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Myxozoans infecting the sharptooth catfish, *Clarias gariepinus* in the Okavango River and Delta, Botswana, including descriptions of two new species, *Henneguya samochimensis* sp. n. and *Myxobolus gariepinus* sp. n.

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Abstract. During a recent investigation of parasites infecting fishes from the Okavango River and Delta, Botswana (southern Africa) fourteen sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae) were examined for the presence of myxozoan infections. Results revealed the presence of two species of the genus *Henneguya* Thélohan, 1895 and one species of the genus *Myxobolus* Bütschli, 1882 infecting this fish host. Two of the sampled fish exhibited large plasmodia of *Henneguya suprabranchiae* Landsberg, 1987 in the cartilage of the accessory breathing organ, another two individuals were infected with *H. samochimensis* sp. n. plasmodia in the gills and another three individuals revealed an infection with *Myxobolus gariepinus* sp. n. plasmodia in the ovaries.

The sharptooth catfish, *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae) is probably the most widely distributed fish species in Africa, with many names such as *C. mossambicus* Peters, 1852 and *C. lazera* Valenciennes, 1840 being recognised as its junior synonyms (Skelton 1993). The economic importance of this fish species has increased greatly in recent years as a result of its extensive use in aquaculture (Skelton and Teugels 1992). Furthermore, natural populations of *C. gariepinus* form a staple diet for many subsistence farmers throughout the African continent. Coinciding with the growing economic value of this fish is the increased interest in its parasite loads and what effect they might hold for the aquaculture industry. One particular group of parasites, the myxozoans, is well known for the diseases they cause in commercially important fish hosts. Fortunately, the pathological species represent merely a fraction of the more than 1350 described species throughout the world (Kent et al. 2001).

In Africa more than 135 species of myxozoans are known to infect freshwater, brackish and marine fishes (Kostoïngue et al. 2001). Seven of these, representing two genera, have been described from *Clarias* Scopoli, 1777 species in Africa (Table 1). *Henneguya clariae* Abolarin, 1971 was the first species to be described from the gills of *C. lazera* in Nigeria by Abolarin (1971). This description also appears to be the first record of the genus *Henneguya* Thélohan, 1892 in Africa. Several years later Landsberg (1987) described *H. laterocapsulata* Landsberg, 1987 and *H. suprabranchiae* Landsberg, 1987 from the skin and suprabranchial organs, respectively, of the same host in Israel. Ashmawy et al. (1989) described *H. branchialis* Ashmawy, Abu-Elwafa, Imam et El-Otifi, 1989 from the gills of *C. lazera* in Egypt. Some dispute has existed regarding the identification of this species and recently Ali (1999) suggested that *H. branchialis* is in fact a synonym of *H. suprabranchiae*. *Myxobolus clarii* Mandour, Galal et Abed, 1993 was described from the testis of *C. lazera* in Egypt by Mandour et al. (1993), afterwhich *M. comoei* Kabré, Sakiti, Marqués et Sawadago, 1995 was described from the gills of *C. anguillaris* Linnaeus, 1758 in Burkina Faso by Kabré et al. (1995). Most recently, Kostoïngue et al. (1999) described *H. fusiformis* Kostoïngue, Fall, Faye et Toguebaye, 1999 from the gills of *C. anguillaris* in Chad.

This paper presents preliminary results of the first investigation into myxozoan parasites infecting the sharptooth catfish, *Clarias gariepinus* in the Okavango River and Delta in Botswana. Two new species, *Henneguya samochimensis* sp. n. and *Myxobolus gariepinus* sp. n. are described, whilst *H. suprabranchiae* is recorded for the first time in southern Africa.

MATERIALS AND METHODS

Fourteen *Clarias gariepinus* specimens were captured and examined for the presence of myxozoan infections. During June and July (1998–2000) as well as in August 2001 fish were sampled using a series of gill nets from the lagoon environments within the Okavango River Panhandle and Delta regions in Botswana. Captured fish were killed using high concentrations of anaesthetic benzocaine (2.5×10^{-5} g/l) (ethyl-4-aminobenzoate), and then identified using Skelton (1993), measured and examined for the presence of myxozoan

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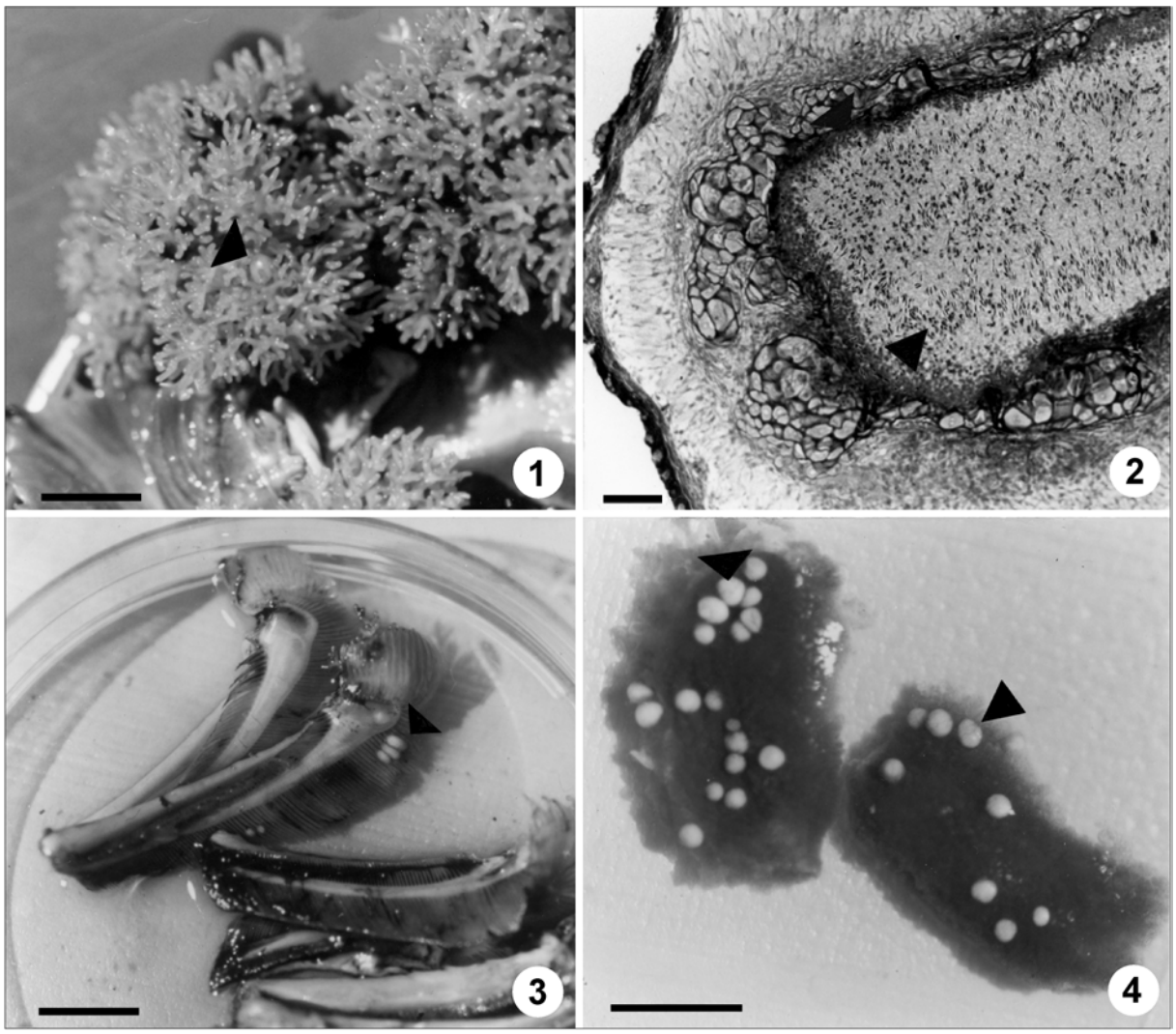
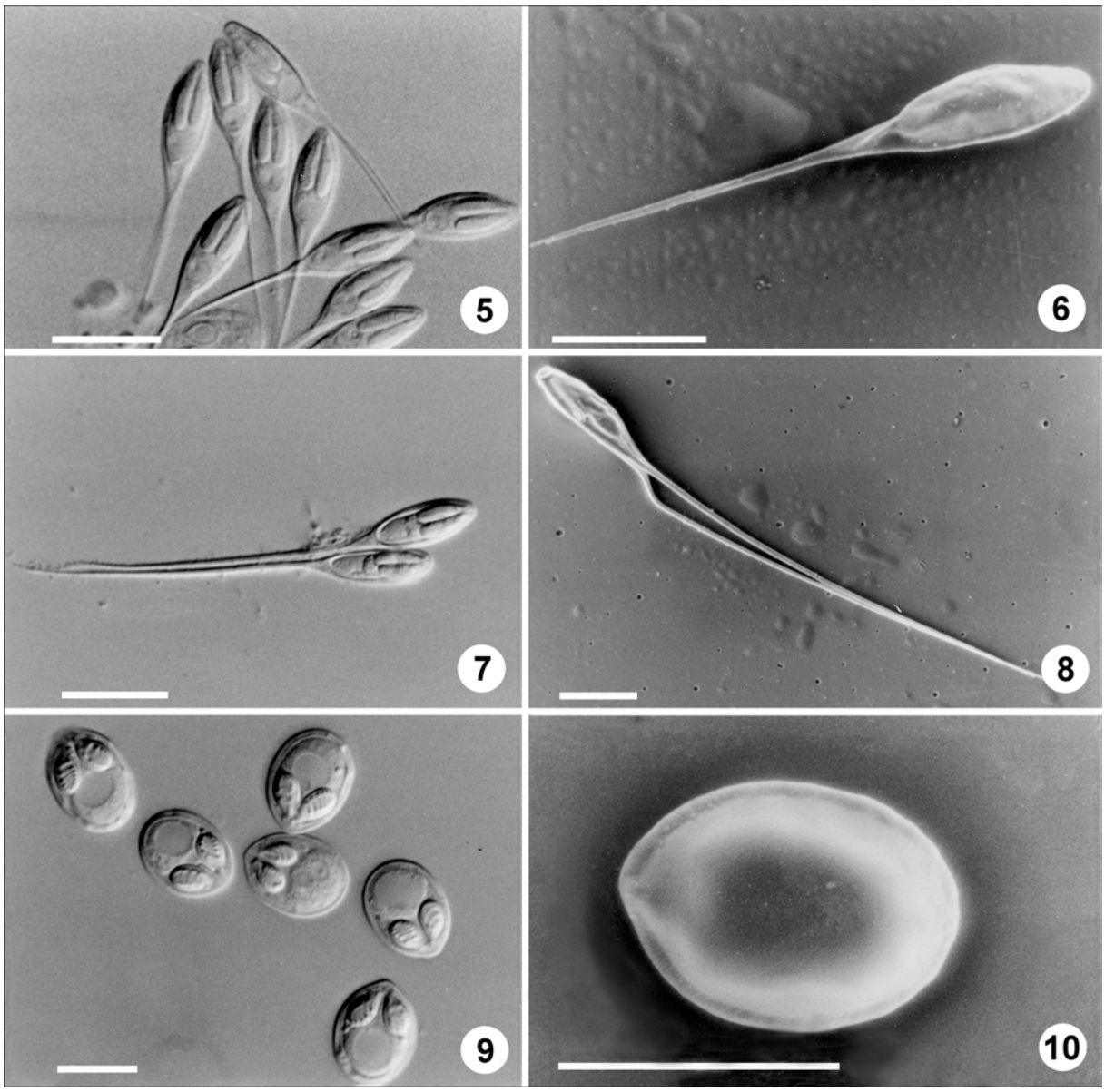


Figs. 1–4. Photographs of myxozoan infections in the organs of *Clarias gariepinus* from the Okavango River and Delta, Botswana. **Fig. 1.** *Henneguya suprabranchiae* Landsberg, 1987 plasmodia in the accessory breathing organ. **Fig. 2.** Histological section through infected accessory breathing organ showing displacement of cartilage by the large *H. suprabranchiae* plasmodia. **Fig. 3.** *Henneguya samochimensis* sp. n. plasmodia in the gills. **Fig. 4.** *Myxobolus gariepinus* sp. n. plasmodia in the ovaries. Scale bars: Figs. 1, 3, 4 = 1 cm; Fig. 2 = 10 µm; arrowheads indicate position of plasmodia.

infections. Due to the remote collection localities, mature myxosporean spores found in plasmodia were fixed in 10% buffered neutral formalin and transported back to the laboratory in the Department of Zoology and Entomology at the University of the Free State, Bloemfontein. There they were photographed using an Axiophot microscope with differential interference contrast on a layer of 0.5% non-nutrient agar. Formalin-fixed spores were dehydrated through a series of ethanol concentrations, critical point dried in a Biorad critical point drier, coated with gold in an Emscope SC 500 sputter coater and viewed using a JEOL Winsem JSM 6400 at 5 or 10 kV. Formalin-fixed spores were measured according to the guidelines provided by Lom and Arthur (1989), with minimum and maximum values of spore measurements provided in micrometres (µm), followed in parentheses by arithmetic mean and standard deviation. Tissue samples of organs containing plasmodia were fixed in Davidson's solution and prepared for histological sectioning using standard techniques. Sections were cut at 7 µm and stained with standard Masson's Trichrome stain. All reference material, in the form of fixed spores or silver-impregnated smears of spores, has been deposited in the parasite collection of the Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa where it has been allocated a reference number. Type material of the new species has been deposited in the collection of the National Museum, Bloemfontein (South Africa) where it has been allocated a NMBP number indicating its place in the National Museum Bloemfontein's Parasite collection.



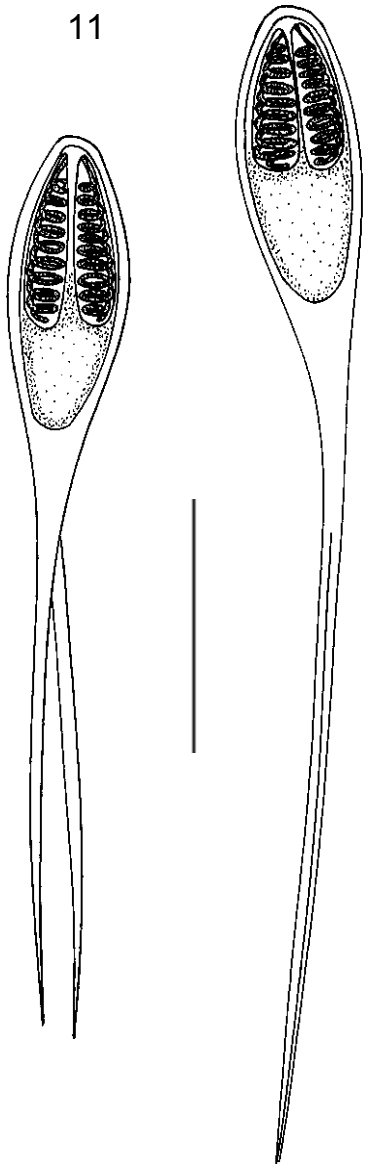
Figs. 5–10. Photomicrographs (Figs. 5, 7, 9) and scanning electron micrographs (Figs. 6, 8, 10) of formalin-fixed spores of myxozoans from the gills and ovaries of *Clarias gariepinus* from the Okavango River and Delta, Botswana. **Figs. 5**, **6.** *Henneguya suprabranchiae* Landsberg, 1987. **Figs. 7**, **8.** *Henneguya samochimensis* sp. n. **Figs. 9**, **10.** *Myxobolus gariepinus* sp. n. Scale bars = 10 µm.

RESULTS AND DISCUSSION

An average of six *Henneguya suprabranchiae* plasmodia was found situated within the tips of cartilage in the accessory breathing organ of two of the 14 individual *C. gariepinus* collected. The second *Henneguya* species was found infecting the primary gill filaments of two *C. gariepinus* specimens, with one to four plasmodia situated in the primary gill lamellae of the infected individuals. Ovaries of three of the captured *C. gariepinus* were infected with plasmodia of the *Myxobolus* species. In each case an average of 16 plasmodia were seen distributed throughout the ovaries.

Henneguya suprabranchiae Landsberg, 1987
Figs. 1, 2, 5, 6, 11

Description of vegetative stages. Sporogonic plasmodia found in cartilage at tips of suprabranchial respiratory organ. Polysporous plasmodia round, yellowish, 2–4 mm in diameter.



Figs. 11, **12.** Myxozoans infecting *Clarias gariepinus* from the Okavango River and Delta, Botswana; microscope projection drawings of formalin-fixed spores. **Fig. 11.** *Henneguya suprabranchiae* Landsberg, 1987. **Fig. 12.** *Henneguya samochimensis* sp. n. Scale bar = 10 µm.

Description of spores (based on 10 formalin-fixed spores from fully mature plasmodia). Spore body elongated to fusiform in valvular view with anterior and posterior ends tapering to blunt points, 16.2–18.2 (17.2 ± 0.5) long. Spore body prolonged by two filiform, thin extensions that are separated. Total length of spores 38.4–43.0 (40.4 ± 1.9). Caudal length 22.2–25.8 (23.2 ± 1.4). Two elongated pyriform polar capsules situated at anterior end of spore lying parallel, 7.5 (7.5 ± 0) long × 1.9 (1.9 ± 0) wide. Widest region of spore is towards posterior ends of polar capsules measuring 5.0–6.3 (6.0 ± 0.5). Polar capsules contain polar filaments with nine coils, filling almost two thirds of spore cavity. Two smooth shell valves visible, two filiform and long expansions clearly separated at base. Narrow sutural ridge visible surrounding spore body.

Host: *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae).

Site of infection: Cartilage of the accessory breathing organ.

Prevalence: 14.3% (2/14).

Localities: Samochima (18º25'26.08"S; 21º54'09.26"E) and Duba (18º58'27.78"S; 22º33'44.22"E) Lagoons, Okavango River and Delta, Botswana.

Material examined: 1999/07/04-13 (spores fixed in 10% buffered neutral formalin).

Remarks. The morphology and measurements of the myxozoan spores found in mature plasmodia at the tips of the accessory breathing organ of *C. gariepinus* conform to the description of *Henneguya suprabranchiae*. This myxosporean species was originally described from the accessory breathing organ of the same host in Israel by Landsberg (1987). As illustrated in Fig. 2, it appears as if the infection may also result in replacement of the cartilaginous tissue in the accessory breathing organ. El-Mansy and Bashtar (2002) found that mass growth of the plasmodium of *H. suprabranchiae* in this organ led to pressure on the host cartilaginous tissue which was subsequently compressed. This is the first record of *H. suprabranchiae* in the Okavango River and Delta in Botswana.

Henneguya samochimensis sp. n. Figs. 3, 7, 8, 12

Description of vegetative stages. One to four large, oval to oblong, mature sporogonic plasmodia found extending into primary gill filaments. Polysporous plasmodia, yellow to whitish, 2–5 mm in length.

Description of spores (based on 10 formalin-fixed spores from mature plasmodia). Mature spore body elongated to oval in valvular view with bluntly pointed narrow anterior end, 12.3–15.0 (13.7 ± 0.8). Posterior end narrowly rounded. Spore body, prolonged by two very filiform, narrow and long extensions, total length 47.0–53.0 (50.3 ± 4.5). Caudal appendages separated from each other, 34.7–35.3 (36.6 ± 3.7) long. Two pyriform to straight polar capsules visible in anterior part of spore. Polar capsules nearly parallel to each other, filling just less than half of spore body cavity 5.0–6.0 (5.6 ± 0.6) long × 1.3–1.9 (1.6 ± 0.3) wide. Widest part of spore found towards posterior ends of polar capsules, measuring 5.0–7.0 (6.0 ± 0.6). Polar capsules contain eight coils in the polar filament. Two smooth shell valves visible that extend into two filiform projections. Narrow sutural ridge visible.

Type host: *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae).

Type locality: Samochima Lagoon (18º25'26.08"S; 21º54'09.26"E), Okavango River, Botswana.

Site of infection: Primary gill lamellae.

P r e v a l e n c e : 14.3% (2/14).

E t y m o l o g y : Named after the type locality.

M a t e r i a l : Syntypes; spores in 10% neutral buffered formalin, 2000/08/12-03 (NMBP 276); spores in 10% neutral buffered formalin, 1999/07/02-33 (NMBP 277) and 1999/07/02-01 (NMBP 278); in the collection of the National Museum, Bloemfontein, South Africa.

Remarks. Significant differences can be seen when comparing the morphology and spore measurements of *H. samochimensis* to that of the other African *Henneguya* species parasitizing *Clarias* hosts (Table 2). *Henneguya samochimensis* differs from *H. clariae*, which has fused caudal appendages (Abolarin 1971) and a much longer total spore length (Table 2). The shape of the spore body of *H. clariae* has an almost sharply pointed anterior end with two unequally-sized polar capsules. *Henneguya samochimensis* differs significantly from *H. fusiformis* since the latter species has a fusiform spore body that contains two polar capsules with one situated behind the other (Kostoïngue et al. 1999). *Henneguya samochimensis* is also distinct from *H. laterocapsulata* in having two polar capsules both positioned next to each other in the anterior of the spore and not having one polar capsule that discharges laterally as in the case of the latter. The caudal appendages of *H. laterocapsulata* extend from a thick caudal base (Landsberg 1987), which is absent in *H. samochimensis*. Furthermore, the caudal appendages of *H. laterocapsulata* are also thick and divergent, curving outwards half way along their length. This is distinctly different from the thin filiform caudal appendages of *H. samochimensis*. Compared to *H. suprabranchiae*, *H. samochimensis* has a much longer average total spore length, whilst the polar capsules of *H. samochimensis* are proportionally smaller than those of *H. suprabranchiae* (Table 2). Morphologically *H. samochimensis* resembles *H. bopeleti* Fomena et Bouix, 1987 described from *Chrysichthys nigrodigitatus* in Cameroon by Fomena and Bouix (1987). The spore body length of *H. samochimensis* is, however, shorter, and the total spore length is longer than that of *H. bopeleti*. Another morphologically similar species is *Henneguya nyongensis* Fomena et Bouix, 1996, found in the gills of *Marcusenius moori* in Chad by the same authors (Fomena and Bouix 1996), but is distinguished from *H. samochimensis* in having very characteristic 'neck-like' appearances at the anterior ends of the polar capsules.

Myxobolus gariepinus sp. n. Figs. 4, 9, 10, 13

Description of vegetative stages. Sporogonic plasmodia found in ovaries. Polysporous plasmodia spherical, whitish, 2–3 mm in diameter.

Description of spores (based on 10 formalin-fixed spores from fully mature plasmodia). In valvular view, spore body ovoid to spherical with anterior end bluntly rounded, 13.7–15.0 (13.9 ± 0.4) long. Widest region of the spore observed towards posterior ends of polar capsules, measuring 10.0–11.2 (10.8 ± 0.5). Two smooth shell valves visible with two pyriform polar capsules of equal size converging in anterior part of spore, 6.0–6.2 (6.2 ± 0.1) long × 3.0–3.7 (3.5 ± 0.13) wide. Five to six coils of polar filament in polar capsules. Intercapsular process absent. Large iodinophilous vacuole present in sporoplasm.

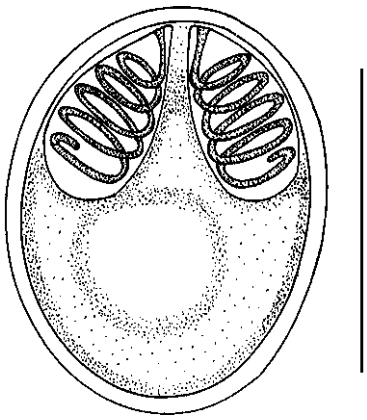


Fig. 13. *Myxobolus gariepinus* sp. n. infecting *Clarias gariepinus* from the Okavango River and Delta, Botswana; microscope projection drawing of formalin-fixed spore. Scale bar = 10 µm.

T y p e h o s t : *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae).

T y p e l o c a l i t y : Samochima Lagoon (18º25'26.08"S; 21º54'09.26"E), Okavango River, Botswana.

S i t e o f i n f e c t i o n : Ovaries.

P r e v a l e n c e : 21% (3/14).

E t y m o l o g y : Species name derived from the type host.

M a t e r i a l : Syntypes; spores in 10% neutral buffered formalin, 1999/07/02-39 (NMBP 279); spores in 10% neutral buffered formalin, 1999/07/02-31 (NMBP 280) and 1999/02/02-02 (NMBP 281); in the collection of the National Museum, Bloemfontein, South Africa.

Remarks. *Myxobolus gariepinus* shows similarities to *M. clarii* Mandour, Galal et Abed, 1993 (Table 1), but differs in having a small blunt point, which appears to be absent in *M. clarii*. The overall shape of *M. gariepinus* also resembles *M. comoei* Kabré, Sakiti, Marqués et Sawadago, 1995 (Table 1) in having a similar almost spherical spore body, the latter species, however, has two polar capsules that take up about half the space in the spore cavity while the polar capsules of *M. gariepinus* only take up about one third of the spore cavity. The small blunt point at the anterior end of *M. gariepinus* is also absent in *M. comoei*. Other morphologically similar species include *M. bilongi* Fomena, Marqués, Bouix et Njiné, 1994, *M. fotoi* Fomena, Marqués et Bouix, 1993 and *M. njinei* Fomena, Bouix et Birgi, 1985. Firstly, *M. gariepinus* is distinct from *M. bilongi*, found in the gills of a *Labeo* sp. by

Table 1. Myxozoan species from *Clarias* Scopoli, 1777 hosts in Africa and Israel.

Species	Host	Organ	Country	Reference
Henneguya branchialis[#]	*C. lazera* *	gills, intestine	Egypt	Ashmawy et al. (1989)
H. clariae	*C. lazera* *	gills	Nigeria	Abolarin (1971)
H. fusiformis	*C. anguillaris*	gills	Chad	Kostoïngue et al. (1999)
H. laterocapsulata	*C. lazera* × *H. bidorsalis*	skin	Israel	Landsberg (1987)
H. samochimensis	*C. gariepinus*	gills	Botswana	present study
H. suprabranchiae	*C. lazera* *	a.b.o.	Israel	Landsberg (1987)
Myxobolus clarii	*C. lazera* *	testis	Egypt	Mandour et al. (1993)
M. comoei	*C. anguillaris*	gills	Burkina Faso	Kabré et al. (1995)
M. gariepinus	*C. gariepinus*	ovaries	Botswana	present study

a.b.o. – accessory breathing organ; # – synonym of *Henneguya suprabranchiae* Landsberg, 1987; * – junior synonym of *Clarias gariepinus* (Burchell, 1822)

Table 2. Comparison of spore measurements (in μm) of the myxosporean species previously described from *Clarias* Scopoli, 1777 hosts in Africa to those collected from *C. gariepinus* (Burchell, 1822) from the Okavango River and Delta in Botswana.

Species	Re	STL	SBL	SBW	CL	PC		No. coils
						L	W	
Henneguya branchialis [#]	1	28.4–41.1 (34.4)	12.7–17.6 (14.7)	4.4–6.4 (5.0)	15.5–23.5	5.9–8.3 (6.9)	1.5–2.9 (2.1)	n/p
H. clariae	2	45.0–107.5 (88.0)	17.5–28.5 (22.0)	5.5–8.5 (6.5)	27.5–89.0 (66.0)	5.0–12.0* 5.5–13.5**	2.5–3.0* 3.0–3.5**	n/p
H. fusiformis	3	59.0–61.0	29.0–33.0	5.0–7.0	28.0–31.0 (30.0)	5.0–6.0	3.0–4.0	n/p
H. laterocapsulata	4	29.0–36.2 (32.7)	13.8–16.0 (14.7)	3.7–5.3 (4.3)	15.2–20.2 (18.0)	4.1–5.3 (4.3)	2.2–3.0 (2.6)	5–6
H. samochimensis (present study)	P	47.0–53.0 (50.3)	12.3–15.0 (13.7)	5.0–7.0 (6.0)	34.7–35.3 (36.6)	5.0–6.0 (5.6)	1.3–1.9 (1.9)	8
H. suprabranchiae	5	30.7–43.3 (37.5)	12.2–14.3 (13.5)	5.6–6.9 (6.4)	18.5–29.0 (24.0)	7.0–8.1 (7.6)	1.8–2.3 (2.1)	9–10
H. suprabranchiae (present study)	P	38.4–43.0 (40.4)	16.2–18.2 (17.2)	5.0–6.3 (6.0)	22.2–25.8 (23.2)	7.5	1.9	9
Myxobolus clarii	6	n/a	9.0–12.0	7.5–10.0	n/a	3.5–5.0	2.0–2.5	n/p
M. comoei	7	n/a	10.0–12.0	8.0–9.0	n/a	4.0–5.0	2.5–3.0	n/p
M. gariepinus (present study)	P	n/a	13.7–15 (13.9)	10.0–11.2 (10.8)	n/a	6.0–6.2 (6.2)	3.0–3.7 (3.5)	5–6

CL – caudal process length; L – length; n/a – not applicable; n/p – not provided by original authors; P – present study; PC – polar capsule; Re – reference; SBL – spore body length; SBW – spore body width; STL – spore total length; W – width; 1 – Ashmawy et al. (1989); 2 – Abolarin (1971); 3 – Kostoïngue et al. (1999); 4 – Landsberg (1987); 5 – Mandour et al. (1993); 6 – Kabré et al. (1995); * – shorter polar capsule; ** – longer polar capsule; # – synonym of *Henneguya suprabranchiae* Landsberg, 1987

Fomena et al. (1994), since the latter myxosporean possesses two polar capsules of unequal sizes. *Myxobolus gariepinus* differs from *M. fotoi* since the latter has an almost completely spherical spore and also according to Fomena et al. (1993) has polar capsules that are subspherical and take up approximately one fourth of the spore cavity. *Myxobolus njinei*, a parasite of various *Barbus* species (Fomena et al. 1994), has a much larger and more oval spores, with two distinctly unequally sized polar capsules taking up almost two thirds of the spore body (Fomena et al. 1985), differing from the more spherical spore body of *M. gariepinus*.

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