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Research article

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Myxobolus opsaridiumi sp. nov. (Cnidaria: Myxosporea) infecting different tissues of an ornamental fish, *Opsaridium ubangiensis* (Pellegrin, 1901), in Cameroon: morphological and molecular characterization

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Abstract. We report a new myxozoan, *Myxobolus opsaridiumi* sp. nov., infecting the ornamental fish *Opsaridium ubangiensis* (Pellegrin, 1901) collected from the Anga River near the city of Yaounde, Cameroon. Plasmodia were found in the skin, muscles and spleen. The overall prevalence of infection was 54.7% (288 parasitized fish out of 526 examined). The myxospores were ovoid to subspherical in frontal view and lenticular in lateral view. The valves were symmetrical and relatively thick, without edge markings. The myxospore measurements were 10.7±0.14 (10–11.5) µm long, 9±0.15 (8–10) µm wide and 6.2±0.7 (5.6–7.2) µm thick. The two ovoid polar capsules were equal in size, converging and opening together at the anterior end, measuring 5±0.07 (4.3–6.0) µm long and 2.7±0.07 (2.2–3.0) µm wide. Polar filaments were coiled from 5 to 7 turns. Histopathological analysis revealed no inflammatory reaction associated with the infection. A BLAST search found that the newly obtained 18 rDNA sequence had a low sequence similarity with available sequences for *Myxobolus* on GenBank. A phylogenetical analysis based on ribosomal DNA partial sequences showed that *M. opsaridiumi* sp. nov. is closely associated with several species of *Myxobolus* infecting cyprinid fish.

Keywords. Myxozoan parasites, fish pathology, new species, Africa, SSU rDNA, phylogeny, histopathology.

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Introduction

Myxozoa are cosmopolitan microscopic cnidarians that live as endoparasites of invertebrate and vertebrate hosts, primarily in aquatic environments (Kent *et al.* 2001; Lom & Dyková 2006; Jiménez-Guri *et al.* 2007). More than 2400 species and 64 genera are recognized (Zhang *et al.* 2013; Atkinson *et al.* 2018; Liu *et al.* 2019; Mathews *et al.* 2020). They are characterized by morphological simplicity and life-cycle complexity (Fiala *et al.* 2015). These parasites are identifiable at the spore stage, particularly as actinospores in invertebrate hosts and myxospores in vertebrates. The myxospore is a multicellular structure composed of one to 15 nematocyst-like polar capsules, each containing coiled, extrusible filaments, and at least one ameboid infective germ or sporoplasm (Kent *et al.* 2001). Infection can be coelozoic, most often in the gallbladder and urinary bladder, or histozoic, targeting skin, muscles, gills or the digestive system. Myxosporean parasites are significant pathogens of fish in both wild and cultured stocks throughout the world, and they are responsible for production losses in farmed fish (Lom & Dyková 2006). Within the class Myxosporea, the genus *Myxobolus* includes approximately 900 described species (Eiras *et al.* 2005, 2014; Kaur & Singh 2012). Most species infect specific organs, but some have been reported to infect several organs in the same host species (Lekeufack & Fomena 2013; Eiras *et al.* 2014).

Until recently, myxosporean species were identified only by the morphological and metric characteristics of the myxospore, organ specificity and tissue tropism (Molnár 1994; Dyková & Lom 2007). However, classical zoological methods make it difficult to distinguish morphologically similar myxosporean species that can infect identical tissues and develop in closely related host species (Molnár *et al.* 2002, 2009; Lisnerová *et al.* 2020). Molecular methods have become increasingly popular tools in parasitological studies to identify myxozoan species (Kent *et al.* 2001; Mansour *et al.* 2014; Liu *et al.* 2016). A combination of morphology and molecular classification, considering host range and tissue specificity, provides a more precise approach to distinguishing valid myxosporeans species from identified taxa (Kent *et al.* 2001; Fiala 2006; Molnár *et al.* 2009).

Opsaridium ubangiensis Pellegrin, 1901 is a benthopelagic cypriniform of the family Cyprinidae. It is the single species of its genus present in Lower Guinea, is widely distributed in coastal basins from Cameroon to Congo and can reach more than 120 mm in length (Stiassny *et al.* 2007). In addition to its high-quality meat and rapid growth, species of the genus *Opsaridium* play an important role in national and international pet trades as ornamental (aquarium) fish. Until now, more than 270 species of Myxosporea have been reported to infect African fishes (Deli *et al.* 2017) and none were known to infect *O. ubangiensis*.

The present study is part of an ongoing investigation to characterize the myxosporean parasites from freshwater fish in Cameroon. Morphological, histological and molecular data were used to describe a new species of *Myxobolus* that infects the skin, muscles and spleen of *O. ubangiensis* from the Anga River in Cameroon.

Material and methods

Sampling examination

A total of 526 specimens of *Opsaridium ubangiensis* were caught with a fine-meshed net from September 2014 to July 2017 from the Anga River at Yaounde (3°51′37″ N, 11°23′27″ E, Centre Region, Cameroon, Central Africa). Sampled fish were immediately transported alive to the laboratory of the Department of Parasitology and Ecology at the University of Yaounde 1 in Cameroon, where they were kept in aerated aquaria for identification and parasitological examination. The standard length of the collected fishes ranged from 32 to 117 mm, and identification was carried out according to Stiassny *et al.* (2007).

In the laboratory, specimens were first examined with the naked eye (eyes, fins, operculum, scales, skin) and then with an Olympus BO61 binocular lens to detect the presence of cysts. After dissection of the hosts, internal organs (gills, heart, liver, kidneys, spleen, gallbladder, gonads and intestines) were removed and examined individually. Some of the recorded cyst contents were identified using an optical microscope with a 100 × objective lens.

Myxospore and histological examinations

Permanent smears of spores were fixed in methanol and stained with May Grünwald-Giemsa. Drawings of fresh spores were carried out using a wild M20 microscope equipped with a camera lucida. Measurements were made on at least 50 spores as proposed by Lom & Arthur (1989). Microphotographs of fresh and stained spores were taken using an Olympus BH-2 microscope equipped with a microphotograph.

For histological studies, tissue samples from infected skin, muscle and spleen were fixed in 10% neutral buffered formalin. The fixed tissue samples were embedded in paraffin wax and then sliced into sections 3 µm thick. Sections were stained with hematoxylin and eosin (H&E) and finally examined and photographed using an Axio Imager Z2 microscope (Carl Zeiss Inc., Berlin, Germany) equipped with a digital camera (Axiocam MRC5, Zeiss). Plasmodia from skin, muscles and spleen were preserved in absolute ethanol for molecular analysis.

Molecular characterization

Molecular analyses were performed in the Department of Zoology at King Saud University. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Partial SSU rDNA gene was amplified using the primers MyxF144 and MyxR1944 (Mansour *et al.* 2013). Polymerase chain reaction (PCR) amplifications were performed in a T100™ Thermal Cycler (Bio-Rad, Singapore). The PCR program consisted of an initial denaturation step for 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 56°C and 120 s at 72°C, and a final extension of 72°C for 10 min. All PCR reactions were performed in a final volume of 30 µl, containing 1 × iProof High Fidelity Master Mix (Bio-Rad, Hercules, CA), 0.2 µM of each primer and 50–100 ng of genomic DNA. The PCR products were sequenced by a commercial sequencing company (Macrogen Inc., Seoul, South Korea) using the same primers used for PCR amplification. A consensus sequence was obtained from both sense and anti-sense strands of samples originating from three different PCR products. This consensus sequence was used to query similar sequences of *Myxobolus* using BLAST in GenBank (Altschul *et al.* 1997). Sequences were aligned by applying the default parameters in ClustalX ver. 2.1.0.12 (Larkin *et al.* 2007). Phylogenetic analyses were performed by Bayesian inference (BI) and maximum likelihood (ML) methods. The BI analyses employed MrBayes ver. 3.2 (Ronquist *et al.* 2012) and the Markov Chain Monte Carlo (MCMC) method for 1 500 000 generations, with two independent runs of four simultaneous MCMC chains (nchains = 4). Trees were saved every 100 generations (sample freq = 100). ML analyses were based on the General Time Reversible model with a gamma-distributed rate and invariant sites (GTR + G + I) using PhyML ver. 3.0 (Guindon *et al.* 2010). The analysis involved 22 nucleotide sequences. Positions containing gaps and missing data were removed.

Results

Phylum Cnidaria Hatschek, 1888
Unranked Subphylum Myxozoa Grassé, 1970
Class Myxosporea Bütschli, 1881
Order Bivalvulida Schulman, 1959
Suborder Variisporina Lom & Noble, 1984
Family Myxobolidae Thélohan, 1892
Genus *Myxobolus* Bütschli, 1882

Myxobolus opsaridiumi sp. nov.
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Figs 1–4; Table 1

Etymology

The specific epithet is related to the host genus name.

Type material

CAMEROON • infected skin, muscle and spleen of *Opsaridium ubangiensis* with plasmodia; Centre Region, Anga River, Yaounde; deposited in parasitological collection of the Zoology Department Museum, College of Science, King Saud University, Saudi Arabia; Myxospsar/12/2018.

Taxonomic summary

Type host

Opsaridium ubangiense Pellegrin, 1901 (Cyprinidae).

Infected tissues

Skin, muscles and spleen.

Prevalence

54.7% (288 parasitized fish out of 526 examined).

Vegetative stages

Ovoid, spherical or ellipsoid plasmodia, variable in size, measuring from 0.3 mm to 2.5 mm in length and 0.2 mm to 1.5 mm in width.

Description of myxospores (Fig. 1)

Mature spores were ovoid to subspherical in frontal view and lenticular in lateral view (Fig. 1A–B). The valves were relatively thick, without edge markings. Intercapsular processes were absent. The spore size was 10.7±0.14 (10–11.5) µm long, 9±0.15 (8–10) µm wide and 6.2±0.7 (5.6–7.2) µm thick. The two ovoid polar capsules were equal in size, converging and opening together at the anterior end of the same pore (Fig. 1A, C–D). They measured 5±0.07 (4.3–6.0) µm in length and 2.7±0.07 (2.2–3.0) µm in width. Polar filaments were coiled from 5 to 7 turns perpendicular to the longitudinal axis of the polar capsules (Fig. 1D). A sporoplasm containing an iodinophilous vacuole of varying shape and size filled the entire space below the polar capsules (Fig. 1A).

Clinical finding and histopathology

Based solely on gross observation of the fish, no signs of disease were observed. Parasitized fish harbored cysts on skin, muscles and spleen. On skin, white cysts up to 2 mm long were collected from the body

flanks of some fish (Fig. 2A). Sections revealed that plasmodia developed in the connective tissue of the dermis beneath the underside of scales (Fig. 2B). Plasmodia were flattened and surrounded by a thin membrane and an internal endoplasm comprising a loosely defined matrix containing developed spores (Fig. 2B).

Some plasmodia were spotted within muscle cells (Fig. 2C). Plasmodia were spindle-shaped, centrally located in the cell and not surrounded by a visible wall. No evidence of inflammation or immune-cell recruitment was seen. The integrity of myofibrils within the infected fibers showed some degree of lysis, with partial loss of myofibrillar details and striations (Fig. 2C–D). These lesions were observed close to plasmodia. Mature spores were scattered in the cytoplasm of infected cells (Fig. 2D).

Infected spleens had plasmodia of up to 2.5 × 1.5 mm (Fig. 3A). They were white, isolated or clustered (Fig. 3B). Some infected spleens were heavily infected and plasmodia were randomly distributed in the

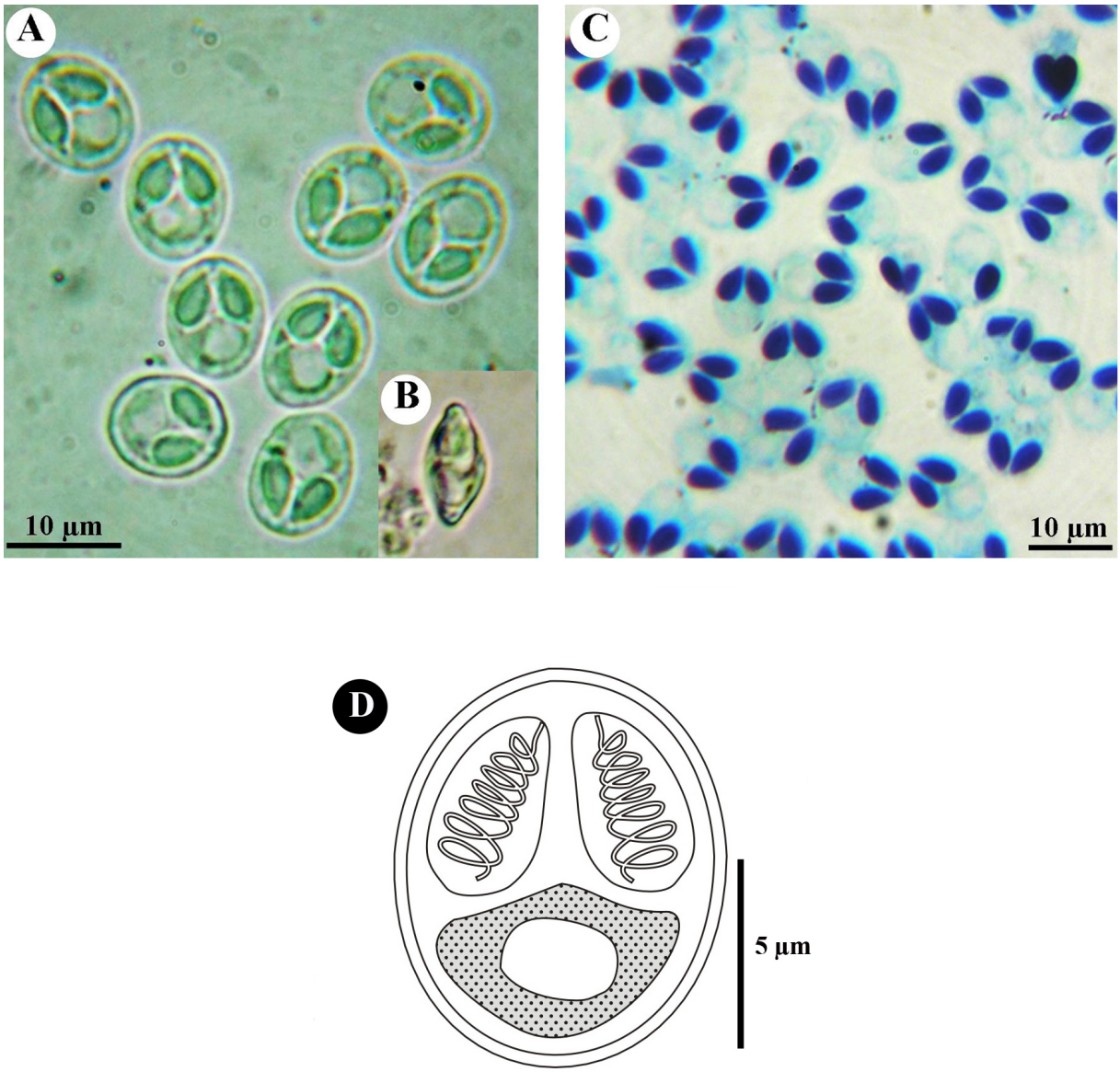

Fig. 1. Photomicrographs of *Myxobolus opsaridiumi* sp. nov. infecting skin, muscle and spleen of *Opsaridium ubangiensis* (Pellegrin, 1901). **A**. Fresh myxospores in frontal view. **B**. Fresh myxospore in lateral view. **C**. Giemsa-stained myxospores. **D**. Diagrammatic drawing of a mature myxospore.

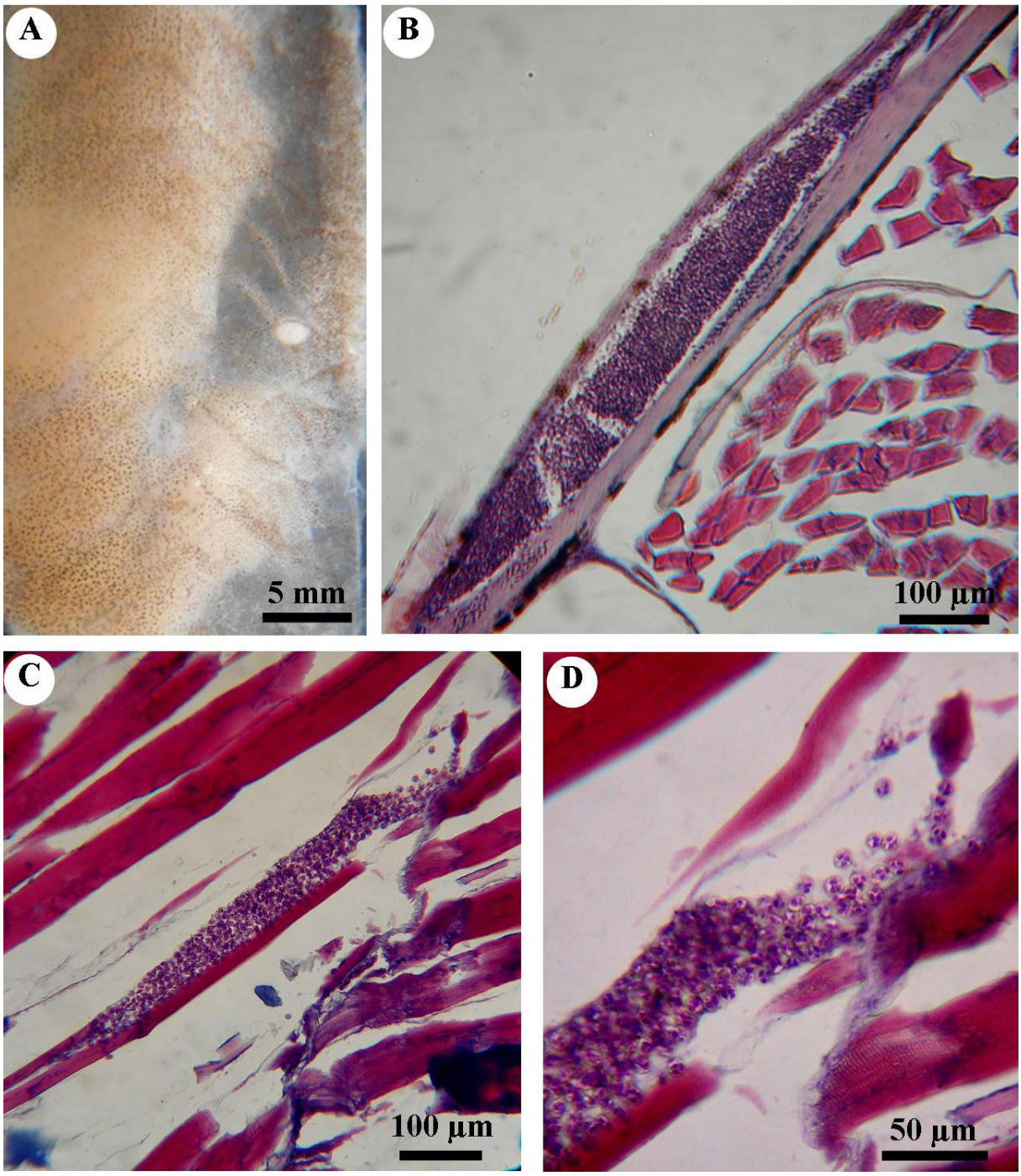

Fig. 2. Photomicrographs of plasmodia of *Myxobolus opsaridiumi* sp. nov. developing on *Opsaridium ubangiensis* (Pellegrin, 1901). **A**. Plasmodium development on the skin. **B**. Histological section stained with hematoxylin and eosin showing plasmodium situated in the dermis. **C**. Plasmodium developing within muscle fibers (hematoxylin and eosin). **D**. Higher magnification of a plasmodium from the muscle fibers.

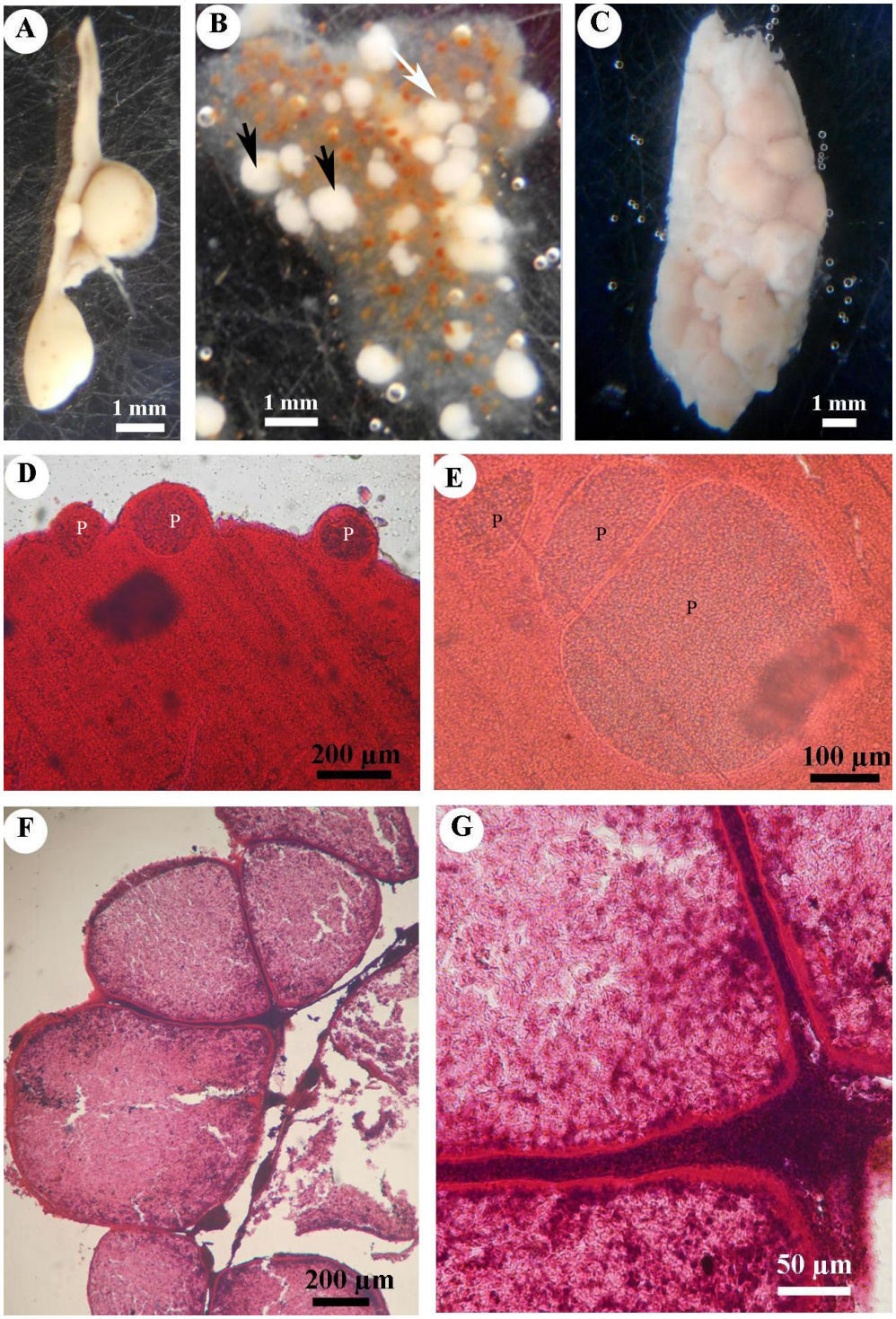

Fig. 3. A–C. Photomicrographs of plasmodia of *Myxobolus opsaridiumi* sp. nov. affecting a spleen of *Opsaridium ubangiensis* (Pellegrin, 1901). **A**. Spleen harbouring large plasmodia. **B**. Whitish plasmodia isolated from each other (black arrows) or arranged in grape-like clusters (white arrow). **C**. Spleen completely filled with plasmodia. – **D–G**. Histological sections stained with hematoxylin and eosin of spleens of *O. ubangiensis* infected with plasmodia of *M. opsaridiumi* sp. nov. **D**. Plasmodia implanted on the external region of the spleen. **E**. Asynchronous development of plasmodia within the spleen. **F**. Mechanical compression of the cells adjacent to the cysts. **G**. Higher magnification of plasmodia showing each surrounded by a wall and full of myxospores. Abbreviation: P = plasmodium.

Table 1. Comparative description of *Myxobolus opsaridiumi* sp. nov. with morphologically similar species (measurements in micrometres). Averages of the parameters measured are followed by minimal and maximal values in brackets. Abbreviations: LS = length of the spore; WS = width of the spore; TS = thickness of the spore (/ means not reported in the species description); PC = relative length of the polar capsules (= means equal; ≠ means unequal); LPC = length of the polar capsules; WPC = width of the polar capsules; FC = number of polar filament coils; IP = intercapsular process (A = absent; P = present).

Species	Host	Site of infection	Country	LS	WS	TS	PC	LPC	WPC	FC	IP	Reference
M. opsaridiumi sp. nov.	*Opsaridium ubangiense*	skin, muscle, spleen	Cameroon	10.7 (10–11.5)	9 (8–10)	6.2 (5.6–7.2)	=	5 (4.3–6)	2.7 (2.2–3)	5–7	A	Present study
M. calcariferum Basu & Haldar, 2003	*Lates calcarifer*	gill	India	6.6 (6.1–7.1)	6.2 (5.7–6.5)	/	=	4.2 (3.8–4.5)	2.3 (2.0–2.7)	4–5	A	Basu & Haldar 2003
M. caudatus Gogebashvili, 1966	*Barbus bynni*	fin	Egypt	17.5 (16–19.2)	12.8 (11–13.6)	/	=	7.4 (6.4–9)	3.8 (3.2–4.5)	8–9	P	Ali *et al.* 2002
M. clarii Mandour *et al.*, 1993	*Clarias lazera*	testis	Egypt	10.6 (9–12.2)	8.7 (7.5–9.9)	/	=	4.1 (3.5–4.8)	2.4 (2.2–2.7)	5	A	Mandour *et al.* 1993
M. franciscoi Eiras *et al.*, 2010	*Prochilodus argenteus*	fins	Brazil	6.4 (6.0–6.9)	6.0 (5.8–6.4)	3.2	=	2	1.5	3	A	Eiras *et al.* 2010
M. gariepinus Reed *et al.*, 2003	*Clarias gariepinus*	ovaries	Botswana	13.9 (13.7–15.0)	10.8 (10.0–11.2)	/	=	6.2 (6.0–6.2)	3.5 (3.0–3.7)	5–6	A	Reed *et al.* 2003
M. heterotisi Boungou *et al.*, 20006	*Heterotis niloticus*	gill	Burkina Faso	12.2 (11.5–13)	9.5 (9.5–10)	/	=	6.4 (6–7)	3.5 (3–4.5)	10	A	Boungou *et al.* 2006
M. insignis Eiras *et al.*, 2005	*Semaprochilodus insignis*	gill	Brazil	14.5 (14–15)	11.3 (11–12)	7.8 (7–8)	=	7.6 (7–8)	4.2 (3–5)	6	P	Eiras *et al.* 2005
M. ouemeensis Sakiti, 1997	*Synodontis gambiensis*	gill	Benin	8.5(7–9.5)	5.1 (4–6)	/	=	3.8 (3–4)	2.3 (2–3.5)	/	A	Sakiti 1997
M. rohitae Haldar *et al.*, 1983	*Labeo rohita*	scale	India	10.6 (9.9–12.1)	9 (8.8–9.9)	/	=	6.6	3.3	5–6	A	Haldar *et al.* 1983
M. sourouensis Boungou *et al.*, 2006	*Heterotis niloticus*	gill	Burkina Faso	11.3 (11–14)	8.8 (8–10)	/	=	5.7 (5–7)	2.3 (2–3.5)	7	A	Boungou *et al.* 2006
M. synodontisi Abakar-Ousman *et al.*, 2006	*Syndontis schall*	gill	Chad	12.1 (11.5–13)	8.9 (8.2–9)	/	≠	7.9 (7–9)* 6.9 (6.2–7)**	2.8 (2.5–3)* 2.6 (2.5–3)**	13-15	A	Abakar-Ousman *et al.* 2006

*Refers to the largest polar capsule.
**Refers to the smallest polar capsule.

whole organ. In these cases, abnormal enlargement of the spleen was evident (Fig. 3C). Histological sections revealed that, for moderately infected spleens, cysts were either fixed to the external region of the organ (Fig. 3D) or completely implanted within it (Fig. 3E). Development of cysts in the spleen was asynchronous (Fig. 3D–F). Atrophy of the adjacent splenic cells surrounding the cyst was likely due to mechanical compression (Fig. 3F–G). Each plasmodium was surrounded by a wall of a monolayer of flat cells (Fig. 3F–G). The central part of the plasmodium was occupied by fully mature spores, with initial stages of development visible in the periphery (Fig. 3G).

Phylogenetic position

Partial SSU rDNA sequences obtained from different organs were 100% identical. The consensus sequence of 1667 base pairs was submitted to GenBank with the accession number MN497413. This sequence did not match any publicly available myxozoan sequence. The sequence with the highest nucleotide similarity, at 91.8%, was for *Myxobolus haichengensis* Chen, 1958 (GenBank entry KY965936), which reportedly infects the gills of *Abbottina rivularis* (Basilewsky, 1855). Similarity with *M. dibombensis* Folefack *et al.*, 2019, a species we recently sequenced from *Labeobarbus batesii* (Boulenger, 1903) in Cameroon, was only 88.6%.

The phylogenetic position of the newly sequenced species was analyzed with maximum likelihood and Bayesian inference methods. Both methods produced an identical topology. *Myxobolus opsaridiumi* sp. nov. occurs in a large clade that includes species infecting cyprinids (Fig. 4). The new species exhibits the highest phylogenetic affinity with *M. haichengensis*, *Myxobolus* sp. (accession number MG253819) from the fins of *Capoeta tinca* (Heckel, 1843) off Anatolia, and *M. squamae* Keysselitz, 1908 infecting the skin of the common barbel *Barbus barbus* (Linnaeus, 1758).

Discussion

Based on morphological features of the myxospores infecting the skin, muscle and spleen of *Opsaridium ubangiensis*, the parasite was assigned to the family Myxobolidae Thelohan, 1892 and the genus *Myxobolus* Bütschli, 1882. To date, no Myxosporidia had been reported to infect *O. ubangiensis*. This is therefore the first myxobolid infection encountered in this cyprinid fish. Using robust morphological, histological, ecological and molecular data, the described Myxozoa was considered a new species, designated as *Myxobolus opsaridiumi* sp. nov.

However, some species of *Myxobolus* infecting other freshwater fishes and showing a similar myxospore morphology, tissue tropism, or geographical location and habitat have been used for comparisons with the new species (Table 1). These species include *Myxobolus caudatus* Gogebashvili, 1966; *M. clarii* Mandour, Galal & Abed, 1993; *M. gariepinus* Reed, Basson & Van As, 2003; *M. heterotisi* Boungou *et al.*, 2006; *M. sourouensis* Boungou *et al.*, 2006; *M. synodontisi* Abakar-Ousman *et al.*, 2006; *M. ouemeensis* Sakiti, 1997; and *M. rohitae* Haldar, Das & Sharma, 1983 (Table 1).

In Egypt, Ali *et al.* (2002) reported *M. caudatus* in the caudal fin of *Barbus bynni* (Forsskål, 1775) (Cyprinidae). *Myxobolus caudatus* differs from our species in that it has a larger myxospore and more developed polar capsules containing more coils of the polar filament (8–9 vs 5–7).

Myxobolus clarii forms cysts in the testis of *Clarias gariepinus* Burchell, 1822 (synonym: *Clarias lazera* Valenciennes, 1840) (Clariidae) in Egypt. The myxospores of this species, although ovoid and of comparable size, have a slightly pointed anterior and a rounded posterior. Its polar capsules are relatively small.

Myzobolus gariepinus, a parasite of the ovaries of *Clarias gariepinus* (Clariidae), which is found in Botswana, forms spores with a small and blunt point at the anterior end and dimensions that exceed the maximum ranges of the spore of the parasite being described, and with more developed polar capsules.

The polar capsules of *M. gariepinus* occupy only approximately a third of the spore cavity, while the spore of the species described here has two polar capsules that occupy close to half the space of the spore cavity.

Boungou *et al.* (2006) described two species of myxosporidia from Burkina Faso with a general shape comparable to that of our parasite, *M. heterotisi* and *M. sourouensis*, which are gill parasites of *Heterotis niloticus* (Cuvier, 1829) (Osteoglossidae). *Myxobolus heterotisi* differs from our species in that it has larger spores and more developed polar capsules (6.41 × 3.53 µm vs 5 × 2.7 µm) that contain a polar filament with more coils (10 vs 5–7). *Myxobolus sourouensis* has longer spores (11–14 µm vs 10–11.5 µm) which are slightly nippled at the anterior end.

In Chad, *Synodontis schall* (Bloch & Schneider, 1801) (Mochokidae) harbors *M. synodontisi* in its gills. Although of comparable shape, the myxospores of this species are longer. They contain more developed polar capsules, which cover the anterior two-thirds of the myxospore cavity, and are sometimes slightly asymmetrical, with a greater number of coils of the filament (13–15 vs 5–7).

In Benin, *Synodontis gambiensis* Günther, 1864 (Mochokidae) harbors *M. ouemeensis* in the cartilaginous tissue of the branchial arch and primary gill filaments. Although comparable in shape, the myxospores of *M. ouemeensis* are less developed, with shorter polar capsules (3–4 µm vs 4.3–6 µm).

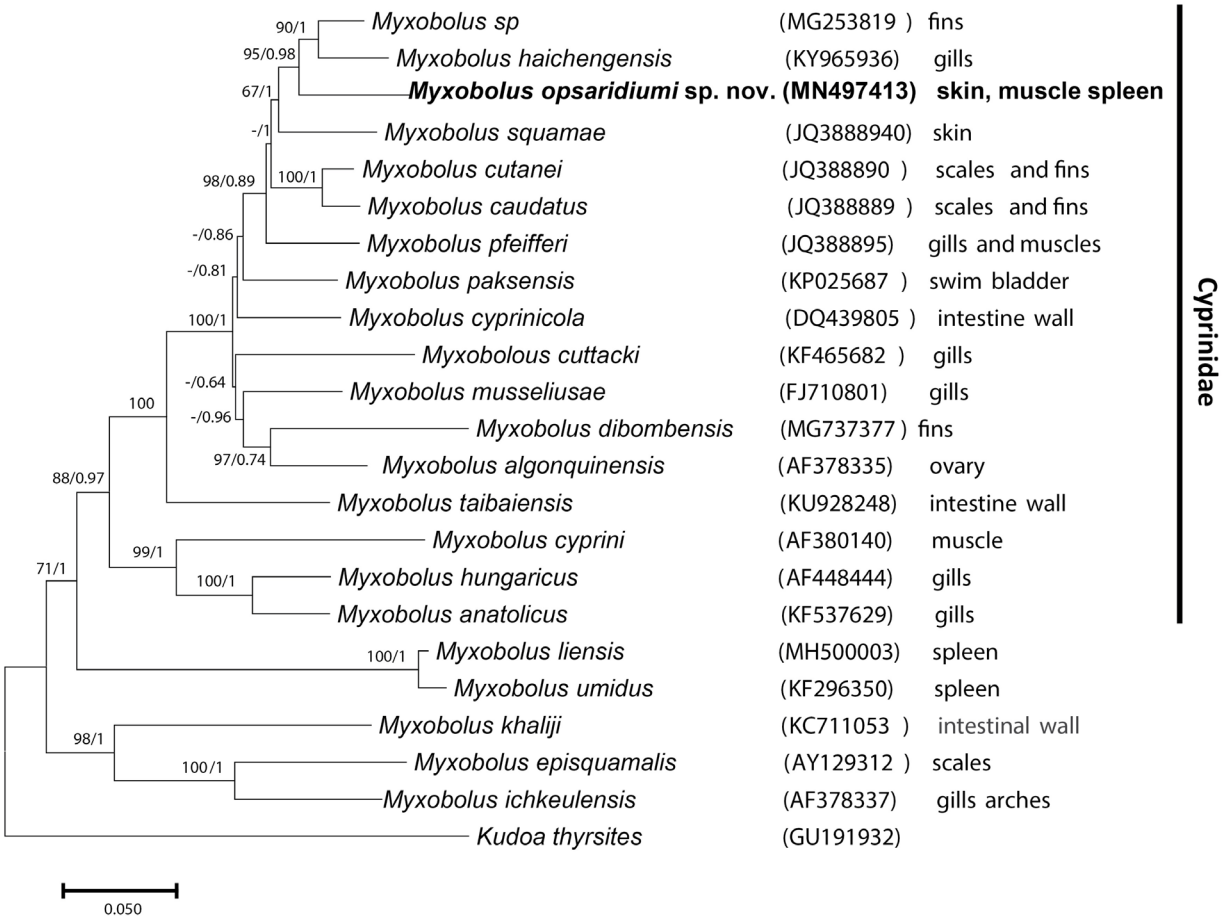

Fig. 4. Maximum likelihood phylogenetic tree based on the SSU rDNA sequences showing the position of *Myxobolus opsaridiumi* sp. nov. (in bold) and related species. Accession numbers and infected tissues are listed adjacent to the species names. Numbers at the nodes represent Bayesian posterior probabilities and ML bootstrap percentages. *Kudoa thyrsites* (Gilchrist, 1924) was used as the outgroup.

Myxobolus rohitae forms plasmodia on the scales of *Labeo rohita* Hamilton, 1822 (Cyprinidae) in India. Although of comparable size (10.6 × 9 vs 10.7 × 9 μm), the spores of this species have an intercapsular process. In addition, its polar capsules are longer (6.6 vs 5 μm) compared to those of the species being described.

Among all compared species, the 18S rDNA sequence of only *M. caudatus* is available in GenBank. This species exhibits 90% sequence similarity with our present species. The newly obtained sequence diverges substantially from that of all previously sequenced species of *Myxobolus*. The maximum similarity was obtained with *M. haichengensis*, which reportedly infects the gill filaments of *Abbottina rivularis* in China. The single species of *Myxobolus* sequenced from Cyprinidae collected in Cameroon was *M. dibombensis* (Lekeufack *et al.* 2019), which exhibited only 83% similarity. The phylogenetic tree did not show strict clustering of selected species of *Myxobolus* according to geographic locality or tissue tropism. It is evident that species infecting cyprinids from around the world and different tissues clustered together in the same clade and can be separated from species infecting other hosts from different families and in some cases by tissue tropism. This data is consistent with previous reports, suggesting that phylogenetic affinity of the fish host provided the strongest co-evolutionary congruence (Fiala 2006; Molnar *et al.* 2011; Zhang *et al.* 2014; Karlsbakk *et al.* 2017; Zhao *et al.* 2020).

In our investigation we found plasmodia of *M. opsaridiumi* sp. nov. in three different organs (skin, muscle and spleen). Studies on the site selection of fish myxosporeans infecting the gills, fins, kidneys and muscle suggest that most myxosporean species have a strict tissue tropism determined by the site of the infected organ used for species identification (Molnár 1994; Molnár & Székely 2014). Myxozoan parasites are considered as one of the most striking examples of parasite radiation, which could explain their high diversity (Holzer *et al.* 2018). This great diversity would be acquired by their success in exploiting new hosts and new geographic distributions (Fiala *et al.* 2015). Adaptation to a new host would be facilitated when the newly exploited species was closely related to the original host within a similar tissue. Another means of radiation is the ability of the same species to explore multiple habitats within the same host and to have the ability to switch in other hosts as well. The selective pressure of the new tissue would be stronger than that of a new related host and is usually associated with evolutionary advantages (Patra *et al.* 2018). This explains the high specificity of many myxozoan genera and species to the infected tissues, as in the cases of species of *Ceratomyxa* Thelohan, 1892 infecting the gallbladder and *Sphaerospora* spp. mostly being parasites of the urinary system.

Myxosporidia infections on the skin are rare but easily observable. The presence of cysts of *M. opsaridiumi* sp. nov. on the skin did not cause an inflammatory reaction in host fish, with proliferation a direct consequence. According to Lekeufack & Fomena (2013), the presence and proliferation of cysts of Myxosporidia on the skin are due to the direct contact of this organ with the immediate aquatic environment of the fish, providing an attachment surface to the actinospores.

The histopathological analysis of the skeletal muscle revealed no signs of inflammation or recruitment of phagocyte cells. The integrity of myofibrils within the infected myofibers exhibited some degree of degeneration, with a partial loss of myofibrillar details and striations. These changes were observed in regions close to the plasmodia. Such histopathological observations in the muscle were similar to those reported by Manrique *et al.* (2015) on *Myxobolus* sp. infecting the skeletal muscle of *Piaractus mesopotamicus* (Adriano *et al.*, 2006) in Brazil. Species of the genus *Myxobolus* were reported in the skeletal muscle of fish (Molnár & Székely 2014). Pathological effects by species of *Myxobolus* reported in cyprinid fish include muscle hypertrophy in *Leuciscus cephalus* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758) and *Abramis brama* (Linnaeus, 1758) as a result of infection by *M. buckei* (Longshaw *et al.*, 2003); skeletal muscle infection by spores of *M. cyprini* Doflein, 1898 in *Cyprinus carpio* Linnaeus, 1758 and *M. pseudodispar* Gorbunova, 1936 in *R. rutilus* reported by Baska (1987). As a result of

M. artus Akhmerov, 1960 infection in the muscle, Ogawa *et al.* (1992) observed damaged growth in *C. carpio*. Most of these pathological changes are responsible for myoliquefaction and the production of meat unfit for consumption (Gilman & Eiras 1998), with the possibility of food poisoning when the meat is consumed raw or undercooked.

Because teleost fish have no medullary cavity in their bones, the spleen and kidney serve as the primary haemopoietic organs (Agius & Roberts 2003), and as fish have no lymph nodes, the spleen plays an essential role in antigen trapping (Press 1998). Although some species of *Myxobolus* reportedly infect the spleen of teleost fish (Eiras *et al.* 2005, 2014), no histological observations have been made in affected spleens. It appears that *M. opsaridiumi* sp. nov. can be responsible for damage in the spleen of *Opsaridium ubangiensis*, as the development of the plasmodia reduces the vessel lumen and in some cases completely obstructs the lumen of the spleen arterioles. A high parasite load could therefore compromise blood circulation and undermine spleen function. Specific patterns of development and the final site of sporogenesis can vary among species of Myxozoa. Without observation of the developing stages it is difficult to determine whether the spores developed at the site of the infection or were carried there by the host's macrophages. This observation led Dyková (1984) to conclude that myxospores are generally transported by macrophages homing in on macrophage centers of hematopoietic organs such as the kidney, spleen and hepatopancreas, where they are destroyed. However, in the present study only vegetative stages of *M. opsaridiumi* sp. nov. were observed in the spleen and no free spores were seen. The spleen is therefore a normal site for *M. opsaridiumi* sp. nov. sporogenesis.

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