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Two new species of *Myxobolus* (Myxozoa: Myxosporea) parasites of *Barbus callipterus* Boulenger, 1907 (Cyprinidae) and *Oreochromis niloticus* Linnaeus, 1758 (Cichlidae) in Cameroon

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ABSTRACT:

In order to assess the Myxosporeans fauna of Cameroon fresh water fishes so as to find the fight strategies, 655 specimens (350 *Oreochromis niloticus* and 305 *Barbus callipterus*) were sampled in Mapé river (Sanaga basin) and examined. Standard methods were used for the sampling of fishes, conservation and microscopy. Morphometric characteristics of the spores were used for species identification. Two new species belonging to the genus *Myxobolus* Büstchli, 1882 were described namely *Myxobolus tchoumbouei* n. sp in *Barbus callipterus* which formed cysts within various organs (fins, skin and operculum); *Myxobolus mapei* n. sp parasite of kidneys and liver in *Oreochromis niloticus* and *Barbus callipterus. Myxobolus tchoumbouei* exhibited very long spores (19.19 x 8.89 µm), pear-shaped with rounded anterior end sometimes flattened. Polar capsules were dissymmetrical. They measured 7.60 x 3.00 µm for the bigger and 7.06 x 2.62 µm for the smaller. *Myxobolus mapei* n. sp had ellipsoidal spores (13.50 x 6.83 µm) with unequal polar capsules. The larger polar capsule (6.44 X 2.88 µm) was about 1.5 times longer than the smaller one (4.13 X 1.61 µm) and filled half of the spiral cavity. The awareness about these parasites is useful to find fighting strategies.

Keywords:

Myxosporeans, *Oreochromis niloticus, Barbus callipterus*, *Myxobolus tchoumbouei* n. sp, *Myxobolus mapei* n. sp, Mapé river, Cameroon.

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INTRODUCTION

Fish is an important source of animal proteins (FAO, 2016). Many factors influence fish productions among which are parasites (Renault and Guichard, 2007). Myxosporeans are primarily fish parasites (Fomena *et al*., 2010; Eiras *et al*., 2010). They affect fish growth (Longshaw *et al*., 2010), their reproduction (Obiekezie and Okaeme, 1990) and are involved in epizooties responsible for massive fish deaths (Gbankoto *et al*., 2001; Feist and Longshaw, 2005). The world of Myxosporean fauna is composed of about 2180 species gathered within 62 genera among which the genus *Myxobolus* Bütschli, 1882 is the most abundant with 792 species (Lom et Diková, 2006). The Myxosporean fauna of Africa fresh water fishes comprises about 240 species (Lekeufack, 2010). Moreover, in Cameroon particularly, 74 species were described. Those species belong to the genera *Myxobolus*, *Henneguya*, *Myxidium*, *Thelohanellus*, *Sphaerospora, Chloromyxum* and *Hoferellus*. The objective of this study is to describe two new species of Myxosporean, parasites of economical and dietary important fishes in Cameroon namely *Myxobolus tchoumbouei* n.sp. and *Myxobolus mapei* n.sp.

MATERIALS AND METHODS

Study site

Fishes were sampled in Mapé river (tributary of Mbam river), Bankim subdivision (6°00'- 6°20' NL and 11°20'-11°40' EL, Adamawa – Cameroon Region, Central Africa). The average altitude is about 724 m. The soil is a mixture of clay and sand. The climate is of tropical Soudano-Guinean type with two seasons: a long rainy season running from March to November and a short dry season from November to March. The annual average temperature is about 23°C and the rainfall varies between 1500 and 2000 mm (Olivry, 1986).

Fish sampling and conservation

A total of 655 specimens of fishes (305 *Barbus callipterus* and 350 *Oreochromis niloticus*) were bought monthly from fishermen during the study period i.e. May 2016 to May 2017. They were captured both at the day and night using fish nets and fishing canes. On the field, specimens were immediately stored at 10% formalin solution and transported to the laboratory for examination.

Identification of myxosporeans

In the laboratory, fishes were identified according to Stiassny *et al*. (2007) and examined according to the method used by Abakar (2006). So, standard and total lengths were measured to the closest millimeter using a slide caliper of stainless brand. Fishes were weighed using Sartorius electronic scale of 0.01g accuracy and were sex determined after dissection.

External organs (fins, skin, scales and eyes) and internal organs (gills, spleen, kidneys, intestines, gall bladder, stomach and gonads) were examined with naked eyes, then with Motic stereoscopic microscope at 10X to look for the macroscopic cysts. Smears of kidneys, spleen and gonads were made and examined at a total magnification of 1000 X with a light microscope in order to look for spores. Cysts were crushed between slide and cover glass in a drop of distilled water and their contents were identified with the light microscope at 1000X. Spores were fixed using methanol, stained with May-Grünwald-Giemsa and snapped with digital camera, Canon Ixus brand. Species were identified according to Lom and Arthur (1989). The measurements are given in the form of mean (minimum - maximum) values.

RESULTS

Myxobolus tchoumbouei n. sp

Vegetative stage

This parasite forms whitish cysts which are ovoid, polysporous and generally macroscopic (1 to 2 mm in diameter). They are embedded in various organs (fins, skin and operculum). A single host can harbor 1 to 7 cysts.

Spores

They are big (19.19 x 8.89 µm), pear-shaped with rounded (Figures 1-6) and sometimes with flattened anterior end. The inter capsular appendix is lacking. Polar capsules are slightly dissymmetrical and of unequal dimensions. The number of polar filament coils ranges from 7 to 10. The sporoplasm is very well developed and occupies two third of spiral cavity.

Measurements: Length (L): 19.19 (17.50 – 25.50) µm; width (l): 8.89 (7.50 – 10) µm; ratio (L/l): 2.15; length of large polar capsule (L'): 7.60 (6 – 8.75) µm; width of large polar capsule (l'): 3 (2.50 – 3.75) µm; ratio (L'/l'): 2.52; length of small polar capsule (L''): 7.06 (5.63 – 8.75) µm; width of small polar capsule (l''): 2.62 (1.88 – 4.50) µm; ratio (L''/l''): 2.71

Host type: *Barbus callipterus* Boulenger, 1907

Location: Fins, operculum, skin, kidneys, liver

Prevalence: 11.15% (34 / 305)

***Myxobolus mapei* n .sp** (Figures 7-10)

Vegetative stage: This myxosporean did not form cysts. Pansporoblasts were found in the kidneys.

Spores: From frontal view**,** spores are ellipsoidal. The polar capsules are dissymmetrical. The larger polar capsule is well developed (6.44 X 2.88 µm) and 1.5 times longer than the smaller one and lies in the half of sporal cavity. The sporoplasm is binucleated and the intercapsular appendix is absent.

Measurements : Length (L): 13.35 (10.50 – 16.50) µm; width (l): 6.83 (6 - 9) µm; ratio (L/l): 1.97 length of large polar capsule (L'): 6.44 (6 – 8.25) µm; width of large polar capsule (l'): 2.85 (2.25 – 4.50) µm; ratio (L'/l'): 2.28; length of small polar capsule (L''): 4.19 (3 – 5.25) µm; width of small polar capsule (l''): 1.61 (1.50 – 2.25) µm; ratio (L''/l''): 2.80.

Hosts: *Oreochromis niloticus* Linnaeus, 1758 and *Barbus callipterus* Boulenger, 1907

Location: liver and kidneys

Prevalence: 3. 43 % for *Oreochromis niloticus* **(**12/350); 9.18 % for *Barbus callipterus* (28 / 305).

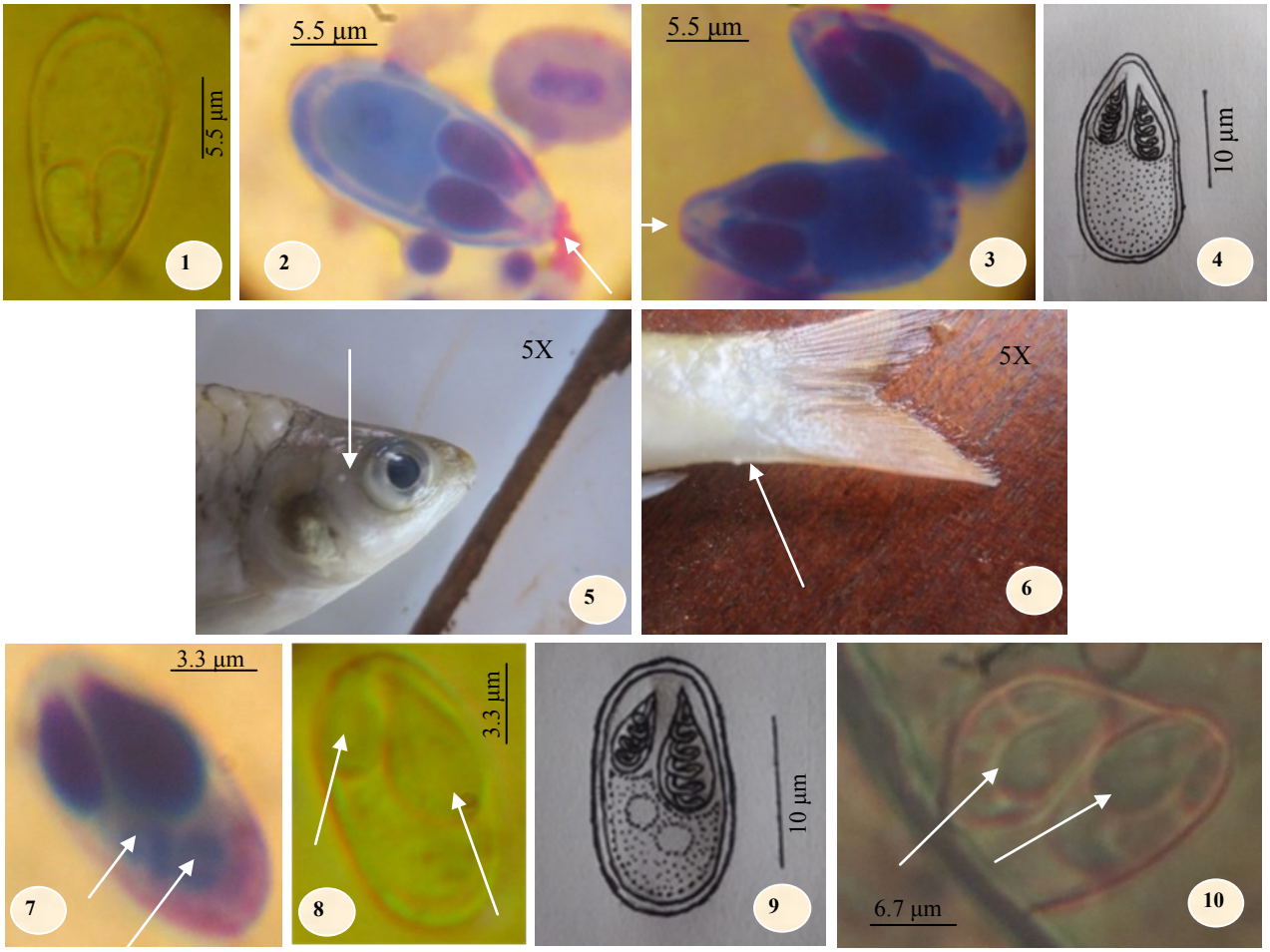
DISCUSSION

Myxobolus tchoumbouei n.sp.

There are relevant differences between *Myxobolus tchoumbouei* n. sp. and four myxosporeans species of the genus *Myxobolus* described throughout the world (Table 1). In fact, Lom *et al*. (1992) described *Myxobolus aureatus* as the fins parasite of *Notropis anogenus, Pimephales notatus* and *P. promelas* in Canada, but that species is shorter (14 -16.6 µm) than our specimen (17.50 – 25.50 µm) and possesses polar

Table 1. Comparison of *Myxobolus tchoumbouei* n.sp. and *Myxobolus mapei* n. sp with five similar species described in Cyprinidae across the world

S. No	Parasite species	Hosts	Infested organs	Countries	References
1	*Myxobolus aureatus* Ward (1919)	*Notropis anogenus* *Pimephales notatus* *P. promelas*	Fins	Canada	Lom *et al.* (1992)
2	*Myxobolus angustus* Kudo (1886)	*Pimephales vigilax*	Gills	Canada	Kudo (1886)
3	*Myxobolus pseudokoi* Li and Desser (1985)	*Luxilus cornutus*	Gills	Canada	Li et Desser (1985)
4	*Myxobolus bilobus* Cone *et al*. (2005)	*Notemigonus crysoleucas*	Gills	Canada	Cone et *al*. (2005)
5	*Myxobolus mbailaoi* Fomena *et al.* (2004)	*Citharinus citharus*	Skin, operculum, intestines	Cameroon	Fomena *et al.* (2004)
6	*Myxobolus tchoumbouei* n. sp	*Barbus callipterus*	Fins, operculum, skin, kidneys, liver	Cameroon	Present study
7	*Myxobolus mapei* n. sp.	*Barbus callipterus* *Oreochromis niloticus*	Kidneys, liver	Cameroon	Present study



Figures 1-10: **Spores and cysts micrographs and lines drawings of different *Myxobolus* species described**
1-6: *Myxobolus tchoumbouei n.sp.*
1: Fresh spore (scale bar: 5.5 µm), dissymmetric polar capsules and well developed sporoplasm
2: Stained spore with May-Grünwald- Giemsa (scale bar: 5.5 µm), rounded anterior end (arrow)
3: Stained spores with May-Grünwald- Giemsa (scale bar: 5.5 µm), flattened anterior end (arrow)
4: Line drawing, scale bar: 10 µm
5: Cyst implanted in the operculum (arrow, white spot) x5
6: Cyst implanted in the skin (arrow, white spot) x5
7-10: *Myxobolus mapei n. sp.*
7: Stained spore with May-Grünwald- Giemsa, two nuclei (arrows), scale bar: 3.3 µm
8: Fresh spore, the bigger size of the large polar capsule compared to the smaller polar capsule (arrows), scale bar: 3.3 µm
9: Line drawing, scale bar: 10 µm
10: Disporal pansporoblast (arrows), scale bar: 6.7µm

capsules which are not only symmetrical but also longer (6.20 -9.4µm). *Myxobolus angustus* described by Kudo (1886) on the gills of *Pimephales vigilax* in Canada is far different from *Myxobolus tchoumbouei* n. sp. by many features: length (14 -15µm), symmetrical polar capsules (8-9.5 X 2.5 -3 µm), less developed polar capsules with iodinophilous vacuole. Moreover, the anterior end of *Myxobolus angustus* is narrow. Li and Desser (1985) described *Myxobolus pseudokoi*, gills parasite of *Luxilus cornutus* in Canada. This species differs from *M. tchoumbouei n.* sp. by its size (11.50 – 14.50 X 6 - 7 µm), symmetrical polar capsules (6 – 7.5 X 2 -3 µm) and the presence of an iodinophilous vacuole. The species resembling remarkably *Myxobolus tchoumbouei n.* sp. is

Myxobolus bilobus, a gill myxosporean of *Notemigonus crysoleucas,* Cyprinidae from Brewer and Opeongo lakes and Algonquin park (Canada). However, there are several diverging characters. *Myxobolus bilobus* cysts are bilobed (where the name originates) in contrast to those of *M. tchoumbouei n.* sp. Moreover, both parasites species infest Cyprinidae fishes belonging to the different genera. *Myxobolus bilobus* seems to be specific to the gills of *Notemigonus crysoleucas* whereas *M. tchoumbouei* n. sp. targets several organs (fins, operculum, skin, kidneys, liver) in *Barbus callipterus. Myxobolus bilobus* spores are shorter *(*20 – 22.10 µm) and less wide (7. 50 – 9.30 µm). Although its polar capsules are dissymmetrical and of unequal sizes like those of *M. tchoumbouei* n. sp, they are however longer, hence, reducing the sporoplasm volume. The lengths of the larger and smaller polar capsule are 9.10 -12 µm and 9.10 -11 µm respectively in *Myxobolus bilobus* against 6 – 8.75 µm and 5.63 – 8.75 µm for *Myxobolus tchoumbouei* n. sp. The rounded and sometimes flattened anterior end of *Myxobolus tchoumbouei* n. sp. makes it very different from *M. bilobus* which rather has a narrower anterior end.

In a nutshell, the parasite of *Barbus callipterus* is probably new and we propose to name it *Myxobolus tchoumbouei* n. sp. in honor to Tchoumboué Joseph, Emeritus Professor in the University of Dschang-Cameroon.

Myxobolus mapei n. sp

In Africa, several species of *Myxobolus* are described. Fomena *et al*. (2004) described *Myxobolus mbailaoi* in Cameroon. This parasite infesting skin, operculum and intestines of *Citharinus citharus* differs from *Myxobolus mapei n. sp.* which does not form cysts and parasitizes two taxonomically distant hosts namely *Oreochromis niloticus* and *Barbus callipterus*. *Myxobolus mbailaoi* spores are less long and wider (11.57 X 7.7 µm) compared to *Myxobolus mapei* n. sp. (13.35 X 6.83 µm). Although having unequal polar capsules, they are less developed than those of *Myxobolus mapei* n. sp. In addition, its sporoplasm has an iodinophilous vacuole lacking in our parasite.

Nchoutpouen and Fomena (2011) described *Myxobolus nchoutnounensis* from *Labeo parvus* in Cameroon. Although this parasite has two dissymmetrical polar capsules, it possesses an intercapsular triangle and iodinophilous vacuole, features missing in *Myxobolus mapei* n. sp. Moreover, the latter does not form cysts. We think that the parasite being described is new and will be called *Myxobolus mapei* n. sp referring to the river where the hosts were captured.

CONCLUSION

The newly described *Myxobolus* species confirm the predominance of the genus *Myxobolus* among Myxosporidia. The available data are useful and will be used to elaborate fighting strategies.

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