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Description and histopathology of *Myxobolus kodjii* sp. nov. and *Myxobolus dzeufieti* sp. nov. (Myxozoa: Myxobolidae) parasites of some Teleost fish from Maga Lake in Cameroon

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Abstract

Examination of some Teleost fish caught in the Maga Lake located in the Far North Region of Cameroon, revealed the presence of two new species of Myxosporidia belonging to the genus *Myxobolus*, of which morphological and histological description is given in the present study. These species are: *Myxobolus kodjii* sp. nov., parasite of the eyes of *Labeo senegalensis* and *Myxobolus dzeufieti* sp. nov., parasite of the skin of *Oreochromis niloticus* and *Tilapia* sp. *M. kodjii* sp. nov. forms ovoid myxospores, with rounded ends, 8.0 (7.0–9.0) µm long, 5.9 (5.5–6.6) µm wide and 3.8 (3.5–4.2) µm thick. Polar capsules are pyriform, equal in size and measure 3.7 (3.2–4.0) µm × 1.6 (1.4–2.0) µm. *M. dzeufieti* sp. nov. forms ovoid myxospores, with the anterior end slightly narrowed, 12.3 (11.4–13.7) µm long, 9.8 (9.2–10.6) µm wide and 5.7 (5.0–6.0) µm thick. Its polar capsules are pyriform and equal in size and measure 4.8 (4.0–5.5) µm × 2.9 (2.5–3.3) µm. These new species of *Myxobolus* are histozoic. The cysts of *M. kodjii* sp. nov. induce a local inflammatory reaction and their implantation in the sclera can affect the sight of the host fish. The presence of the cysts of *M. dzeufieti* sp. nov. on the skin did not cause an inflammatory reaction in host fish.

Keywords: *Myxobolus, Morphology, Histopathology, Fish, Freshwater, Maga*

1. Introduction

Myxosporidia are microscopic Cnidaria, spores forming parasites and wordwide distributed. Predominantly fish parasites (Lom and Dyková, 2006; and Atkinson *et al*., 2018), some species have been found in Trematodes (Freeman and Shinn, 2011), Crustaceans (Korczynski, 1998), Amphibians (Mutschmann, 2004), Reptiles (Johnson, 1969), Birds, (Bartholomew *et al*., 2008), Mammals (Friedrich *et al*., 2000) and immunodeficient humans (Boreham *et al*., 1998; and Moncada *et al*., 2001).

Spores morphology is the major criterion used for the identification and description of new species of Myxosporidia with additional criteria being vegetative stage characteristics, host specificity, organ specificity and geographic location (Lom and Arthur, 1989; Lom and Dyková, 1992; Molnár, 1994; Molnár, 2002 and

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Molnár and Eszterbauer, 2015). Eszterbauer (2004) believes that in case of high similarity in the morphology of spores of various species or morphological diversity of spores within a given species, molecular data can be used for further clarification. To date, more than 2,400 species of Myxosporidia belonging to 64 genera are recognized worldwide (Eiras *et al*., 2011; Zhang *et al*., 2013; Fiala *et al*., 2015; Wagner, 2016; and Eiras *et al*., 2021). The numerically larger genus *Myxobolus* is represented by about 1027 species (Eiras *et al*., 2005, 2014, and 2021).

In freshwater fish, species of the genus *Myxobolus* are generally histozoic. Some are host and / or organ specific, but others are found in several host species and / or organs (Lom and Dyková, 2006). Although it is widely known that many *Myxobolus* species cause asymptomatic infections in fish, there is increasing evidence that some species are highly pathogenic and cause various damages to their hosts (Dyková and Lom, 2007). These damages, able to weaken or kill fish can result in severe epizootics (Okaeme *et al*., 1988; and Arsan and Bartholomew, 2008).

During a general study of the Myxosporidia parasites of some Teleosts of great nutritional and economic importance from the Maga Lake in Cameroon, two new species belonging to the genus *Myxobolus* were identified. Morphological and histological data were used to describe these species, which are parasites of the eyes in *Labeo senegalensis* Boulenger, 1902 and of the skin in *Oreochromis niloticus* Linneaus, 1758 and *Tilapia* sp..

2. Materials and methods

The fish examined were sampled in the Maga Lake between March 2016 and June 2017. This lake is located 77 km from the town of Maroua, in the Department of Mayo-Danay (Far North Region of Cameroon). Fish specimens were caught in an area of the lake corresponding to the geographical coordinates 10°47'58"–10°48'57" North latitude and 14°54'07"–15°00'39" East longitude. Lake Maga has a water capacity of 600 million m^3 at its coastal fill and a total surface of 39,000 ha (Sighomnou *et al*., 2002). The climate in Maga is of sahelo-sudanian type, characterized by a long dry season from October to April and a short rainy season from May to September. The average temperature is 28°C (Leumbe Leumbe *et al*., 2015).

The fish examined were caught with a 1 cm × 1 cm mesh gill net and fixed with a 10% formalin solution. In the laboratory, the identification made following the keys proposed by Lévêque *et al*. (1992) and Stiassny *et al*. (2007) revealed that the species sampled are *Labeo senegalensis* Boulenger, 1902 (Cyprinidae), *Oreochromis niloticus* Linneaus, 1758 (Cichlidae) and *Tilapia* sp. (Cichlidae).

The external organs (scales, skin, fins, opercula, eyes) of each fish specimen were inspected, using an Olympus BO61 binocular lens, for any potential Myxosporidia cysts. Subsequently, the gills, digestive tract, heart, gall bladder, spleen, gonads and kidney were removed and examined individually. The contents of the plasmodia were examined under a 100× microscope objective. Smears from the muscle and all internal organs were examined under the microscope. The contents of the gallbladder, urinary bladder and gas bladder were also examined using a microscope. Spores smears were fixed with pure methanol, stained with May-Grünwald-Giemsa and examined under the light microscope. Drawings of fresh spores were performed using a Wild M-20 microscope equipped with a camera Lucida. Measurements were carried out on 50 unstained spores using an objective micrometer.

For histopathological studies, eyes and skin fragments bearing myxosporidia cysts were dehydrated in a series of alcohol baths of increasing concentrations (70%, 95% and 100%). After clearing in xylene baths, the preparations were included in paraffin and finally sliced into sections of 5 to 8 μm thick with a Reichert-Jung 2030 microtome. The sections were then deparaffinised, stained with haematoxylin and eosin, covered with a cover glass and examined under microscope.

Microphotographs of the parasitized organs, histological sections, fresh and stained spores were taken using an Olympus BH-2 microscope equipped with a microphotograph device.

3. Results

Myxobolus kodjii sp. nov.

Host: *Labeo senegalensis* Valenciennes, 1842 (Cyprinidae).

Organ infected: eyes.

Prevalence: 19.33% (46 fish parasitized out of 238 examined).

Vegetative stages: ovoid cysts, measuring 180–700 μm long and 125–550 μm wide have been observed. They are implanted in the sclera of the eye (Figure 1A). Infections can be unilateral or bilateral. In a parasitized fish, 3 to 93 cysts can be counted per eye and up to 167 per fish specimen.

Histopathology: Frontal section of the fish eyeball revealed implantation of Myxosporidia cysts in the sclera. In contrast to healthy fish (Figure 1B), in parasitized fish, the cyst occupies the entire width of the sclera (Figure 1C), induce hyperplasia and swelling (arrow) of the tissue. High magnification of the section of parasitized tissue shows that in the plasmodia, mature spores are located in the medial area (asterisk) and immature spores are located in the peripheral area (arrows) in a gelatinous substance (Figure D). In addition, monocytes are abundant at the periphery of the cyst (Figure 1E).

Myxospores: Small in size (7.0–9.0 μm × 5.5–6.6 μm), mature myxospores are ovoid with both ends rounded (Figure 2A). In lateral view, they are biconvex (Figure 2B) and 3.8 (3.5–4.2) μm thick. The largest width of the spore is obtained at the base of polar capsules. The valves are thick (Figure 2C). The polar capsules are pyriform and equal in size (Figure 2C); they are 3.7 (3.2–4.0) μm long and 1.6 (1.4–2.0) μm wide, and occupy the front half of the spore cavity. Within each polar capsule, the filament form 5–6 coils arranged perpendicularly to the longitudinal axis (Figure 2D). A sporoplasm fill the rest of the spore cavity.

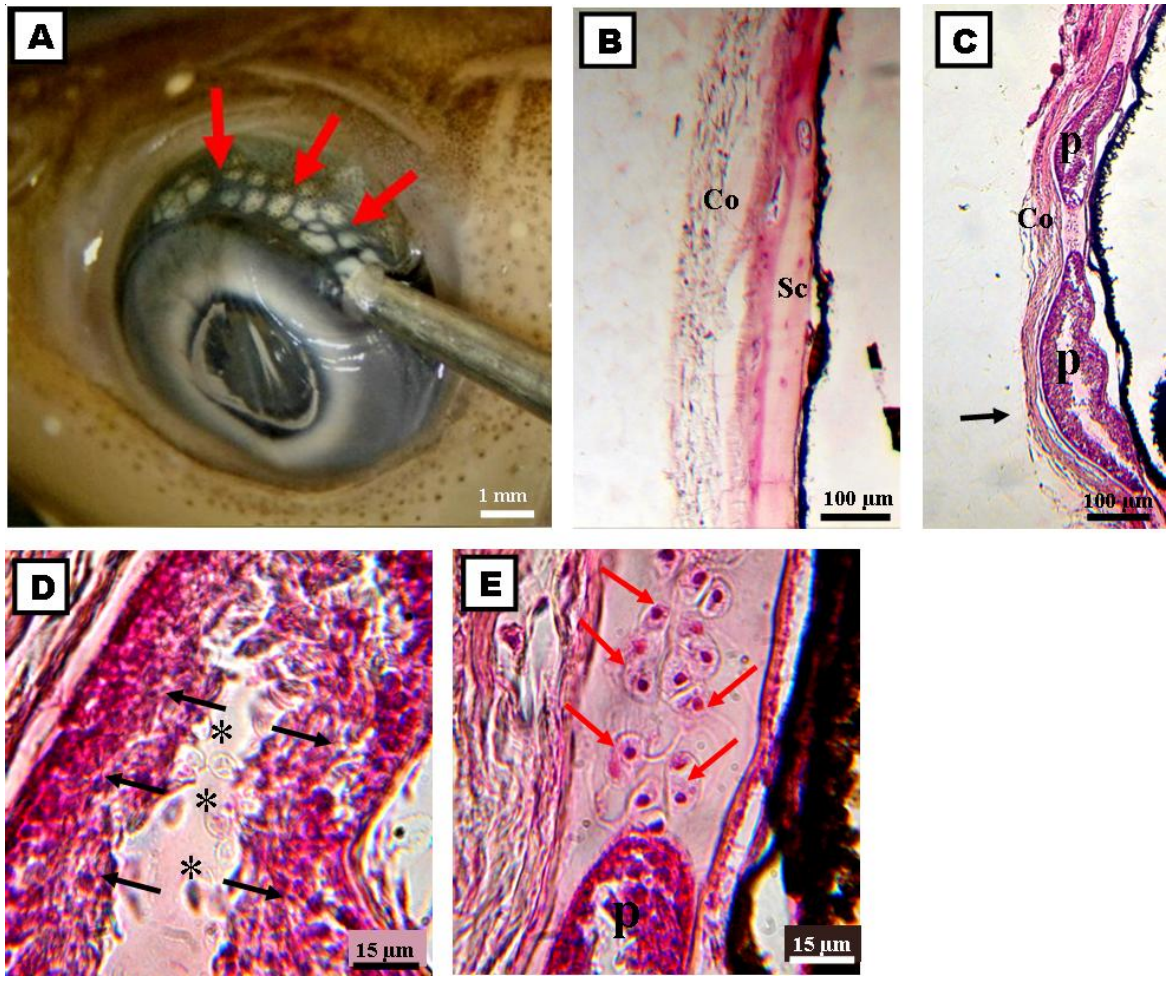

Figure 1: **Microphotographs of plasmodia of *Myxobolus kodjii* sp. nov. in the eye of *Labeo senegalensis*. Clustering of plasmodia within the sclera (red arrows) (A). Histological section of the eye of *L. senegalensis* showing a section of a non-parasitized sclera (B). Histological section of a portion of infected eye showing two plasmodia within the sclera: observe the swelling of the sclera (black arrow) (C). Plasmodium revealing the location of mature spores (asterisks) in the medial part and immature spores (black arrows) at the periphery (D). Observe the influx of monocytes (red arrows) at the periphery of the plasmodium (E). (Sc = sclera, Co = conjunctiva, P = plasmodium).**

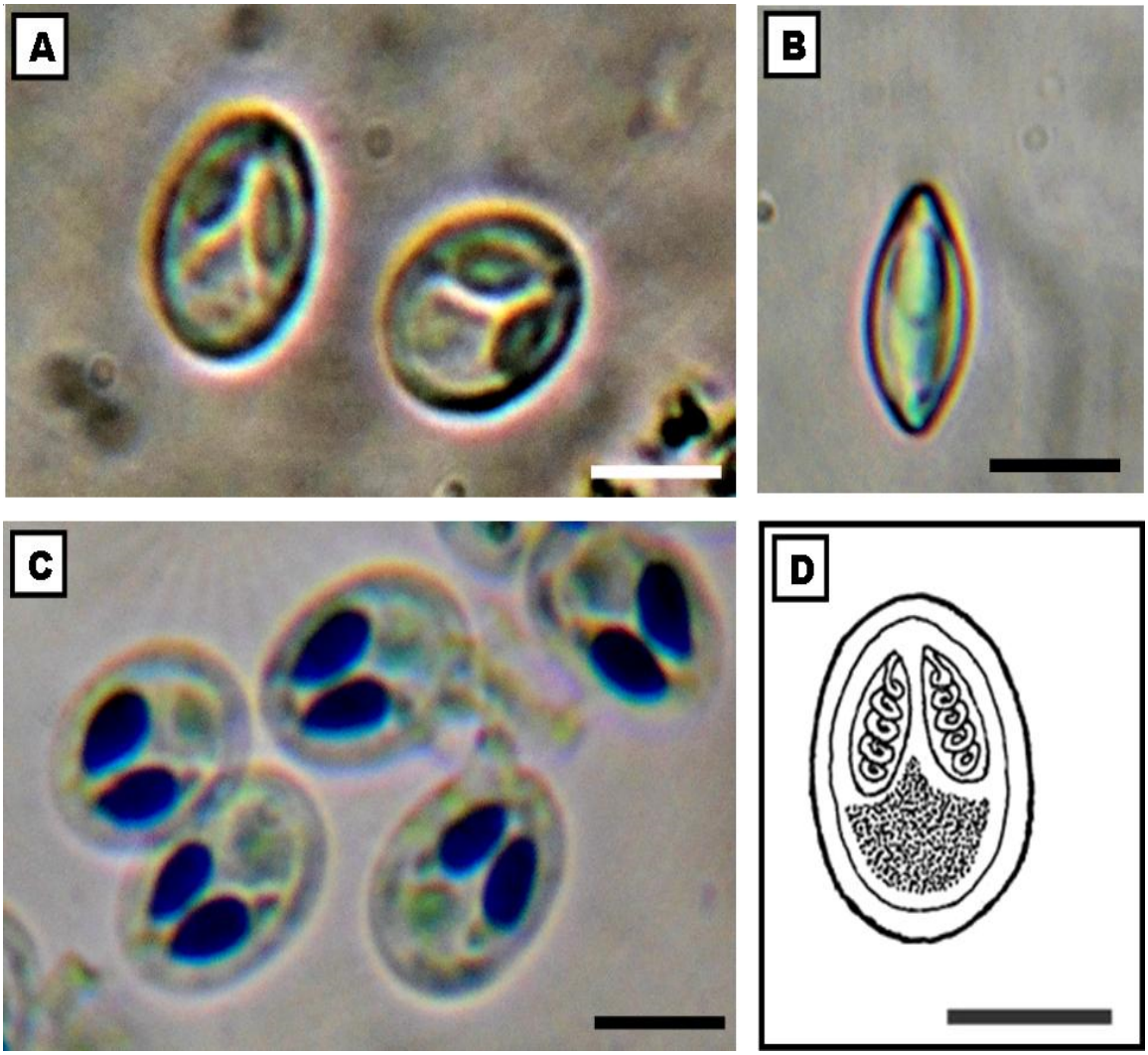

Figure 2: Microphotographs of *Myxobolus kodjii* sp. nov. (A-C): unstained spores in frontal view (A); unstained spore in lateral view (B); stained spores with May-Grünwald-Giemsa (C). Line drawing of the mature spore (D). Scale bar: 5 µm

***Myxobolus dzeufieti* sp. nov.**

Hosts: *Oreochromis niloticus* Linneaus, 1758 and *Tilapia* sp. (Cichlidae).

Organ infected: skin.

Prevalences: 33.3% (08 parasitized fish out of 24 examined) in *O. niloticus* and 8.8% (03 parasitized host individuals out of 34 examined) in *Tilapia* sp.

Vegetative stages: This *Myxobolus* species forms ovoid plasmodia (Figure 3A) observable with naked eyes and arranged anarchically in the fish skin. They measure 1000–1500 µm × 170–250 µm. In a parasitized fish specimen, 5 to 17 cysts can be counted.

Histopathology: The plasmodia are implanted in the connective tissue of the dermis (Figure 3B-C). No sign of an immune response due to the presence of these nodules was observed in the host fish.

Myxospores: Medium in size (11.4–13.7 µm × 9.2–10.6 µm), the myxospore is regularly ovoid in frontal view. The anterior end is slightly narrowed while the posterior end is broad and rounded (Figure 4A). View laterally, the spore is biconvex (Figure 4B) and 5.7 (5.0–6.0) µm thick. The largest width is observed at the base of polar capsules. Valves are smooth. Intercapsular appendix is absent. The polar capsules are ovoid and of equal size (Figure 4C). They measure 4.8 × 2.9 µm on average. In each, the filament forms 5-7 coils (Figure 4D).

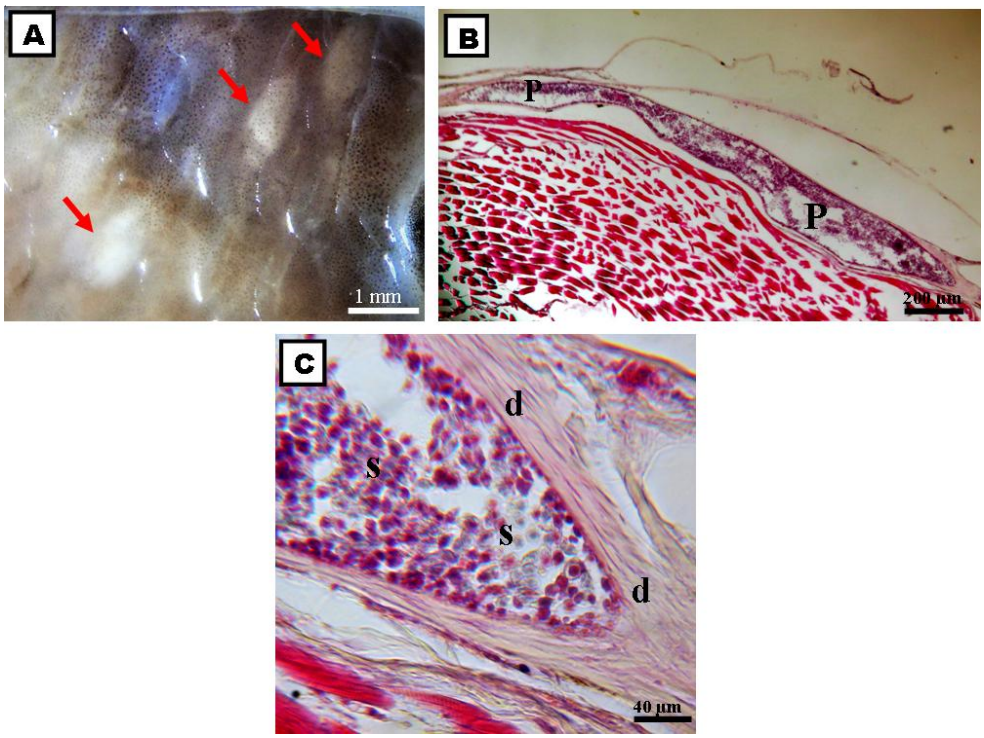

Figure 3: Microphotographs of plasmodia of *Myxobolus dzeufieti* sp. nov. developing in the skin of *Oreochromis niloticus* and *Tilapia* sp. Plasmodia implanted in the skin (red arrows) (A). Histological section of a portion of the skin bearing a plasmodium stained with haematoxylin and eosin (B). High magnifyed section of the skin showing the implantation of a plasmodium within the dermis (C). (d= dermis; p = plasmodium; s = spore).

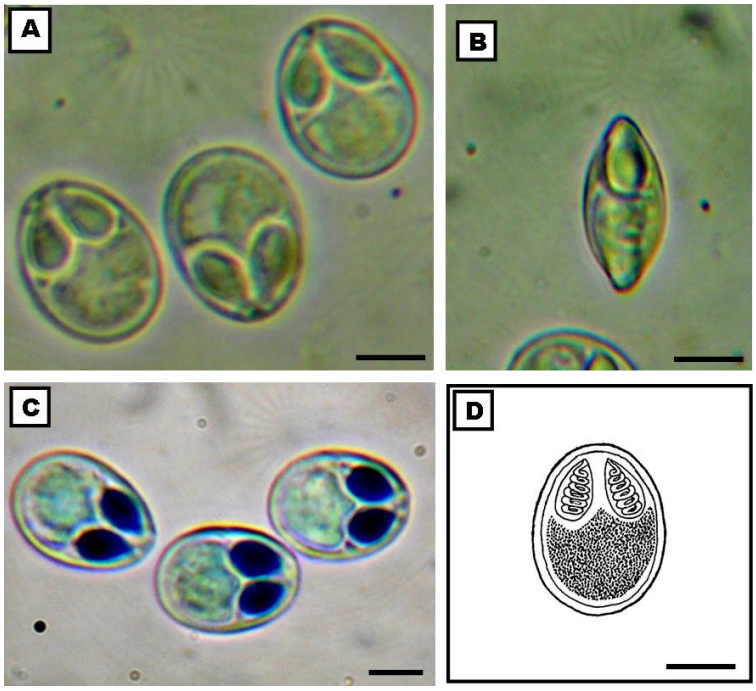

Figure 4: **Microphotographs of *Myxobolus dzeufieti* sp. nov. (A-C): unstained spores in frontal view (A); unstained spore in lateral view (B); stained spores with May-Grünwald-Giemsa (C). Line drawing of the mature spore (D). Scale bar: 5 µm**

4. Discussion

4.1. Myxobolus kodjii sp. nov.

Out of 1027 *Myxobolus* species described worldwide, 22 have been described in the eyes of freshwater fish (Eiras *et al*., 2005; Eiras *et al*., 2014; and Eiras *et al*., 2021). From these species, 04 produce spores with a morphology comparable to that of the parasite being described; these are: *Myxobolus couesii* Fantham *et al*. (1939) (host: *Couesius plumbeus* in Canada); *Myxobolus occularis* Abu-El-Wafa, 1988 (parasite of *Tilapia* sp. in Egypt); *Myxobolus corneus* Cone *et al*. (1990) (host: *Lepomis macrochirus* in USA) and *Myxobolus cordeiroi* Adriano et al. (2009) (host: *Zungaro jahu* in Brazil). However, the spores of the present parasite are less developed (7.0-9.0 × 5.5-6.6 µm) compared to those of *M. couesii* (10.4-13.2 × 7.7-9.4 µm). Our parasite differs from *M. occularis* by having narrower spores (5.9 µm vs. 8.5 µm on average). Compared to *M. corneus*, our spores are less developed (8.0 × 5.9 µm vs. 9.4 × 8.0 µm on average); in addition, the polar capsules of *M. corneus* are wider (2.4 µm vs. 1.6 µm on average). The tapered anterior end of the spore and the presence of valvular folds in *M. cordeiroi* distinguish it from our species.

The size and ovoid shape of the spore of the present parasite are similar to those of some parasites species describes on diverse organs of freshwater fish. These are *Myxobolus episquamalis* Egusa *et al*. (1990), a parasite of the scales of *Mugil cephalus* in Japan; *Myxobolus zillii* Sakiti *et al*. (1991), a gills parasite in *Tilapia zillii* in Benin); *Myxobolus testicularis* Tadjari *et al*. (2005), a testicular parasite in *Hemiodopsis microlepis* in Brazil; *Myxobolus dermiscalis* Kaur *et al.* (2016), a parasite of the scale of *Labeo rohita* in India and *Myxobolus nigerae* Dar *et al*. (2016), a gill parasite in *Schizothorax niger* in India (Table 1). Our spores are less wide than those of *M. zillii* (5.9 µm vs. 7.5 µm on average). Compared to *M. testicularis*, our species forms spores that are significantly narrower (5.9 µm vs. 7.2 µm on average). The presently describe species differs from *M. episquamalis* with the following characters: absence of truncation at the anterior end of spores, absence of valvular folds and less developed polar capsules (3.7 × 1.6 µm vs. 4.4 × 2.2 µm on average). *M. dermiscalis* differs from the present species in having less developed spores (5.8-7.8 µm × 4.0-6.0 µm). The spores of *M. nigerae* are less developed (6.7 × 5.0 µm on average) and the suture line is sinuous.

All these differences lead us to think that we are in the presence of a new species of Myxosporidia. We propose the name *Myxobolus kodjii* referring to Mr. KODJI Etienne whose contribution was remarkable in this work during fish sampling.

The sclera plays a crucial role in the eyesight. The swelling of the sclera induced by the cysts of *M. kodjii* materializes the deformation of the structure of this tissue. The deformation of the sclera in a fish can lead to myopia, hyperopia and other types of eye pathologies (Flitcroft, 2012). The direct consequence of these pathologies is impaired vision. Adriano *et al.* (2009) reported that the extension of the corneal epithelium following the development of plasmodia of *Myxobolus cordeiroi* in *Zungaro jahu* in Brazil affects the refractive power of the cornea. According to Cavin *et al.* (2012), the release of spores following the rupture of plasmodia of *Myxobolus ali* in the sclera of *Cyclopterus lumpus* in the USA, causes local inflammatory reactions. Furthermore, the implantation of cysts of *M. ali* in the sclera of *C. lumpus* in Canada induces a specific immune response (Gendron *et al*., 2020). Thus, the influx of monocytes to the site of implantation of plasmodia of *M. kodjii* in *L. senegalensis* would reflect the phagocytosis reaction triggered by the presence of these parasitic cysts.

4.2. Myxobolus dzeufieti sp. nov.

Based on general spore morphology, the parasite found in the skin of *Oreochromis niloticus* and *Tilapia* sp. is comparable to some species of the genus *Myxobolus* parasites of freshwater fish. These parasites species are : *M. galilaeus* Landsberg (1985); *M. dahomeyensis* Sakiti *et al.* (1991); *M. sarotherodoni* Sakiti *et al*. (1991); *M. camerounensis* Fomena *et al*. (1993); *M. sourouensis* Boungou *et al.* (2006); *M. gariepinus* Reed *et al.* (2003); *M. opsaridiumi* Lekeufack-Folefack *et al.* (2021) (Table 2).

In Israel, Landsberg (1985) described *M. galilaeus* in the melano-macrophage centres of the kidney and spleen of *Sarotherodon galilaeus*. This species differs from ours by the anterior end of it spore which is as wide as the posterior end and the presence of valvular folds.

In Benin, Sakiti *et al*. (1991) described *M. dahomeyensis* (parasite of the ovaries of *S. melanotheron*, *T. zillii* and Tilapia hybrid) and *M. sarotherodoni* (parasite of the cartilaginous tissue and blood vessels of the gill arches in *S. melanotheron*). Compared to the spores of these two *Myxobolus* species, the spores of the parasite under description are significantly larger with more developed polar capsules. In addition, the polar capsules of *M. dahomeyensis* and *M. sarotherodoni* are pyriform.

Table 1: Comparative description of *Myxobolus kodjii* sp. nov.with morphologically similar species (measurements in micrometer)

Species	Hosts	Sites of infection	Country	LS	WS	TS	PC	LPC	WPC	FC	IP	Ref.
M. kodjii sp. nov.	*L. senegalensis*	Eye	Cameroon	8.0(7–9)	5.9(5.5–6.6)	5.9(5.5–6.6)	=	3.7(3.2–4.0)	1.6(1.4–2.0)	5–6	A	Present study
M. cordeiroi	*Zungaro jahu*	Eye	Brazil	10.8 ±0.5	7.2 ±0.2	5.5±0.2	=	5.2 ±0.3	1.5 ±0.2	5–6	A	Adriano *et al.*, 2009
M. corneus	*Lepomis macrochirus*	Eye	USA	9.4(8.0–10.5)	8.0 (6.5–9)	/	=	5.3 (4.0–5.5)	2.4 (2.5 - 3)	7–8	P	Cone *et al.*, 1990
M. couesii	*Couesius plumbeus*	Eye	Canada	10.4–13.2	7.7–9.4	/	=	4.1–5.5	1.4 - 3.2	/ /	A	Fantham *et al.*, 1939
M. dermiscalis	*Labeo rohita*	Scales	India	5.8–7.8	3.9–5.9	/	=	3.9–5.9	1.8 - 3.8	5–6	A	Kaur *et al.*, 2016
M. episquamalis	*Mugil cephalus*	Scales	Japan	8.6(7.5–9.5)	6.8(6.0–7.5)	5.1(4.5–5.5)	=	4.4(3.8–5.0)	2.2(2.0–3.0)	/ /	A	Egusa *et al.*, 1990
M. nigerae	*Schizothorax niger*	Gills lamellae	India	6.6(6.3–6.9)	5 (4.8–5.2)	/	=	3.3(3.1–3.5)	1.6(1.5–1.7)	5	A	Dar *et al.*, 2016
M. occularis	*Tilapia* sp.	Eye	Egypt	9.6	8.5	/	=	5.6	3.4	/ /	A	Negm-Eldim *et al.*, 1988
M. testicularis	*Hemiodopsis microlepis*	Testis	Brazil	8.6(8.2–9.1)	7.2(6.7–7.5)	2.7(2.4–3.0)	=	3.5(3.3–3.8)	1.7(1.3–2.0)	5–6	A	Tadjari *et al.*, 2005
M. zillii	*Tilapia zillii*	Gills	Benin	9.8(8–11)	7.5(6–8)	/	=	5.1(4–6)	2.5(2–3)	/ /	P	Sakiti *et al.*, 1991

Note: LS: length of spore; WS: width of spore; TS: thickness of the spore; PC: relative length of the polar capsules (=: equal; '": unequal); LPC: length of polar capsules; WPC: width of polar capsules; FC: number of polar filament coils; IP: intercapsular process (A: absent; P: present).

Table 2: Comparative description of *Myxobolus dzeufieti* sp. nov.with morphologically similar species (measurements in micrometer)

Species	Hosts	Sites of infection	Country	LS	WS	TS	PC	LPC	WPC	FC	IP	Ref.
M. dzeufieti sp. nov.	*O. niloticus* and *Tilapia* sp.	Skin	Cameroon	12.3(11.4–13.7)	9.8(9.2–10.6)	5.7(5.0–6.0)	=	4.8(4.0–5.5)	2.9(2.5–3.3)	5–7	A	Present study
M. camerounensis	*O. niloticus*	Gills, integument	Cameroon	16.8(14–22)	11.9(10–16)	/	=	6.8 (6–8)	3.9 (2.6–4.5)	6–7	A	Fornena, *et al.*, 1993
M. dahomeyensis	*S. melanotheron*, *T. zillii* and *Tilapia* hybrid	Ovaries	Benin	9.3(6.5–12)	7.1 (6–8)	/	=	3,6(2–4.5)	2,2 (1–3)	4–5	A	Sakiti *et al.*, 1991
M. galilaeus	*Sarotherodon galilaeus*	Kidneys, spleen	Israel	11.9(10.3–13.1)	9.1(7.9–10.0)	6.5(5.8–7.0)	=	3.5(3.1–4.0)	2.8(2.3–3.1)	4–5	A	Landsberg, 1985
M. gariepinus	*Clarias gariepinus*	Ovary	Botswana	13.9(13.7–15.0)	10.8(10–11.2)	/	=	6.2(6.0–6.2)	3.5 (3.0–3.7)	5–6	A	Reed *et al.*, 2003
M. kainjiae	*O. niloticus* and *S. galilaeus*	Ovaries	Nigeria	8.9(8.1–10)	6.6(6.5–6.7)	/	=	2.4(2.2–2.6)	1.4(1.2–1.5)	3–4	A	Obiekezie and Okaeme, 1990
M. opsaridiumi	*Opsaridium ubangiense*	Skin, muscles and spleen	Cameroon	10.7(10–11.5)	9.0(8–10)	6.2(5.6–7.2)	=	5.0(4.3–6)	2.7(2.2–3)	5–7	A	Lekeufack-Folefack *et al.*, 2020
M. sarotherodoni	*S. melanotheron*	Gills	Benin	11.4(9–13)	8.6(7.5–10)	/	=	3.1(2–4)	2.4(2–3)	/ /	A	Sakiti *et al.*, 1991
M. sourouensis	*Heterotis niloticus*	Gills	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

Note: LS: length of spore; WS: width of spore; TS: thickness of the spore; PC: relative length of the polar capsules (=: equal; '": unequal); LPC: length of polar capsules; WPC: width of polar capsules; FC: number of polar filament coils; IP: intercapsular process (A: absent; P: present).

Although *M. camerounensis* develops plasmodia in the skin of *Oreochromis niloticus* in Cameroon, this parasite differs from our species by the more developed size of its spores (16.8 × 11.9 μm vs. 12.3 × 9.8 μm on average) and polar capsules (6.8 × 3.9 μm vs. 4.8 × 2.9 μm on average).

Myxobolus sourouensis develops large plasmodia in the gills of *Heterotis nilotius* (Arapaimidae) in Burkina Faso. In addition to the host species, this Myxosporidia differs from the species being described by the affected organ and it less developed spores(11.3 × 8.8 μm vs. 12.3 × 9.8 μm on average) containing longer polar capsules (5.7 μm vs. 4.8 μm on average).

In Botswana, *M. gariepinus* develops plasmodia in the ovaries of *Clarias gariepinus* (Clariidae). This Myxosporidia differs from the parasite of *O. niloticus* and *Tilapia* sp. captured in the Maga reservoir in that its spores (13.9 × 10.8 μm in average) and polar capsules (6.2 × 3.3 μm in average) are much more developed.

Lekeufack-Folefack *et al*. (2021) described *M. opsaridiumi* in the skin, muscle and spleen of *Opsaridium ubangiense* (Cyprinidae) in Cameroon. The spore of the present parasite described from Cichlidae is significantly larger (12.3 × 9.8 μm vs. 10.7 × 9.0 μm on average) compared to that of *M. opsaridiumi*.

Considering the above differences, we believe that we are in the presence of a new species and propose to name it *Myxobolus dzeufieti* sp. nov. as a sign of sympathy to Professor DZEUFIET DJOMENI Paul Désiré whose contribution was remarkable in the histopathology part of this work.

The skin of the fish produces mucus that helps it to glide in the water, plays an important role in the water regulation by the organism and protects it from infection. Skin infections caused by Myxosporidia in fish are easy to detect (Lekeufack-Folefack *et al*., 2021). Heavy infestation with Myxosporidia plasmodia can lead to the rejection of parasitized fish by potential consumers. Zhang *et al*. (2010) revealed that sub-epidermal development of plasmodia of *Myxobolus turpisrotundus* is responsible for the unsightly appearance of *Carassius auratus gibelio* in China. The lack of inflammatory response in fish affected by *M. dzeufieti* corroborates the observations of Zhang *et al.* (2010) and Lekeufack-Folefack *et al.* (2021).Furthermore, these authors believe that the absence of inflammatory response would have as direct consequence, the proliferation of the parasite stages on the fish host.

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