



Morphological and molecular characterization of *Myxobolus dibombensis* sp. n. (Myxozoa: Myxobolidae), a parasite of the African carp *Labeobarbus batesii* (Teleostei: Cyprinidae) from Dibombe River, Cameroon

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Abstract

Myxobolus dibombensis sp. n. (Cnidaria: Myxosporea: Bivalvulida) is described from the fins of the African carp, *Labeobarbus batesii*, based on morphological and molecular data. Prevalence of infection was 51.9% (67/129). Ovoid to spherical cyst-like plasmodia were found in the intrasegmental region and among the fin rays. No pathological changes were found in the fish host tissue surrounding the cyst-like plasmodia. Mature myxospores were ovoid in frontal view and lenticular in lateral view, with slightly truncated anterior and rounded posterior ends. Myxospores measured 16.8 (15.8–18.0) μm long and 11.4 (10.0–13.0) μm wide. There was a triangular intercapsular appendix measuring 3.8 (2.6–4.5) μm long. Polar capsules were ovoid and slightly unequal in size, occupying approximately one-third of the myxospore length. The larger polar capsule measured 7 (6–8) μm long and 3.6 (3–4) μm wide, while the smaller one measured 5.8 (4.8–7.0) μm long and 3 (2–4) μm wide. The larger polar capsule contained nine to 11 filament coils, whereas the smaller one contained seven to nine coils. SSU rDNA gene sequence of *M. dibombensis* sp. n. did not match any sequences available in the GenBank. The similarity with available *Myxobolus* spp. sequences ranged from 65 to 81%. The novel species clustered with *M. algonquinensis*, which infects the cyprinid *Luxilus cornutus* from Canada.

Keywords *Myxobolus* · African carp · SSU rDNA · Phylogeny · Fish diseases

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Introduction

Myxosporeans are a group of microscopic metazoans, which are primarily parasites of freshwater and marine fishes, and some other vertebrates (Sitjà-Bobadilla et al. 2016). These parasites are characterized by multicellular myxospore, composed of one to 15 nematocyst-like polar capsules each containing a polar filament, and one or many amoeboid sporoplasms (Kent et al. 2001; Whipps et al. 2003). There are 2200 nominal myxosporean species classified into 64 genera and 17 families, essentially based on morphological characteristics of myxospores with some fragment data on their SSU rDNA sequence (Lom and Dyková 2006). Detailed morphological characterization supplemented with molecular data is important for describing new species and for amendment of the known species. Within the myxosporea, *Myxobolus* is the most speciose genus with more than 900 described species (Liu et al. 2016), of which more than 90 nominal species have

been reported to infect Cyprinid fishes (Liu et al. 2016; Kato et al. 2017). Cyprinidae is the largest freshwater fish family with about 375 genera and 3060 known species that includes carps, minnows, and other related species (Froese and Pauly 2015). African cyprinids are represented by 526 species (Stiassny et al. 2007). Of them, the African carp, *Labeobarbus batesii* (Boulenger, 1903) is widespread throughout southern Cameroon and north Gabon forested areas, and possibly as far north as Chad (Moelants 2010). *Labeobarbus batesii* has a high nutritional value that make it a suitable candidate for aquaculture (Tiogué et al. 2013). To the best of our knowledge, no myxosporeans species are known from *L. batesii* in Africa. In the present study, we describe a new species of *Myxobolus* from the fins of *L. batesii* using spore morphometrics and SSU rDNA sequence data.

Materials and methods

During the present study, 129 specimens of *L. batesii* (Cyprinidae) were caught using gill net during a period from November 2012 to June 2017 in the Dibombe River at N'lohe village (4° 74'–4° 78' N, 9° 79'–9° 82' E, Cameroon, Central Africa). The fish were transported to the laboratory of Parasitology and Ecology of the University of Yaounde 1 in Cameroon for identification and parasitological examination. The standard length of the collected fishes ranged from 65 to 180 mm, and fish were identified according to Stiassny et al. (2007) and Vivien (2012). Fishes were grossly examined macroscopically and then under an Olympus Bo 61 stereomicroscope for the presence of myxosporean cysts on external organs such as eyes, fins, operculum, scales, and skin. After dissection, internal organs (kidney, bile duct, liver, gall bladder, digestive tract, spleen, heart, gonads, urinary duct, urethras, and muscles) were thoroughly examined. Some of the cysts were crushed between glass slides and coverslips, and the myxospore contents were identified using a $\times 100$ objective of an IVYMEN light microscope. Permanent smears of myxospore were fixed in methanol and stained with May Grünwald-Giemsa. Drawings of fresh myxospores were carried out using wild M-20 microscope equipped with a camera lucida. Measurements were carried out on at least 50 fresh myxospores as proposed by Lom and Arthur (1989). Fresh and stained myxospores were photographed using an Olympus BH-2 microscope equipped with camera.

For histological studies, infected fins were fixed in 10% neutral buffered formalin. The fixed fins were then cut into 3- μ m-thick sections after embedding in paraffin wax and processed for histology. Sections were stained with hematoxylin and eosin (H&E) and examined and photographed using Axio Imager Z2 microscope (Carl Zeiss Inc., Berlin,

Germany) equipped with digital camera (Axiocam MRC5, Zeiss).

For molecular analysis, genomic DNA was extracted from cyst-like plasmodia preserved in absolute ethanol using the DNeasy Blood and 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 reactions (PCRs) were performed in a thermocycler (Techne TC-Plus Satellites, Staffordshire, UK). 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 54 °C and 120 s at 72 °C, and a final extension of 7 °C for 10 min. PCR reactions were performed in a final volume of 30 μ l, containing 1 \times iProof™ High Fidelity Master Mix (Bio-Rad, Hercules, CA), 0.2 μ M of each primer and 50–100 ng of genomic DNA. Thereafter, 3 μ l of the PCR products were electrophoresed on 1% agarose gel in 0.5 \times Tris/borate/EDTA (TBE) buffer, stained with ethidium bromide, and visualized using a UV-transilluminator system. The PCR products were purified using A ExoSAP-IT™ (USB, Afymetrix) treatment and sequenced by the commercial sequencing company (Macrogen Inc., Seoul, South Korea) using the same primers used for PCR.

The contiguous sequence was obtained from forward and reverse sequences originating from three different PCR products. This sequence was used to query similar *Myxobolus* sequences using BLAST in the GenBank (Altschul et al. 1997). *Myxobolus* spp. sequences were selected for analysis based on infected host family and geographic distribution. A genetically distant Multivalvulida sequence was selected as an outgroup. Sequences were aligned applying the default parameters in ClustalX 2.1.0.12, (Larkin et al. 2007).

Phylogenetic analyses were performed by Bayesian inference (BI) and maximum likelihood (ML) methods. The BI analyses were performed using MrBayes version 3.2 (Ronquist et al. 2012) using Markov Chain Monte Carlo (MCMC) for 2,000,000 generations with two independent runs of four simultaneous MCMC chains (nchains = 4). Trees were saved every 100 generations (samplefreq = 100). The first 25% of the samples were discarded from the cold chain.

Maximum likelihood analyses were based on the General Time Reversible model with a gamma-distributed rate and invariant sites (GTR+G+I). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, followed by selection of the topology with superior log likelihood value. The tree with the highest log likelihood (– likelihood (– 16,689.8910) was selected. The analysis involved 44 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5%

alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 1377 positions in the final dataset. Phylogenetic analyses were conducted with inferred nodal support based on 1000 bootstrap replicates using MEGA7 (Kumar et al. 2016).

Results

During the survey of myxosporean fauna in the River at N'lohe, numerous whitish ovoid or spherical cyst-like plasmodia were observed infecting the African carp, *L. batesii*. The cysts were of variable sizes (210–600 × 125–410 µm), scattered in all the fin rays and in between fin rays and filled with mature myxospores. External examination of infected fishes did not show any signs of abnormalities such as ecchymoses or deformities. Rupture of cyst-like plasmodia released mature spores morphologically typical of the genus of *Myxobolus*.

Family Myxobolidae Thélohan, 1892

Genus *Myxobolus* Bütschli, 1882

Myxobolus dibombensis sp. n.

Type host: *Labeobarbus batesii* (Boulenger, 1903) (Cyprinidae)

Site of infection: All fins

Prevalence: 51.9% (67 parasitized fish out of 129 examined)

Type locality: N'lohe, 4° 74'–4° 78' N, 9° 79'–9° 82' E (Littoral Region in Cameroon)

Type-material: Fins infected with plasmodia were deposited in the parasitological collection of the Zoology Department Museum, College of Science, King Saud University, Riaydh, Saudi Arabia, with 163 number (Myx/12/2018). Partial sequence of the small subunit ribosomal DNA was deposited in GenBank under the accession numbers MG737377.

Etymology: The specific epithet is related to the Dibombe River, where the host fish was captured.

Vegetative stages: Ovoid or spherical cyst-like plasmodia of variable sizes infecting the fin rays.

Myxospores (Figs. 1 and 2):

Mature myxospores are ovoid in frontal view (Figs. 1A, C and 2a) and lenticular in lateral view (Figs. 1B and 2b), with slightly truncated anterior end and rounded posterior. Myxospore are 16.8 (15.8–18) µm long and 11.4 (10–13) µm wide. Shell valves are thick and symmetric with six to eight valvular folds at the posterior end of the myxospore (Fig. 1A). The myxospores are characterized by the presence of a conspicuous triangular intracapsular appendix of 2.9 (2.2–3.8) µm long (Figs. 2a and 3a). Polar capsules are ovoid and slightly unequal (Fig. 2c). The larger polar capsule is 7 (6–8) µm long and 3.6 (3–4) µm wide with nine to 11 filament coils while the smaller one is 5.8 (4.8–7) µm long and 3 (2–4)

µm wide with seven to nine filament coils (Fig. 3a, b). The sporoplasm is binucleated and contains an iodophilous vacuole of varying shape and size (Figs. 2a and 3a).

Histology: Some plasmodia were located in the intrasegmental region, inside the tube-like lumen of the two hemisegments constituting the cartilaginous fin rays (Fig. 3a, b). While, in between the fin rays, the plasmodia were found under the epidermis and surrounded by a thin layer of connective tissue (Fig. 3d). No pathological changes were found in the cartilaginous structure of the hemisegments and no inflammatory reaction was recorded in the dermis.

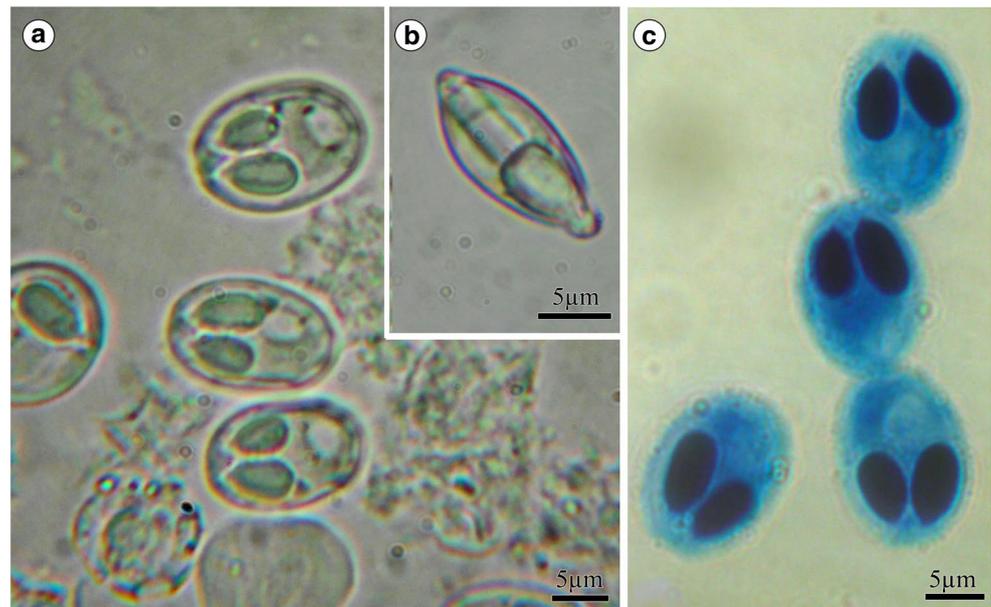
Molecular analysis: The partial sequence of 1676 bp of the SSU rDNA obtained from *M. dibombensis* sp. n. was deposited in GenBank under the accession number MG737377. This sequence did not match with any of the *Myxobolus* sequences available in the universal database. The GC content was 47.8%. Pairwise alignment of the obtained sequence showed that the maximum similarity was 81.5% with *Myxobolus algonquinensis* Xiao and Desser, 1997 infecting ovary of the golden shiner *Notemigonus crysoleucas* from a lake in Canada. The similarity with other selected *Myxobolus* spp. sequences was between 65 and 80%.

Phylogenetic trees: Phylogenetic trees based the on ML and BI yielded a similar topology with some differences in support values at some nodes (Fig. 4). *Myxobolus dibombensis* sp. n. was positioned sister to *M. algonquinensis* within the lineage grouping three *Myxobolus* species, *Myxobolus musseliasae*, *Myxobolus tsangwuensis*, and *Myxobolus basilamellaris* which infect different tissues of the common carp, *Cyprinus carpio* Linnaeus, 1758 from different geographic localities. This subclade occurs in a larger clade grouping *Myxobolus* spp. reported to infect cyprinid fishes. Thus, of the 44 species of *Myxobolus* selected in this phylogenetic analysis, 37 infect 14 fish species belonging to the family Cyprinidae. Most of these *Myxobolus* species were collected from different tissues of the same species host as *C. carpio* (7 species), *Abramis brama* (5 species), and *Carassius auratus* (4 species). Species of *Myxobolus* infecting marine fishes from other families such as the Mugilidae, form a well-supported independent clade.

Discussion

Fundamentally, with some exception, the various *Myxobolus* species described outside Africa could be considered as independent species because of the topographical barriers and should not have to be compared with species described from Africa (Ali et al. 2002). However, in the present study, some *Myxobolus* species from outside Africa were considered owing to their presence in a similar host species. Out of more than 900 *Myxobolus* species described, only ten species closely resemble the present parasite and could be compared. These

Fig. 1 Photomicrographs of *Myxobolus dibombensis* sp. n. isolated from the fin rays of *Labeobarbus batesii*. Fresh myxospores in frontal (A), lateral view (B), and Giemsa-stained myxospores (C)



species are the following: *Myxobolus vedavatiensis* Seenapa and Manohar, 1981 and *M. algonquinensis* which are non-African species, whereas *Myxobolus njinei* Fomena et al. 1985; *Myxobolus dossoui* Sakiti et al. 1991; *Myxobolus bilongi* Fomena et al. 1994; *Myxobolus caudatus* Ali et al. 2002; *Myxobolus intestinalis* Ali et al. 2002; *Myxobolus charii* Fomena et al. 2004, *Myxobolus ngassami* Lekeufack et al. 2017; and *Myxobolus sessabai* Lekeufack et al. 2017 are all African species (Table 1).

Myxobolus vedavatiensis that infect the gills of *Cirrhina mrigala* in India; differs in having slightly smaller myxospores, lacking the sutural folds and the larger polar capsule was nearly double in length as that of the smaller one (6.2 µm vs 3.9 µm). *Myxobolus algonquinensis* forms

cysts in the ovaries of *N. crysoleucas* (Cyprinidae) in Canada and its myxospores are clearly distinguishable from those of the present species owing to the absence of truncation at the anterior end, lack of the sutural folds, smaller intercapsular process and symmetrical polar capsules. *M. ngassami* which parasitizes many organs (e.g., fins, kidneys, eyes, operculum, and skin) of the cyprinid fish *Barbus callipterus* in Cameroon differs in having quite shorter myxospores (10.5 µm vs 16.8 µm) with equal polar capsules. *M. njinei* forms cysts in the gills of several cyprinid fish species (*Barbus camptacanthus*, *B. guirali*, and *B. martorelli*) in Cameroon differs in lacking sutural marking and intercapsular process and having equal polar capsules. Myxospores of *M. dossoui* parasitizing the gill arch of *Tilapia zillii*,

Fig. 2 Line drawing in frontal (a) and lateral (b) view of *Myxobolus dibombensis* sp. n

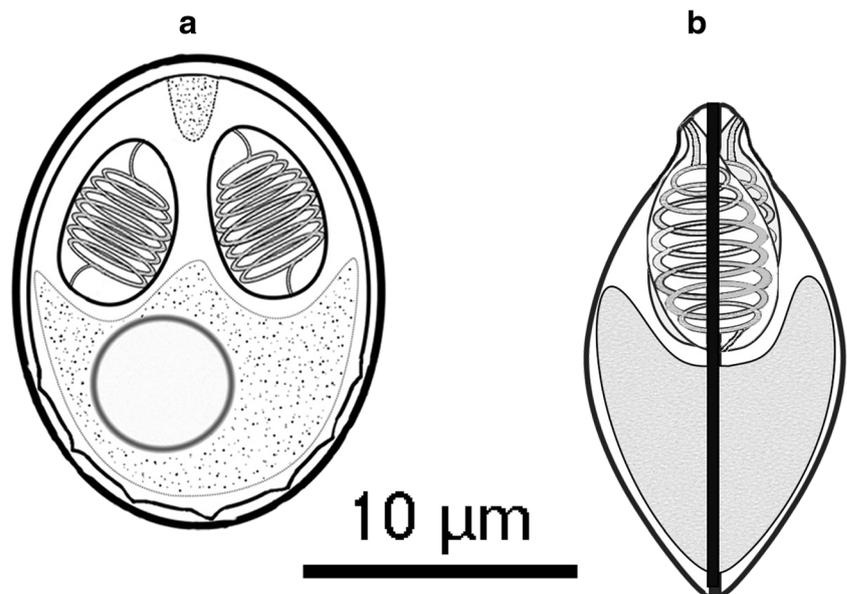
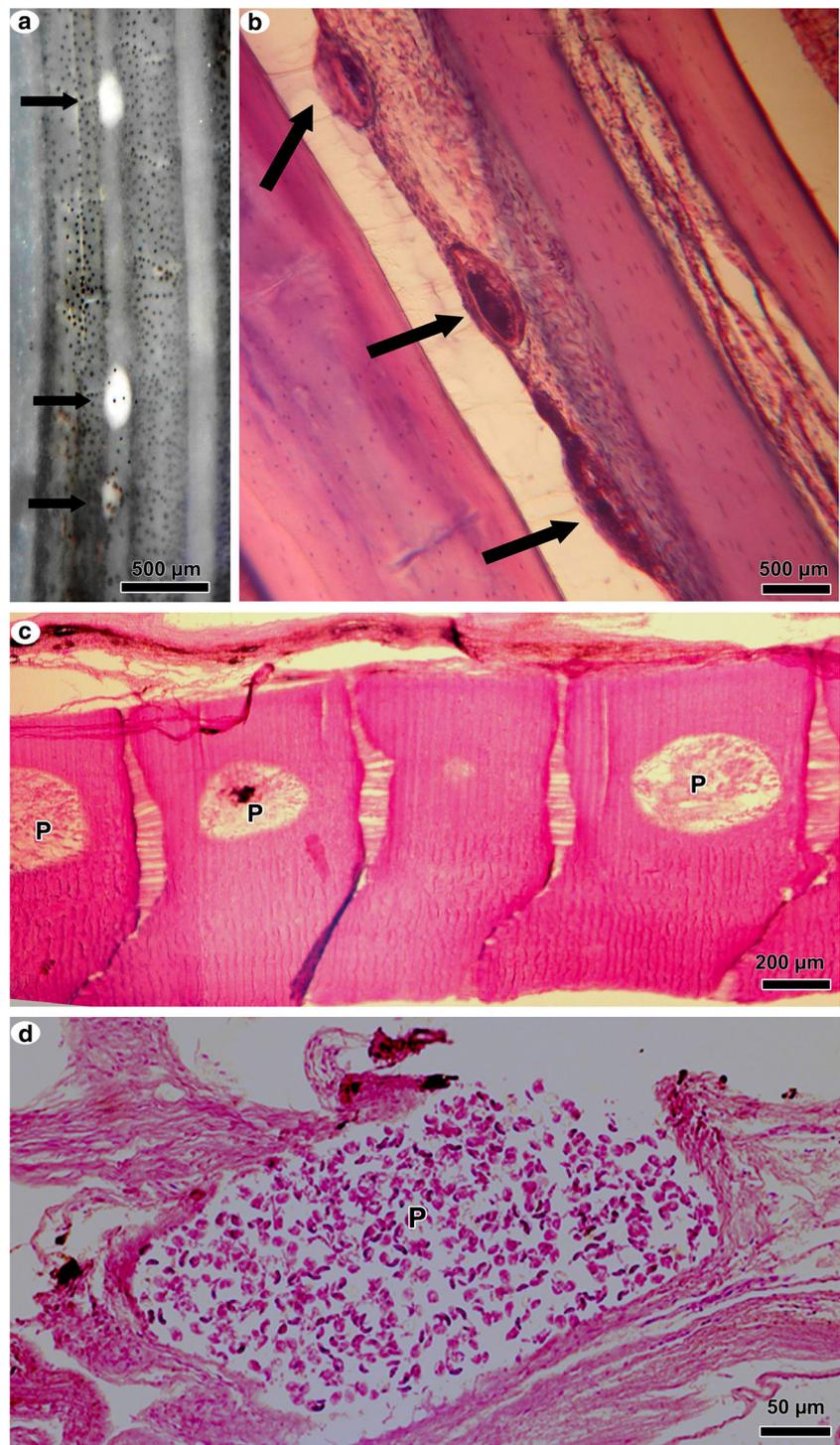


Fig. 3 Photomicrographs of *Myxobolus dibombensis* sp. n. infecting the fin rays of *Labeobarbus batesii*. **a** Fresh preparation of fin showing plasmodia in the intrasegmental region (arrows); **b–d** Histological sections stained with H&E of fins rays showing the plasmodia in the intrasegments of rays (P or arrows)



Hemichromis fasciatus, and *Tilapia* hybrid in Benin can be easily distinguished by their smaller and spherical myxospores with fewer polar filament coils (7–9 vs. 9–11). *M. bilongi*, a parasite of *Labeo* sp. (Cyprinidae) in Cameroon differs from the parasite described here by its subspherical myxospores, the lack of the sutural folds and fewer polar filament coils in the smaller polar capsule (6–7 vs 7–9).

Similarly, the longer elliptical myxospores and longer equal polar capsules with fewer polar filament coils differentiated *M. caudatus* (parasite of the fin of *Barbus bynni* in Egypt) from the present species. *M. intestinalis*, a parasite of the intestine of *Barbus bynni* (Cyprinidae) in Egypt, forms myxospore whose dimensions (12–13.6 × 8–9.6 μm) are less than the minimum ranges of the myxospore of

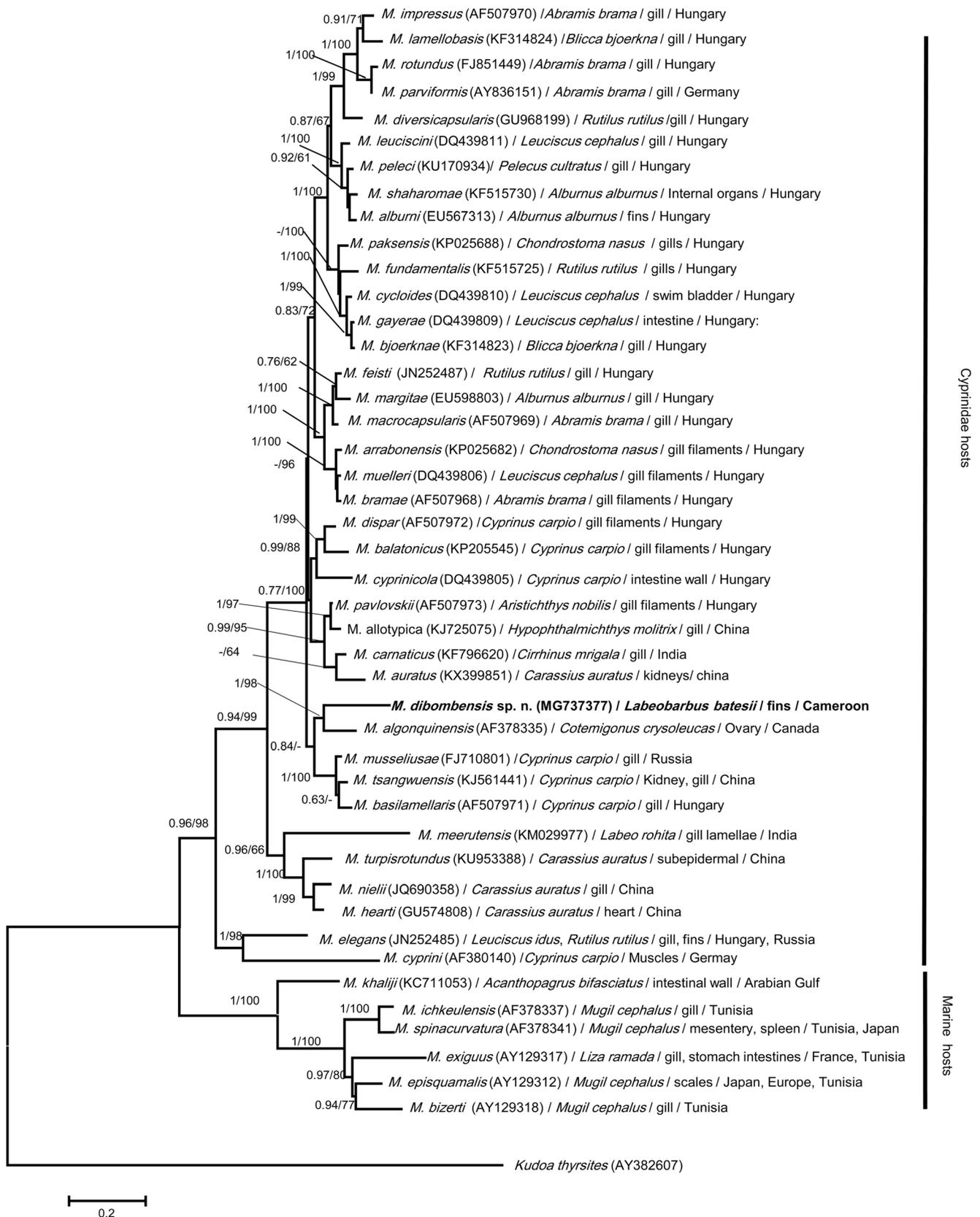


Fig. 4 Maximum likelihood phylogenetic tree based on the SSU rDNA sequences showing the position of *Myxobolus dibombensis* sp. n. (in bold) and related species. Accession numbers, host species, infection

site, and geographic origin are listed adjacent to the species name. Numbers at the nodes represent Bayesian posterior probabilities and ML bootstrap percentages. *Kudoda thyrsites* was used as outgroup

Table 1 Comparative measurements of *Myxobolus dibombensis* sp. n. with morphologically similar species

<i>Myxobolus</i> species	Host	Country	Spore length	Spore width	Polar capsule length	Polar capsule width	Number of filament coils	Reference
<i>M. dibombensis</i> sp. n.	<i>Labeobarbus batesii</i>	Cameroon	16.8 (15.8–18.0)	11.4 (10.0–13.0)	7 (6–8) 5.8 (4.8–7.0)	3.6 (3–4) 3 (2–4)	9–11 7–9	Present study
<i>M. algonquinensis</i>	<i>Notemigonus crysoleucas</i>	Canada	14.7 (13.6–15.4)	10.9 (10.1–12.1)	5.3 (5.1–5.5)	2.7 (2.5–2.9)	4–6	Xiao and Desser (1997)
<i>M. bilongi</i>	<i>Labeo</i> sp.	Cameroon	15.3 (14–17)	12.2 (11.3–14.5)	7.4 (6.5–8) 5.3 (4.1–6.5)	4.8 (4–6) 3.5 (3–4)	9–10 6–7	Fomona et al. (1994)
<i>M. caudatus</i>	<i>Barbus bynnii</i>	Egypt	17.5 (16–19.2)	12.8 (11–13.6)	7.4 (6.4–9)	3.8 (3.2–4.5)	8–9	Ali et al. (2002)
<i>M. charii</i>	<i>Citharus citharus</i>	Chad	14 (13–15.5)	11.4 (10.5–12)	7.6 (6.4–8.5)	3.8 (3.2–4.2)	8–10	Fomona et al. (2004)
<i>M. dossoui</i>	<i>Tilapia zilli</i> , <i>Hemichromis fasciatus</i> and <i>Tilapia hybrid</i>	Benin	9.9 (8.5–11)	9.2 (8–10.5)	5.5 (4.5–6.5) 4.3 (3–5.5)	3.1 (2.5–5) 3.1 (2–3.5)	7–9 5–6	Sakiti et al. (1991)
<i>M. intestinalis</i>	<i>Barbus bynnii</i>	Egypt	12.5 (12–13.6)	8.8 (8–9.6)	7.7 (7.2–8)	3.3 (3.2–3.6)	5–6	Ali et al. (2002)
<i>M. ngassami</i>	<i>Barbus callipterus</i>	Cameroon	11.5 (10.7–12.8)	9.4 (8.3–10.5)	5.0 (4–5.6)	2.8 (2.1–3.4)	7–10	Lekeufack et al. (2017)
<i>M. njinei</i>	<i>Barbus camptacanthus</i> , <i>B. batesii</i> , <i>B. guirali</i> , <i>B. martoreli</i>	Cameroon	16.17 (14–20)	13.46 (11.6–18.5)	7.81 (6.5–9)	4.59 (3.5–5.4)	7–8	Fomona et al. (1985)
<i>M. sessabai</i>	<i>Barbus callipterus</i>	Cameroon	13.4 (12.6–14)	10.8 (9.9–11.5)	5.9 (5–6.3)	3.4 (2.5–3.8)	8–10	Lekeufack et al. (2017)
<i>M. vedavattensis</i>	<i>Cirrhina mrigala</i>	India	13.8 (13–15)	9.2 (8–10)	6.2 (6–7) 3.9 (3–5)	3.4 (3–4) 2.6 (2–3)	–	Seenapa and Manohar (1981)

Mean values of spore dimensions are given in μm and range in parentheses

M. dibombensis sp. n. (15.8–18 × 10–13 µm) with less number of polar filament turns (5–6 vs. 9–11). The typically ovoid myxospores with reduced intercapsular process and equal polar capsules differentiate *M. charii* (parasite of the skin of *Citharinus citharus* in Chad) from the species detected in the present study. Finally, *M. sessabai*, the parasite of the skin and kidneys of *Barbus callipterus* (Cyprinidae) in Cameroon differs in having smaller ovoid myxospores with equal polar capsules.

To the best of our knowledge, the SSU rDNA sequence of *M. dibombensis* sp. n. is the first one for a myxosporean species from fishes in the rivers of Cameroon and is the first myxosporean reported to infect *Labeobarbus batesii* in Africa. Molecular data show at least 20% of nucleotide difference between *M. dibombensis* sp. n. and the rest of the *Myxobolus* spp. According to the molecular analysis, the most related species was *M. algonquinensis*, reported from the golden shiner *N. crysoleucas* in Canada.

Overall, the results of morphological and molecular analyses presented here were sufficient to establish the present species as new to the science and given the name *M. dibombensis* sp. n. which is derived from the Dibombe River, where the host fish was captured.

According to Becerra et al. (1983), teleost fin is an organ of simple structure, composed of epithelium, connective tissue, endothelial elements, and the chondrifying collagenous substance of the fin rays. Molnár (2002) suggested that teleost fin is one of the most common sites of establishment of eukaryotic parasites and possibly of other pathogens. This author also suggested that, because of their different tissue affinities, myxosporeans could occur in diverse locations even within this organ with a relatively simple structure. The establishment of *M. dibombensis* sp. n. in the fin rays and in between the fin rays indicated that organ specificity gains priority over tissue specificity. The histological findings indicate that *M. dibombensis* sp. n. causes only minor local changes on the fins of *L. batesii*, because no pathological changes were found in the cartilaginous structure of the hemisegments and no inflammatory reaction was recorded in between the fin rays (Fig. 3). According to Molnár and Székely (2003), the pathological importance of changes caused by myxosporeans greatly depends on the parasite species, intensity of parasite colonization, the size of plasmodia, the type of the attacked organ. Parasites colonizing vital organs such as the gills, the brain, or the kidney have much higher pathogenicity (Halliday 1976; Dyková and Lom 1978; Sanaullah and Ahmed 1980; Camus et al. 2017). Accordingly, *M. dibombensis* sp. n., by forming cysts on the fins, cannot be considered a highly pathogenic species, and the fin lesions caused could be considered negligible. However, the rupture of *M. dibombensis* sp. n. cysts implanted in the fin rays and in between the fin rays of *L. batesii* makes it susceptible to secondary bacterial, fungal, and viral infections. Finally, the gross damage caused on

L. batesii fins can result in overall weakness and slow swimming movements.

Phylogenetic analysis shows the clustering of *Myxobolus* spp. according to the taxonomy of the host and placed unambiguously *M. dibombensis* sp. n. with a strong support, in a clade grouping species infecting Cyprinidae. This clade is included within the “clade 1” recently suggested by Karlsbakk et al. (2017). The clustering of most of selected species occurred mainly according to the family, followed by the genus, species, tissue tropism, and geographic origin. This is in accordance with preceding phylogenetic analyses using the SSU rDNA gene of myxosporean parasites, where strong correlation between the molecular evolution of Myxosporea and the taxonomic affiliation of the infected fish host, its environment, tissue tropism, and geographic distribution have been revealed (Andree et al. 1999; Kent et al. 2001; Eszterbauer 2004; Holzer et al. 2004; Fiala 2006; Karlsbakk et al. 2017). More sequencing effort of other *Myxobolus* species infecting Cyprinid and non-Cyprinid fishes from Cameroon should help in understanding the influence of the taxonomy of the host or the environment in the evolution of these parasites.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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