Research Article

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***Myxobolus pseudowulii* sp. n. (Myxozoa: Myxosporea), a new skin parasite of yellow catfish *Tachysurus fulvidraco* (Richardson) and redescription of *Myxobolus voremkhai* (Akhmerov, 1960)**

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Abstract: Two species of *Myxobolus* Bütschli, 1882 were found in yellow catfish *Tachysurus fulvidraco* (Richardson). A species of *Myxobolus* infecting the gills was morphologically identified as *Myxobolus voremkhai* (Akhmerov, 1960) and it was characterised here with additional morphological and molecular data. The other species of *Myxobolus* infecting the host's skin did not conform to any known myxosporean species. It is characterised by the presence of round, black or milky white plasmodia with black spots. Myxospores are pyriform in frontal view and lemon-shaped in lateral view, measuring 12.9–16.2 µm (14.6 ± 0.7 µm) in length, 8.1–10.8 µm (9.4 ± 0.5 µm) in width, and 6.1–8.1 µm (7.0 ± 0.4 µm) in thickness. Two ampullaceous polar capsules are slightly unequal in size, larger polar capsule 7.2–9.5 µm (7.9 ± 0.4 µm) long by 3.0–3.9 µm (3.5 ± 0.2 µm) wide, smaller capsule 6.9–8.0 µm (7.4 ± 0.3 µm) long by 2.9–3.9 µm (3.4 ± 0.2 µm) wide. Polar filaments are coiled with seven to nine turns. Histologically, the plasmodia develop in the stratum spongiosum of skin dermis, resulting in epithelial cell shedding and immunological cell infiltration. Given the morphological and molecular differences between this species and other species of *Myxobolus*, we proposed the name of *Myxobolus pseudowulii* sp. n. for this parasite from the skin of yellow catfish. Interestingly, some spores of the new species possess *Henneya*-like caudal appendages. Phylogenetically, *M. pseudowulii* sp. n. and *M. voremkhai* infecting yellow catfish group together in one clade with other parasites of Siluriformes, indicating that parasites clustering according to the fish host order may be an important factor affecting the evolution of species within the *Myxobolus* clade.

Keywords: phylogeny, morphology, histology, ssrRNA, China

Myxosporeans are an economically important microscopic metazoan endoparasites infecting mainly fish and infrequently amphibians, reptiles, birds and mammals (Lom and Dyková 2006, Fiala et al. 2015). Owing to their negative impact on fish, myxosporeans have attracted much attention (Lom and Dyková 2006, Liu et al. 2010a, Fiala et al. 2015, Velasco et al. 2016). To date, approximately 2,300 myxosporean species have been described based on spore morphology (Fiala et al. 2015). However, due to incredible species diversity and uniformity of spores, which are used for species identification, distinguishing the morphologically similar species based on spore morphology alone has resulted in misidentification of several cryptic species in the past (Lom and Dyková 2006, Liu et al. 2010b, 2011, 2013, Fiala et al. 2015). In China, several morphologically similar myxosporean species infecting different tissues and organs of different hosts were misidentified as the same

species (Chen and Ma 1998). Therefore, it is crucial to validate the already described myxosporean species and identify cryptic and new myxosporean species by combination of spore morphology, biological traits (host species/family specificity, organ specificity, tissue tropism), and molecular data (Liu et al. 2014a, Thabet et al. 2016, Velasco et al. 2016).

Yellow catfish *Tachysurus fulvidraco* (Richardson) (Siluriformes: Bagridae) is an economically important fish cultured in China with an annual production over 300 million kilograms (Fishery Bureau in Ministry of Agricultural 2016). According to Chen and Ma (1998), approximately 24 myxosporeans have been found from different tissues and organs of yellow catfish in China. Although most of the species do not cause severe diseases, several species have been reported as serious pathogens responsible for mass mortality or loss of economic value of yellow cat-

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fish (Chen and Ma 1998), such as *Unicauda pelteobagrus* Ma, 1998 infecting the gills, and *Myxidium pseudobagrusi* Chen et Hsieh, 1984 infecting the kidney.

An investigation was conducted on the myxosporean diversity of yellow catfish. Here, we report a novel species of *Myxobolus* Bütschli, 1882 infecting the skin of yellow catfish and redescribe *Myxobolus voremkhai* (Akhmerov, 1960) infecting the gills of this fish host.

MATERIALS AND METHODS

Fish samples

One yellow catfish (18.0 cm in length) collected from a fish farm in Yingcheng City, Hubei Province, with several plasmodia (cysts) in the skin and gills was transported to the Laboratory of Fish Diseases in College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei Province, China in June 2016. The fish was held in aquaria in the laboratory and euthanised with 0.2 mg/ml tricaine methanesulfonate (MS-222, Sigma, St. Louis, MO, USA) prior to dissection.

Morphological methods

Gross microscopic examinations of the organs of yellow catfish for myxosporean infections were conducted within 24 h after its transportation. The plasmodia containing myxospores consistent with those of species of the genus *Myxobolus* were collected from the skin and gills. Fresh spores were measured as reported by Lom and Arthur (1989) using an Olympus BH2 microscope equipped with an ocular micrometre. Mean and standard deviations of each spore dimension were obtained from fresh mature spores ($n = 60$). Digitised images were obtained from fresh wet mounts by a Nikon Eclipse 80i microscope. Line drawings were made based on digitised images. All measurements are given in micrometres (μm) unless otherwise indicated.

Histological observation

Tissue samples from organs containing developing and mature plasmodia were fixed in Bouin's solution, embedded in paraffin wax, sectioned at 5–6 μm and stained with haematoxylin and eosin.

DNA isolation and sequencing

Genomic DNA was extracted using BioTeke™ Genomic DNA extraction kit (BioTeke Co., Ltd., Beijing, China) according to the manufacturer's protocol. PCR amplification of the small subunit ribosomal RNA (ssrRNA) gene was performed with primers 18s of Hillis and Dixon (1991) and 18r of Whipps et al. (2003) in a 50 μl volume of reaction solution, which consisted of approximately 100 ng of extracted genomic DNA, 1×*Taq* Buffer (MBI Fermentas, Vilnius, Lithuania), 2.5mM MgCl_2 , 0.2 mM dNTPs (MBI Fermentas), 2 μM each primer, and 2.5 U of *Taq* DNA polymerase (MBI Fermentas) in MilliQ purified water. The cycling conditions were as follows: an initial denaturation at 94 °C for 7 min, followed by 35 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 60 s, and a terminal extension at 72 °C for 10 min. PCR products were purified using the AxyPrep™ DNA Gel Extraction Kit (AxyGen Bio Co., Ltd., Hangzhou, China) and cloned into PMD-19T vector system (Takara, Otsu, Japan). Then two positive clones were selected and sequenced in both directions on the

Fig. 1. Yellow catfish *Tachysurus fulvidraco* (Richardson) infected with *Myxobolus pseudowulii* sp. n. in the skin. Arrows show the plasmodia in the skin.

Fig. 2. Photomicrograph of fresh spores of *Myxobolus pseudowulii* sp. n. from *Tachysurus fulvidraco* (Richardson). **A** – frontal view; **B** – lateral view. Arrows show the membrane sheath.

ABI PRISM® 3730XL DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). Forward and reverse sequence segments were aligned in BioEdit (Hall 1999) and a contiguous sequence was deposited in GenBank. A standard nucleotide-nucleotide BLAST search was conducted to query posted sequences.

Phylogenetic analysis

To evaluate the relationship of the current species to myxobolids available in GenBank, 52 sequences matching the top BLAST hits were aligned using the software MAFFT v. 7.271 with default parameters (Kato and Standley 2013). *Myxidium cuneiforme* Fujita, 1924 was designated as outgroup. Phylogenetic analyses were carried out on 1,185 character alignment. Optimal evolutionary models for maximum likelihood (ML) and Bayesian analyses were determined by jModeltest (Posada 2008) using the Akaike information criterion as the general time reversible model (GTR+I+G). Nucleotide frequencies were estimated from the data (A = 0.2935, C = 0.1795, G = 0.2639, T = 0.2632); six rates of nucleotide substitution were [AC] = 1.0145, [AG] = 3.6442, [AT] = 1.9003, [CG] = 0.6191, [CT] = 5.6391, [GT] = 1.0000;

Fig. 3. Line drawing of fresh spores of *Myxobolus pseudowulii* sp. n. from *Tachysurus fulvidraco* (Richardson).

the proportion of invariable sites was 0.3070, and the gamma distribution was 0.5490 as estimated with six rate categories. ML analysis was performed using PhyML v. 3.1 (Guindon et al. 2010). Bootstrap confidence values were calculated with 1,000 replicates. Bayesian analysis was conducted in MrBayes (Ronquist and Huelsenbeck 2003) under an evolutionary model, with 10^6 generations, tree sampled every 100 generations and the first 25% of trees was discarded as burn-in as analysed by the Tracer v 1.6 (Drummond and Rambaut 2007). Trees were initially examined in Figtree v.1.4.3 (Drummond and Rambaut 2007), then edited and annotated in Adobe Illustrator (Adobe Systems Inc., San Jose, CA, USA).

RESULTS

Myxobolus pseudowulii sp. n.

Figs. 1–4

ZooBank number for species:

urn:lsid:zoobank.org:act:ACAA06F7-15E0-49B8-97D4-28F910E86184

Description. Plasmodia round, black or milky white with black spots, 0.65–1.09 mm in diameter. Histozytic in skin (Fig. 1). Myxospores (Figs. 2, 3) pyriform in frontal view and lemon shaped in lateral view, 12.9–16.2 (14.6 ± 0.7) long, 8.1–10.8 (9.4 ± 0.5) wide and 6.1–8.1 (7.0 ± 0.4) thick. Most spores surrounded by oval membrane sheath. Two ampullaceous polar capsules slightly unequal in size, with larger polar capsule 7.2–9.5 (7.9 ± 0.4) long by 3.0–3.9 (3.5 ± 0.2) wide, and smaller capsule 6.9–8.0 (7.4 ± 0.3) long by 2.9–3.9 (3.4 ± 0.2) wide. Polar filaments coiled with 7–9 turns. Sutural line straight and distinct (Fig. 3). Some spores with caudal appendages 2.2–4.5 long (Fig. 4).

Histology. The plasmodia of *Myxobolus pseudowulii* sp. n. developed in the stratum spongiosum of skin dermis (Fig. 7A), which contained the spores and some late developmental stages (Fig. 7B). A number of melanocytes were observed to surround the plasmodia (Fig. 7B,C). This

Fig. 4. Photomicrograph of spores of *Myxobolus pseudowulii* sp. n. with caudal appendages (arrows) from *Tachysurus fulvidraco* (Richardson).

infection resulted in epithelial cells shedding of the skin and the inflammatory responses of immunological cell infiltration were also observed (Fig. 7D).

Type host: Yellow catfish *Tachysurus fulvidraco* (Richardson) (Siluriformes: Bagridae).

Locality: Fish farm in Yingcheng City, Hubei, China (30°55'N; 113°34'E; June 2016).

Site of infection: Skin.

Type material: Mature spores fixed in 5% formalin were deposited in National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences (Coll. No. CFPM201603).

Etymology: The species is named for the similarity of its spore shape to that of *Myxobolus wulii* Landsberg et Lom, 1991.

Remarks. In terms of spore morphology, *M. pseudowulii* differed not only from all the species of *Myxobolus* infecting yellow catfish, but also from myxobolids from phylogenetically closely related fish species, albeit being morphologically similar to *M. pfrille* Landsberg et Lom, 1991, *M. kiangsiensis* Chen in Chen et Ma, 1998 and *M. aureatus* Ward, 1919 (Table 1). Oval spores of *M. pfrille* are different from those of *M. pseudowulii* in their shape. *Myxobolus kiangsiensis* can be distinguished from *M. pseudowulii* by its eggplant-like polar capsules. The polar capsules of *M. aureatus* are smaller than those of *M. pseudowulii* and the melon seed-like spore of *M. aureatus* are also different from those of the present species

Fig. 5. Photomicrograph of fresh spores of *Myxobolus voremkhai* (Akhmerov, 1960) from *Tachysurus fulvidraco* (Richardson). **A** – frontal view; **B** – lateral view.

Fig. 6. Line drawing of fresh spores of *Myxobolus voremkhai* (Akhmerov, 1960) from *Tachysurus fulvidraco* (Richardson).

Fig. 7. Histology of skin with plasmodia of *Myxobolus pseudowulii* sp. n. from *Tachysurus fulvidraco* (Richardson). **A** – plasmodium developing in the stratum spongiosum of dermis; **B**, **C** – number of melanocytes surrounding the plasmodium; black arrows show the melanocytes and white arrow shows the plasmodium membrane; **D** – epithelial cells shedding of the skin (white arrow) and lymphocytes (black arrows). *Abbreviations:* P – plasmodium; sc – stratum compactum; ss – stratum spongiosum.

Fig. 8. Phylogenetic tree generated from Bayesian analysis of ssrRNA gene sequences of *Myxobolus pseudowulii* sp. n. and *Myxobolus voremkhai* Landsberg et Lom, 1991 and related myxobolids. GenBank accession numbers are listed adjacent to species names. Support values at branching points are listed as: Bayesian posterior probabilities/bootstrap values from maximum likelihood analysis. Dashes are shown for values under 50%. **Abbreviations:** AB – arterial bulb; B – brain; BA – bulbus arteriosus; F – fins; G – gills; H – head; I – intestine; K – kidney; L – liver; M – muscle; MO – medulla oblongata; NT – neural tissue; S – serosa; SC – spinal cord; SK – skin; SL – scales; SP – spleen.

in spore shape. While molecular biological methods have become the mainstream in myxosporean species identification (Kaur and Attri 2015, Mansour et al. 2015, Özer et al. 2016), to our best knowledge, no molecular studies on *M. pfrille*, *M. kiangsiensis*, *M. gigi* (Fujita, 1927) and *M. aureatus* have been conducted to compare them molecularly with *M. pseudowulii* so far.

Myxobolus voremkhai (Akhmerov, 1960) Landsberg et Lom, 1991

Figs. 5, 6

Syn.: *Myxobolus pseudorasbora* Akhmerov, 1960

Redescription (based on material from China). One plasmodium oval, milky white, 0.86 mm in diameter. Histozoic in gills. Myxospores (Figs. 5, 6) elongated pyriform in frontal view, lemon-shaped in lateral view, 13.1–15.5 (14.5 ± 0.5) long, 7.1–8.6 (7.8 ± 0.3) wide and 5.6–7.2

(6.3 ± 0.4) thick. Most spores surrounded by approximately oblong membrane sheath with horizontally straight end. Two ampullaceous polar capsules, slightly unequal in size, with larger polar capsule 6.3–7.4 (6.8 ± 0.3) long by 2.2–3.3 (2.8 ± 0.2) wide, and smaller capsule 5.8–7.1 (6.5 ± 0.3) long by 2.2–3.3 (2.8 ± 0.2) wide. Polar filaments coiled with 5–7 turns. Sutural line straight and distinct (Fig. 6).

Histology not available.

Type host: Yellow catfish *Tachysurus fulvidraco* (Richardson) (Siluriformes: Bagridae).

Type locality: Amur River basin, Russia.

Other locality: Fish farm in Yingcheng City, Hubei, China (30°55'N; 113°34'E; June 2016).

Site of infection: Gills.

Voucher specimens: Mature spores fixed in 5% formalin were deposited in National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences with associated collection number CFP201604.

Table 1. Comparison of *Myxobolus pseudowulii* sp. n. and *M. voremkhai* with the *Myxobolus* species infecting yellow catfish *Tachysurus fulvidraco* (Richardson) and its closely related fish species.

Parasite	Spore length	Spore width	Spore thickness	Polar capsule length	Polar capsule width	Infected organ	Host
<i>M. aureatus</i> Ward, 1919	13.7–14.2 (13.9)	6.8–7.0 (6.9)	5.6	5.8–6.0 (5.9)	2.1–2.2 (2.1)	gills, kidney	<i>T. fulvidraco</i> , <i>T. nitidus</i> (Sauvage et Dabry de Thiersant)
<i>M. chulkiangensis</i> Ma, 1998	10.4–11.2 (10.6)	7.0–8.0 (7.8)	7.0	4.8–5.6 (5.3)	3.2–4.0 (3.5)	urinary bladder	<i>T. nitidus</i>
<i>M. grig^a</i> (Fujita, 1927)	12.0	6.0	5.0	7.0	-	kidney	<i>T. fulvidraco</i>
<i>M. haichengensis</i> Chen, 1998	10.2–12.0 (11.4)	7.6–9.6 (8.7)	6.2–6.6 (6.4)	4.2–6.0 (5.2)	2.4–3.6 (2.9)	kidney	<i>T. fulvidraco</i>
<i>M. huamaensis</i> Liu, 2014	9.6–11.0 (10.3)	6.0–7.2 (6.8)	-	3.6–3.8 (3.7)	2.2–2.4 (2.3)	intestine	<i>T. fulvidraco</i>
<i>M. kawabatae^a</i> (Fujita, 1927)	16.0	6.0	8.0	12.0; 9.0	3.0; 3.0	kidney	<i>T. fulvidraco</i>
<i>M. kiangsiensis</i> Chen in Chen et Ma, 1998	10.8–15.6 (12.4)	6.6–8.4 (7.6)	5.2–6.0 (5.4)	6.0–8.4 (6.6)	3.3	intestine	<i>T. fulvidraco</i>
<i>M. macrocapsularis</i> Reuss, 1906	12.0–12.6 (12.1)	6.6–7.6 (7.2)	6.0–6.2 (6.1)	5.4–6.0 (5.7)	2.2–2.4 (2.1)	urinary bladder	<i>T. fulvidraco</i>
<i>M. mtielleri</i> Bütschli, 1882	10.8–12.0 (11.5)	6.0–8.4 (7.0)	4.5–5.0	4.2–6.0 (4.9)	2.2–2.4 (2.3)	gills, kidney, spleen, brain, urinary bladder, gall bladder	<i>T. fulvidraco</i> , <i>T. nitidus</i> , <i>Pseudobagnus vachellii</i> (Richardson)
<i>M. ochowensis</i> Chen in Chen et Ma, 1998	12.0–13.2 (12.8)	8.4–10.2 (9.5)	6.6–7.2 (7.1)	6.0–7.4 (6.6); 6.0–6.6 (6.2)	3.0–3.6 (3.2); 2.4–3.0 (2.0)	dorsal fins	<i>T. fulvidraco</i>
<i>M. oviformis</i> Thélohan, 1892	10.8–12.0 (11.2)	8.4–9.6 (9.0)	6.0–6.6 (6.3)	4.8–6.0 (5.6)	2.6–2.8 (2.7)	gills	<i>T. fulvidraco</i>
<i>M. pelteobagnus</i> Ma et Zhao, 1998	10.4–12.0 (11.3)	7.2–8.0 (7.6)	4.0–4.8 (4.3)	4.8–5.6 (5.2)	2.4–2.8 (2.5)	kidney	<i>T. nitidus</i>
<i>M. permagnus</i> Wegener, 1910	18.0–19.2 (18.1)	9.2–12.0 (10.2)	8.4	9.6–10.8 (9.7)	3.5–4.0	gills	<i>T. fulvidraco</i>
<i>M. pfrille</i> Landsberg et Lom, 1991	12.0–14.4 (12.9)	8.4–10.2 (9.5)	7.0	5.0–6.0 (5.6)	3.6	intestine	<i>T. fulvidraco</i>
<i>M. physophilus</i> Reuss, 1906	12.0–13.0 (12.5)	8.4–9.6 (9.0)	8.4	6.6–8.4 (7.2)	2.8–3.6 (3.1)	gills, kidney, fins, urinary bladder	<i>T. fulvidraco</i> , <i>T. nitidus</i>
<i>M. tunicatus^b</i> Akhmerov, 1960	16.0	6.5	-	6.5–7.0	2.6–2.8	urinary bladder, kidney	<i>T. fulvidraco</i>
<i>M. twistus</i> Chen in Chen et Ma, 1998	16.2–17.4 (16.7)	8.4–9.6 (8.9)	5.4–6.2 (5.9)	7.4–9.0 (8.1)	2.6–3.4 (3.0)	kidney	<i>T. fulvidraco</i>
<i>M. uniporus</i> Fujita, 1927	10.4–12.0 (11.6)	6.7–8.4 (7.4)	4.8–6.0 (5.2)	4.8–5.4 (5.1)	2.4–2.9 (2.7)	gills	<i>T. fulvidraco</i>
<i>M. vescus</i> Akhmerov, 1960	8.6–11.2 (10.1)	4.8–6.3 (5.6)	3.1–3.8 (3.3)	4.4–5.6 (5.1)	1.0–1.4 (1.2)	gills, stomach, kidney	<i>T. fulvidraco</i> , <i>P. vachellii</i>
<i>M. voremkhai</i> (Akhmerov, 1960)	13.8–16.8 (14.9)	7.2–8.4 (7.8)	4.8–6.0	6.6–9.2 (8.0)	2.3–3.4 (2.7)	gills, fins, kidney, liver, urinary bladder, eyes, gall bladder, gonads, swim bladder, spleen	<i>T. fulvidraco</i> , <i>T. nitidus</i> , <i>P. vachellii</i>
<i>M. voremkhai^c</i>	13.1–15.5 (14.5 ± 0.5)	7.1–8.6 (7.8 ± 0.3)	5.6–7.2 (6.3 ± 0.4)	6.3–7.4 (6.8 ± 0.3); 5.8–7.1 (6.5 ± 0.3)	2.2–3.3 (2.8 ± 0.2); 2.2–3.3 (2.8 ± 0.2)	gills	<i>T. fulvidraco</i>
<i>M. pseudowulii</i> sp. n. ^d	12.9–16.2 (14.6 ± 0.7)	8.1–10.8 (9.4 ± 0.5)	6.1–8.1 (7.0 ± 0.4)	7.2–9.5 (7.9 ± 0.4); 6.9–8.0 (7.4 ± 0.3)	3.0–3.9 (3.5 ± 0.2); 2.9–3.9 (3.4 ± 0.2)	skin	<i>T. fulvidraco</i>

^a data from Fujita (1927); ^b data from Eiras et al. (2005); ^{c, d} data from present study and other data from Chen and Ma (1998); dashes – no data.

Remarks. In a morphological comparison with *Myxobolus* species infecting yellow catfish and its closely related fish species (Table 1), the present species could be distinguished from all of them by the size of myxospores except *M. voremkhai*. The present species is identical to *M. voremkhai* in morphological and morphometric characters. Moreover, the present species infecting the gills of yellow catfish is similar to the description of *M. voremkhai* (syn. *Myxobolus pseudorasbora* Akhmerov, 1960) in Chen and Ma (1998). Above all, despite the absence of comparable molecular data of *M. voremkhai*, the present species of *Myxobolus* can be identified as *M. voremkhai*. In addition, we supplement the molecular data on *M. voremkhai* in the present research, which will facilitate its species identification in further research.

Sequence analysis

A total of 2,032 and 2,020 bases of the ssrRNA gene of *Myxobolus pseudowulii* sp. n. (KY229918) and *Myxobolus voremkhai* (KY229919) were generated, respectively, and the contiguous sequences were deposited in GenBank. A BLAST search indicated that both the ssrRNA gene sequences of *M. pseudowulii* and *M. voremkhai* did not match any available sequences in GenBank and they shared 92% similarity with each other. ML and BI analyses resulted in congruent tree topologies with different nodal supports in certain cases. *M. pseudowulii* and *M. voremkhai* clustered together in the phylogenetic trees and were sisters to *Myxobolus* sp. (JN616264) and *Unicauda pelteobagrus* (KC193254) which all grouped within a mixed clade consisting of species of *Myxobolus*, *Unicauda* Davis, 1944 and *Hennegoides* Lom, Tonguthai et Dyková, 1991 (Fig. 8).

DISCUSSION

In the present paper, we presented morphological and molecular data for two species of *Myxobolus* from yellow catfish: *Myxobolus pseudowulii* sp. n. from the skin and *Myxobolus voremkhai* from the gills, which were identified based on spore morphology, biological traits, and molecular data. In the present study, the morphological and molecular analysis revealed that the species from the skin of yellow catfish is a novel species of *Myxobolus*. The species of *Myxobolus* infecting the gills of yellow catfish in the

present paper is identified as *M. voremkhai* based on the identical morphology and infection site. Interestingly, the membrane sheath surrounding most *M. voremkhai* mature spores and some spores present in the plasmodium were not reported in its original species description (Akhmerov 1960). This membrane sheath variation was also reported in *Thelohanellus wuhanensis* Xiao et Chen, 1993 and *Thelohanellus macrovacuolaris* Liu, Zhai et Gu, 2016 (see Liu et al. 2014b, 2016). We speculate that different spore maturity might affect the existence of membrane sheath. Phylogenetic analyses placed *M. pseudowulii* and *M. voremkhai* in the mixed clade composed of species of the genera *Myxobolus*, *Unicauda* and *Hennegoides*. The non-monophyly of the genus *Myxobolus* has been reported many times to cluster some species from other genera into the *Myxobolus* clade, especially the species from the genus *Henneguya* Thélohan, 1892 characterised by the caudal appendages on the spores (Fiala 2006, Bartošová et al. 2009, Fiala and Bartošová 2010, Liu et al. 2010a).

Interestingly, some spores of *Myxobolus pseudowulii* with *Henneguya*-like caudal appendages were observed in the present study. *Myxobolus* spores with caudal appendages have been reported in several previous studies (Shulman 1966, Bahri 2008, El-Mansy 2005, Liu et al. 2010a, 2013, 2014a), indicating that species of *Myxobolus* have the genetic capacity to develop *Henneguya*-like caudal appendages, and the caudal appendages may not be a valid character to distinguish *Myxobolus* with *Henneguya*. Tissue tropism was found to be an important feature for general clustering of myxozoans (Fiala 2006, Carriero et al. 2013, Shin et al. 2014), but this trend was not observed for clustering of species within the *Myxobolus* clade in the present study. *M. pseudowulii* and *M. voremkhai* infecting the siluriform fish grouped together in one clade with other parasites of Siluriformes, indicating that the fish host order may be a more important factor that may have played a more important role in the evolution of species within the *Myxobolus* clade than tissue tropism.

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