https://doi.org/10.48047/AFJBS.7.7.2025.6660-6689

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Phenotypic and molecular characterization of *Myxobolus moliwensis* sp. nov. (Cnidaria: Myxosporea), a parasite of *Labeobarbus habereri* (Steindachner, 1914) from the Moliwè River in Limbé, Cameroon

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Volume7, Issue7 , July 2025

Received : 15 May2025

Accepted : 05 Jun 2025

Published : 09 July 2025

Doi :10.48047/AFJBS.7.7.2025.52-70

AbstractBackground: The genus *Myxobolus* encompasses a diverse group of myxozoan parasites that represent significant threats to the health of freshwater fish populations, impacting both biodiversity and aquaculture productivity. These parasites are known to induce a range of pathological conditions, including gill infections, which can impair respiratory function in infected fish. Despite the increasing concern over myxozoan parasites, the diversity of *Myxobolus* species, particularly within African freshwater systems, remains poorly characterized.

Methods: Specimens of *Labeobarbus habereri* were collected from the Moliwè River, Cameroon. Parasitological examination included gross morphological assessment, histopathological analysis, and light microscopy to characterize the cysts and myxospores. Molecular identification was conducted through sequencing of the small subunit ribosomal DNA (SSU rDNA).

Results: Infected fish exhibited large, whitish cysts (1.9–2.5 mm in length, 60–1400 µm in width) located on the gill filaments. The mature myxospores were ovoid (8.7 ± 0.1 µm in length, 7.0 ± 0.08 µm in width), with two equal, apically oriented polar capsules measuring 4.4 (3.5-5.0) µm in length and 2.4 (1.8-3.1) in width. Molecular identification, based on SSU rDNA sequencing, confirmed *Myxobolus moliwensis* sp. nov. as novel species. Phylogenetic analysis positioned *M. moliwensis* sp. nov. within a sub-clade of gill-infecting *Myxobolus* species, showing a close phylogenetic relationship to *M. anatolicus* and *M. tambroides*.

Conclusion: The identification of *Myxobolus moliwensis* sp. nov. significantly enhances our understanding of myxozoan diversity in African freshwater ecosystems. This study highlights the value of integrating both morphological and molecular approaches for accurate species identification and offers important contributions to the taxonomy of fish parasites, particularly within freshwater environments.**Keywords :** *Labeobarbus habereri* ; Myxosporea ; Fish parasites ; Morphological characterization ; SSU rDNA ; Histopathology ; Phylogenetic analysis

1. Introduction

Fish are crucial source of animal protein and provide essential nutrients that play a vital role in human nutrition (Fariya et al., 2022). The Cyprinidae family, with over 3,000 described species, is the most diverse group of freshwater fish (Imoto et al., 2013; Eschmeyer and Fong, 2015; Froese and Pauly, 2017; Tao et al., 2019). Certain species within this family, such as *Labeobarbus habereri* (Steindachner, 1912), are of significant economic importance in aquaculture, recreational fishing, and fisheries, serving as a critical protein source for humans (Skelton, 2001; Thai et al., 2007; Collins et al., 2012). The livelihoods of many people in Africa, particularly those in Moliwè and surrounding areas in Cameroon, a developing country, rely heavily on *L. habereri*. Disease outbreaks, however, represent a major threat to the economic viability of aquaculture (Bondad-Reantaso et al., 2005). These outbreaks can negatively affect the productivity of *L. habereri* in both natural and artificial aquatic systems, thereby jeopardizing the livelihoods of local communities that depend on this resource in Moliwè and its neighboring areas.

Myxozoa are a significant group of parasitic organisms that have considerable economic and nutritional implications due to their impact on both wild and cultured fish populations (Kent et al., 2001). The subphylum Myxozoa is a morphologically and genetically diverse group of microscopic parasites, comprising approximately 20% of all Cnidarian species (Atkinson et al., 2018). Their complex life cycle involves interactions with both vertebrates, primarily fish, and invertebrates such as annelid worms, in both marine and freshwater environments (Eszterbauer et al*.,* 2015; Holzer et al., 2018). Myxozoans have been reported to infect almost every tissue and organ of fish, often showing some degree of tissue specificity (Mitchell, 1977; Lom and Dyková, 1992; Molnár, 1994).

Myxosporeans are traditionally identified based on the final developmental stage found in the vertebrate host: the myxospore (Lom and Dyková, 2006). To date, spore morphology has been the primary criterion for classifying the subphylum Myxozoa into its various classes, orders, families, and genera (Grasse, 1970; Lom and Arthur, 1989; Lom and Dyková, 1992). However, using morphological characteristics alone for species identification presents a challenge at the species level, as some myxospores are small and lack consistent, measurable traits. This limitation makes it difficult to make accurate taxonomic decisions based on morphology alone (Lom and Dyková, 2006). The advent of DNA sequencing has provided an additional, powerful tool for species identification (Fiala et al., 2015). Combining morphological and molecular characterization of myxosporeans aids in the development of a

comprehensive taxonomic database, which can be valuable for future studies on the ecology of these parasites. This approach is crucial for evaluating their impact on host populations and formulating appropriate management strategies.

In this study, we describe a new species of *Myxobolus* from Cameroon, based on an analysis of tissue tropism, myxospore morphology and dimensions, and SSU rDNA sequence data. This represents the first documentation of a Myxozoan species parasitizing *Labeobarbus habereri* and serves as an initial step towards a more comprehensive faunistic and taxonomic investigation of Myxozoa in the Southwest Region of Cameroon.

2. Materials and methods

2.1. Fish collection

Specimens of *Labeobarbus habereri* (Steindachner, 1914), were collected from the Moliwè River (4°03'669" N latitude; 9°14'736" E longitude), near Limbé, the capital of the Fako Division in the Southwest Region of Cameroon. Fish were captured using nets with a mesh size of 1 cm × 1 cm. After capture, the specimens were stored in coolers to maintain their integrity before being transported to the Parasitology and Ecology Laboratory at the University of Yaoundé I for further analysis. All fish collection protocols were approved by the ethical committee of the University of Yaoundé I.

2.2. Acquisition of morphological and histological data

Upon arrival at the laboratory, fish were euthanized by immersion in an overdose of clove oil (Sigma-Aldrich, USA). Dissection of the gills revealed cysts on the filaments, indicating infection. Infection intensity was assessed using the gill cyst index as described by Kaur and Attri (2015). The average number of cysts on one side of the gill was determined and categorized as follows: light (1–5 cysts), moderate (6–10 cysts), heavy (11–20 cysts), or severe (21 or more cysts). Some cysts were ruptured between a slide and coverslip, and their contents were examined at 1000× magnification using a optic ivymen system microscope (Comecta-Ivymen, Spain) to identify myxospores. Other cysts were preserved in Eppendorf tubes containing 100% ethanol (Merck, Germany) for later analysis. Fresh spores were photographed and measured using an Amscope microscope (AmScope, USA) connected to a computer. Spore drawings were made using a Wild M-20 microscope (Wild Heerbrugg, Switzerland) equipped with a camera lucida. Myxospore identification was carried out according to the phenotypic characteristics outlined by Lom and Arthur (1989).

For histological analysis, gills with infested filaments were fixed in a 10% formalin solution (Sigma-Aldrich, USA) and embedded in molten paraffin (Sigma-Aldrich, USA). Tissue sections (4–8 µm) were obtained using a Reichert-Jung 2030 microtome (Reichert-Jung, Austria), stained with Hematoxylin and Eosin (Sigma-Aldrich, USA), and examined using the same optic microscope for morphological analysis.

2.3. Acquisition of molecular data

DNA was extracted from myxospores preserved in ethanol using the QIAgen DNeasy Tissue and Blood Extraction Kit (Qiagen, Germany), following the manufacturer's protocol. The 18S ribosomal DNA (rDNA) gene was amplified with the following primers: MC5F (5'-CCTGAGAAACGGCTACCACATCCA-3') and MC3R (5'-GATTAGCCTGACAGATCACTCCACGA-3').

Polymerase chain reaction (PCR) was performed in a final volume of 20 µL, containing 7 µL of purified water, 10 µL of Master Mix (Thermo Fisher Scientific, USA), 1 µL of each primer, and 1 µL of DNA template. The PCR amplification profile was as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 60 seconds, annealing at 60°C for 60 seconds, and elongation at 72°C for 120 seconds. A final elongation step was performed at 72°C for 5 minutes. Amplification efficiency was confirmed by electrophoresis of 5 µL of the PCR product on a 1.5% agarose gel stained with thiazole orange (Sigma-Aldrich, USA). The amplicons were purified using a QIAgen PCR Clean-Up Kit (Qiagen, Germany) and sequenced at Inquaba Biotech (South Africa). The obtained sequences were visualized, assembled, and edited using BioEdit software (Hall, 1999), and ambiguous bases were resolved using the corresponding ABI chromatograms.

A total of 29 sequences of the 18S rDNA subunit from myxosporean species of the genus *Myxobolus* were selected for analysis. These sequences were obtained through a standard nucleotide-nucleotide BLAST (blastn) search on the National Center for Biotechnology Information (NCBI) website. Sequences from five recently described myxosporean species from Africa (Lekeufack-Folefack et al., 2019; Lekeufack-Folefack et al., 2021; Feudjio-Dongmo et al., 2022; Lekeufack-Folefack et al., 2022) were included. *Sphaerospora epinepheli* Supamattaya, Fischer-Scherl, Hoffman & Boonyaratpalin, 1991, was chosen as the outgroup. All sequences were aligned using BioEdit software (Hall, 1999).

2.4. Phylogenetic analyses

Bayesian inference and maximum likelihood methods were used to perform phylogenetic analyses. Bayesian inference was conducted using MrBayes software (Ronquist et al., 2012), with 1 million generations and a sampling frequency of 50. The average standard deviation of split frequencies was 0.001882 at the end of the analysis. The optimal evolutionary model was determined using jModelTest [28] based on the Akaike Information Criterion (AIC), with the GTR+I+G model being selected. Maximum likelihood estimates were obtained using IQ-TREE software (Nguyen et al., 2015), with 1000 bootstrap replicates. The phylogenetic trees were visualized using FigTree v1.3.1 (Rambaut, 2009) and edited in Adobe Illustrator (Adobe Systems, San Jose, USA).

3. Results

3.1. Taxonomic summary

Phylum: Cnidaria Hatschek, 1888
Subphylum: Myxozoa Grassé, 1970
Class: Myxosporea Bütschli, 1881
Order: Bivalvulida Shulman, 1959
Suborder: Platysporina Kudo, 1919
Family: Myxobolidae Thélohan, 1892
Genus: *Myxobolus* Bütschli, 1882
Species: *Myxobolus* moliwensis sp. nov.
Type host: *Labeobarbus habereri* (Steindachner, 1914) (Cyprinidae)
Site of infection: gills

Typical location: Moliwè River, Limbe, Fako Division, South-west Region, Cameroon (Latitude: 4°03'669'' N, Longitude: 9°14'736'' E).

Prevalence : 23.3% (117 infected individuals out of 760 examined).

Gill plasmodia index and infection intensity: Mean ± SD of 8.37 ± 1.18, corresponding to a moderate intensity of infection.

Type material : Formalin-fixed gills infected with cysts were preserved in 50 mL Eppendorf tubes and deposited in the parasitological collection of the Laboratory of Parasitology and Ecology, Faculty of Sciences, University of Yaoundé I, Cameroon (Catalogue Number: Myxo/2025/LPE-001). Additionally, a partial sequence of the small subunit ribosomal DNA (SSU rDNA, 882 bp) was deposited in GenBank under the accession number PV123175.

Etymology: The specific epithet is derived from the Moliwè River, the location where the host fish was captured.

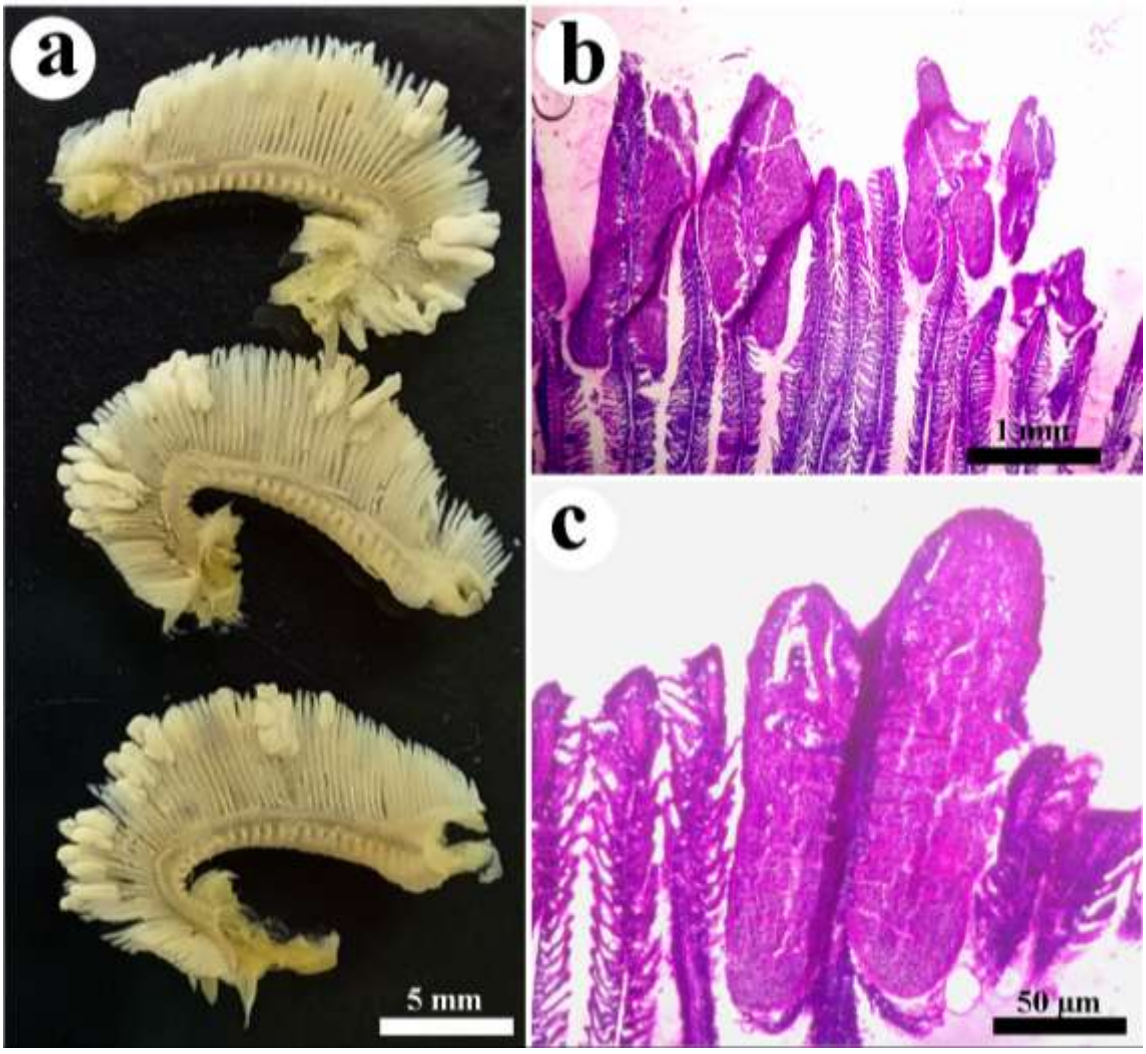

Figure 1. Photomicrographs of the gills of *Labeobarbus habereri* showing cysts of *Myxobolus moliwensis* sp. nov. a: Fresh preparation showing whitish cysts implanted on the apical parts of the gill filaments. **b:** Histological section of gill tissue stained with hematoxylin and eosin (H&E), illustrating cyst development on the gill filaments. **c:** Histological section stained with H&E, revealing cysts encompassing multiple gill lamellae and causing displacement of adjacent structures.

3.2. Vegetative stages

Large, whitish cysts of varying shapes and sizes were observed on the gills of *Labeobarbus habereri* (Figure 1a). These cysts primarily developed apically on the gill filaments but extended proximally to the base of the filaments in some cases (Figure 1a). The cysts measured between 1.9 and 2.5 mm in length and ranged from 60 to 1400 µm in width. A maximum of 27 cysts were identified per parasitized fish, with both sides of the gills being equally affected. The development of these cysts was asynchronous (Figure 1a).

3.3. Histological examination

Histological analysis revealed that the large cysts associated with the parasite infecting *L. habereri* were polysporous and these cysts encompassed several gill lamellae, causing structural displacement and possibly impairing gill function (Figures 1b, c).

3.4. Morphology of myxospores

Mature myxospores were ovoid in shape. In the frontal view, the anterior pole appeared slightly narrowed and displayed an amorphous, homogeneous mass at its apex, while the posterior end was broad and rounded (Figures. 2a, b). In the sutural view, the myxospores exhibited a biconvex structure with thick, smooth valves. The suture line, which extended from the anterior to the posterior ends, was more pronounced in the posterior region (Figures. 2c, d). At the posterior pole, each valve possessed a distinct groove parallel and adjacent to the suture line, and the widest part of the spore was located at its mid-length. The myxospores contained two pyriformes polar capsules that were equal in size, apically oriented, and slightly converging anteriorly. These capsules occupied the anterior half of the spore cavity and each contained 4–6 coils of the polar tubule arranged obliquely to the long axis of the capsule (Figure. 2f). No intercapsular appendix was present. The posterior half of the spore cavity was filled with a finely granular, homogeneous mass of sporoplasm. This sporoplasm extended slightly into the intercapsular space, forming a seamless transition between the anterior and posterior regions of the spore cavity.

Remarks:

The parasite species collected from the gills of *Labeobarbus habereri* closely resembles several myxobolid species reported globally, particularly in terms of size and morphology. These include *Myxobolus branchilateralis* Molnár, Eszterbauer, Marton, Székely & Eiras, 2012, found at the base of gill filaments of *Barbus barbus* (Linnaeus, 1758)

in Hungary; *M. opsaridiumi* Lekeufack-Folefack, Tchoutezo-Tiwa, Al-Tamimi, Fomena, Al-Omar & Mansour, 2021, parasitizing the skin, muscles, and spleen of *Opsaridium ubangiense* Pellegrin, 1901 in Cameroon; *M. tambroides* Székely, Shaharom, Cech, Mohamed, Zin, Borkhanuddin, Ostoros & Molnár, 2012, identified on the gill lamellae of *Tor tambroides* (Bleeker, 1854) in Malaysia; *M. lalbaghensis* Banerjee Bandyopadhyay, Göçmen & Mitra, 2011, isolated from the gills of *Labeo bata* (Hamilton, 1822) in India; *M. calcariferum* Basu & Halder, 2003, found within the gill lamellae of *Lates calcarifer* (Bloch, 1790) in India; *M. anatolicus* Pekmezci, 2014, from the gills of *Capoeta tinca* (Heckel, 1843) in Turkey; *M. eirasianus* Cech, Molnár & Székely, 2012, located on the gills of *Blicca bjoerkna* (Linnaeus, 1758) in Hungary; and *M. goreensis* Fall, Kpatcha, Diebakate, Faye & Toguebaye, 1997, found in the gill filaments of *Mugil cephalus* (Linnaeus, 1758) in Senegal (Table 1). Notably, *M. branchilateralis*, collected from young *Barbus barbus* in the Danube River, forms spores averaging 9.7 µm × 8.2 µm, with larger polar capsules (5.6 µm × 3 µm) and a thin mucous envelope, features that differentiate it from the current species, whose spores measure 8.7 µm × 7.0 µm on average. Similarly, *M. opsaridiumi* exhibits more developed ovoid spores (10.7 µm × 9 µm) and polar tubules with 5–7 coils perpendicular to the polar capsule axis. *Myxobolus tambroides* produces longer spores (9.9 µm vs. 8.7 µm on average) and larger pear-shaped polar capsules (5.7 µm vs. 4.4 µm on average), with knob-like structures between capsule bases. *Myxobolus lalbaghensis* forms blunt-ended spores and pear-shaped polar capsules with an anterior tip. While *M. calcariferum* spores are almost spherical (6.6 µm × 6.2 µm on average), and *M. anatolicus* spores are significantly elongated (10.1 µm on average), both differ from the species under study. The resemblance to *M. eirasianus* is weak due to the pyriform shape and larger dimensions of the latter's spores (12.8 µm × 9.6 µm) and polar capsules (5.9 µm × 3.5 µm). Lastly, *M. goreensis* spores (10 µm on average) are larger and have pyriform polar capsules with perfectly round anterior openings, contrasting the bluntly pointed anterior ends of the presently described species.

3.5. Molecular characterization

A partial sequence of the 18S ribosomal RNA gene (rDNA), comprising 882 base pairs, was successfully amplified and sequenced. The sequence exhibited a GC content of 45.9%. Comparative analysis using the BLASTn tool demonstrated that this sequence is novel and distinct from all known myxosporean sequences available in public genomic databases.

The highest sequence similarities were observed with *Myxobolus tambroides* (95.59%) and *Myxobolus anatolicus* (94.79%).

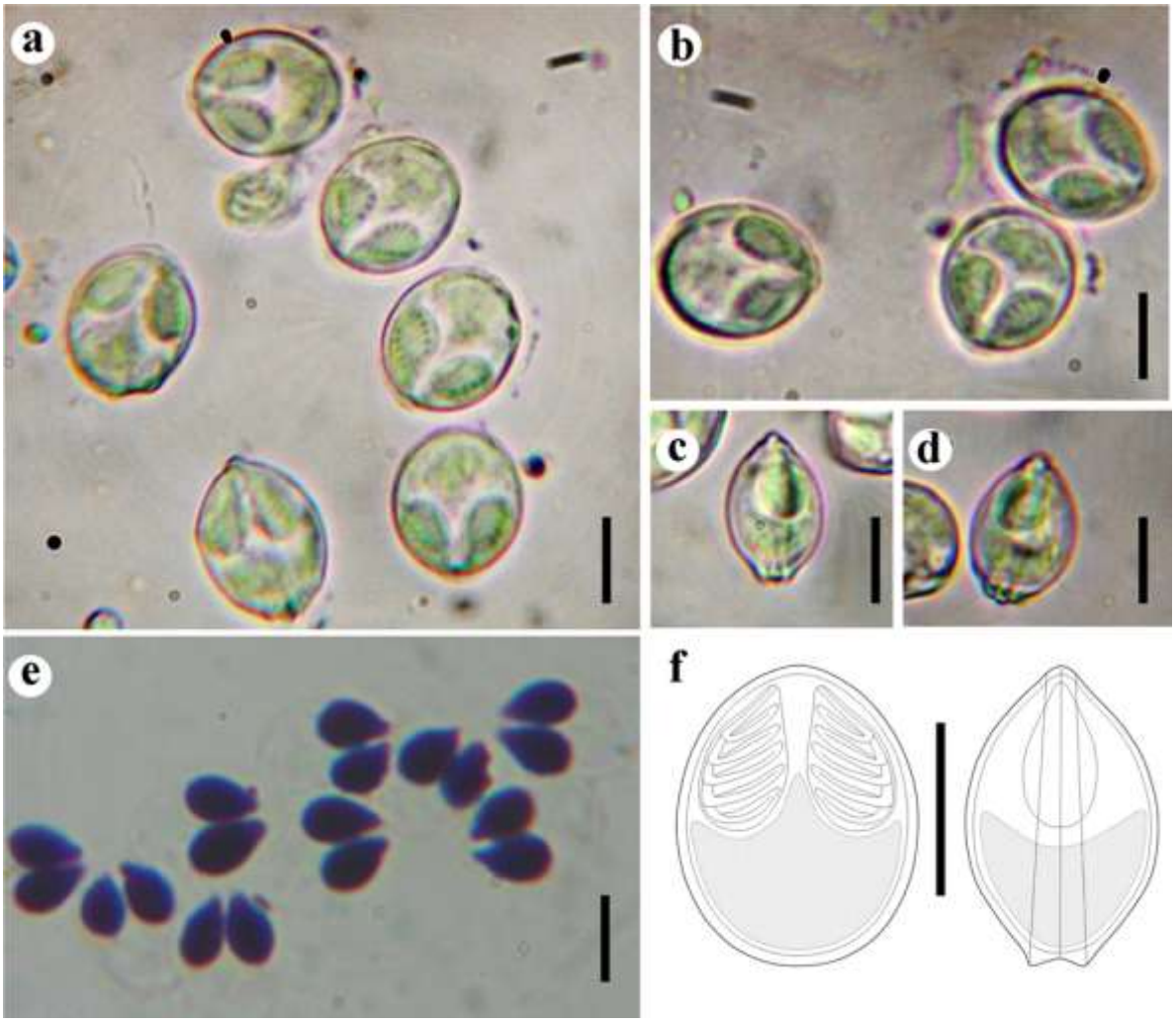

Figure 2. Photomicrographs and illustrations of mature myxospores of *Myxobolus moliwensis* sp. nov. a – b: Myxospores in frontal view. **c – d:** Myxospores in lateral view. **e:** Myxospores stained with May-Grünwald-Giemsa highlighting structural details. **f:** Diagrammatic representation of the myxospore of *Myxobolus moliwensis* sp. nov. in frontal and lateral views. **Scale bars**: 5 µm.

3.6. Phylogenetic analyses

Phylogenetic analyses based on SSU rDNA sequences, including the newly obtained in this study, consistently yielded robust topologies using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods (Fig. 3). The resulting phylogenetic tree classified the parasite species into two primary clades (Fig. 3). Clade A contains species that parasitize the fins, skin, spleen, and scales of African Cypriniformes within the family Cyprinidae, while Clade B encompasses species that infect the gills, oral cavity, intestines, and muscles of Cypriniformes (Cyprinidae) from Europe, America, and Asia. The parasite species identified

on the gills of *Labeobarbus habereri* is positioned within Clade B, specifically within a subclade comprising gill-parasitizing species of Cyprinidae. This species forms a distinct subclade with *Myxobolus anatolicus* and *Myxobolus tambroides*, which infect *Copoeta tinca* and *Tor tambroides*, respectively. Importantly, the grouping of these species is not influenced by their geographical distribution but is instead driven by shared morphological and genetic characteristics, underscoring the relevance of these factors in their evolutionary relationships.

4. Discussion

Myxosporeans represent a highly diverse group of parasitic organisms, with taxonomy historically reliant on the morphological features of the myxospore (Fiala et al., 2015). However, significant morphological similarities among species have often complicated accurate identification (Liu et al., 2013). To address this challenge, a holistic approach that integrates spore morphology, tissue tropism, host specificity, and molecular data has been recommended (Cech et al., 2012; Atkinson et al., 2015). This integrated methodology was applied in the present study to characterize a histozoic myxosporean species isolated from the gills of *Labeo habereri*. The phenotypic and molecular analyses indicate that this parasite species does not match any previously described species. Consequently, we propose this novel parasite as a new species, designated *Myxobolus moliwensis* sp. nov. The specific epithet, "moliwensis," honors the Moliwe River, where the host fish was collected.

The cysts of *M. moliwensis* sp. nov. were observed at the tips of the gill filaments, where they caused engulfment of the gill lamellae. This process resulted in the destruction of these structures and significant gill deformation, potentially impairing their physiological functions. The gills, as critical respiratory organs, play a central role in oxygen exchange, and their dysfunction can lead to reduced gas exchange efficiency and heightened susceptibility to environmental stressors. Our findings align with Dyková and Lom (1978), who reported that myxosporean infections on gills often induce epithelial hyperplasia, leading to tissue destruction and lamellar deformation. Similarly, Oğuz and Oğuz (2019) noted that vegetative stages of myxosporeans can disrupt gill architecture, causing lamellar fusion as a defense mechanism. While this fusion provides a protective barrier, it reduces the respiratory surface area, exacerbating the physiological impact on the host.

Table 1. Comparative chart between *Myxobolus moliwensis* sp. nov. (in bold) with morphologically similar species (Mean values of spore dimensions are given in µm and range in parentheses)

Myxobolus spp.	Spore mesurement			Polar capsule mesurement		Relative length of the polar capsules	Number of coils of polar filament	Infestation site	Host species	Country	Genbank Accession number	Reference
	Length	Width	Thickness	Length	Width							
***M. moliwensis* sp. nov.**	**8.7±0.1 (8-9.7)**	**7.0±0.08 (6.6-8.1)**	**5.8±0.2 (5.6 – 6.2)**	**4.4±0.06 (3.5-5.0)**	**2.4±0.05 (1.8-3.1)**	**=**	**4-5**	Gills	***Labeobarbus habereri***	**Cameroon**	**PV123175**	**Present study**
M. branchilateralis	9.7 ± 0.4 (9.4–10.4)	8.2 ± 0.6 (7.5–9.1)	6.4 ± 0.7 (5.2–7.2)	5.6± 0.4 (5.3–6.8)	3 ± 0.3 (2.5–3.3)	=	6	Gills	*Barbus barbus*	Hungary	JQ388888	Molnár *et al*., 2012
M. opsaridiumi	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	Skin, spleen muscles,	*Opsaridium ubangiense*	Cameroon	MN497413	Lekeufack-Folefack *et al*., 2021
M. tambroïdes	9.9± 0.43 (8.8–10.6)	7.4±0.24 (6.8–7.9)	7.2±0.38 (7–7.9)	5.7±0.7 (5–7)	2.6±0.1 (2.4–2.9)	=	5-6	Gills	*Tor tambroïdes*	Malaisia	JX028236	Székely *et al*., 2012
M. lalbaghensis	9.2 ± 1.0 (7.6- 11.9)	6.8 ± 1.1 (5.1-8.5)	/	5.1 ± 0.7 (4.2-6.8)	2.3 ± 0.3 (1.7- 2.5)	=	4-5	Gills	*Labeo bata*	India	/	Banerjee *et al*., 2011
M. calcariferum	6.6 ± 0.3 (6.1-7.1)	6.2 ± 0.2 (5.7-6.5)	/	4.2 ± 0.2 (3.8-4.5)	2.3 ± 0.2 (2.0-2.7)	=	4-5	Gills	*Lates calcarifer*	India	/	Basu & Halder, 2003
M. anatolicus	10.1 ± 0.4 (9.4 -10.7)	6.9 ± 0.2 (6.6 - 7.2)	4.5 ±0.36 (4.4 -4.6)	4.6 ± 0.4 (4.4 - 4.8)	2.1 ± 0.1 (2 - 2.3	=	5-6	Gills	*Copoeta tinca*	Turkey	KF537629	Pekmezci 2014
M. eirasiianus	12.8 ± 0.3 (12.6–13.5)	9.6 ± 0.42 (9–10.3)	7.9 ± 0.5 (7.2– 9)	5.9 ± 0.28 (5.4–6.3)	3.5 ± 0.1 (3.2–3.7)	=	6	Gills	*Blicca bjoerkna*	Hungary	JF311900	Cech *et al*., 2012
M. goreensis	/	10.9±0.68 (10-13)	/	4.1±0.4 (4-5)	3.0±0.2 (2-4)	=	6	Gills	*Mugil cephalus*	Senegal	/	Fall *et al*., 1997

The arithmetic means of the measured variables are followed in brackets by the minimum and maximum values

/ : No data available ; = : equal

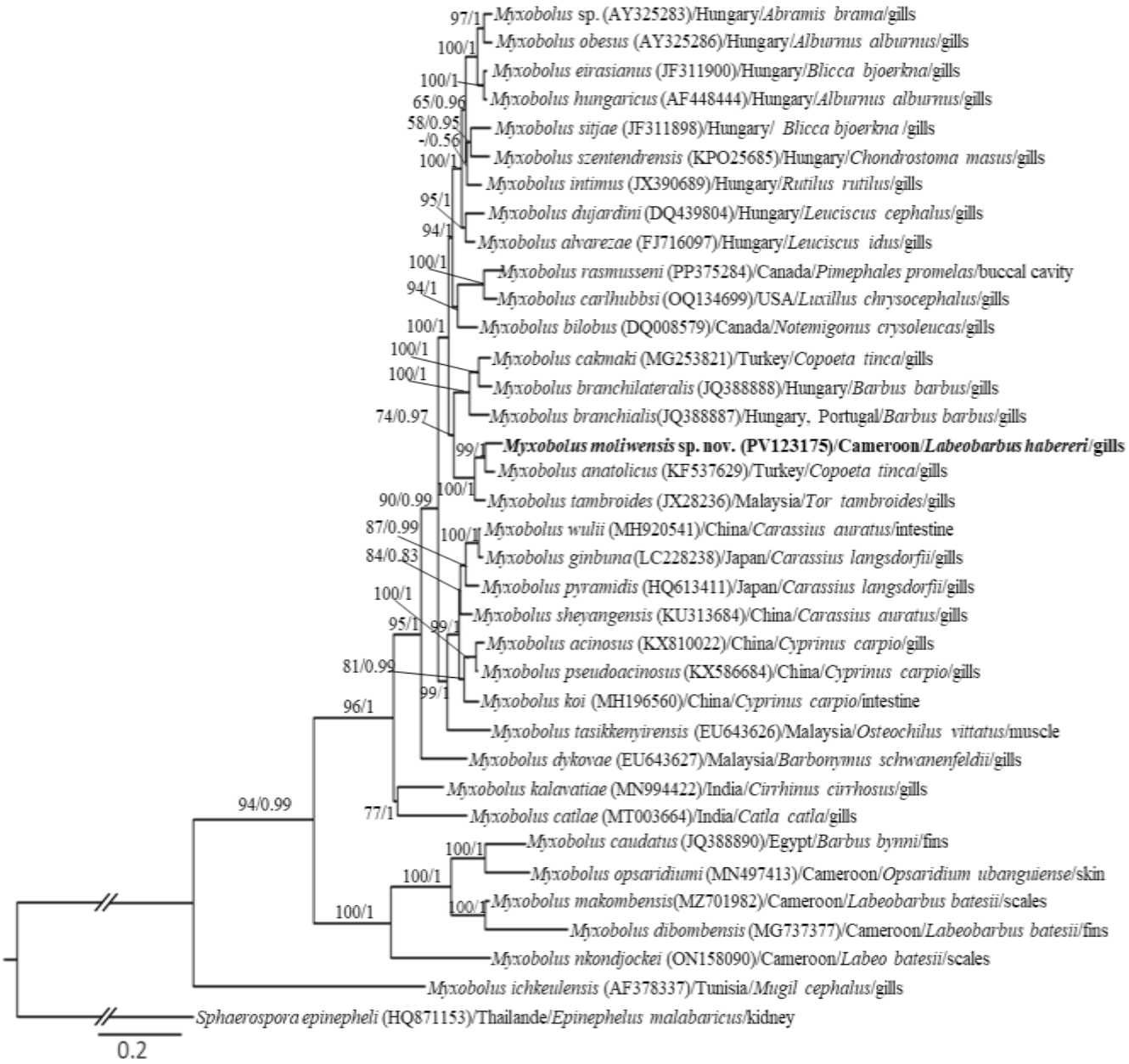

Figure 3. Phylogenetic tree based on Bayesian inference and maximum likelihood methods of the SSU rDNA of *Myxobolus* species related to *Myxobolus moliwensis* sp. nov (indicated in bold). Species names are accompanied by their corresponding GenBank accession numbers in bracket, locality, host species, and parasitized organ. The numbers on the branch nodes represent posterior probability/bootstrap support; dashes indicate bootstrap values below 50. *Sphaerospora epinepheli*was used as outgroup. The double slash (//) symbolizes artificial shortening of the branches for visualisation

The gill cysts index in this study revealed a moderate infection intensity caused by *M. moliwensis* sp. nov., suggesting that while the infection does not reach critical levels, it has notable implications for host health. Moderate infections of similar nature have been reported, by Kaur and Attri (2015) in *Henneguya bicaudi* infections of *Cirrhinus mrigala* in India, highlighting the global prevalence of such parasitic impacts on fish populations.

Phylogenetic analyses positioned *M. moliwensis* sp. nov. within Clade B, forming a subclade with *Myxobolus anatolicus* and *Myxobolus tambroides*, both of which parasitize species within the Cyprinidae family. This subclade is characterized by oval-shaped spores, a feature shared by the three species and reinforcing their phylogenetic affinity. Previous studies (Liu et al., 2019; Abdel-Gaber et al., 2017) have similarly demonstrated a correlation between spore morphology and phylogenetic relationships. Interestingly, the subclade of oval-spored *Myxobolus* species is phylogenetically linked to another clade of gill parasites infecting Cyprinidae in Turkey and Malaysia. This observation supports findings by Shin et al. (2014) and Abdel-Gaber et al. (2017), who proposed that Myxobolidae phylogeny is strongly influenced by tissue tropism, resulting in clusters of species with highly specific host-site affinities. Additionally, the grouping of Cypriniform parasites within Clade B emphasizes the phylogenetic significance of host order and family, corroborating previous research by Rocha et al. (2019) and Vieira et al. (2024).

5. Conclusion

This study highlights the significance of employing integrated, multidimensional approaches to the classification of myxosporeans, encompassing both morphological and genetic analyses. A comprehensive understanding of myxosporean diversity is vital for elucidating the evolutionary dynamics of these parasites and their intricate interactions with aquatic hosts. Such insights into host-parasite relationships are critical for devising effective management and conservation strategies for impacted fish species, ultimately supporting the preservation of aquatic biodiversity.

Conflicts of interest : The authors declare no conflicts of interest regarding the publication of this paper.

Acknowledgements : The authors express their gratitude to the Faculty of Science at the University of Yaoundé I and Mboalab Biotech Yaoundé–Cameroon for providing the necessary facilities to complete this work.

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