
**Abstract**

Examination of some Teleost fishes captured in the Maga detention lake located in the Far North Region of Cameroon, revealed the presence of three new species of Myxosporidia of the genera *Myxidium* Bütschli, 1882 and *Myxobolus* Bütschli, 1882 of which complete description is given in the present study. These species are: *Myxidium tetraodoni* sp. nov., parasite of the urinary bladder of *Tetraodon lineatus* Linnaeus, 1758 (Tetraodontidae) that form ellipsoidal spores with a turgid middle part and rounded ends. They measured 11.6 (10.5-12.5) μm long × 8.2 (7.2-9.6) μm broad; the spherical polar capsules are of equal size and measure 3.7 (3.0-4.3) μm. *Myxidium anisocapsularis* sp. nov., a parasite of the gall bladder of *Distichodus engycephalus* Günther, 1964 (Distichodontidae) form spindle-shaped and elongated spores, that measure 15.2 (14.0-16.2) μm long × 6.0 (5.0-6.7) μm broad; its polar capsules are quite unequal and respectively measure 6.0 (5.0-6.5) × 3.3 (3.0-3.8) μm for the larger and 4.7 (4.0-5.5) × 3.0 (2.3-3.3) μm for the smaller. *Myxobolus magai* sp. nov., a gill parasite of *Labeo batesii* Boulenger, 1911 (Cyprinidae) form ovoid spores with the anterior end larger with small protuberance, that measure 10.6 (9.0-12.0) × 6.3 (5.5-7.0) μm. Its polar capsules measure 2.8 (2.4-3.4) × 2.3 (2.0-3.0) μm.

**Keywords** Myxooza; *Myxidium tetraodoni* sp. nov.; *Myxidium anisocapsularis* sp. nov.; *Myxobolus magai* sp. nov.; Parasite; Freshwater fish; Cameroon

**Introduction**

Due to the increasing needs for animal protein production worldwide, fish industry is a major activity in human populations today [1]. However, fishes are vulnerable to various parasitic infections, including Myxosporidiosis. Myxosporidia can infect numerous fish organs and cause a wide variety of damages [2]. The extent of damage to host tissues and organs depend on the parasite species, its life cycle, infection intensity, and host response [3]. Many pathogenic species can be responsible of severe epizootics in fish farms and hatcheries. Indeed, in natural and artificial environments, Myxosporidia can cause the loss of production and death of fishes. Some parasitized fishes are unsightly and considered unfit for human consumption. Human health can be affected by Myxosporidia when immunodepressed people are contaminated with infected fish [4].

Knowledge of fish parasites is a prerequisite for a rapid diagnosis of the pathogen responsible for epizootic diseases. Early diagnosis can lead to preventive measures that remain the best way to reduce epidemics [5]. Thus it is necessary to record and report new parasites and pathological conditions when they are discovered because such information may be useful in the future as a baseline data for assessing the ecosystems health in the face of global warming [6]. Based on the morphological and metric characteristics of the spores, the number of Myxosporidia described around the world is estimated at 2200 species belonging to 64 genera and 17 families [7].

Eiras et al. [8,9] provided a synopsis of 905 species of *Myxobolus* Bütschli [10]; this genus has the largest global distribution and the high number of Myxosporidia species. With more than 232 species, the genus *Myxidium* Bütschli [10] is numerically the second genus of Myxosporidia [11]. Species of the genus *Myxidium* are predominantly coelozoic (rarely histozoic) infecting the gall bladder, the urinary bladder and the urinary tubules of the kidneys [2,12,13]. The number of Myxosporidia parasites of African freshwater fish is estimated at about 270 species [14,15]. Those of the freshwater fishes in Cameroon are represented by about 80 species, with forty of them belonging to the genus *Myxobolus* and nine to the genus *Myxidium* [14,16,17]. Taxonomic studies and description of new species of Myxosporidia throughout the world suggested that the diversity of species in this group of parasites is largely underestimated.

During a study of Myxosporidia parasites of teleosts of great importance of food and economic in Cameroon, we found three new species whose complete descriptions are given in the present paper. These species are: *Myxidium tetraodoni* sp. nov. a parasite of *Tetraodon lineatus* Linnaeus, 1758 (Tetraodontidae), *Myxidium anisocapsularis* sp. nov. found in *Distichodus engycephalus* Günther, 1964 (Distichodontidae) and *Myxobolus magai* sp. nov. a gill parasite of *Labeo batesii* Boulenger, 1911 (Cyprinidae).
Figure 1: Microphotograph (optical microscopy) of a Trophozoite of *Myxidium tetraodoni* sp. nov. with a granular endoplasm, the spores are formed in pairs within a pansporoblast. Scale bar: 5 μm.

Figure 2: Microphotograph (optical microscopy) of fresh spore of *Myxidium tetraodoni* sp. nov. polar capsules are spherical. Scale bar: 5 μm.

Figure 3: Microphotograph (optical microscopy) of spores of *Myxidium tetraodoni* sp. nov. stained with May-Grünwald-Giemsa. Scale bar: 5 μm.

Figure 4: Microphotograph (optical microscopy) of fresh spore of *Myxidium anisocapsularis* sp. nov. polar capsules are unequal. Scale bar: 5 μm.
Material and Methods

Fish examined were sampled in the Maga detention lake from March to May 2016. Maga is a small village of the Mayo-Danay Division (situated between latitude 10° and 13° North and longitude 14° and 16° East), in the Far North Region of Cameroon. The Maga reservoir lake is located at 77 km from the city of Maroua and covers an area of 39000 hectares. The climate in this locality is the Sahelo-Sudanian type, which is 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 [18]. Maga is located in a floodplain and its vegetation consists of the shrub savannah (weakly wooded) and the herbaceous steppe with grass. Its soil, predominantly clay-sandy, take a clayey-silty shade around the lake [18].

Fish were captured using gill nets. In the field, once the fish were caught, a buttonhole was made on the ventral surface of each host individual. The latter were immediately immersed in a 10% formalin solution contained in a plastic container, before being brought to the laboratory. Systematic position of sampled fishes was taken from Lévêque et al. [19,20]. The species captured are: Tetraodon lineatus (Tetraodontidae), Distichodus engycephalus (Distichodontidae) and Labeo batesii (Cyprinidae).
In the laboratory, the fish were firstly examined with naked eye (eyes, fins, operculum, scales, skin) and then with the Olympus BO61 binocular stereoscopic microscope for the presence of cysts. After dissection of the fish hosts, internal organs such as gills, heart, liver, kidneys, spleen, gallbladder, gonads, intestine and urethra were taken individually and examined. The contents of the cysts were identified with the objective 100X of an IVYMEN light microscope. The content of the gall bladder, urinary bladder and swim bladder was also examined between glass and glass cover slide under the microscope. Smears of kidneys, spleen, liver, gonads, heart and urethra were carefully examined at the 40X objective of the microscope. Spore smears were fixed with methanol and stained with May-Grünwald-Giemsa. Drawings of fresh spores were performed using a Wild M-20 microscope equipped with a camera Lucida. Measurements were carried out on 50 spores using an objective micrometer. Variables taken into account are those proposed by Lom et al. [21]. Microphotographs of spores were performed using an Olympus BH-2 microscope equipped with a microphotograph device.

Results

*Myxidium tetraodoni* sp. nov.

**Vegetative form:** Circular and polysporous plasmodia are found in the urinary bladder of the host. Ectoplasm is clear and smooth. In the granular endoplasm, spores are formed in pairs within pansporoblasts (Figure 1).

**Spores:** Of small size (11.6 μm length on average), spores are ellipsoidal with a bulging middle part and rounded ends (Figures 2-11). Shell valves striated, one can count 9 to 12 fine striations on each (Figure 11). Polar capsules are spherical and equal (Figures 3 and 11). Within each of them, 4 to 5 coils of polar filament can be found (Figure 10). A granular sporoplasm is located between polar capsules.

**Measurements of the spore:** (Table 1).

**Host:** *Tetraodon lineatus* Linnaeus, 1758 (Tetraodontidae).
**Location:** Maga (in the reservoir lake) in Cameroon (Central Africa).

**Location in the host:** Urinary bladder

**Prevalence:** 76.9% (20 individuals fish parasitized out of 26 examined).

### Table 1: Comparative description of Myxidium tetraodoni sp. nov. with morphologically similar species (measurements in micrometre).

<table>
<thead>
<tr>
<th>Myxidium species</th>
<th>Host</th>
<th>Site of infection</th>
<th>Country</th>
<th>LS</th>
<th>WS</th>
<th>PC</th>
<th>LPC</th>
<th>WPC</th>
<th>FC</th>
<th>StV</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>M. tetraodoni</td>
<td>Tetraodon lineatus</td>
<td>urinary bladder</td>
<td>Cameroon</td>
<td>11.6 (10.5–12.5)</td>
<td>8.2 (7.2–9.6)</td>
<td>= 3.7 (3–4.3)</td>
<td>3.7 (3–4.3)</td>
<td>= 4–5</td>
<td>9–12</td>
<td>Present study</td>
<td></td>
</tr>
<tr>
<td>M. brienomyri</td>
<td>Brienomyrus brachyistus</td>
<td>gall bladder</td>
<td>Cameroon</td>
<td>13.7 (12.2–16.2)</td>
<td>6.5 (5.5–9)</td>
<td>= 4.2 (3.5–5)</td>
<td>4.2 (3.5–5)</td>
<td>= 3–5</td>
<td>6–12</td>
<td>Fomena et al. [22]</td>
<td></td>
</tr>
<tr>
<td>M. latesi</td>
<td>Lates niloticus</td>
<td>gall bladder</td>
<td>Chad</td>
<td>15.4 (15–16)</td>
<td>8.3 (8–9)</td>
<td>= 3.3 (3–3.5)</td>
<td>3.3 (3–3.5)</td>
<td>= ±</td>
<td>Kostoïngué et al. [23]</td>
<td></td>
<td></td>
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<td>M. macrocapsulare</td>
<td>Scandirinius erythrophthalmus Grunnian Aplodinotus</td>
<td>gall bladder</td>
<td>Europe America</td>
<td>10–12</td>
<td>6 = 3–4</td>
<td>3–4 = ±</td>
<td>± 3–4</td>
<td>6–12</td>
<td>Auerbach [24]</td>
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<td></td>
</tr>
<tr>
<td>M. macrocheili</td>
<td>Catostomus macrocheilus</td>
<td>gall bladder</td>
<td>North America</td>
<td>11.7 (10.0–14.4)</td>
<td>6.6 (5.6–8.0)</td>
<td>= 4.0 (3.0–5.5)</td>
<td>3.5 (2–4.5)</td>
<td>= 4–6</td>
<td>9–11</td>
<td>Mitchell [26]</td>
<td></td>
</tr>
<tr>
<td>M. moxostomalis</td>
<td>Myxostoma sp.</td>
<td>gall bladder</td>
<td>USA</td>
<td>8.5–10.5</td>
<td>5–6 = 3</td>
<td>3 = 3</td>
<td>10</td>
<td>Fomena et al. [22]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. nyongensis</td>
<td>Barbus jae, B. aspilus, B. guirali, B. martorelli</td>
<td>gall bladder</td>
<td>Cameroon</td>
<td>12.3 (10.8–14.4)</td>
<td>6.4 (4.7–9.4)</td>
<td>= 3.2 (2.0–4.5)</td>
<td>3.2 (2.0–4.5)</td>
<td>= ±</td>
<td>12</td>
<td>Mitchell [26]</td>
<td></td>
</tr>
</tbody>
</table>

Averages of the parameters measured are followed by minimal and maximal values in brackets.

LS: length of the spore; WS: width of the spore; PC: relative length of the polar capsules (=: equal); LPC: length of the polar capsules; WPC: width of the polar capsules; FC: number of polar filament coils; StV: Number of striations on shell valves; ±: absent or not reported in the species description; ±: shell valves striations are present, but the number was not reported in the species description.

**Myxidium anisocapsularis sp. nov.**

**Vegetative form:** Not observed; spores, sometimes abundant, were found free in the bile (Figure 12).

**Spores:** Of medium size (15.2 μm long on average), they are spindle-shaped, elongated (2.5 times longer than wide), with a turgid medial part and acuminate extremities (Figures 4 and 5). Each valve carries 8 to 11 longitudinal striations (Figures 5 and 13). The suture line is straight. The polar capsules are piriform and quite unequal (Figures 6 and 13). The larger polar capsule contains 5 to 6 loops of the filament and the smaller 4 to 5 (Figure 12). A finely granular sporoplasm is located in the extra-capsular space.

**Measurements of the spore:** (Table 2).

**Host:** Distichodus engycephalus Günther, 1964 (Distichodontidae).

**Location:** Maga (in the reservoir lake) in Cameroon (Central Africa).

**Location in the host:** Gall bladder.

**Prevalence:** 15.38% (04 parasitized fish out of 26 examined).
Located between the secondary gill lamellae.

Spores: of small size (10.6 μm long on average), mature spores are narrow (Figures 7, 8, 14 and 15). They occupy the 1/3 of the wider part of the spore (Figure 14). The iodinophilous vacuole is found in a developed sporoplasm (Figures 7 and 15). Each of them contains 4 to 5 coils of polar filament (Figure 14).

Measurements of the spore: (Table 3).

Host: Labeo batesii Boulenge, 1911 (Cyprinidae).

Location: Maga (in the reservoir) in Cameroon (Central Africa).

Location in the host: gills.

Prevalence: 28.5% (04 parasitized fish out of 14 examined).

<table>
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<th>species</th>
<th>Host</th>
<th>Site of infection</th>
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<th>LS</th>
<th>WS</th>
<th>PC</th>
<th>LPC</th>
<th>WPC</th>
<th>FC</th>
<th>IP</th>
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<tr>
<td>Myxobolus magai sp. nov.</td>
<td>Labeo batesii</td>
<td>Gills</td>
<td>Cameroon</td>
<td>10.6 (9.0–</td>
<td>12.0)</td>
<td>6.3 (5.5–7.0)</td>
<td>2.8 (2.4–3.4)</td>
<td>2.3 (2.0–3.0)</td>
<td>4–5</td>
<td>A</td>
<td>Present study</td>
</tr>
<tr>
<td>Myxobolus cichlidatum</td>
<td>Oreochromis niloticus; Sarotherodon galilaeus</td>
<td>Gills, fins, eye, kidneys, spleen, liver</td>
<td>Chad</td>
<td>15.0 (13.5–16)</td>
<td>9.6 (8.5–10.8)</td>
<td>5.8 (5.2–6.5)</td>
<td>3.2 (2.8–3.5)</td>
<td>4–5</td>
<td>P</td>
<td>Abakarousman [31]</td>
<td></td>
</tr>
<tr>
<td>Myxobolus eirasi</td>
<td>Cirrhina mirgata</td>
<td>Caudal fin</td>
<td>India</td>
<td>8.4–8.8 (8.6)</td>
<td>6.5–6.9 (6.7)</td>
<td>3.1–3.3 (3.2)</td>
<td>1.4–1.7 (1.5)</td>
<td>3–4</td>
<td>A</td>
<td>Kaur et al. [32]</td>
<td></td>
</tr>
<tr>
<td>Myxobolus galilaeus</td>
<td>Sarotherodon galilaeus</td>
<td>Kidneys, spleen</td>
<td>Israel</td>
<td>11.9 (10.3–13.1)</td>
<td>9.1 (7.9–10.0)</td>
<td>3.5 (3.1–4.0)</td>
<td>2.8 (2.3–3.1)</td>
<td>4–5</td>
<td>A</td>
<td>Landsberg [30]</td>
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<tr>
<td>Myxobolus gandolomensis</td>
<td>Tilapia guineensis</td>
<td>Kidneys</td>
<td>Senegal</td>
<td>11.3 (10–12)</td>
<td>10.3 (9–12)</td>
<td>3.8 (9–6)</td>
<td>3.8 (3–5)</td>
<td>-</td>
<td>A</td>
<td>Fall et al. [33]</td>
<td></td>
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<tr>
<td>Myxobolus nounensis</td>
<td>Sarotherodon galilaeus; Tilapia mariae</td>
<td>Spleen, kidneys</td>
<td>Cameroon</td>
<td>14.3 (13–15)</td>
<td>12.8 (11.5–14)</td>
<td>5.8 (5–6)</td>
<td>4.5 (4–5)</td>
<td>4–5</td>
<td>P</td>
<td>Fomena et al. [35]</td>
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</tbody>
</table>
**Discussion**

*Myxidium tetraodoni* sp. nov.

By the spore shape, this myxosporidia come close to some species previously described in Africa and other continents. *Myxidium brienomyri* Fomena et al. [22], develops large trophozoites in the gall bladder of *Brienomyrus brachyistus* (Mormyridae) in Cameroon. Spores of this species are longer (13.7 μm on average) and less wide (6.47 μm on average); although spherical, the polar capsules are more developed (4.2 μm diameter on average).

*Myxidium nyongensis* Fomena et al. [22], develop circular and large trophozoites in the gall bladder of *Barbus jae*, *B. aspilus*, *B. guirali* and *B. martorelli* (Cyprinidae) in Cameroon. Its spores are ellipsoidal with rounded ends but longer (12.38 μm on average) and less wide (6.47 μm on average) compared to those of the species in description.

The spores of *Myxidium latesi* Kostoïngué, Faye et al. [23] (host: *Lates niloticus* in Chad) are oval, with pointed ends. They are considerably more developed (15.44 μm long × 8.33 μm wide) compared to those of the present species.

*Myxidium macrocapsulare* Auerbach [24] (host: *Scardinius erythrophthalmus* in Europe and *Grunnian Aplodinotus* in America) forms free spores in the gall bladder with slightly curved ends pointed in the opposite directions. These spores are narrower (5.3 μm-6.8 μm) with polar capsules rather ovoid (4.6 μm × 3.8 μm on average).

The shape of spores of *Myxidium moxostomatis* Kudo [25], recalls that of the species we are describing; however, this parasite of the gall bladder of *Myxostoma* sp. in America forms much smaller spores (8.5 μm-10.5 μm × 5-6 μm).

The spores of *Myxidium macrocheili* Mitchell [26] (host: *Catostomus macrocheilus* in North America) have comparable length with those of our parasite (11.7 μm long on average); however, they are less wide (6.6 μm on average) with ovoid polar capsules which measure 4 × 3.5 μm on average.

The parasite of *Tetraodon lineatus*, which differ from other species by numerous characteristics, is probably new. We propose the name *Myxidium tetraodoni* sp. nov. referring to the generic name of the fish host. This is the first time a Myxosporidia is described in *Tetraodon lineatus*.
**Myxidium anisocapsularis** sp. nov.

The only *Myxidium* species so far described in African Distichodontidae is *Myxidium distichodi* Kostoïngué, [23], a parasite of the gall bladder of *Distichodus engycephalus* in Chad. The spores dimensions of *M. distichodi* (16.3 (16-17) μm × 6.5 (6-7) μm) are comparable to those of the species being described but their polar capsules are equal (constant character).

The general form of spores of the species in description recalls that of *Myxidium parachannae* Sakiti [27], parasite of the gall bladder of *Parachanna obscura* in Benin. However, the spores of *M. parachannae* are much longer (21 μm-25 μm) and less wider (3 μm-5 μm).

*Myxidium birgii* Fomena et al. [22], found in the gall bladder of *Aphyosemiom bivittatum* (Cyprinodontidae) in Cameroon form fusiform spores, with striated valves and a bulging medial part, but longer (17.7 μm-22.5 μm long). The spores shape of *Myxidium mendehi* Fomena et al. [28], recalls that of the species we are describing; however, this parasite of the kidneys of *Barbus guirali* and *B. martorelli* (Cyprinidae) form smaller spores (9.9 μm × 4.1 μm in average).

The spores of *Myxidium camerounensis* Fomena et al. [22], although fusiform with a bulging medial part, are longer (22.04 μm on average), with equal polar capsules containing 7 to 9 loops of polar filament.

The spores of *Myxidium petrocephali* Fomena et al. [22] (host: *Petrocephalus simus* in Cameroon) are slightly arched, more developed (24.14 μm × 8.05 μm on average) with symmetrical and larger polar capsules (10.3 μm × 4.3 μm on average).

*Myxidium aydai* Abdel-Baki [29], parasite of *Caesio suevicus* in Egypt produces spores measuring 23 μm long on average with symmetrical polar capsules measuring 7.2 μm on average.

Out of all Myxosporidian species of the genus *Myxidium* described so far in the world, no one form spores with unequal polar capsules. We believe that the parasite of *Distichodus engycephalus* is new and propose the name *Myxidium anisocapsularis* sp. nov., refering to the asymmetry observed on its polar capsules.

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**Myxidium anisocapsularis** sp. nov.

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Sarotherodon galilaeus in Chad. Although ovoid with a wider anterior end, the spores of this species, differ from those of our species by the following characteristics: larger size (15 μm × 9.6 μm on average), more developed polar capsules (5.8 μm × 3.2 μm on average), presence of an intercapsular appendix.

The spores of Myxobolus eirasii Kaur et al. [32] (parasite of Cirrhina mirgala in India), are morphologically comparable to those of our parasite, but shorter (8.4 μm-8.8 μm) with smaller polar capsules (1.4 μm-1.75 μm).

Fall et al. [33] described Myxobolus gandiolensis in the kidneys of Tilapia guineensis in Senegal. This Myxosporidia differs from the species in description by its wider spores (10.3 (9-12) μm) and its spherical polar capsules.

In India, Myxobolus yogendrai (Tripathi, 1952) Landsberg et al. [34], parasitize Cirrhina mirgala; its, although ovoid with a wider anterior end, possess an intercapsular triangle and four valvular folds.

Myxobolus nounenensis Fomena et al. [35], is a parasite of the spleen and kidneys of Sarotherodon galilaeus and Tilapia mariae (Cichlidae) in the Noun River in Cameroon. Spores of this species are more developed (14.3 μm × 12.8 μm on average), same as its polar capsules (5.8 μm × 4.5 μm on average) and a well-developed intercapsular appendix is present.

These differences lead to think that the parasite of Labeo batesii is a new species. We propose the name Myxobolus magari sp. nov., referring to the locality of Maga where the fish host were captured.

Conclusion

Although Africa has one of the most diverse ichthyological fauna, and the genus Myxidium is the second major group of Myxosporidia, the description of Myxidium tetraodoni sp. nov. and M. anisocapsularis sp. nov. Only brings up to 18 the number of species of the genus Myxidium hitherto found with unequal polar capsules. Only 11 species of the genus Myxidium (Myxosporea: Myxidiidae), from the western chorus of this continent, and to 11 the number of species of the genus described in freshwater fishes of Cameroon. M. tetraodoni is the first Myxosporidia described in fishes of the family Tetraodontidae and M. anisocapsularis is the only species of the genus Myxidium hitherto found with unequal polar capsules. Species of the genus Myxidium are generally coelozoic and rarely histozoic in fish host. The two new species of Myxidium described in the present work are coelozoic. These coelozoic Myxosporidia are generally less pathogenic than histozoic species, their pathogenic action seems to be less evident apart from the occlusion of bile and urinary ducts. In Africa, the species of Myxidium that are identified so far affect hosts belonging to 12 families. In decreasing importance order, these host families can be classified as: Cyprinidae (5 species); Claroteidae (2); Distichodontidae (2); Mochokidae (2); Mormyridae (2); Anabantidae (1); Aplocheilidae (1); Chanidae (1); Cichlidae (1); Claridae (1); Latidae (1); Tetraodontidae (1). Myxosporidia of the genus Myxobolus are generally known as histozoic parasites in freshwater fish. They form cysts of varying size in various organs in their host (gills, fins, stomach wall, intestinal wall, skin, heart, liver, operculum, eyes ...). Eiras et al. [8,9] estimated at about 905 species the number of Myxobolus described in fishes around the world. 33.25% of these species affect the gills in their hosts. The description of M. magari sp. nov. in the gills of Labeo batesii confirms the preference of this organ by the Myxosporidia of the genus Myxobolus.

Compliance with Ethical Standards

Animals used followed a protocol approved and authorized by Institutional Animal Care and Use Committee at Animals Biology and Physiology Department, Faculty of Science, University of Yaoundé I, Cameroon.

Acknowledgement

The authors are thankful to the Faculty of Science, University of Yaoundé I, Cameroon, for providing all the facilities to complete this work.

Conflict of Interest Statement

The authors declared: There is no conflict of interest.

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