ISSN 1996-3351

Asian Journal of **Biological** Sciences



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Asian Journal of Biological Sciences

ISSN 1996-3351 DOI: 10.3923/ajbs.2017.110.120



Research Article Three New Species of *Myxobolus* (Myxosporea: Myxobolidae), Parasites of *Barbus callipterus* Boulenger, 1907 in Cameroon

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Abstract

Background and Objective: Fish are vulnerable to various parasitic infections, out of which Myxozoa is emerging as the major group. *Myxobolus* Bütschli, 1882 is the largest genus within the Myxozoan fish parasites. The aim of the present study was to study Myxosporidia parasites of *Barbus callipterus* Boulenger, 1907. **Materials and Methods:** Cyprinid *Barbus callipterus*, sampled in the Sessaba river at Lebamzip in Cameroon was examined in the present study. Classic fishing methods, fish autopsy and search for myxosporidia parasites were used. Identification of parasite species was based on morphometric characteristics of spores. The arithmetic mean (m) values and interval confidence (2 Sm) of spore parameters was calculated. **Results:** Results were expressed as follow: m±2 Sm (minimal value-maximal value). The study revealed the presence of three new species of the genus *Myxobolus. Myxobolus ngassami* sp. nov. was found in fin, operculum, skin, eye and kidneys of the host. Its spores were ovoid, an intercapsular appendix was present and its polar capsules were unequal. *Myxobolus sanagaensis* sp. nov. was found in the heart with ovoid spore and unequal polar capsules. The spores of *Myxobolus sessabai* sp. nov. were found in skin and kidneys of the host. They were ovoid, with an intercapsular appendix and equal polar capsules. **Conclusion:** The study revealed that Myxosporidia parasite of *Barbus callipterus* was made of three new species: *Myxobolus ngassami* sp. nov., *Myxobolus sanagaensis* sp. nov. and *Myxobolus sessabai* sp. nov. The predominance of the genus *Myxobolus ngassami* sp. nov. Myxobolus ngassami sp. nov. and *Myxobolus sessabai* sp. nov. The predominance of the genus *Myxobolus ngassami* sp. nov., *Myxobolus sanagaensis* sp. nov. and *Myxobolus sessabai* sp. nov. The predominance of the genus *Myxobolus* in the fauna of Myxosporidia parasites of fish was confirmed.

Key words: Myxobolus ngassami sp. nov, Myxobolus sanagaensis sp. nov, Myxobolus sessabai sp. nov, fish parasite

Received: February 26, 2017

Accepted: May 16, 2017

Published: June 15, 2017

Citation: Lekeufack Folefack Guy Benoît, Defoueng Nza Alex Sorel and Fomena Abraham, 2017. Three new species of *Myxobolus* (Myxosporea: Myxobolidae), parasites of *Barbus callipterus* Boulenger, 1907 in Cameroon. Asian J. Biol. Sci., 10: 110-120.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cameroon offers a full spectrum of tropical natural environments. Its dense and diversified hydrographic network covers an area of 294 040 km²¹. Within this large freshwater ecosystem, Vivien¹ listed 603 fish species. In the context of such fish diversity, many species are economically very important in fisheries and some are involved in fish farming projects. However, these fish also constitute a favourable biotope for the development of a large number of parasites, including Myxosporidia²⁻⁴.

Worldwide, the first bibliographical references in the study of Myxosporidia parasite of fish refer to the works of Jurine⁵. Numerous studies have shown that Myxosporidia constitute an important and very diverse fauna among freshwater fish parasites⁶⁻¹³.

Based on the morphological and the metric characteristics of the spores, the worldwide Myxosporidia fauna is now estimated at 2200 species belonging to 64 genera and 17 families¹⁴. The African freshwater fish Myxosporidia fauna is estimated at about 270 species^{3,4}. These Myxosporidia have been described in the following countries: Benin, Botswana, Burkina Faso, Cameroon, Egypt, Ghana, Morocco, Nigeria, Uganda, Senegal, Chad and Tunisia. They belong to the genera *Chloromyxum* Mingazzini, 1890; *Henneguya* Thelohan, 1892; *Hoferellus* Berg, 1898; *Kudoa* Meglisch, 1947; *Myxidium* Bütschli, 1882; *Myxobilatus* Davis, 1944; *Myxobolus* Bütschli, 1882; *Ortholinea* Schulman, 1962; *Sinusolinea* Davis, 1917; *Sphaerospora* Thelohan, 1892; *Thelohanellus* Kudo, 1933; *Triangula* Chen and Hsieh, 1984; *Zchokkella* Auerbach, 1910.

In Cameroon, the available data on Myxosporidia parasites of fish are those of Fomena^{2,15}, Fomena and Bouix¹⁶⁻²², Fomena *et al.*²³⁻²⁹, Folefack³, Nchoutpouen⁴; Nchoutpouen and Fomena³⁰. From the above works, it appears that in this country, the fauna of Myxosporidia parasites of fish is represented by 77 species belonging to the following genera: Myxobolus, Henneguya, Hoferellus, Myxidium, Thelohanellus, Sphaerospora and Chloromyxum. In this central African country, the study of Myxosporidia parasites of the Cyprinid fish has focused on the genus Barbus Cuvier and Cloquet, 1816; Raiamas Jordan, 1919; Labeo Cuvier, 1817 and Labeobarbus Rüppell, 1836^{4,23,30}. It is important to record and report new parasites and new pathological conditions when they are discovered because such information may be useful in the future as baseline data for assessing ecosystem health in the face of threats from global warming.

This study provided the description of *Myxobolus ngassami* sp. nov., *Myxobolus sanagaensis* sp. nov. and *Myxobolus sessabai* sp. nov. parasites of *Barbus callipterus* Boulenger, 1907 (Cyprinidae).

MATERIAL AND METHODS

Data on host fish and study site: Barbus callipterus Boulenger, 1907 (Cyprinidae) is an endemic fish from Central and Western Africa. This gregarious and omnivorous fish has a maximum standard length of 65 mm³¹. Fifty specimens of Barbus callipterus with standard length varying between 42 and 65 mm were harvested from April, 2015 to June, 2016 in the Sessaba river (a sub tributary of the Sanaga river) at Lebamzip, a small village of the district of Sa'a (Department of Lékié, Central Region). This village is elevated at 605 m, between 4°15' 20" and 4°16' North latitude and 11°28' and 11°29' longitude East. The climate is the equatorial transition type in this locality, which is characterized by two rainy seasons (September to mid-November and mid-March to June) and two dry seasons (mid-November to mid-March and July to August) of unequal importance. The mean annual rainfall is 1645 mm³². The primitive vegetation is the rainforest or hemi-ombrophilous forest³³.

Fishing and fish conservation: Fish capture was carried out using the fishing rod. In the field, once the fish were caught, a buttonhole was made on the abdominal region of each host individual. The latter were immediately immersed in a 10% formalin solution that is contained in a plastic container. Systematic position of sampled fishes was taken from Stiassny *et al.*³¹.

Fish dissection and parasites collection: In the laboratory, the fish were first examined with the naked eye (eyes, fins, operculum, scales and skin) and then with the Olympus BO61 stereoscopic microscope, to detect the presence of cysts. After dissection of the hosts, all organs (gills, heart, liver, kidneys, spleen, gallbladder, gonads, intestine and urethra) were also taken individually and examined. The content of these cysts were identified with the objective 100X of an YVYMEN optical microscope. The content of the gallbladder was examined between slide and cover glass under the microscope. The smears of the kidney, spleen, liver, gonads, heart and urethra were mounted on slides and examined at the 40X objective of the microscope.

Stainning, drawing, measurement and photography of parasites: Spore smears were fixed with methanol and then stained with May-Grünwald-Giemsa. Drawings of fresh spores were performed using a Wild M-20 microscope equipped with a camera Lucida. The measurements were carried out on at least 40 spores using an objective micrometer. The variables taken into account are those proposed by Lom and Arthur³⁴. Microphotographies of spores were performed using an Olympus BH-2 microscope in the laboratory of parasitology and ecology of the University of Yaounde 1, Cameroon.

Statistical analysis: The arithmetic mean values of the various parameters measured (spore and its components) were calculated. Results were expressed as follow: $m \pm 2$ Sm (X_{min} - X_{max}) with m = Mean values, 2 Sm = Confidence interval, X_{min} and X_{max} = Minimal and maximal values of the parameter considered.

Let a sample of n specimens (n \geq 40), the variable X denotes a series of measurements and m the mean. Following Folefack³, the mean is calculated using the equation:

$$m = \frac{\sum_{i=1}^{n} x_i}{n}$$

The standard deviation (S) is the square root of the variance S^2 (estimate of variance), the later is expressed as:

$$S^2 = \frac{\sum (x-m)^2}{n-1}$$

where, n-1 is the number of degrees of freedom.

The confidence interval of the mean is 2 Sm, Sm been the standard error of the average calculated by the equation:

$$S_m = \frac{S}{\sqrt{n-1}}$$

For statistical analysis, the level of security retained is 95%.

RESULTS

Myxobolus ngassami sp. nov.

Vegetative form: Whitish plasmodia are observed implanted between the bony rays of the fins, on the operculum, on the skin and in the sclera of the eye. Ocular infestations are uni or bilateral. Plasmodia are ovoid or elongated, measured $315 \times 205 \ \mu m$ on average and are polysporous. A host

individual carries up to five cysts. Vegetative stages are not observed in the kidneys but isolated or grouped spores are found:

- **Spore:** Spores are ovoid in front view (Fig. 1a-d). The valves are thick. At the anterior end, there was an intercapsular appendix of 2.3 ± 0.04 (2-2.8) µm long on average (Fig. 1a, c). Five to six valvular folds are visible at the posterior half of the spore (Fig. 1a, c). The polar capsules are ovoid and slightly dissymmetrical (Fig. 1b, c). The largest surpassing the mid-length of the spore and contains a filament with 7-10 coils. In the small polar capsule, there are 5-7 coils of the filament (Fig. 1c). The sporoplasm often contains an iodinophilous vacuole of varying shape and size (Fig. 1a, d)
- Measurements: Measurements are given in Table 1
- Host: Barbus callipterus Boulenger, 1907 (Cyprinidae)
- Location: Lébamzip (Center region)
- Location in the host: Fins, kidneys, eyes, operculum and skin
- Prevalence: 52% (26 parasitized fish out of 50 examined).
- Type material: Glass slides with stained spores (syntype) and cysts preserved in 10% buffered neutral formalin are deposited in the parasitological collection of the Laboratory of Parasitology and Ecology, Faculty of Science, University of Yaounde 1, Cameroon (No. Myxo/2017/LPE-001)
- **Etymology:** The species is dedicated to Professor NGASSAM Pierre of the University of Yaounde 1

Myxobolus sanagaensis sp. nov.

Vegetative form: In the host, the parasite forms whitish, polysporated, ovoid or subspherical cysts in the heart auricles. They vary in size and are 140-270 μ m long and 65-195 μ m wide. On a host individual, one can count 1-3 cysts:

- **Spore:** Medium in size, the spores are ovoid with both rounded ends (Fig. 2a-d). The valves have 5-6 folds at the posterior end of the spore (Fig. 2a, d). There is no intercapsular triangle. The polar capsules are piriform shape and slightly unequal (Fig. 2b, d). The filament has 8-10 coils in the larger polar capsule and 6-7 coils in the smaller polar capsule (Fig. 2d). The sporoplasm is granular and often contains an iodinophilous vacuole (Fig. 2a, d)
- Measurements: Measurements are given in Table 1
- Host: Barbus callipterus Boulenger, 1907 (Cyprinidae)
- Location: Lébamzip (Center region)

Table 1: List of different	species of Myxosporidia of th	ne genus Myxobolus described	in the fishes of t	he genus <i>Barbus</i> (m	easurements in mici	ometer	()				
Parasite species	Host species	Infestation site	Country	SL	SW	Ъ	LPC	WPC	ΡF	ICA	Reference
M. ampullaceus	B. kolus	Fins	India	9.8 (8.6-10.7)	7.1 (6.4-7.9)	0	5.8 (5.0-6.4)	2.8 (2.5-2.9)	5-6	۷	Kumari ⁵¹
M. azerbajdzanicus	B. lacerta cyri	Gills	Caucasus	18.4-20	13.8-15.7	0	6.1-7.3	5.2-5.9	/	٩	lbragimov ⁵²
M. barbi	B. ticto	Skin	India	12.6-13.5	9.0	0	3.6-4.5	2.7	/	٨	Tripathi ⁵³
M. branchialis	B. barbus borysthenicus	Gills	Ukraine	6.8-8.4	5.8-6.4	0	2.5-3.2	1.6-2.0	/	۷	Markevitsch ⁵⁴
M. branchilateralis	B. barbus	Gills	Hungary	9.9 (9.4-10.4)	8.4 (7.5-9.1)	0	3.0 (4.9-6.0)	3 (2.5-3.3)	9	A	Molnar <i>etal.</i> 55
M. bulbocordis	B. sharpeyi	Heart	Iran	19 (17.3-19.6)	15.3 (13.8-15.5)	0	8.4 (8.1-9.2)	5.8 (5.2-6.3)	/	٩	Masoumian <i>et al</i> ⁴⁶
<i>M. ngassami</i> n. sp.	B. callipterus	Fins, kidneys, eye,	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*	٩	Present study
		opercules and integument					4.4 (3.5-5.1)**	2.4 (1.7-3)**	5-7**		
M. caudatus	B. bynni	Caudal fin	Egypt	17.5 (16-19.2)	12.8 (11-13.6)	0	7.4 (6.4-9)	3.8 (3.2-4.5)	8-9	٩	Ali <i>et al.</i> ⁵⁶
M. cutanei	B. barbus bocagei	Scales	Spain	11.5 (10.5-12.5)	8.9 (8.0-9.7)	0	5.2 (4.0-6.5)	2.7 (2.0-3.5)	8-10	٩	Alvarez-Pellitero and
											Gonzalez-Lanza ⁵⁷
M. egypticus	B. bynni	Intestine	Egypt	12.5 (12.0-13.6)	8.8 (8.0-9.6)		7.7 (7.2-8.0)	3.3 (3.2-3.6)	5-6	٩	Ali <i>et al.</i> ⁵⁶
M. etsatsaensis	B. thamalanensis	Gills	Botswana	13.0 (12.8- 15.0)	6.8 (6.2-8.0)	¥	7.5 (7.0-8.0)*	2.3 (1.2-2.5)*	7-8*	Ч	Reed <i>et al</i> ⁵⁸
							**/	**/	**/		
M. fahmii	B. bynni	Gills	Egypt	11.0 (10.8-12.0)	7.1 (6.4-8.0)	0	6.8 (6.4-7.2)	3.2 (2.8-3.8)	6-7	۷	Ali <i>et al.</i> ⁵⁶
M. nyongana	<i>B. aspilus.</i> and	Gills	Cameroon	10.8 (7.3-13.0)	6.0 (5.0-7.0)	0	5.9 (5.0-7.0)	1.9 (1.4-2.5)	6-9	A	Fomena and Bouix ²¹
)	B. camptacanthus										
M. hyderabadense	B. pinnauratus	Gills	India	10.1 (9.3-11.5)	5.9 (5.0-8.0)	0	5.8 (5.0-7.3)	2.2 (1.4-3.0)	8-9	٨	Kumari ⁵¹ , Landsberg
M immediate	andred a	Fine and aille		10 5 13 7	11 00		E E 6.0	7 a c	,	<	and Lom ²² Misschnichanla ⁶⁰
w.impressus	b. barbus	rins and gills	UKraine	10.51-C.01	11-7.6		0.0-C.C	2.0-4		¥	MIROSONICHENKO**
M. indiae	B. sarana	Gills	India	13.7 (12.4-15.0)	7.3 (6.4-8.6)	0	5.9 (5.7-7.1)	2.1 (1.4-2.5)	8-10	A	Kumari ⁵¹ , Landsberg
											and Lom ⁵⁹
M. intestinealis	B. bynni	Intestine	Egypt	12.5 (12-13.6)	8.8 (8-9.6)	0	7.7 (7.2-8)	3.3 (3.2-3.6)	5-6	Р	Ali <i>et al.</i> ⁵⁶
M. iranicus	B. luteus	Spleen	Iran	13.6 (13.2-14.0)	8.9 (7.5-9.2)	\$	7.3 (6.9-7.5)*	3.3 (2.9-3.5)*	7*	٩	Molnar <i>et al</i> / ⁶¹
							7.0 (6.6-7.2)**	/**	e**		
<i>M. irinae</i>	B. capito conocephalus	Kidneys	Central Asia	9.4-10.6	7-7.7	0	4-5.9	2.4-3		٩	Daniyarov ⁶²
M. karuni	B. grypus. and B. luteus	Gills	Iran	14.1 (13.0-14.9)	10.2 (9.7-10.4)	0	6.2 (6.5-7.5)	3.4 (3.2-3.9)	10-11	٨	Masoumian <i>et al.</i> ⁶³
M. koli	B. kolus	Fins	India	8.4 (7.1-9.6)	6.0 (5.0-6.4)	*	4.3 (3.9-4.6)*	2.8 (2.1-3.1)*	/	٨	Kumari ⁵¹
							2 (1.4-2.1)**	1.2 (0.7-1.4)**			
M. laterobranchialis	B. barbus	Gills	Hungary	9.9 (9.4-10.4)	8.4 (7.5-9.1)	0	3 (4.9-6)	3 (2.5-3.3)	9	A	Molnar <i>et al.</i> 55
<i>M. sanagaensis</i> n. sp.	B. callipterus	Heart	Cameroon	9.9 (9.4 - 11)	6.8 (6.1 - 7.3)	*	4.6 (4.1 - 5.1)*	4 (3.2 - 4.3)*	8-10*	۷	Present study
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Parasite species	Host species	Infestation site	Country	SL	SW	РС	LPC	WPC	ΡF	ICA	Reference
M. mesopotamiae	B. grypus	Fins	lran	9.2 (8.9-9.4)	8.1 (7.8-8.5)	0	3.8 (3.6-4.2)	2.5 (2.4-2.7)	7	A	Molnar <i>et al</i> . ⁶¹
M. njinei	B. camptacanthus, B. cuirali and B. martorelli	Gil arch connective tissue	Cameroon	16.17 (14-20)	13.46 (11.6-18.5)	0	7.81 (6.5-9)	4.59 (3.5-5.4)	7-8	٩	Fomena <i>et al.</i> ²³
M. nkolyaensis	B. jae	Caudal muscles	Cameroon	9.0 (8.0-11.0)	8.3 (7.2-11.5)	0	4.4 (3.5-5.5)	3.0 (2.2-3.5)	/	۷	Fomena and Bouix ¹⁸
M. nodulointestinalis	B. sharpeyi. and B. luteus	Smooth muscles layer of	lran	12.6 (11.7-13)	8.1 (7.8-9.1)	0	3.6 (2.6-3.9)	2.4 (2.2-2.6)	4-5	٩	Masoumian <i>et al.</i> ⁴⁶
		intestine wall									
M. oloi	B. aspilus, B. camptacanthus, B. camptacanthus,	Gill arch epithelium, gullet and kidneys	Cameroon	9.3 (6.3-11.5)	7.2 (5.1-9.4)	*	5.7 (4.0-7.0)* 3.9 (2.2 - 5)**	3.1 (1.8-4.0)* 2 (1.5 -2.5)**	4-5* 3**	۷	Fomena and Bouix ¹⁸
	B. guirali and B. martorelli										
M. osmaniae	B. punjaubensis	Liver and intestine	India	13.5 (12.0-15.0)	8.6 (7.1-10.0)	*	5.6 (5.0-7.1)* 2.6 (2 1-3 6)**	3.2 (2.9-3.9)* 2 5 (1 4-2 9)**	5-6* /**	A	Kumari ^{sı}
Al avaidatio			Conthe Africa	20.01	0 1 5		11 1 4*	0 4*		<	$\Gamma = m + h = m 64$
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M. paludinosus	B. paludinosus	Gills	Botswana	12.0 (11.2-13.7)	8.6 (7.5-10.0)	0	5.7 (5.0-6.8)	2.4 (2.0-2.5)	6-7		Reed <i>et al.</i> ⁵⁸
M. persicus	B. grypus, B. sharpeyi	Gills	Iran	100 (9.1-10.4)	73 (65-78)	*	5.1 (4.5-5.8)*	27 (26-32)	6-7*	٩	Masoumian <i>et al.</i> ⁶³
	and <i>B. luteus</i>						48 (45-51)**		7-8**		
M. pfei eri	B. barbus	Muscles gills, Kidneys and spleen	~	10-13	9-12.2	0	5-5.7	/	~	٩	Thelohan ⁶⁵
M. pinnaurati	B. pinnauratus	Gills	India	9.6 (8.0-11.4)	7.0 (6.5-7.9)	\$	4.4 (3.6-6.4)*	1.9 (1.1-2.1)*	/	A	Kumari ⁵¹
							3.1 (2.9-5)**	1.6 (1.1 -2.1) **			
M. saranai	B. sarana	Gills	India	6.4-7	4.5-5	*	3.5*	1.5*	/	A	Tripathi ⁵³ , Landsberg
							1.5**	1**			and Lom ⁵⁹
<i>M. sessabai</i> n. sp.	B. callipterus	Skin and kidneys	Cameroon	13.4 (12.6-14)	10.8 (9.9-11.5)	0	5.9 (5-6.3)	3.4 (2.5-3.8)	8-10	٩	Present study
M. shadgani	B. rajanorum	Gills	lran	13.9 (13.3-14.1)	13.7 (13.3-14.1)	\$	8.2 (7.9-8.3)*	5.3 (4.9-5.5)*	*0	٩	Molnar <i>et al</i> ⁶¹
							7.9 (7.6-8.1)**	5.2 (4.6-5.4)**	7**		
M. sharpeyi	B. sharpeyi	Gill cartillage	lran	9.6 (9.2-9.8)	8.1 (8.6-7.5)	0	3.6 (3.3-4.0)	2.8 (2.2-2.4)	5	٩	Molnar <i>et al</i> . ⁶¹
M. tisae	B. barbus	Kidneys	Hungary	9-10	8	*	5.5-6.5*	3-3.5*	7-8*	٩	Lom ⁶⁶
							4.5-5.5**	3-3.5**	6-7**		
M. valdogeli	B. brachicephalus	Gills	Central Asia	7.5-9.5	6-6.5	0	4-4.5			Р	Dogel ⁶⁷ , Landsberg
											and Lom ⁵⁹

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Fig. 1(a-d): Photomicrographs and line drawings of spores of *Myxobolus ngassami* sp. nov., (a) Fresh spores (Scale bars: 10 μm),
 (b) Stained spores with May-Grünwald-Giemsa (Scale bars: 10 μm) and (b-d) Line drawings of spores (Scale bars: 5 μm)



Fig. 2(a-d): Photomicrographs and line drawings of spores of *Myxobolus sanagaensis* sp. nov., (a) Fresh spores (Scale bars: 10 μm), (b) Stained spores with May-Grünwald-Giemsa (Scale bars: 10 μm) and (c, d) Line drawings of spores (Scale bars: 5 μm)

- Location in the host: Heart
- **Prevalence:** 18% (9 parasitized fish out of 50 examined)
- **Type material:** Glass slides with stained spores (syntype) and cysts preserved in 10% buffered neutral formalin are deposited in the parasitological collection of the Laboratory of Parasitology and Ecology, Faculty of Science, University of Yaounde 1, Cameroon (No. Myxo/2017/LPE-002)
- **Etymology:** The species is related to the Sanaga basin in which the host fish were caught

Myxobolus sessabai sp. nov.

Vegetative form: Whitish and spherical cysts are implanted under the skin. They are polysporated and measured $350 \times 345 \,\mu\text{m}$ on average. On a host individual, one can count 1-6 cysts. In the kidneys, the spores are isolated or grouped:



Fig. 3(a-d): Photomicrographs and line drawings of spores of *Myxobolus sessabai* sp. nov., (a) Fresh spores (Scale bars: 10 μm),
 (b) Stained spores with May-Grünwald-Giemsa (Scale bars: 10 μm) and (c, d) Line drawings of spores (Scale bars: 5 μm)

- Spore: Medium in size, they are ovoid with two rounded ends (Fig. 3a-d). There are 6-7 valvular folds at the posterior end of the spore (Fig. 3a, c). At the anterior end, there is a well-developed intercapsular process mesuring 2.6±0.08 (2.1-3.2) µm long (Fig. 3a, c). Polar capsules are piriform and symmetrical (Fig. 3b). In each of them, 8-10 coils of the filament are counted (Fig. 3c). The sporoplasm contains a large iodinophilous vacuole (Fig. 3a, d)
- Measurements: Measurements are given in Table 1
- Host: Barbus callipterus Boulenger, 1907 (Cyprinidae)
- Location: Lébamzip (Center region)
- Localization in the host: Skin, kidneys
- **Prevalence:** 22% (11 fish parasitized out of 50 examined)
- Type material: Glass slides with stained spores (syntype) and cysts preserved in 10% buffered neutral formalin are deposited in the parasitological collection of the Laboratory of Parasitology and Ecology, Faculty of Science, University of Yaounde 1, Cameroon (No. Myxo/2017/LPE-003)
- **Etymology:** The species is related to the Sessaba river in which the host fish were captured

DISCUSSION

According to Eiras *et al.*^{11,13}, there are many Myxosporidia of the genus *Myxobolus* having an intercapsular appendix and unequal polar capsules described in freshwater fishes.

*Myxobolus dossoui*³⁵, parasite of cartilaginous tissue of the gill arches of *Tilapia zillii*, *Hemichromis fasciatus* and

Tilapia hybrid in Benin, forms spores with unequal polar capsules and an intercapsular appendage. However, these spores are sub-spherical and less developed ($9.9 \times 9.2 \mu m$ on average).

While having an intercapsular triangle and asymmetric polar capsules, *Myxobolus bilongi*²⁵, parasite of *Labeo* sp. in Cameroon, forms larger and spherical spores ($15.3 \times 12.2 \mu m$ on average), with the anterior end slightly flattened.

With the general form and the components of the spore, the parasite in description approaches *Myxobolus analfinus*³⁶ *M. debsantus*³⁶ and *M. tripurensis*³⁷. *Myxobolus analfinus* foud in the fins of *Heteropneustes fossilis* in India, forms spores with a narrowed anterior end and a suture line free of folds. *Myxobolus debsantus* described on the fins of hybrid carp, Catla-Rohu (*Catla catla*× *Labeo rohita*) differs from the species in description by its less developed spores (9.0×8.4 µm on average), strong dissymmetry between polar capsules. *Myxobolus tripurensis* was described in the gills of *Labeo calbasu* in India. Spores of this Myxosporidia are less developed (8.1-9.4×6.5-7.0 µm), with 9 shell valve folds in the posterior quarter, less developed polar capsules (4.2×2.3 µm on average for the larger and 2.7×1.7 µm for the smaller). The intercapsular appendage is crescent-shaped.

*Myxobolus burkinei*³⁸, a parasite of *Labeo coubie* in Burkina-Faso, forms spores with an intercapsular triangle and unequal polar capsules. This species differs from the parasite being described by its clearly dissymmetrical polar capsules (6.19 and 4 μ m on average respectively for the larger and the smaller) and the lack of folds on shell valves.

Seenappa and Manohar³⁹ described *Myxobolus vedavatiensis* in the gills of *Cirrhina mrigala* (Cyprinidae) in

India. Although the general shape of spores, the presence of unequal polar capsules, the presence of an intercapsular triangle approximates this parasite to the species in description but *M. vedavatiensis* forms longer spores (13-15 μ m), similarly. The suture line does not show fold. The larger polar capsule is more developed (6-7 × 3-4 μ m).

Spores of *Myxobolus haldari*⁴⁰, parasites of numerous Cyprinid fishes (*Cirrhina mrigala, Labeo dyocheilus, L. rohita* and *L. bata*) in India are less developed ($9.31 \times 7.95 \mu$ m on average) with shorter polar capsules (4.31 and 2.95μ m on average respectively for the larger and smaller), compared to 5.0 and 4.4 µm obtained for our species.

*Myxobolus buckei*⁴¹, parasite of Cyprinid fish (*Leuciscus cephalus, Abramis brama* and *Rutilus rutilus*) in the United Kingdom, forms ovoid spores with an intercapsular appendix and shell valve folds at the posterior end. Spores of this Myxosporidia are however longer (14 μ m on average) with equal polar capsules.

These differences lead to the creation of a new species for the parasite of *B. callipterus* and we propose the name *Myxobolus ngassami* sp. nov., as a sign of honour to Professor NGASSAM Pierre, a lecturer at the University of Yaounde 1.

Several species of Myxosporidia have been described in the heart of freshwater fish throughout the world.

*Myxobolus hearti*⁴² is a parasite the heart of *Carassius auratus auratus* in China. Its polar capsules are unequal and there is no intercapsular appendage. Spores of this species are ovoid but larger (14.8×11.2 µm on average) compared to those of the species in description.

*Myxobolus dogieli*⁴³, *M. cordis*⁴⁴ and *M. heteromorpha*⁴⁵ parasites of the heart in their hosts, form spherical spores and are devoid of intercapsular triangle. Spores of these parasites are more developed (9-16×8-15 μ m, 12×10 μ m and 10.9×9.5 μ m, respectively) and their polar capsules are unequal.

*Myxobolus bulbocordis*⁴⁶ was found in the heart of *Barbus sharpeyi* in Iran. The spores of this Myxosporidia, although ovoid with shell valve folds are quite distinguished from those of the species in description by the following characteristics: Relatively large size ($19 \times 15.3 \mu m$ on average), presence of an intercapsular triangle, equal polar capsules.

*Myxobolus oloi*¹⁸ parasites the heart, gills, esophagus and kidneys of *Barbus aspilus*, *B. camptacanthus*, *B. guirali* and *B. martorelli* in Cameroon. Despite the comparable spore dimensions and the presence of unequal polar capsules, the parasite stands away from the species in description by the absence of folds on shell valves.

Seenappa and Manohar³⁹ described *Myxobolus vedavatiensis* in the gills of *Cirrhina mrigala* in India. Spore of

this *Myxobolus* are more developed $(13-15 \times 8-10 \mu m)$ has an intercapsular triangle are devoid of shell valve folds, its polar capsules are more developed $(6.2 \times 3.4 \mu m \text{ and } 3.9 \times 2.6 \mu m \text{ on average respectively for the larger and the smaller}).$ *Myxobolus bhadrensis*³⁹ is a parasite the muscle of*Labeo rohita*in India. The spore of this Myxosporidia is similar to that of the species presently described with its shape, the absence of an intercapsular triangle and the asymmetry observed in polar capsules. However,*M. bhadrensis*differs from the parasite of*Barbus callipterus* $by the absence of folds on shell valves at the posterior end of spore and its less developed polar capsules (4.5 and 2.5 <math>\mu$ m in length respectively for the larger and the smaller).

These differences lead one to think that the parasite of *Barbus callipterus* is new. The name *Myxobolus sanagaensis* sp. nov. is propose, referring to the Sanaga basin in which the host fish were caught.

Myxosporidia species with equal polar capsules and intercapsular triangle have been described in Africa and around the world.

*Myxobolus njinei*²³, a parasite of *Barbus camptacanthus*, *B. guirali* and *B. martorelli* in Cameroon, forms larger spores $(16.17 \times 13.46 \,\mu\text{m} \text{ on average})$ with apical truncation.

*Myxobolus parvus*⁴⁷ forms cysts in the gills of *Mugil cephalus* in India. Although having an intercapsular triangle and equal polar capsules, its spores are clearly distinguishable from those of the species we are describing by the following characteristics: intercapsular triangle less developed, lack of folds on the suture line, smaller spores ($6.3 \times 4.8 \mu m$ on average).

Sarkar⁴⁸ described *Myxobolus meglitschus*, a parasite forming cysts in gill lamellae of *Notopterus notopterus* in India. Although ovoid, spores of this species are less developed ($8.8 \times 6.96 \mu$ m on average). Its smaller polar capsules ($4.42 \times 2.07 \mu$ m on average) contain only 5-7 coils of the filament.

Spores of *Myxobolus gayerae*⁴⁹, parasite of the intestine of *Leuciscus cephalus* L. (Cyprinidae) in Hungary are ellipsoidal, with shell valve folds at the posterior end. They have an intercapsular triangle and unequal polar capsules. Compared to our species, *M. gayerae* has larger spores $(15.1 \times 12.7 \,\mu\text{m on average})$, its piriform polar capsules contain only 6 coils of the filament.

In Brazil, Carriero *et al.*⁵⁰ described *Myxobolus pantanalis* in the gills of *Salminus brasiliensis*. Spores of this species are ovoid but less developed ($9.3 \times 6.5 \mu m$ on average), devoid of intercapsular triangle, its polar capsules are equal but contain only 4-5 coils of the filament. Sakiti *et al.*³⁵ described *Myxobolus dossoui* in the gills of various cichlids (*Tilapia zillii, Hemichromis fasciatus* and *Tilapia* hybrid) in Benin. This parasite forms spores of comparable morphology with an intercapsular triangle. However, they are quite small ($9.9 \times 9.2 \mu m$ on average) with unequal polar capsules.

The parasite of *Barbus callipterus*, which differs from known Myxosporidia by many aspects, is new. We propose the name *Myxobolus sessabai* sp. nov. to recall the Sessaba River in which the host fish were captured.

CONCLUSION

Myxosporidia recorded in this study belong to the genus *Myxobolus*. Morphometric characteristics of spores indicate new species for which the names *Myxobolus ngassami* sp. nov., *Myxobolus sanagaensis* sp. nov. and *Myxobolus sessabai* sp. nov. are proposed. The infection with the three parasites species described does not cause observed significant economic loss because the infection was only found in wild Cyprinid. However, it seems to be dangerous if the infection occur in fish ponds. Further research will focus on the epidemiology of Myxosporidia parasites of cultured *Barbus callipterus*.

SIGNIFICANCE STATEMENT

This study provides the description of *Myxobolus ngassami* sp. nov., *Myxobolus sanagaensis* sp. nov. and *Myxobolus sessabai* sp. nov. parasites of *Barbus callipterus* Boulenger, 1907 (Cyprinidae). In Cameroon, 7 species of parasites of the genus *Myxobolus* are now known in six species of fish of the genus *Barbus*. The identified parasites affect many organs. The rupture of cysts implanted under these organs can provoke wound that would constitute entry door for secondary pathogens (bacteria and fungi).

ACKNOWLEDGMENT

The authors are greatly indebted to Prof. AJEAGAH Gideon AGHAINDOUM who linguistically corrected the manuscript.

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