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FISH PARASITOLOGY - ORIGINAL PAPER

Morphological variation in *Myxobolus drjagini* (Akhmerov, 1954) from silver carp and description of *Myxobolus paratypicus* n. sp. (Cnidaria: Myxozoa)

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Abstract

There is uncertainty in the identification of *Myxobolus drjagini*, the causative agent of silver carp twist disease, in the literature. An investigation of fish parasites in Lake Taihu, China, revealed several *Myxobolus drjagini*–like myxosporeans infecting the subcutaneous tissue of the head skin, the olfactory and oculomotor nerves in the cranial cavity, and the intrafilamental epithelium of the gills of silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844). Myxospores from the head skin and the nerves were identified as conspecific to *M. drjagini* based on morphological and molecular data; although the spores from each of the two organs presented morphological variations. SSU rDNA sequence analysis revealed that the sequence of *M. drjagini* previously deposited in GenBank (AF085179) was invalid. Myxospores from the gills were identified as *Myxobolus paratypicus* n. sp. The spores were oval, asymmetric in frontal view, 13.8 (12.9–14.9) μm long, 9.9 (9.2–11.1) μm wide, and 7.0 μm thick. Two pyriform polar capsules were unequal in size (ratio above 4:1) with slightly converging anterior ends, and the posterior end of the large polar capsule extended beyond the middle of the spore. The large polar capsule was 7.5 (6.2–8.2) μm long and 5.0 (4.2–5.6) μm wide; the small polar capsule was 2.7 (2.1–3.6) μm long and 1.4 (1.1–1.9) μm wide. Polar filaments were coiled with 7–8 turns in the large polar capsule. The SSU rDNA sequence of *M. paratypicus* n. sp. was not identical to that of any myxozoan available in GenBank and showed highest similarity with *M. drjagini* (96%) and *Myxobolus pavlovskii* (95%) collected from bighead carp and silver carp, respectively.

Keywords Myxozoan · *Myxobolus* · Silver carp · Brain · SSU rDNA

Introduction

Myxobolus Butschli, 1882, is the most species-rich genus in the family Myxobolidae (Cnidaria: Myxozoa), consisting of approximately 800 species (Eiras et al. 2005; Zhang et al. 2010), and some myxobolids cause severe diseases in wild and farmed fishes, e.g., *Myxobolus cerebralis*, *M. drjagini*, and *M. honghuensis* (Wu et al. 1975; Sarker et al. 2015; Xi et al. 2011, 2013). *Myxobolus* species have been defined traditionally based on spore shape and polar capsules. The limited morphological characteristics and morphological variations between species make identification difficult and have impelled research to consider host preference, tissue tropism, and molecular sequence in species characterization (Atkinson et al. 2015). More than 300 named species in this genus have been reported from freshwater fishes in China (Chen and Ma 1998); however, the validity of some species needs to be reevaluated because they were recorded from a wide range of fish hosts or various organs and lack molecular sequence information.

Myxobolus drjagini (Akhmerov 1954) Schulman, 1962, synonym *Disparospora drjagini*, was originally described from the head skin of silver carp *Hypophthalmichthys molitrix* (Cuvier et Valenciennes, 1844) in the Amur River (named Heilong Jiang in China). *M. drjagini* was a common parasite of the silver carp with strict host specificity in Europe, and it was thought to have spread from eastern Asia to Europe

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through association with its imported fish host, silver carp (Molnár 1994; El-Mansy and Molnár 1997). Based on the available data in the literature, *M. drjagini* was only reported from the head skin of the silver carp in Europe (Molnár and Eszterbauer 2015); however, it was widely recorded in silver carp and bighead carp *Aristichthys nobilis* (Richardson, 1845) in China, infecting a wide range of fish organs, e.g., the gill arch, skin, eye socket, heart, brain, brain-lymph, kidney, swim bladder, intestine, gall bladder, body cavity, fin, and liver (Wu et al. 1975; Chen and Ma 1998). Heavy infection destroyed the nerves in the cranial cavity causing "twist disease" and massive mortality of silver carp (Wu et al. 1975). Meanwhile, it is worth noting that in the same organs of the silver carp and bighead carp, several morphologically similar myxosporeans were also found, such as *M. atypicus* Li et Nie, 1973, from the central nerves; *M. hypophthalmichthydis* (Dogiel et Achmerov, 1960) Chen et Ma, 1998, from the head skin; *M. nobillis* Li et Nie, 1973, and *M. aristichthys* Nie et Yin, 1973, from the gills, intestine, and kidney (Chen and Ma 1998). However, it is unclear whether these different species in the literature truly occurred in the same organs, or the records included misidentification. To answer this question, it is necessary to collect new material and characterize these species based on morphological characteristics and DNA sequences. The results will facilitate our understanding of myxosporean diversity.

During the recent fish parasite investigation, several *M. drjagini*–like myxosporeans were found in the gill, the head skin, and the cranial cavity of silver carp. In this report, we identify and characterize these myxosporeans based on morphological and molecular analyses.

Materials and methods

Sample collection and morphometric analyses

From October 2016 to June 2017, 23 silver carps, body length 26–32 cm, were netted from Lake Taihu, China (31° 25′ N, 120° 17′ E), and transferred on ice to the laboratory for parasitological examination. Macroscopic examination of the skin, gills, liver, intestine, kidney, and brain was conducted with naked eyes and a dissecting microscope. Plasmodia found were isolated separately and observed with wet-mount slides using an Olympus CX-31 microscope equipped with a digital camera, and then, spores on the slide were collected in a 1.5-mL centrifuge tube and stored at − 20 °C for molecular analysis. Archival specimens of silver carp with twist disease, collected from Lake Taihu in the 1980s, stored in formalin and kept at the Freshwater Fisheries Research Center, were also examined. The plasmodia in the cranial cavity were removed, rinsed with water, and observed under a light microscope. Morphometric data of at least 30 fresh mature spores from each myxosporean found in the silver carp were obtained according to the guidelines of Lom and Dyková 2006. All measurements are given in micrometers (μm). Line drawings of spores were based on the digitized images.

DNA isolation and sequencing

Myxosporean genomic DNA was extracted using a QIAamp® DNA Micro kit (Qiagen, Germany) following the manufacturer's instructions. The small subunit ribosomal DNA (SSU rDNA/18S rDNA) was amplified with primers ERIB1 and ERIB10 (Barta et al. 1997). The PCR product was purified using a Sangon Biotech® sanorep Column DNA Extraction kit and commercially sequenced via the primer walking method (Sangon Biotech, Shanghai, China). DNA sequence chromatograms were assembled and inspected with SeqMan (Lasergene package, DNAStar Inc., Madison, WI). One plasmodium from each organ, the head skin, the cranial cavity, and the gill filaments, was separately sequenced. Attempts to amplify the SSU rDNA from the archival specimen stored in formalin failed. Assembled sequences were deposited in GenBank and compared with other myxozoan sequences available in NCBI database using BLASTn search.

Phylogenetic analyses

The SSU rDNA sequences of closely related myxozoans were downloaded from GenBank and aligned using the Clustal X 1.8 program with the default setting (Thompson et al. 1997). Phylogenetic analyses were conducted with Bayesian inference (BI) and maximum likelihood (ML) methods. The appropriate nucleotide substitution model was estimated based on the lowest BIC (Bayesian Information Criterion) score, and the ML phylogenetic trees were inferred with MEGA 7.0 (Kumar et al. 2016). Branch support was computed with 1000 replicates of bootstrap analyses. Bayesian analyses were conducted under the same ML model with MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck 2003) by running four independent MCMCMC analyses with four chains and 5 million generations. Posterior probability values were used as support for the Bayesian topology. The phylogenetic trees were all rooted on the external group of *Myxidium lieberkuehni* and *Chloromyxum legeri*.

Results

In 23 seemingly healthy silver carps, myxosporean plasmodia were found in the head skin (subcutaneous tissue, Fig. 1c) (5/23, 21.7%), the olfactory and oculomotor nerves (Fig. 1a, b) (3/23, 13%), and the gill filaments (Fig. 1d) (2/23, 8.7%). Myxospores from the cranial cavity of archival (Fig. 2a-c) and the newly collected specimens (Fig. 2d–f)

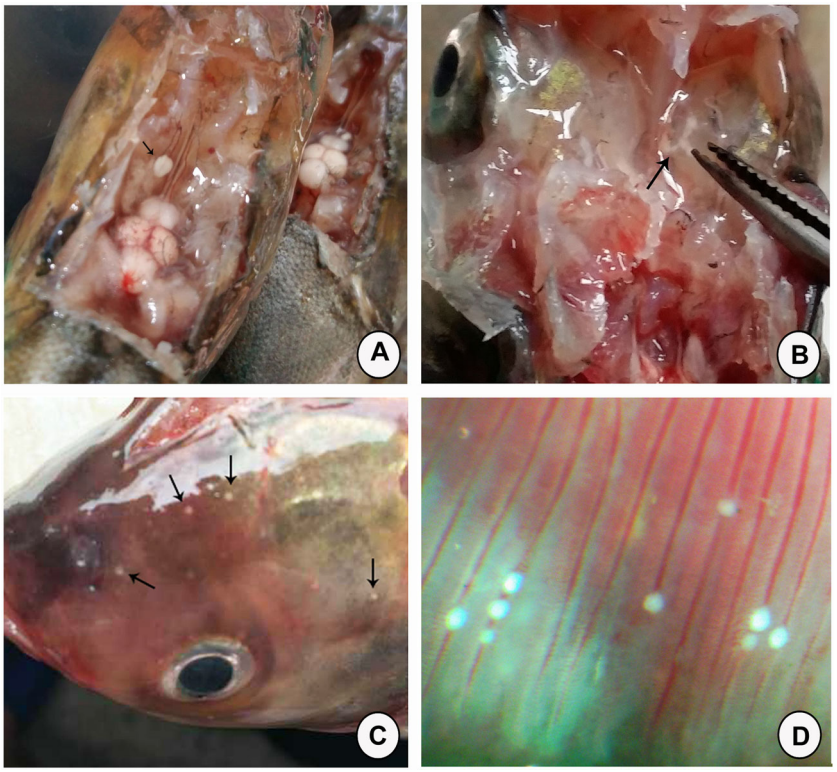

Fig. 1 Plasmodia of *Myxobolus drjagini* (**a**–**c**) and *Myxobolus paratypicus* n. sp. (**d**) in silver carp. **a**, **b** Plasmodia located on the olfactory nerve and oculomotor nerve. **c** Plasmodia located in the subcutaneous tissue of the head. **d** Plasmodia located in the gill filament

showed consistent morphological characteristics (Table 1) and were considered to be conspecific with *Myxobolus drjagini*, as described by Akhmerov (1954), although morphological variation associated with host tissues (cranial cavity vs. head skin) was observed. Myxospores from the epithelium of the gill filament also showed similar characteristics to *M. drjagini*, but differed markedly from the *M. drjagini* in the polar capsules, and are described as a new species in this report.

Description of *Myxobolus drjagini* (Akhmerov 1954) Schulman, 1962

Myxospores from plasmodia in the nervous tissue (Fig. 2d–f, Fig. 3a, b). Spores (fresh material from three plasmodia, $n = 26$) elliptical, asymmetric in frontal view, and fusiform in sutural view, 12.8 (11.8–14.0) μm long, 8.9 (8.3–9.6) μm wide, and 7.1 μm thick. Two pyriform polar capsules unequal in size, ratio almost 2:1, with convergent points; openings situated far from the long axis of spore, near the small polar capsule side. The large polar capsule markedly leaning towards the small polar capsule, 5.8 (5.2–6.5) μm long and 3.3 (2.9–3.7) μm wide; the small polar capsule 3.0 (2.3–3.7) μm long and 1.7 (1.4–2.0) μm wide. Polar filaments coiled with 7–8 turns in the large polar capsule. Sutural folds not visible. Posterior end of polar capsule near the middle of spore body.

Myxospores from plasmodia in the head skin (Fig. 2g–i, Fig. 3c). Spores (from three plasmodia, $n = 20$) asymmetric in frontal view and fusiform in sutural view, with slightly tapered posterior end, 13.9 (12.6–14.7) μm long, 8.4 (7.5–9.9) μm wide, and 6.5 μm thick. Two pyriform polar capsules unequal in size, with convergent apexes; apexes situated to the side of the long axis of spore. Large polar capsule 5.2 (4.7–5.8) μm long and 3.7 (3.1–4.2) μm wide, small polar capsule 3.1 (2.3–3.5) μm long and 1.9 (1.2–2.6) μm wide. Polar filaments coiled with 7–8 turns in the large polar capsule. Posterior end of polar capsule does not reach the middle of spore body.

Remarks: spores isolated from the archival specimens, which were fixed in formalin (Fig. 2a–c, Table 1), had similar morphological characteristics and morphometric data as the fresh spores collected in this report from the cranial cavity of silver carp. A few spores were found with 3–5 sutural folds. Spores from the head skin closely resembled *M. drjagini* as described by Akhmerov 1954 (Fig. 3d); however, they differed from the spores from the cranial cavity in being more elongated and having a large polar capsule with a smaller incline. SSU rDNA sequences of the specimens collected in this report from the head skin (1937 bp, GenBank: MH119078) and cranial cavity (1945 bp, GenBank: MH119079) were 99.9% similar.

Taxonomic summary

Type host: *Hypophthalmichthys molitrix* (Cuvier et Valenciennes, 1844) (Cypriniformes: Cyprinidae)

Type locality: Amur River, China

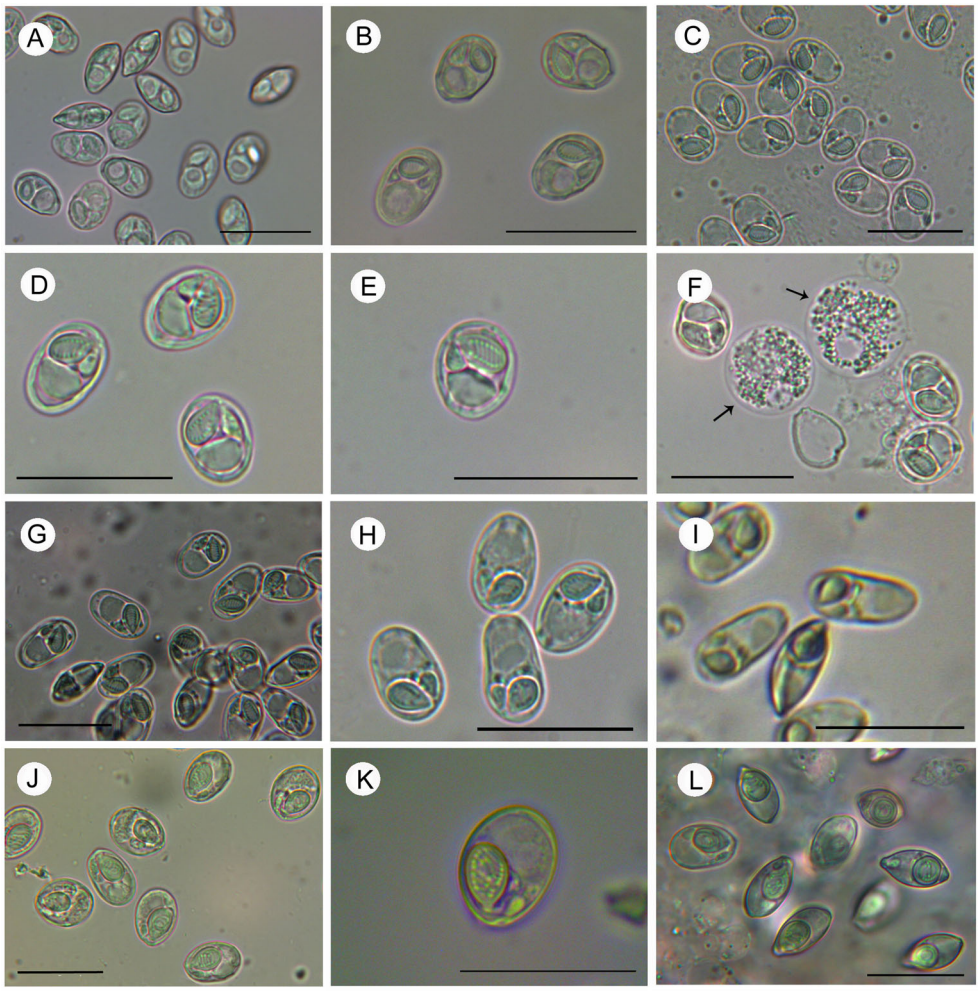

Fig. 2 Light photomicrographs of myxospores of *Myxobolus drjagini* (**a**–**i**) and *Myxobolus paratypicus* n. sp. (**j**–**l**) infecting the silver carp. Notes: formalin-fixed spores from the cranial cavity of the archival specimen collected in the 1980s (**a**–**c**), fresh spores from the olfactory nerve (**d**–**f**) (arrows in f indicate presporogonic stages), the head subcutaneous tissue (**g**–**i**), and fresh spores from the gill filament (**j**–**l**). Scale bars = 20 μm

Other localities: Lake Balaton, Hungary; Lake Taihu, Zhejiang, and Hubei provinces, China.

Site of infection: histozoic, oval plasmodia in the head skin (subcutaneous tissue) and cranial cavity (olfactory and oculomotor nerves)(Fig. 1a–c)

Prevalence: in the 23 silver carp examined, plasmodia of *M. drjagini* were observed in the head skin of five individuals (21.7%), and, three of the five infected individuals also had plasmodia in the cranial cavity (13%)

Materials deposited: myxospores fixed in 10% formalin were deposited in the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (SCM20160610–11). The SSU rDNA sequence of *Myxobolus* drjagini was deposited in GenBank (MH119078, head skin; MH119079, cranial cavity).

Description of *Myxobolus paratypicus* n. sp.

Myxospores (Fig. 2j–l, Fig. 3e, f): mature fresh spores (from 3 plasmodia, $n = 20$) oval, asymmetric in frontal view, and fusiform in sutural view, 13.8 (12.9–14.9) μm long, 9.9 (9.2–11.1) μm wide, and 7.0 μm thick. Two unequal, pyriform polar capsules, ratio > 4:1, with slightly converging anterior ends; openings situated near long axis of spore. Large polar capsule 7.5 (6.2–8.2) long and 5.0 (4.2–5.6) wide, small polar capsule 2.7 (2.1–3.6) μm long and 1.4 (1.1–1.9) μm wide. Polar filaments coiled with 7–8 turns in the large polar capsule. Posterior end of polar capsule extends beyond the middle of spore body. Shell valves smooth. Sutural folds difficult to discern.

Remarks: the new species *Myxobolus paratypicus* n. sp. was similar in spore shape and measurements to "*Myxobolus drjagini*", a species from the gills of silver carp described in a previous report of fish myxosporeans from Lake Taihu (Ma 1993; Fig. 3g, h; Table 1)). However, *M. paratypicus* n. sp. could be distinguished from *M. drjagini* described here in the size and arrangement of its polar capsules.

Several myxosporeans with two polar capsules of unequal size have been reported from the silver carp, such as *M. pavlovskii* (Akhmerov, 1954) Landsberg et Lom, 1991; *M. hypophthalmichthydis* (Dogiel et Achmerov, 1960) Chen et Ma, 1998; *M. nobillis* Li et Nie, 1973; *M. aristichthys* Nie et

Table 1 Morphometric data of *Myxobolus drjagini* and *Myxobolus paratypicus* n. sp. and species with unequal polar capsule reported from the silver carp *Hypophthalmicthys molitrix*

Species	Infected organ	SL	SW	ST	LPL	LPW	SPL	SPW	NC	Reference
M. drjagini[a]	Brain	12.4 (11.7–13.3)	8.9 (8.4–9.9)	6.3	5.5 (4.9–6.2)	3.3 (2.8–3.8)	3.2 (2.6–4.4)	1.9 (1.4–3.0)	7–8	This study
M. drjagini	Brain	12.8 (11.8–14.0)	8.9 (8.3–9.6)	7.1	5.8 (5.2–6.5)	3.3 (2.9–3.7)	3.0 (2.3–3.7)	1.7 (1.4–2.0)	7–8	This study
M. drjagini	Head skin	13.9 (12.6–14.7)	8.4 (7.5–9.9)	6.5	5.2 (4.7–5.8)	3.7 (3.1–4.2)	3.1 (2.3–3.5)	1.9 (1.2–2.6)	7–8	This study
M. drjagini	Head skin	14.0	9–10	6.0	6.0	3.0	4.0	2.0–2.5	–	Akhmerov 1954
M. paratypicus n. sp.	Gill	13.8 (12.9–14.9)	9.9 (9.2–11.1)	7.0	7.5 (6.2–8.2)	5.0 (4.2–5.6)	2.7 (2.1–3.6)	1.4 (1.1–1.9)	7–8	This study
M. paratypicus n. sp.[b]	Gill	13.2 (12.1–13.6)	8.8 (7.6–9.1)	6.7 (6.8–7.8)	7.6 (6.8–7.8)	5.0 (4.6–5.3)	2.1 (2.0–3.0)	1.3 (1.0–1.5)	–	Ma 1993
M. atypicus	Gill	14.7 (13.2–16.8)	10.5 (8.5–10.8)	7.0–8.4	8.4 (7.4–9.6)	5.8 (5.4–6.0)	5.0 (3.6–5.4)	3.0 (2.4–3.6)	6–7	Chen and Ma 1998
M. nobillis	Gill	12.7 (12.0–14.0)	10.6 (10.2–12.0)	6.6–6.8	7.4 (7.2–7.5)	5.4 (4.8–5.8)	3.7 (3.2–4.5)	2.3 (2.2–2.5)	9	Chen and Ma 1998
M. hypophthalmichthydis	Head skin, intestine	13.9 (12.0–15.0)	10.6 (9.6–11.4)	8.4 (7.9–8.9)	6.4 (4.8–7.2)	3.6 (3.2–3.8)	3.5 (3.0–3.6)	1.9 (1.4–2.4)	5–6	Chen and Ma 1998
M. aristichthys	Heart	17.4 (16.8–18.0)	13.3 (12.0–14.4)	9.8 (9.6–10.2)	11.5 (10.8–12.0)	8.3 (7.8–9.0)	3.6 (3.0–4.8)	1.8 (1.6–2.2)	6–8	Chen and Ma 1998

All measurements are in μm. *SL*, spore length; *SW*, spore width; *ST*, spore thickness; *LPL*, larger polar capsule length; *LPW*, larger polar capsule width; *SPL*, smaller polar capsule length; *SPW*, smaller polar capsule width; *NC*, number of coils in larger polar filament

[a] Archival specimen collected in 1980s and stored in formalin

[b] The myxosporean in literature was incorrectly identified as *Myxobolus drjagini* (Ma 1993)

Yin, 1973; and *M. atypicus* Li et Nie, 1973. However, *M. paratypicus* n. sp. differed from *M. pavlovskii* and *M. hypophthalmicthys* with a higher size ratio of the two polar capsules. Moreover, the polar capsules *M. aristichthys* were larger than those of *M. paratypicus* n. sp., and the large polar capsule almost occupied 1/2–2/3 of the spore body. The spore apex of *M. nobillis* was tapered, which was different from that of the round shape of *M. paratypicus* n. sp. This new species closely resembled *M. atypicus*, but the small polar capsule of the latter was larger than this new species (Table 1).

Taxonomic summary

Type host: *Hypophthalmichthys molitrix* (Cuvier et Valenciennes, 1844)

Type locality: Lake Taihu (31° 25′ N, 120° 17′ E), Wuxi, China

Other localities: unknown

Site of infection: histozoic, oval plasmodia in the epithelium of the gill filament (Fig. 1d)

Prevalence: 8.7% (2/23)

Materials deposited: spores fixed in 10% formalin were deposited in the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (SCM20160612).

The SSU rDNA sequence of *Myxobolus paratypicus* n. sp. (1939 bp) was deposited in GenBank under the accession number MH119080.

Etymology: the species is named with reference to the morphological similarity to *Myxobolus atypicus* Li et Nie, 1973. The new specific name is the combination of "para" and "atypicus".

Molecular data and phylogenetic analysis

Sequence alignment revealed the SSU rDNA sequences of *M. drjagini* and *M. paratypicus* n. sp. generated in this report showed 94.7% similarity (Table 2) and therefore differed by more than the common intraspecific sequence variation (< 1%; Zhao et al. 2013). A BLAST search in GenBank indicated that the sequences of *M. drjagini* and *M. paratypicus* n. sp. were not identical to any myxosporean sequences available, but all closely aligned with myxosporeans collected from the bighead carp and silver carp, *M. carnaticus*, *M. pavlovskii*, and *M. allotypica* (91.3%–95%) (Table 2). Surprisingly, the sequences of *M. drjagini* reported here and that deposited in GenBank (AF085179) showed only 64% identity. In a previous study, Molnár (2011) also questioned the

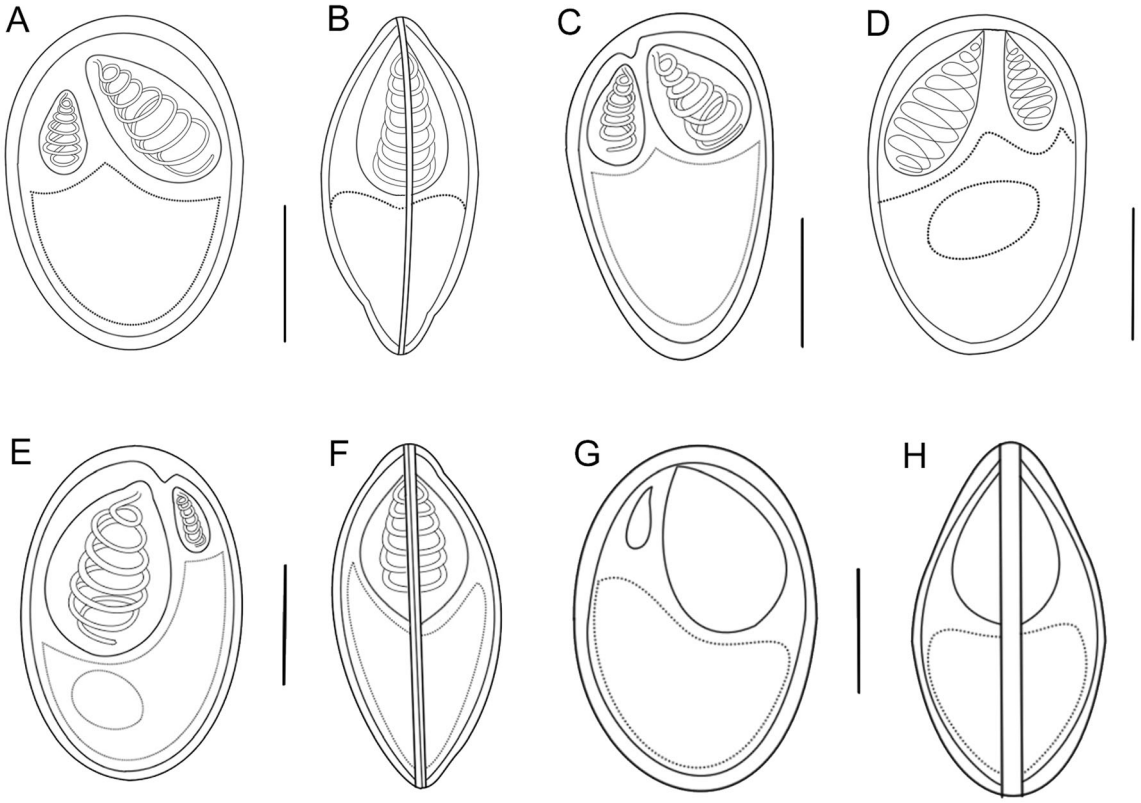

Fig. 3 Line drawings of *Myxobolus drjagini* (**a**–**d**) and *Myxobolus paratypicus* n. sp. (**e**–**h**) from silver carp. Notes: the spores from the olfactory nerve (**a**, **b**), from the head subcutaneous tissue (**c**), drawn based on the report of Akhmerov 1954 (**d**), collected from the head skin of silver carp, and (**g**, **h**) drawn based on the report of Ma 1993. Scale bars = 5 μm

sequence reliability of *M. drjagini* (AF085179, spelled incorrectly in GenBank). Phylogenetic analysis further suggested that *M. drjagini* and *M. paratypicus* n. sp. were two closely related species forming a subclade with robust branch support (Fig. 4) and showed the lowest genetic distance between *M. pavlovskii* and *M. allotypica* from the silver carp and bighead carp. The *M. drjagini* (AF085179) sequenced by Andree et al. (1999) was clustered in a far and unrelated clade, consisting of *M. insidiosus*, *M. cerebralis*, and *M. squamalis*, which were collected from trout.

Discussion

Most myxosporeans are organ- and tissue-specific parasites (Molnár 2011; Molnár and Eszterbauer 2015); however, *Myxobolus drjagini* seemed to be an exception infecting a wide range of organs of the silver carp according to the records in the literature, e.g., the kidney, swim bladder, liver, gall bladder, intestine, brain, brain-lymph, gill arch, skin and fin (see Chen and Ma 1998). In this report, *M. drjagini* was only found at two typical infection sites, the subcutaneous tissue of the head skin and the nerves of the cranial cavity of silver carp,

Table 2 Similarity matrix for the 18S rDNA sequences of *Myxobolus* species from the silver carp and bighead carp

Species	1	2	3	4	5	6
*Myxobolus drjagini**		0.001	0.039	0.061	0.049	0.091
*Myxobolus drjagini***	2		0.039	0.061	0.049	0.091
Myxobolus paratypicus n. sp.	76	76		0.057	0.051	0.093
Myxobolus allotypica	93	93	87		0.028	0.082
Myxobolus pavlovskii	95	94	99	42		0.055
Myxobolus carnaticus	176	176	179	124	109	

The lower triangle matrix shows the pairwise number of base differences, while the upper triangle shows the pairwise differences in the term of percentage

*From the brain of silver carp

**From the head skin of silver carp

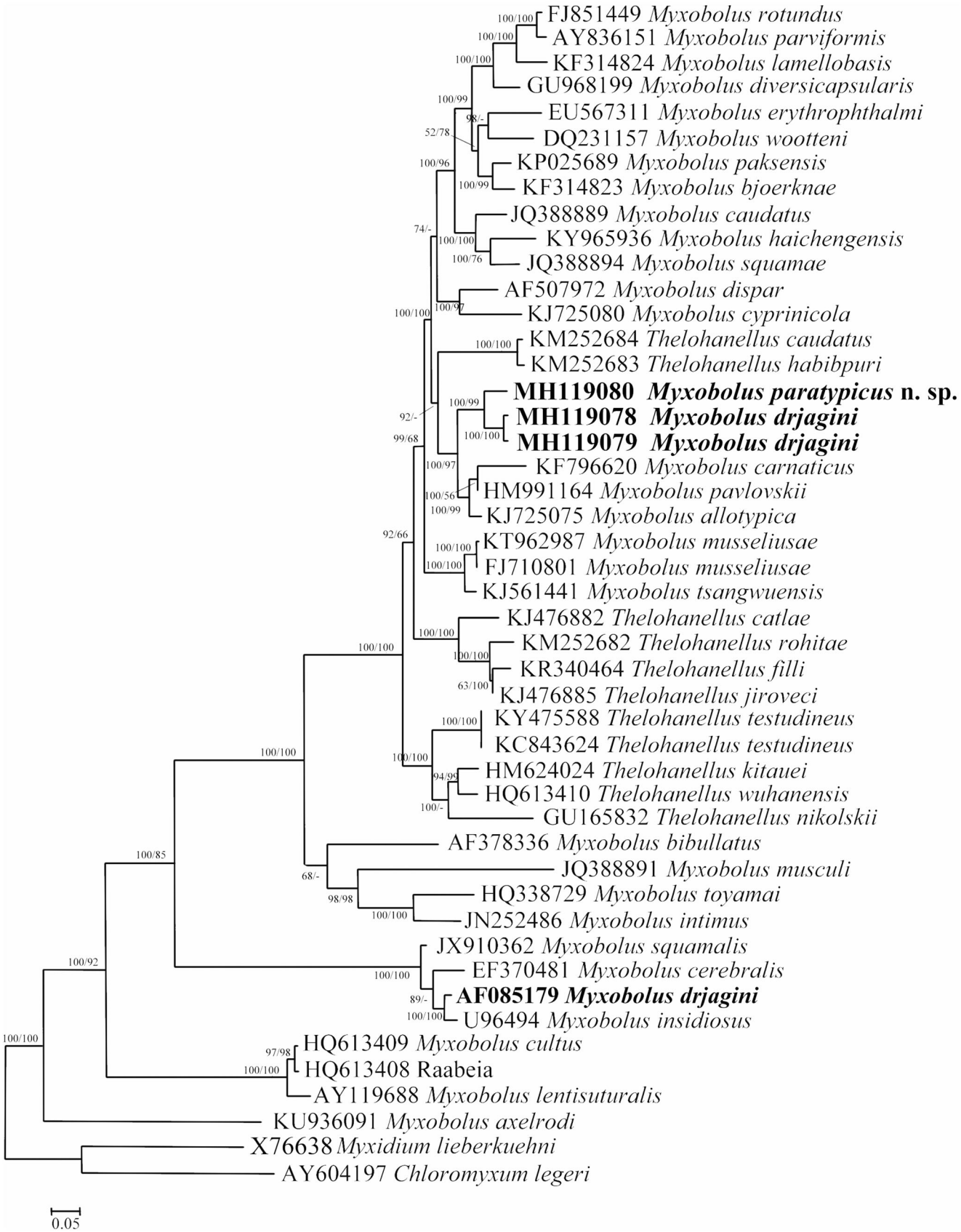

Fig. 4 Phylogenetic tree of myxozoans inferred using Bayesian inference (BI) and maximum likelihood (ML) analyses with SSU rDNA sequences. Values at the branches are posterior probability for BI and bootstrap support for ML

and was identified based on morphological characteristics and DNA sequences. However, spores from the two sites differed in spore shape and arrangement of their polar capsules (Figs. 2 and 3); these variations could be a response to differences in nutrition and immunological responses associated with different host organs.

The lack of distinguishable morphological characteristics and intraspecific spore variation makes myxosporean identification difficult. DNA sequences, mostly SSU rDNA, have been widely used to identify and characterize myxosporean species (Kato et al. 2017; Camus et al. 2017). To date, hundreds of myxosporean sequences have been obtained and deposited in the GenBank database. However, some sequences derived from poorly identified samples and atypical hosts or organs should be used cautiously in sequence analyses (Molnár 2011). Molnár (2011) placed the SSU rDNA sequence of "*M. drjagini*" (AF085179) determined by Andree et al. (1999) into an uncertain group. In this study, the newly obtained sequences showed very low similarity (64%) with that of Andree et al. (1999) and clustered separately into far, unrelated clades (Fig. 4). Therefore, the previous sequence of *M. drjagini* (AF085179) was confirmed here to be invalid and may have been detected from a contaminated or mixed sample. *M. drjagini* is distributed widely in wild water bodies in China (Wu et al. 1975; Chen and Ma 1998) and is a severe threat to the feral and cultured silver carp stock. The valid SSU rDNA sequence of *M. drjagini* detected in this report will facilitate further epidemiological investigation.

Fixation of myxosporean specimens can affect the morphological dimensions of spores, and 10% neutral formalin was a commonly used fixative because it produced the least shrinkage of spores (Parker and Warner 1970). In this study, spores of *M. drjagini* from the archival specimen fixed in formalin since 1980s, showed little shrinkage compared with the fresh spores examined here, e.g., spore length 12.4 μm vs. 12.8 μm, spore width 8.9 μm vs. 8.9 μm, spore thickness 6.3 μm vs. 7.1 μm, larger polar capsule length 5.5 μm vs. 5.8 μm. However, it is difficult to extract high-quality DNA for PCR from formalin-fixed specimens, and the trial here to amplify the SSU rDNA from the archival specimen failed. In future studies, it would be a useful procedure to respectively fix partial specimen in formalin and ethanol for morphological and molecular analyses.

Tissue tropism is an important characteristic used for distinguishing myxosporeans, and many species show tissue tropism in spore development in their fish host (Atkinson et al. 2015; Molnár and Eszterbauer 2015). In the 23 silver carp examined here, *Myxobolus paratypicus* n. sp. was found in the gill filament, while *M. drjagini* was only observed in the subcutaneous tissue of the head skin and the nerves of the cranial cavity. DNA sequence analysis showed high similarity (96%) and revealed that they were separate yet closely related species. *Myxobolus drjagini* has been reported from more than ten organs of the silver carp in China, e.g., the gill arch, eye socket, skin, heart, brain, kidney, swim bladder, intestine, gall bladder, body cavity, fin, and liver (Wu et al. 1975; Chen and Ma 1998). However, in the current study, *M. drjagini* was found only in the head skin and the brain. Due to the small sample size examined (23 individuals) and low prevalence of infection, further extensive investigation is needed to uncover *M. drjagini* occurrence in fish host organs.

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Compliance with ethical standards

Animal experiments were approved by the Ethical Committee of the Freshwater Fisheries Research Center and followed the national guidelines for the care and use of vertebrate animals.

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

References

Akhmerov AK (1954) On closely related species of a new myxosporean genus. Dokl Akad Nauk SSSR 97:1101–1103 **in Russian**

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(1):68–74

Atkinson SD, Bartošová-Sojková P, Whipps CM, Bartholomew JL (2015) Approaches for characterizing myxozoan species. In: Okamura B, Gruhl A, Bartholomew J (eds) Myxozoan evolution, ecology and development. Springer International Publishing, Cham

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 domestic fowl inferred using complete small ribosomal DNA sequences. J Parasitol 83:262–271

Camus AC, Dill Jennifer A, Rosser TG, Pote LM, Griffin MJ (2017) *Myxobolus axelrodi* n. sp. (Myxosporea: Myxobolidae) a parasite infecting the brain and retinas of the cardinal tetra *Paracheirodon axelrodi* (Teleostei: Characidae). Parasitol Res 116:387–397

Chen QL, Ma CL (1998) Fauna Sinica. Invertebrata, Vol 15. Myxozoa, Myxosporea. Science Press, Beijing

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

El-Mansy A, Molnár K (1997) Extrapiscine development of *Myxobolus drjagini* Akhmerov, 1954 (Myxosporea, Myxobolidae) in oligochaete alternative hosts. Acta Vet Hung 45:427–438

Kato E, Kasai A, Tomochi H, Li YC, Sato H (2017) Four *Myxobolus* spp. (Myxosporea: Bivalvulida) from the gill lamellae of common carp (*Cyprinus carpio*) and Japanese silver crucian carp (*Carassius*

langsdorfii) in the western part of Japan, with the description of three new species (*M. tanakai* n. sp., *M. paratoyamai* n. sp., and *M. ginbuna* n. sp.). Parasitol Res 116:2427–2441. https://doi.org/10.1007/s00436-017-5545-4

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870–1874

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

Ma C (1993) Myxosporidia of the freshwater fishes from Taihu Lake (2). J Chongqing Teacher College (Natural Science Edition) 10(1):1–17 **(in Chinese with English abstract)**

Molnár K (1994) Comments on the host, organ and tissue specificity of fish myxosporeans and types of their intrapiscine development. Parasitol Hung 27:5–20

Molnár K (2011) Remarks to the validity of GenBank sequences of *Myxobolus* spp. (Myxozoa, Myxosporidae). Acta Parasitol 56(3): 263–269. https://doi.org/10.2478/s11686-011-0054-6

Molnár K, Eszterbauer E (2015) Specificity of infection sites in vertebrate hosts. In: Okamura B, Gruhl A, Bartholomew J (eds) Myxozoan evolution, ecology and development. Springer International Publishing, Cham

Parker JD, Warner MC (1970) Effects of fixation, dehydration and staining on dimensions of myxosporidan and microsporidan spores. J Wildl Dis 6:448–456

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformat 19(12):1572–1574

Sarker S, Kallert DM, Hedrick RP, El-Matbouli M (2015) Whirling disease revisited: pathogenesis, parasite biology and disease intervention. Dis Aquat Org 114:155–175

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25(24):4876–4882. https://doi.org/10.1093/nar/25.24.4876

Wu PH, Chang ZH, Chang J, Chen YS (1975) Twist disease of *Hypophthalmichthys molitrix* in Hangchow region of Chekiang Province. Acta Zool Sin 21(2):190–196 **in Chinese with English abstract**

Xi BW, Xie J, Zhou QL, Pan LK, Ge XP (2011) Mass mortality of pond-reared *Carassius gibelio* (Bloch) caused by *Myxobolus ampullicapsulatus* in China. Dis Aquat Org 93:257–260

Xi BW, Zhang JY, Xie J, Pan LK, Xu P, Ge XP (2013) Three actinosporean types (Myxozoa) from the oligochaete *Branchiura sowerbyi* in China. Parasitol Res 112:1575–1582

Zhang JY, Yokoyama H, Wang JG, Li AH, Gong XN, Ryu-Hasegawa A, Iwashita M, Ogawa K (2010) Utilization of tissue habitats by *Myxobolus wulii* Landsberg et Lom, 1991 in different carp hosts and disease resistance in allogynogenetic gibel carp: Redescription of *M. wulii* from China and Japan. J Fish Dis 33:57–68

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