myxosporidia based on genetic distance. J Chongqing Normal Univ 31:31–34

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Schlegel M, Lom J, Stechmann A, Bernhard D, Leipe D, Dyková I, Sogin ML (1996) Phylogenetic analysis of complete small subunit ribosomal RNA coding region of *Myxidium lieberkuehni*: evidence that myxozoa are metazoa and related to the Bilateria. Arch Protistenkd 147:1–9

Shulman SS (1966) The myxosporidian fauna of the USSR. Nauka Publs, Moscow and Leningrad, pp 1–504 (translated by Sharma S. 1990)

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313

Székely C, Molnár K, Cech G (2015) Description of *Myxobolus balatonicus* n. sp. (Myxozoa: Myxobolidae) from the common carp *Cyprinus carpio* L. in Lake Balaton. Syst Parasitol 91:71–79

Wang XZ, Li JB, He SP (2007) Molecular evidence for the monophyly of East Asian groups of Cyprinidae (Teleostei: Cypriniformes) derived from the nuclear recombination activating gene 2 sequences. Mol Phylogenet Evol 42:157–170

Wang XZ, Gan XN, Li JB, Mayden RL, He SP (2012) Phylogenetic analysis and systematic significance of Cyprinidae (Teleostei: Cypriniformes) based on Bayesian method and maximum likelihood method. Sci China Life Sci 42:648–661

Whipps CM, Kent ML (2006) Phylogeography of the cosmopolitan marine parasite *Kudoathyrsites* (Myxozoa: Myxosporea). J Eukaryot Microbiol 53:364–373

Whipps CM, Zhao YJ (2015) Synopsis of the species of the genus *Sphaeromyxa*, Thélohan, 1892 (Myxosporea: Bivalvulida: Variisporina: Sphaeromyxidae). Syst Parasitol 92:81–99

Yakovchuk TA (1979) A new species of the genus *Myxobolus* (Myxosporidia: Myxobolidae) from the gill filaments of the carp. Parazitologiia 13:635–636 (In Russian)

Zhang JY, Wang JG, Li AH, Gong XN (2010) Infection of *Myxobolus turpisrotundus* sp. n. in allogynogenetic gibel carp, *Carassius auratus* *gibelio* (Bloch), with revision of *Myxobolus rotundus* (s. l.) Nemeczek reported from *C. auratus auratus* (l.). J Fish Dis 33:625–638

Zhang B, Gu ZM, Liu Y (2018) Morphological, histological and molecular characterization of three *Myxobolus* species (Cnidaria: Myxosporea) from silver carp *Hypophthalmichthys molitrix* Valenciennes and bighead carp *Hypophthalmichthys nobilis* Richardson in China. Parasitol Int 67:509–516

Zhao YJ, Ma CL, Song WB (2001) Illustrated guide to the identification of pathogenetic protozoa in maricuture—II diagnostic methods for the myxosporean. J Ocean Univ Qingdao 31: 681–688

Zhao YJ, Sun CY, Kent ML, Deng JL, Whipps CM (2008) Description of a new species of *Myxobolus* (Myxozoa: Myxobolidae) based on morphological and molecular data. J Parasitol 94:737–742

Zhao YJ, Li NN, Tang FH, Dong JL (2013) Remarks on the validity of *Myxobolus ampullicapsulatus* and *Myxobolus honghuensis* (Myxozoa: Myxosporea) based on SSU rDNA sequences. Parasitol Res 112:3817–3823