Morphological, histological and molecular characteristics of Myxobolus spp. (Cnidaria: Myxozoa) infecting the kidney of silver carp in Lake Taihu

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Abstract

Myxozoans are common microscopic endoparasites in fish, and some are highly pathogenic to their wild and farmed fish hosts. In the present study, myxosporeans infection in the kidney of silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) from Lake Taihu, was investigated, and two dominate species, *Myxobolus lieni* (Nie & Li, 1973) and *Myxobolus varius* (Achmerov, 1960), with infection prevalence 60.2% and 35.2% respectively, were well characterized based on morphological, histopathological and DNA sequence data. *M. lieni* formed small roundish plasmodia in the epithelial cells of renal tubules. The mature myxospores appeared suborbicular, slightly flat in frontal view and fusiform shaped in sutural view. Dispersed myxospores of *M. varius* were found in the renal interstitium without forming plasmodia structures and enclosed within melano-macrophage centers. The spore appeared elliptical in frontal view, with wider anterior than posterior and shuttle shaped in sutural view. Interestingly, the occurrence of myxozoans in the kidney detected with SSU rDNA PCR and clone sequencing, revealed co-infection of five *Myxobolus* species. BLASTn search indicated SSU rDNA gene sequences obtained here were not identical to any sequence available in GenBank. Phylogenetic analyses showed that the five *Myxobolus* detected here were clustered together, forming a separate clade of cyprinid-infecting myxozoans.

Introduction

Myxozoans are widespread endoparasitic cnidarians of aquatic vertebrates and invertebrates, and most have been described from marine and freshwater fishes (Rosser et al. 2016). Until now, over 2500 named species have been described using morphological characteristics of myxospores (Okamura et al. 2018; Saha et al. 2018; Naldoni et al. 2019, Liu et al. 2019). While many myxozoans cause asymptomatic or subclinical infection in fish hosts, some species could induce serious diseases and mortality, e.g., *Myxobolus cerebralis*, the causative agent of whirling disease in salmonids; *Sphaerospora dykovae*, the pathogen of acute swim bladder inflammation disease in common carp; and *Myxobolus honghuensis*, the cause of swollen pharynx in gibel carp (Lom and Dyková 2006; Schmidt-Posthaus et al. 2015; Xi et al. 2015; Yang et al. 2022).

Silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844), a filter-feeding freshwater fish and indigenous cyprinid in East Asia (Zhao et al. 2018). It has been introduced worldwide for aquaculture and stocked in lakes and reservoirs to prevent cyanobacterial blooms. However, in North America, it entered and became established in local rivers, raising more and more concern about its threat to water ecosystems. In China, silver carp is an important cultured fish, which is extensively reared in earthen ponds, lakes and reservoirs, and its annual production is approximately 4.5 million tons.

Myxosporean infections are significant threats to silver carp health (Wu et al. 1979; Marton and Eszterbauer 2011; Xi et al. 2019). Until now, over 70 species have been described from different organs of silver carp (Chen and Ma 1998; Zhang et al. 2018). Unfortunately, most myxozoans from silver carp in literature were simply recorded with spore morphological characteristics, while there is limited information on tissue tropism and DNA sequences. Myxosporeans are common parasites of fish kidney, and most have specific site of sporogony (Molnár 2007; Molnár and Eszterbauer 2015). In this study, we report the myxosporeans found in the kidney of silver carp from Lake Taihu, based on morphological characteristics, histological and molecular characteristics.

Materials And Methods

Fish sampling and microscopic examination

Between June and December 2018, 108 silver carp originated from Lake Taihu, China (31°32'N, 120°13'E) were collected and examined for parasites. Fish were transported to the laboratory immediately on ice, dissected and inspected with a routine procedure. Wet mount smears of kidney were observed under the microscope (Olympus CX31) equipped with a digital camera. Morphological and morphometric data of myxospores found in the tissues were obtained according to the guidelines of Lom and Dyková (2006). Line drawing sketches of the myxospores were made based on the digitized images. All measurements were given in micrometers (μm) unless otherwise indicated.

Histopathological section

In order to determine the exact location and distribution of myxosporeans in the kidney tissue, fresh samples were cut (1–3 cm), fixed in 4% buffered paraformaldehyde, and embedded in paraffin. Sections (6–8 μm) were made and stained with hematoxylin and eosin (H&E).

DNA extraction and sequencing

After microscopic examination, the same kidney smear was collected in a 1.5-mL centrifuge tube and used for DNA extraction. DNA was extracted with a QIAamp® DNA Micro Kit (Qiagen, Germany) according to the manufacturer's instructions. Partial SSU ribosomal DNA (rDNA) sequence was amplified with primers ERIB1 and ERIB10 (Barta et al. 1997). The PCR products were purified using a SanPrep Column DNA Gel Extraction Kit (Sangon Biotech, Shanghai, China), cloned into the pMD-18T vector system (Takara), and then 10 positive clones of each sample were picked out. The clones were sequenced using the universal primers M13F & R, and a walking primer (5'-CTTCTTTCATTTATTAAATCGGGAC-3') with an ABI 3730XL DNA analyzer (Applied Biosystems). The contiguous sequences were assembled and inspected using the software SeqMan (Lasergene, DNAStar).

Sequences alignment analysis showed a mixed infection of myxosporeans in kidney. To confirm the correspondence of myxospore and sequence, two dominant myxospores were sorted and directly sequenced as follow: kidney tissue was grounded and diluted with saline until a few myxospores were visible on slide under the microscope. Individual myxospores (n = 10) from each species were collected using a 20-μL pipette tip. DNA was extracted with lysis buffer for microorganism to direct PCR (Takara) following the manufacturer's instructions. The partial SSU rDNA sequences were amplified using a nested PCR assay with external primers ERIB1 and ERIB10, and internal primers MyxospecF and MyxospecR (Fiala 2006). The PCR products were purified and sequenced directly using the internal primers.

Phylogenetic analysis

The SSU rDNA partial sequences of myxosporeans obtained in this study were deposited in to GenBank (1994–2020 bp, MK371243–MK37124348). Homologous sequences were determined by performing BLASTn search at NCBI, and 46 closely related myxosporean sequences were downloaded from yhe NCBI database. Sequences alignment was conducted using the Clustal X 1.8 program (Thompson et al. 1997) and MEGA 7.0 software with the default setting (Kumar et al. 2016). Phylogenetic trees were conducted with Bayesian inference (BI) and maximum likelihood (ML) methods. The best nucleotide substitution model (GTR + F + I + G4) was estimated with Modelfinder according to BIC (Bayesian Information Criterion). ML tree was constructed using IQ-TREE (Nguyen et al. 2015), and branch support was estimated with 1000 bootstrap replicates. BI analyses were conducted using the software MrBayes ver. 3.1.2 with parameter settings nruns = 4, nst = 6, rates = invagamma, and ngen = 5,000,000. Posterior probability values were used as support for the Bayesian topology. *Zschokkella* sp. (Holzer, 2004), *Hoferellus gilsoni* (Holzer, 2003) were chosen as outgroups.

Results

A high prevalence of myxosporean infection (78.7%, 85/108) was determined in the kidney of silver carp. Two dominant myxosporeans were found under microscope and were identified as *Myxobolus lieni* and *Myxobolus varius*. The infection prevalence of *M. lieni* and *M. varius* were 60.2% (65/108) and 35.2% (38/108), respectively; meanwhile, a co-infection of the two species were found in 18 silver carp (16.7%, 18/108). Morphological, histological, and molecular characteristics of the two species were described as follows.

Morphological characteristics of *Myxobolus lieni* (Nie & Li, 1973)

Mature spores (Fig. 1a–d) appeared suborbicular and slightly flat in frontal view, fusiform shaped in sutural view, measuring 7.33 ± 0.22 (6.62–7.96) in length, 7.51 ± 0.14 (6.93–8.23) in width (n = 50), and 5.78 ± 0.13 (5.5–6.08) in thickness (n = 10). Two equal polar capsules, pear-shaped with apophysis at top end, measuring 4.29 ± 0.34 (3.81–4.82) in length, 2.92 ± 0.13 (2.56–3.29) in width, and polar tubules coiled 4–5 turns.

Type host

Silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844)

Host size

22.0–35.0 cm

Site of infection

histozoic, plasmodia found in the intraepithelium of renal tubular (Fig. 2a–c)

Prevalence

60.2% (65/108)

Locality

Lake Taihu, Wuxi, China.

Vouch specimens

Infected tissues fixed in 4% buffered paraformaldehyde were deposited in Freshwater Fisheries Research Center, Chinese Academy of Fishery Science (accession no. MTR20181116)

Morphological characteristics of *Myxobolus varius* (Achmerov, 1960)

Mature spores (Fig. 1e–h) appeared to be ellipse in frontal view, with wider anterior than posterior, and shuttle shaped in sutural view, measuring 11.03 ± 0.005 (10.24–12.11) in length, 7.34 ± 0.055 (6.36–7.67) in width (n = 30), and 5.49 (5.19–5.71) in thickness (n = 5). Two equal polar capsules are pyriform with apophysis at top end, measuring 4.66 ± 0.08 (4.32–4.94) in length, 2.64 ± 0.03 (2.40–2.98) in width, polar tubules coiled 4–5 turns.

Type host

Silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844)

Host size

20.1–36.4 cm

Site of infection

histozoic, renal interstitium, plasmodia not found

Prevalence

35.2% (38/108)

Locality

Lake Taihu, Wuxi, China

Vouch specimens

Infected tissues fixed in 4% buffered paraformaldehyde were deposited in Freshwater Fisheries Research Center, Chinese Academy of Fishery Science (accession no. MTR20181117)

Histology

No significant pathological changes were observed in the sections of infected fish kidney. Small roundish plasmodia of *M. lieni*, containing several mature myxospores, were found within the epithelial cells of the renal tubules (Fig. 2a–c). Dispersed myxospores of *M. varius* were found in the renal interstitium without forming plasmodia structures and enclosed within melano-macrophage centers (Fig. 2d).

Molecular data

Partial SSU rDNA sequences of *M. lieni* (1994 bp, GenBank accession MK371243) and *M. varius* (2019 bp, MK371244) were obtained; and in the 20 PCR clones of kidney tissues, eight clones showing sequence similarity 99.5–99.9% were identified as *M. lieni*, six with 99.9–100% similarity were identified as *M. varius*. Meanwhile, six clones showing only 88.8–97.2% similarity, which significantly beyond the common intraspecific variation < 2% (Table 2). Here we tentatively assigned these sequences as *Myxobolus* sp1 (1 clone, MK371245), *Myxobolus* sp2 (1 clone, MK371246), *Myxobolus* sp3 (4 clones, MK371247 and MK371248).

Table 1
Comparative data for spore measurements (means ± standard deviation in micrometres) of *Myxobolus lieni* and *Myxobolus varius* and morphologically sim species

Species	Host	Site in host	SL	SW	PCL	PCW	TS	PTC	Reference
M. lieni	*Hypophthalmichthys molitrix*	Kidney	7.33 ± 0.22 (6.62–7.96)	7.51 ± 0.14 (6.93–8.23)	4.29 ± 0.34 (3.81–4.82)	2.92 ± 0.13 (2.56–3.29)	5.78 ± 0.13 (5.5–6.08)	4–5	Present study
M. lieni Nie et Li, 1973	*Hypophthalmichthys molitrix* *Hypophthalmichthys nobilis*(Richardson, 1845)	Kidney, gills, Urinary bladder	7.2–7.4	7.2–7.4	3.8(3.6–4.2)	2.8 (2.6–3.0)	4.8–5.0	5	Chen and Ma, 1998
M. artus	*Hypophthalmichthys molitrix*	Spleen, Intestine, Kidney	6.0 (5.4–6.6)	8.1 (7.2–8.4)	3.4 (3.1–3.6)	2.7 (2.6–2.8)	5.6 (5.5–5.7)	4–5	Chen and Ma, 1998
M. parvus	*Hypophthalmichthys molitrix* *Hypophthalmichthys nobilis*	Almost all organ	6.1 (5.8–6.4)	6.0 (5.6–6.2)	3.2 (3.0–3.4)	2.3 (2.2–2.4)	4.2	4–5	Chen and Ma, 1998
M. varius	*Hypophthalmichthys molitrix*	Kidney	11.03 ± 0.005 (10.24–12.11)	7.34 ± 0.06 (6.36–7.67)	4.66 ± 0.08 (4.32–4.94)	2.64 ± 0.03 (2.40–2.98)	5.49 (5.19–5.71)	5	Present study
M. varius Achmerov, 1960	*Hypophthalmichthys molitrix*	Kidney, Intestine	10.9(9.0–13.0)	7.3(6.0–8.5)	5.2 (4.1–6.0)	2.7 (2.2–3.0)	5.6 (4.5–6.5)	5–6	Chen and Ma, 1998
M. ellipsoides	*Hypophthalmichthys molitrix* *Hypophthalmichthys nobilis*	Almost all organ	11.0 (9.6–12.0)	7.3 (7.0–7.8)	4.9 (4.5–5.4)	3.0 (2.4–3.2)	6.0	5	Chen and Ma, 1998
M. pronini	*Carassius auratus gibelio*	Mesentery, Abdominal cavity	14.7 ± 0.24 (13.8 – 15.6)	9.6 ± 0.65 (9.0 – 13.3)	5.3 ± 0.16 (4.8 – 5.6)	3.0 ± 0.12 (2.9 – 3.4)	6.6 ± 0.16 (6.2 – 7.2)	5–6	Liu et al. 20

Abbreviations: *SL* spore length, *SW* spore width, *ST* spore thickness, PCL polar capsule length, *PCW* polar capsule width, *PTC* polar tubules coil.

Table 2
SSU rDNA sequence similarity (%) between *Myxobolus* spp. isolated from kidney of silver carp (p-distance).

	M. lieni	*Myxobolus* sp1	*Myxobolus* sp2	*Myxobolus* sp3	*M. varius*
M. lieni	99.5–99.9				
Myxobolus sp1	96.7–97.2	-			
Myxobolus sp2	89.2–89.5	88.4	-		
Myxobolus sp3	89.1–89.6	87.5–87.8	89.2–89.5	99.2–100	
M. varius	90.3–90.7	88.8–88.9	90.7–90.6	93.1–93.8	99.9–100

BLASTn analyses showed the new obtained sequences were not identical to any sequences available in GenBank. The SSU rDNA sequence of *M. lieni* has a relative higher identity with *Myxobolus intimus* (JF311899), *Myxobolus pronini* (MH329619), *Myxobolus pavlovskii* (MG520368), *Myxobolus abitus* (MG520367), *Myxobolus linzhiensis* (KY965935), 86.8–83.5%; *M. varius* has a relative higher identity with *Myxobolus pavlovskii* (MG520368), *Myxobolus kiuchowensis* (MG520366), *Myxobolus abitus* (MG520367), *Myxobolus drjagini* (MH119078), 86.2–85.9%.

Bayesian inference (BI) and maximum likelihood (ML) analyses conducted with SSU rDNA all demonstrated similar phylogenetic topology of myxozoans examined (Fig. 3). *M. lieni* and *M. varius*, as well as the 3 unidentified *Myxobolus* in the kidney of silver carp, formed a separate clade of closely related to cyprinid-infecting myxozoans. However, this clade was away from the species recently detected from the other organs of silver carp and bighead carp, such as *M. pavlovskii*, *M. kiuchowensis*, *M. drjagini*, *M. paratypicus* and *M. abitus* (Chen and Ma 1998).

Discussion

Myxobolus lieni (Nie & Li, 1973) was firstly reported from the kidney and spleen of silver carp in a list of myxosporeans from Lake Huama (Hubei, China) without a detailed description in 1964. Subsequently, it was formally published in the monograph of Chen (1973), and cited in the synopsis of *Myxobolus* species by Eiras et al. (2005). However, a new *Myxobolus* species found from *Brycon orthotaenia* in South America was named as *lienis* in recently, which refers to the site of infection in fish host (splean in latin: lien) (Naldoni et al. 2019). Although the two specific names were extremely similar and raised a question about the priority, we tentatively considered them all to be valid as the etymological motivation of *lieni* was not available in its original description.

The myxospores of *M. lieni* found here showed consistent morphological characteristics, measurements, plasmodia and host organ with the original description in the literature. It resembled *Myxobolus artus* (Achmerov, 1960) and *Myxobolus parvus* (Schulman, 1962), all infecting the kidney of silver carp. However, *M. artus* and *M. parvus* have smaller spore size (Table 1), while *M. artus* has much flatter spore shape, the ratio of polar capsule length (PCL) to spore length (SL) in *M. parvus* is much bigger. Furthermore, SSU rDNA sequence analysis also demonstrated that *M. lieni* examined had lower than 77% similarity with *M. artus* (FJ710799) and *M. parvus* (KX242161).

The myxospores of *M. varius* examined in this study also showed consistent morphological characteristics, measurements and host with the original description in the literature (Chen and Ma 1998). It differed from the morphometrically related species *Myxobolus ellipsoides* (Thélohan, 1892) by having elliptical myxospores with a wider anterior, while the latter has an oval-spore. Additionally, *M. ellipsoides* was reported from many organs of fish hosts (Chen and Ma 1998), thus *M. ellipsoides* may represent a cryptic species complex. *M. varius* closely resembled *Myxobolus pronini* found in the abdominal cavity of *Carassius auratus gibelio*. However, *M. pronini* had a much larger spore (Liu et al. 2016). In addition, BLASTn analysis further demonstrated that there was no identical myxozoan sequence available in GenBank to *M. varius*, and the highest similarity was not above 86.3%.

Myxozoans are commonly found in fish kidney and have different development patterns and infection sites in this organ (Roberts 1975; Dykova 1984; Molnár and Kovács-Gayer 1985; Holzer and Schachner 2001; Molnár 2007; Abdel-baki et al. 2015). Until now, over eight *Myxobolus* species have been recorded in kidney of silver carp, such as *Myxobolus bramaeformis* (Akhmerov, 1960), *Myxobolus changshingenis* (Chen & Ma, 1998), *Myxobolus gourdiformis* (Li & Nie, 1973), *Myxobolus latus* (Shulman, 1962), *M. lieni*, *Myxobolus nephroides* (Li & Nie, 1973), *Myxobolus nobillis* (Li & Nie, 1973) and *M. varius*. However, the information regarding specific sites or tissue in kidney and sporogenesis sites of those *Myxobolus* was not recorded in the literature. Histological analysis showed that *M. lieni* examined here formed small plasmodia, containing 2–4 mature myxospores and located inside epithelial cells of the renal tubules (Fig. 2). This type of development is a common characteristic of species of *Myxobilatus* or *Hoferellus* (Molnár 2007). Although the pathogenicity of *M. lieni* cannot be precisely assessed here, the release of mature myxospores in the epithelium might induce serious damage to the renal tubules. Moreover, dispersed myxospores of *M. varius* were observed in the renal interstitium without plasmodial formation and surrounded by large masses of hemosiderin (Fig. 2). It was considered that these myxospores were translocated to the kidney from other organs and captured by macrophages (Molnár 2007).

PCR assay is more sensitive than microscopic examination, particularly in cases with low or mixed infection. In this study, PCR further demonstrated that, except *M. lieni* and *M. varius*, other three *Myxobolus* species were also co-occurring in the kidney of silver carp, although no visible plasmodia or spores of those species were observed. This phenomenon is mostly attributed to the low abundance or presporogonic stage of those species in the kidney. Several myxozoan co-infections in fish blood and organs also have been reported in previous research. For example, Holzer et al. (2014) detected multi-species infection in swim bladders of common carp suffering from Swim Bladder Inflammation (SBI) by sequencing clones of non-specific PCR products.

Phylogenetic analyses based on the SSU rDNA sequences revealed that myxozoans from cyprinid hosts formed separate clades distinct from other fish hosts in the family Catostomidae, Anguillidae and Salmonidae, whereas myxozoans in the same clade did not have strict organ preference (Fig. 3). Interestingly, the five *Myxobolus* determined here from kidney of silver carp were clustered in a sperate clade, and formed sister species which shared an ancestral species.

What caused the sympatric occurrence of those close related species, sympatric speciation or a secondary encounter of species originating from allopatry? To better understand the differences and the evolutionary relationship between the different *Myxobolus* species in further study, the analysis of marker genes other than ribosomal RNA gene sequences, the SSU and LSU, is also necessary.

Declarations

Author contribution

Bingwen Xi designed the study. Qingjie Zhou wrote the manuscript. Qingjie Zhou, Zeyi Cao and Zhipeng Gao carried out the experiment. Bingwen Xi and Kai Liu provided technical support. All authors reviewed the manuscript.

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Data availability

No other data and material are provided.

Ethics approval

Animal experiments were approved by the Ethical Committee of Nanjing Agricultural University and followed the national guidelines for the care and use of vertebrate animals.

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare no competing interests.

References

1. Abdel-baki, A. A. S., Abdel-haleem, H. M., Sakran, T., Zayed, E., Ibrahim, K. E. & Alquraishy, S. (2015). Two *Myxobolus spp.* infecting the kidney of nile tilapia (oreochromis niloticus) in the river nile at beni-suef govenorate, egypt, and the associated renal changes. Parasitology Research, *114*(3), 1107–1112. https://doi.org/10.1007/s00436-014-4282-1
2. Barta, J. R., Martin, D. S., Liberator, P. A., Dashkevicz, M., Anderson, J. W., Feighner, S. D., Feighner, S. D., Elbrecht, A., Perkins-Barrow, A., Jenkins, M. C., Danforth, H. D., Ruff, M. D. & Profous-Juchelka, H. (1997). Phylogenetic relationships among eight Eimeria species infecting domestic fowl inferred using complete small ribosomal DNAsequences. The Journal of Parasitology, *83*(2), 262–271. https://doi.org/10.2307/3284453
3. Chen, C. L. (1973). *An illustrated guide to the fish disease and causative pathogenic fauna and flora in the Hubei Province*. Science press, Beijing, (In Chinese).
4. Chen, Q. L. & Ma, C. L. (1998). *Myxozoa, Myxosporea. Fauna Sinica*. Science Press, Beijing, pp. 528 (In Chinese).
5. Dykova, I. (1984). The role of melanomacrophage centres in the tissue reaction to myxozoan infections of fishes. [cyprinic species, myxosporeans, *Sphaerospora renicola*, *Mitraspora cyprini*, *Thelohanellus*, *Hoferellus cyprini*, *Myxobolus*] *Bulletin of the European Association of Fish Pathologists*, *65*(4).
6. Eiras, J., Molnár, K. & Lu, Y. S. (2005). Synopsis of the species of *Myxobolus* Bütschli, 1882 (Myxozoa: Myxosporea: Myxobolidae). Systematic Parasitology, *61*(1), 1–46. http://doi.org/10.1007/s11230-004-6343-9
7. Fiala, I. (2006). The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. International Journal for Parasitology, *36*(14), 1521–1534. https://doi.org/10.1016/j.ijpara.2006.06.016
8. Holzer, A. S. & Schachner, O. (2001). Myxosporea and macrophage centers in chub (*Leuciscus cephalus*) quantitative interaction seems to be focused on *Myxobolus cyprini*. *Parasitology*, *122*(1), 55–62. https://doi.org/10.1017/s003118200000706x
9. Holzer, A. S., Hartigan, A., Patra, S., Pecková, H. & Eszterbauer, E. (2014). Molecular fingerprinting of the myxozoan community in common carp suffering Swim Bladder Inflammation (SBI) identifies multiple etiological agents. Parasites & Vectors, *7*(1) 398–407. https://doi.org/10.1186/1756-3305-7-398
10. Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054
11. Liu, X. H., Batueva, M. D., Zhao, Y. L., Zhang, J. Y., Zhang, Q. Q., Li, T. T. & Li, A. H. (2016). 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. Parasites & Vectors, *9*(1), 562–573. https://doi.org/10.1186/s13071-016-1836-3
12. Liu, X. H., Zhang, D. D., Yang, C. Z. & Zhao, Y. J. (2019). Morphological and molecular identification of *Myxobolus parakoi* sp. nov. (Myxozoa: Myxobolidae), from *Cyprinus carpio* in Chongqing China. *Zootaxa*, *4657*(1), 117–126. https://doi.org/10.11646/zootaxa.4657.1.4
13. Lom, J. & Dyková, I. (2006). Myxozoan genera: definition and notes on taxonomy, life cycle terminology and pathogenic species. Folia Parasitologica, *53*(1),1–36. https://doi.org/10.14411/fp.2006.001

14. Marton, S. & Eszterbauer, E. (2011). The development of *Myxobolus pavlovskii* (Myxozoa: Myxobolidae) includes an echinactinomyxon-type actinospore, Folia Parasitologica, *58*(2), 157–163. https://doi.org/10.14411/fp.2011.015
15. Molnár, K. & Kovács-Gayer, E. (1985). The pathogenicity and development within the host fish of *Myxobolus cyprini* Doflein, 1898. Parasitology, *90*(3), 549–555. https://doi.org/10.1017/S0031182000055530
16. Molnár, K. (2007). Site preference of myxozoans in the kidneys of Hungarian fishes. Diseases of Aquatic Organisms, *78*(1), 45–53. https://doi.org/10.3354/dao01827
17. 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, Switzerland, pp. 295–313. https://doi.org/10.1007/978-3-319-14753-6_16
18. Naldoni, J., Zatti, S. A., Silva, M. R. M., Maia Antonio, A. M. & Adriano, E. A. (2019). Morphological, ultrastructural, and phylogenetic analysis of two novel *Myxobolus* species (Cnidaria: Myxosporea) parasitizing bryconid fish from São Francisco River, Brazil. Parasitology International, *71*, 27–36. https://doi.org/10.1016/j.parint.2019.03.009
19. Nguyen, L. T., Schmidt H. A., von Haeseler, A. & Minh B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution, *32*, 268–274. https://doi.org/10.1093/molbev/msu300
20. Okamura, B., Hartigan, A. & Naldoni, J. (2018). Extensive Uncharted Biodiversity: The Parasite Dimension. Integrative and Comparative Biology, *58*, 1132–1145. https://doi.org/10.1093/icb/icy039
21. Roberts, R. J. (1975). Melanin containing cells of teleost fish and their relation to disease. *In*: Ribelin, W. E., Migaki, G. (Eds). The Pathology of Fishes. University of Wisconsin Press, Madison. pp. 399–428.
22. Rosser, T. G., Griffin, M. J., Quiniou, S. M. A., Alberson, N. R., Woodyard, E. T., Mischke, C. C., Greenway, T. E., Wise, D. J. & Pote, L. M. (2016). *Myxobolus ictiobus* n. sp. and *Myxobolus minutus* n. sp. (Cnidaria: Myxobolidae) from the gills of the smallmouth buffalo *Ictiobus bubalus* Rafinesque (Cypriniformes: Catostomidae). Systematic Parasitology, *93*(6), 565–574. https://doi.org/10.1007/s11230-016-9636-x
23. Saha, M. & Bandyopadhyay, P. K. (2018). Identification of a new myxosporean parasite *Thelohanellus indiana* n. sp. (Myxosporea: Myxobolidae) isolated from three major organs of goldfish, *Carassius auratus* L. highlighted with its morphological and SSU Rdna sequence based molecular description. Microbial Pathogenesis, *122*, 191–199. https://doi.org/10.1016/j.micpath.2018.05.038
24. Schmidt-Posthaus, H. & Wahli, T. (2015). Host and environmental influences on development of disease. *In*: Okamura B., Gruhl A., Bartholomew J. (Eds.), *Myxozoan Evolution*, *Ecology and Development*. Springer International Publishing, Cham, Switzerland, pp. 281–293. https://doi.org/10.1007/978-3-319-14753-6_15
25. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, *25*(24), 4876–4882. https://doi.org/10.1093/nar/25.24.4876
26. Wu, B. H., Wang, S. X., Chen, Y. S., Lu, C. C. & Huang, L. P. (1979). On the pathogenic agent of the twist disease of the silver carp in Hangzhou region of the Zhejiang province, Acta Zoologica Sinica, *25*(1), 0–57(In Chinese).
27. Xi, B. W., Zhou, Z. G., Xie, J., Pan, L. K., Yang, Y. L. & Ge, X. P. (2015). Morphological and molecular characterization of actinosporeans infecting oligochaete *Branchiura sowerbyi* from Chinese carp ponds. Diseases of Aquatic Organisms, *114*(3), 217–228. https://doi.org/10.3354/dao02859
28. Xi, B. W., Zhao, X., Li, P. & Xie, J. (2019). Morphological variation in *Myxobolus drjagini* (Akhmerov, 1954) from silver carp and description of *Myxobolus paratypicus* n. sp. (Cnidaria: Myxozoa). Parasitology Research, *118*(7), 2149–2157. https://doi.org/10.1007/s00436-019-06350-6.
29. Yang, K., Zhai, K. X., Xi B. W. & Xi, J. (2022). Tissue tropism of *Myxobolus honghuensis* in diseased and asymptomatic gibel carp (*Carassius auratus gibelio*). Journal of Fisheries of China, *46*(6), 1085–1093. https://doi.org/10.11964/jfc.20201012446
30. Zhao, Z. G., Dong, S. L. & Xu, Q. Y. (2018). Effects of starvation on the breathing and feeding of filter-feeding silver carp (*Hypophthalmichthys molitrix Val.*). Aquaculture, *495*, 241–246. https://doi.org/10.1016/j.aquaculture.2018.05.040
31. Zhang, B., Gu, Z. M. & 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. Parasitology International, *67*(4), 509–516. https://doi.org/10.1016/j.parint.2018.04.011

Figures

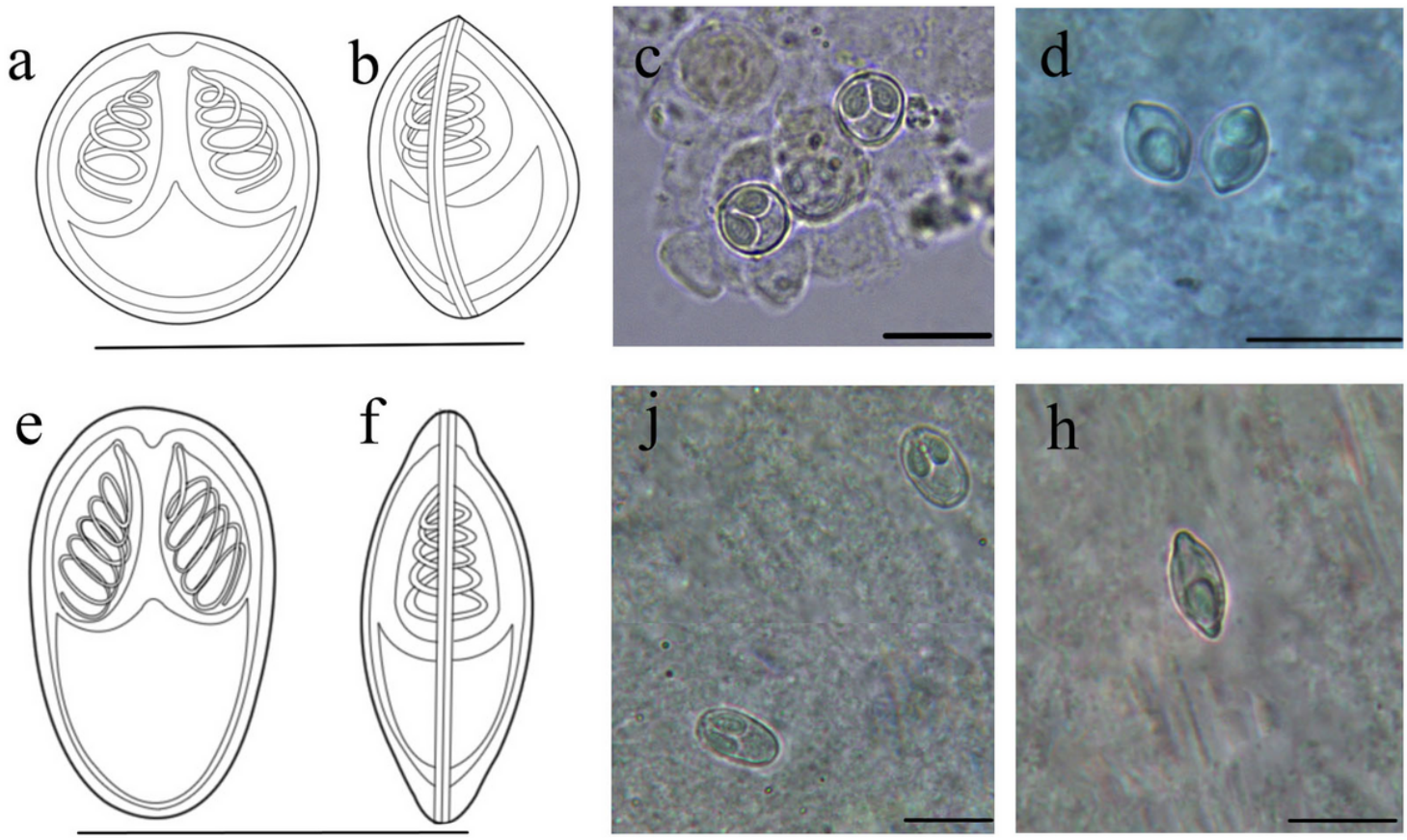

Figure 1

Schematic drawing and fresh mature spores of *M. lieni* (Nie et Li, 1973) (a-d) and *M. varius* (Achmerov, 1960), (e-h) from *Hypophthalmichthys molitrix*. Scale bar = 10 μm.

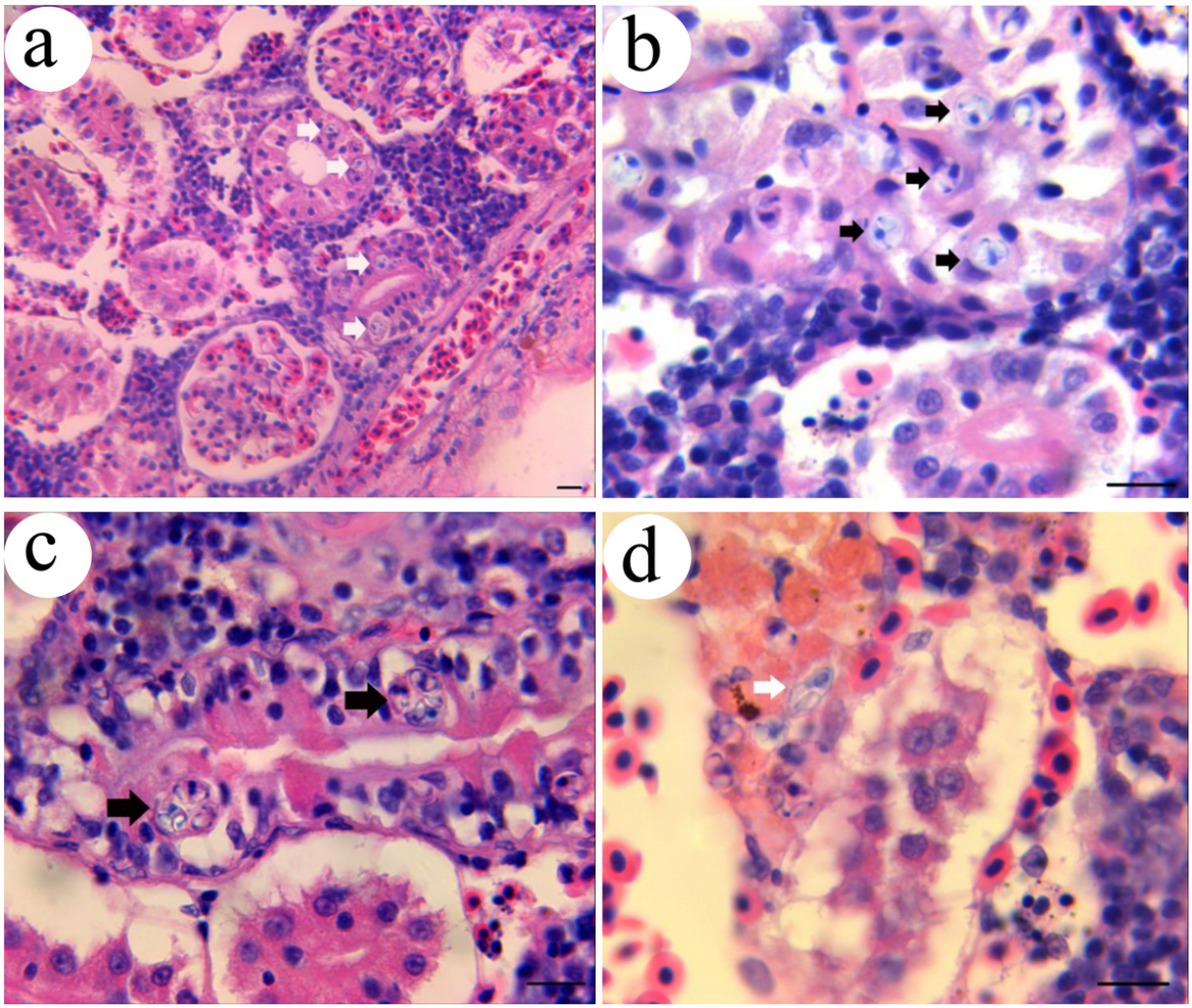

Figure 2

Histopathological sections of *Hypophthalmichthys molitrix* kidney infected with *M. lieni* (Nie & Li, 1973) (a-c) and *M. varius* (Achmerov, 1960) (d). Scale bar = 10 µm.

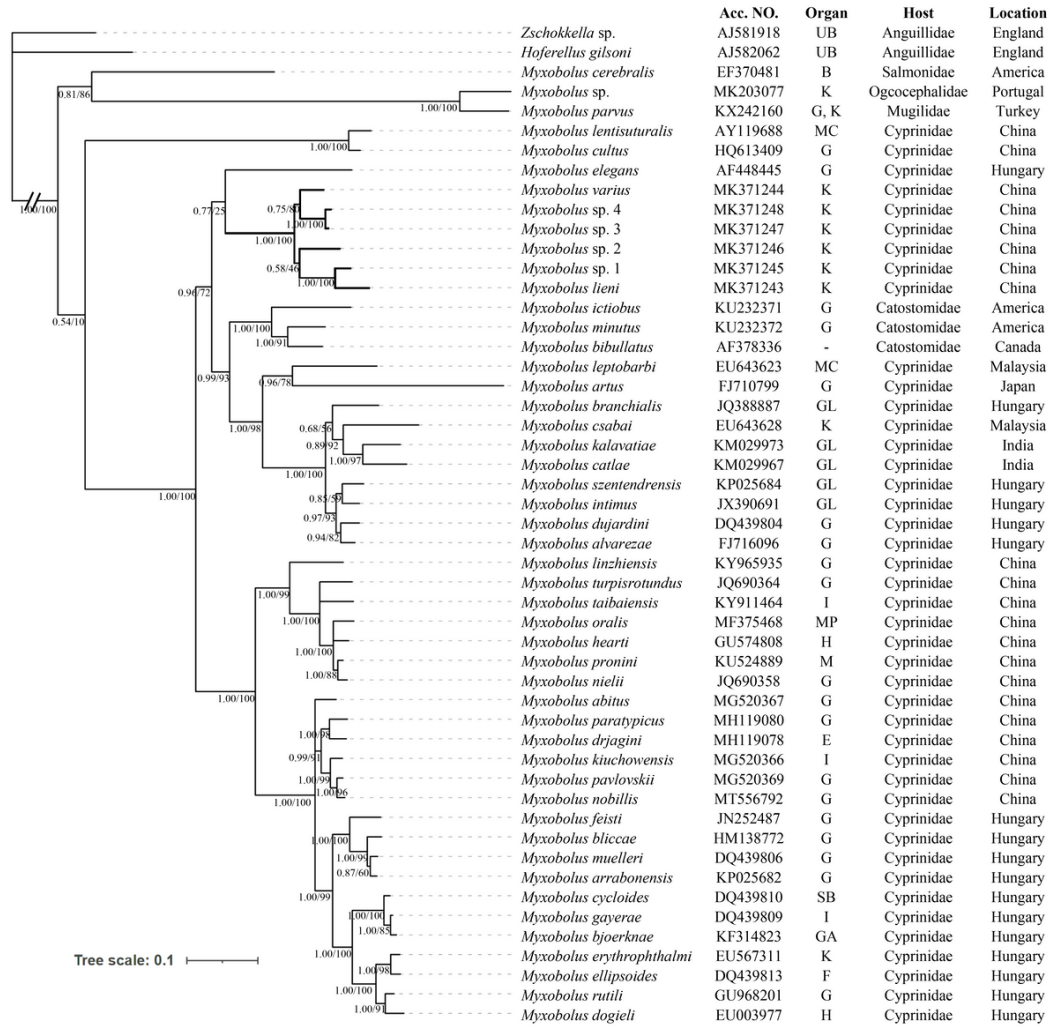

Figure 3

Bayesian inference trees of myxozoans constructed with the SSU rDNA sequences. Numbers near the nodes shows the posterior probability of BI and bootstrap values of ML, respectively. Information of GenBank accession number, infection site, host and locality follow their specie name. Abbreviations: B - brain; E - encephalocoele; F - fin; G - gills; GA - gill arch; GB - gall bladder; GL - gill lamellae; H - heart; I - intestine; K - kidney; M - mesentery; MP- palate in the mouth; MC - muscle cells; S-skin; SB - swim bladder; UB - urinary bladder. Asterisk (-): data was not provided.