Myxobolus species (Myxozoa), parasites of fishes in the Okavango River and Delta, Botswana, including descriptions of two new species

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Abstract. Fieldwork was conducted in 1998 and 1999 in the Okavango River and Delta and a total of 275 fishes representing 31 species were examined for the presence of myxosporean parasites. A total of seven myxosporeans of the genus Myxobolus Bütschli, 1882 were found infecting the fishes. Two new species namely Myxobolus etsatsensis sp. n. from Barbus thamalakanensis Fowler, 1935 and M. paludinosus sp. n. from Barbus paludinosus Peters, 1852 are described. Myxobolus africanaus Fomena, Bouix et Birgi, 1985, M. camerounensis Fomena, Marqués et Bouix, 1993, M. hydrocyni Kostoiugue et Toguèbaye, 1994, M. nyongana (Fomena, Bouix et Birgi, 1985) and M. tilapiae Abolarin, 1974 are recorded for the first time in Botswana and descriptions of these species are provided.

Myxosporean research in Africa dates back to the late 19th century with Gurley (1893) being one of the earliest authors referring to the continent. The African continent boasts over a 100 myxosporean species from freshwater, brackish and marine fishes of which 84 infect primarily freshwater fishes (Fomena and Bouix 1997) and this number is continuously growing. When comparing the known African myxosporeans to the more than 1,300 species described worldwide, it is evident that for a huge continent with such high fish diversity, a large gap exists in the knowledge on the occurrence and distribution of these parasites.

In southern Africa little research has been conducted on myxosporean parasites of fish, with only a few publications appearing largely on marine myxosporeans from South Africa such as Fantham (1919, 1930), Gilchrist (1924), Paperna et al. (1987) and Ali (2000). The only record ever of a freshwater myxosporean from Botswana is that of Peters (1971), commenting on Boullenger (1911) who published a brief note on an anabantid showing a mouth-brooding habit from the Okavango River. According to Peters (1971), Boullenger commented the following: “On examining a female, about 5 ins. long, I found seven or eight eggs about one line in diameter, closely packed on each side in a cavity behind the gills, entirely covered by operculum”. While conducting comparative studies on the ethology of African Anabantidae, Peters (1971) examined the rounded bodies, which did look like eggs, and discovered that they were in fact mature plasmodia from a myxosporean infection.

Now, 30 years later, the results of the first investigation into myxosporean parasites infecting fishes in the Okavango River and Delta are presented. Over a period of two years (1998 and 1999) a total of 275 fishes from the Okavango Delta, representing 31 species and 9 families were examined for the presence of myxosporean parasites. This paper reports on the occurrence of seven myxosporeans of the genus Myxobolus Bütschli, 1882 found infecting eight different host fish species in the Okavango River and Delta, Botswana.

MATERIALS AND METHODS

Fieldwork was conducted in the Okavango River and Delta in Botswana during June and July in both 1998 and 1999. Fishes were collected using hand nets, cast nets, sein nets and a series of gill nets from mainstream and lagoon environments. Live fishes were taken back to a mobile field laboratory where they were kept in aerated aquaria. The fishes were identified and anaesthetised with a dosage of benzocaine sufficient to kill them. Standard techniques when working with myxosporeans requires the observation and photography of live spores, but due to the isolated collection localities mature myxosporean spores found in plasmodia were fixed in 10% buffered neutral formalin. Due to the formalin fixation of the spores, some structures could not be observed in the material, such as intercapsular appendices and iodinophilous vacuoles. Since most of the plasmodia in the present study were very small, spores from a number of plasmodia were measured. However, in all cases the plasmodia were obtained from the same host specimen. The fixed spores were photographed using a Zeiss Axioskop microscope with differential interference contrast on a layer of 0.5% non-nutrient agar and were measured according to the guidelines provided by Lom and Arthur (1989). Minimum and maximum values of spore measurements are provided in micrometres (µm), followed in parentheses by the arithmetic mean and standard deviation. Permanent preparations were made by impregnating myxospore spores with silver nitrate. Myxoboli described in the
present study have only been compared to African species, as the Okavango River, which forms part of the Upper Zambesi System, is a pristine habitat with no introductions or translocations of fishes. 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 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.

RESULTS

*Myxobolus africanus* Fomena, Bouix et Birgi, 1985, *M. camerounensis* Fomena, Marquès et Bouix, 1993, *M. hydrocyni* Kostoïngue et Toguebaye, 1994, *M. nyongana* (Fomena, Bouix et Birgi, 1985) and *M. tilapiae* Abolarin, 1974 were all collected for the first time in Botswana. Two species that did not conform to the description of any known African species were also collected and are subsequently described as *M. etsatsaensis* sp. n. and *M. paludinosus* sp. n.

*Myxobolus africanus* Fomena, Bouix et Birgi, 1985 Figs. 1, 8, 15

**Description of vegetative stages:** Only sporogenic plasmodia found within secondary gill lamella. Polysporous plasmodia spherical, whitish, 1 mm in diameter.

**Description of spores** (based on 11 spores from fully mature plasmodia): In valvular view, spore body slightly elongate to ovoid with anterior end bluntly pointed and posterior end rounded, 16.2-17.5 (16.7 ± 0.67) in length. Two rounded pyriform polar capsules of equal size situated in anterior part of spore measuring 6.8-7.5 (7.3 ± 0.32) long × 3.8-3.6 (3.6 ± 0.42) wide. Five to six coils visible in polar filament.

**Hosts:** *Oreochromis andersonii* (Castelnau, 1861), *Tilapia ruweti* (Poll et Thys van den Audenarde, 1965). **Site of infection:** Gill arch and buccal cavity. **Locality:** Xaro and Etsatsa Mainstreams in the Okavango River and Delta (Botswana).

**Material examined:** 1998/09-08-04 (spores from *O. andersonii* fixed in 10% buffered neutral formalin) and 1998/07-24-25 (spores from *T. ruweti* fixed in 10% buffered neutral formalin).

**Remarks:** This species conforms to the description of *M. camerounensis* originally described by Fomena et al. (1993) from the gills of *Oreochromis niloticus* in Cameroon. There are currently 11 *Myxobolus* species parasitising cichlids in Africa (Baker 1963, Abolarin 1974, Landsberg 1985, Faisal and Shalaby 1987, Sakiti al. 1991, Fomena et al. 1993). *Myxobolus camerounensis* is most similar to *M. homeosporus* Baker, 1963, but differs in having ovoid spores that are not slightly elongated. The polar capsules of *M. homeosporus* are also slightly smaller and thus although the spore dimensions are similar, the polar capsules of *M. camerounensis* take up more space in the spore body.

The presence of *M. camerounensis* on the gill arches and buccal cavities of *O. andersonii* and *T. ruweti* provide two new host records for this myxosporean species. These records increase the number of fish hosts infected by *M. camerounensis* to three, all of which are cichlids. The presence of this species in the Okavango River and Delta in Botswana is also a new geographic record for the species.

*Myxobolus homeosporus* Baker, 1963

**Description of vegetative stages:** Sporogenic plasmodia found within epithelial cells of gill operculum as well as in gill arch cartilage. Polysporous plasmodia spherical, whitish, 1 mm in diameter.
Figs. 1-7. Myxobolus Bützchli, 1882 species collected from the Okavango River and Delta, Botswana; microscope projection drawings of formalin-fixed spores. Fig. 1. Myxobolus africanus Fomena, Bouix et Birgi, 1985 from the gills and fins of Hepsetus odoe (Bloch, 1794). Fig. 2. Myxobolus camerounensis Fomena, Marqués et Bouix, 1993 from the gill arch of Oreochromis andersonii (Castelnau, 1861). Fig. 3. Myxobolus hydrocyi Kostoïngue et Toguebaye, 1994 from the gills of Hydrocynus vittatus Castelnau, 1861. Fig. 4. Myxobolus nyongana (Fomena, Bouix et Birgi, 1985) from the gills of Barbus poechii Steindachner, 1911. Fig. 5. Myxobolus cf. tilapiae Abolarin, 1974 from the buccal cavity of Tilapia rendalli rendalli (Boulenger, 1896). Fig. 6. Myxobolus etsatsaensis sp. n. from the gills of Barbus thalakamensis Fowler, 1935. Fig. 7. Myxobolus paludinosus sp. n. from the gills of Barbus paludinosus Peters, 1852. Scale bar = 10 µm.
Description of spores (based on 10 spores from fully mature plasmodia): Spore body ovoid in valvular view, with anterior and posterior end rounded, 8.7-10.1 (9.9 ± 0.38) in length. Widest region of spore observed towards posterior ends of polar capsules, 6.2-7.5 (7.2 ± 0.39) in width. Two shell valves visible with sutural ridge passing around edge of spore. Shell valves smooth with two slender polar capsules of equal size situated anteriorly, 3.7-5 (4.5 ± 0.63) long × 1.0-1.2 (1.2 ± 0.13) wide. Number of coils in polar filament within polar capsules difficult to observe.

Host: *Hydrocynus vittatus* Castelnau, 1861.
Site of infection: Gill operculum and gill arch.
Locality: Xaro and Etsatsa mainstreams, Okavango River and Delta (Botswana).
Material examined: No. 1999/07/08-14 (spores fixed in 10% buffered neutral formalin).

Remarks: The overall spore morphology and dimensions of this species conforms to that of *Myxobolus hydrocyni* originally described from the gills of *Hydrocynus forskalii* in Chad by Kostoïngue and Barbus *Hydrocynus vittatus* Castelnau, 1861.

Description of vegetative stages: Sporogonic plasmodia found within secondary gill lamellae. Polysporous plasmodia small and rounded, whitish, 0.5 mm in diameter.

Description of spores (based on 12 spores from fully mature plasmodia): In valvular view, spor body is teardrop-shaped to ovoid with anterior tapering to blunt point, 11.0-11.2 (11.2 ± 0.26) in length. Widest region of spore observed towards centre of sporoplasm, 6.1-7.0 (6.5 ± 0.31) in width. Two smooth shell valves visible as well as narrow sutural ridge, which is slightly broader at posterior end of spore. Two polar capsules of occasionally unequal size situated in anterior part of spore, 3.0-5.5 (4.4 ± 0.79) long × 1.25-2.5 (1.6 ± 0.44) wide. Polar filament coils seven times within polar capsules.

Host: *Barbus poechii* Steindachner, 1911.
Site of infection: Secondary gill lamellae.
Locality: Etsatsa Mainstream, Okavango River and Delta (Botswana).
Material examined: 1999/07/08-13 (spores fixed in 10% buffered neutral formalin).

Remarks: The morphology of these spores conforms to the description of *M. nyongana*, which was originally described from the gills of *Barbus jae* by Fomena et al. (1985). Other *Myxobolus* species found in *Barbus* hosts in Africa are *M. njinei* Fomena, Bouix et Birgi, 1985, *M. nkolyaensis* Fomena et Bouix, 1994 and *M. oloi* Fomena et Bouix, 1994. *Myxobolus nyongana* differs from these three in the following ways. *Myxobolus njinei* has a spherical to ovoid spore with rounded posterior and anterior ends. The spores are also much larger than the spores of *M. nyongana*. *Myxobolus nkolyaensis* has an almost spherical spore with sub-spherical polar capsules, as well as a reduced sporoplasm and occasionally a third polar capsule in the spore body (Fomena and Bouix 1997). *Myxobolus oloi* has an oval spore body with asymmetric polar capsules containing four to five coils in the polar filaments and these spores are also smaller in overall dimension to that of *M. nyongana*.

This represents both a new geographical and host record for *M. nyongana* found in the gills of *B. poechii* in Botswana.

*M. nyongana* (Fomena, Bouix et Birgi, 1985) Fomena et Bouix, 1997 Figs. 4, 11, 17
Syn.: *Myxobolus barbi* Fomena, Bouix et Birgi, 1985

Description of vegetative stages: Sporogonic plasmodia found within the buccal cavity. Polysporous plasmodia, rounded, whitish, 0.5 mm in diameter.

Description of spores (based on 10 spores from fully mature plasmodia): In valvular view, spor body oblong to oval with anterior and posterior ends bluntly rounded, 14.0-15.5 (15.0 ± 0.39) in length. Widest region of spore observed towards centre of spor body, 12.0-12.6 (12.3 ± 0.27) in width. Two smooth shell valves visible with narrow sutural ridge surrounding spore. Two almost spherical to pyriform polar capsules of equal size situated in anterior part of spore, 3.8-5.0 (4.6 ± 0.55) long × 3.0-4.0 (3.5 ± 0.44) wide. Polar filaments have four to six coils within polar capsules.

Host: *Tilapia rendalli* rendalli (Boulenger, 1896).
Locality: Samochina Lagoon, Mohembo Floodplains, Okavango River and Delta (Botswana).
Site of infection: Buccal cavity.
Material examined: 1998/06/21-25 (spores fixed in 10% buffered neutral formalin).

Remarks: This species is preliminarily identified as *Myxobolus cf. tilapiae*. Abolarin (1974) provides various different drawings of this species, of which only
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Figs. 8-14. Myxobolus Bütschli, 1882 species collected from the Okavango River and Delta, Botswana; differential interference contrast micrographs of formalin-fixed spores. 

**Fig. 8.** Myxobolus africanus Fomena, Bouix et Birgi, 1985 from the gills and fins of *Hepsetus odoe* (Bloch, 1794).

**Fig. 9.** Myxobolus camerounensis Fomena, Marqués et Bouix, 1993 from the gill arch of *Oreochromis andersonii* (Castelnau, 1861).

**Fig. 10.** Myxobolus hydrocygni Kostoiingue et Toguebaye, 1994 from the gills of *Hydrocynus vittatus* Castelnau, 1861.

**Fig. 11.** Myxobolus nyongana (Fomena, Bouix et Birgi, 1985) from the gills of *Barbus poecilii* Steindachner, 1911.

**Fig. 12.** Myxobolus cf. *tilapiae* Abolarin, 1974 from the buccal cavity of *Tilapia rendalli rendalli* (Boulenger, 1896).

**Fig. 13.** Myxobolus etsatsaensis sp. n. from the gills of *Barbus thamalakanensis* Fowler, 1935.

**Fig. 14.** Myxobolus paludinosus sp. n. from the gills of *Barbus paludinosus* Peters, 1852. Scale bars = 10 µm.

one resembles the species collected from the Okavango in Botswana. More material from the type host and type locality of *M. tilapiae* would have to be examined to determine whether this species does show such great variation. *Myxobolus tilapiae* is similar to *M. heterosporus* (Baker, 1963) type (I) in overall spore shape. The polar capsules of *M. heterosporus* are, however, more pyriform, compared with the more spherical polar capsules of *M. tilapiae*. *Myxobolus tilapiae* is similar to *M. polycentropsi* Fomena, Bouix et Birgi, 1985 and *M. synodonti* Fomena, Bouix et Birgi, 1985, parasites of *Polycentropsis abbreviata* and *Synodontis batesii* respectively. The former myxosporean species, *M. polycentropsi*, is similar to *M. tilapiae* in having anterior and posterior ends that are both bluntly rounded. The polar capsules of *M. polycentropsi* are, however, more pyriform (Fomena et al. 1985), compared to the almost spherical ones in *M. tilapiae*. Finally, *Myxobolus synodonti* is distinct from *M. tilapiae* in having the anterior end slightly more tapered than the more rounded posterior end. The polar capsules of *M. synodonti* are much larger and elongated, compared to the more spherical polar capsules of *M. tilapiae*.

This represents both a new geographical and host record for *M. tilapiae*.

**Myxobolus etsatsaensis** sp. n. Figs. 6, 13, 19

**Description of vegetative stages:** Polysporous plasmodia found within secondary gill lamellae, whitish, very small and rounded.

**Description of spores** (based on 9 spores from fully mature plasmodia): In valvular view, spores extremely elongated, pyriform to teardrop-shaped, with anterior end tapering sharply to blunt point and posterior end rounded, 12.8-15.0 (13.0 ± 0.94) in length. Two smooth
shell valves visible. Sutural ridge passes around entire spore and is slightly broader at the posterior end. Two extremely elongated, pyriform polar capsules of unequal length, situated almost parallel to one another in anterior half of spore, 7.0-8.0 (7.5 ± 0.35) long × 1.25-2.5 (2.3 ± 0.43) wide. Polar filaments contain seven to eight coils in polar capsules. Widest part of spore observed towards posterior of polar capsules, 6.2-8.0 (6.8 ± 0.65) in width. A small sporoplasm situated in posterior half of spore.

_Ty pe_ host: _Barbus thamalakanensis_ Fowler, 1935.

_Site of infection:_ Secondary gill filaments.

_T ype m ater i al:_ Holotype, spores in 10% neutral buffered formalin, 1998/07/25-22A (NMBP 221) and paratypes, spores in 10% neutral buffered formalin, 1998/07/25-22B (NMBP 274) and 1998/07/25-22C (NMBP 275) in the collection of the National Museum, Bloemfontein, South Africa.

_Re marks:_ As already mentioned, four _Myxobolus_ species, namely _M. njinei_ Fomena, Bouix et Birgi, 1985, _M. nkolyaensis_, _M. oloi_, and _M. oliv_., have been described from _Barbus_ hosts in Africa. _Myxobolus etsatsaensis_ can easily be distinguished from these species because of its characteristic elongated to pyriform spore body. The closest resemblance is with _M. nyongana_ described by Fomena et al. (1985) from the gills of _Barbus jae_. _Myxobolus etsatsaensis_ has a much less extended spore body.

Other morphological similarities are found with _M. amieti_ Fomena, Bouix et Birgi, 1985, _M. beninensis_ Sakiti, Blanc, Marquès et Bouix, 1991, _M. kriebiensis_ Fomena et Bouix, 1994 and a _Myxobolus_ sp. described by Obiekezie and Okaeme (1990). Compared to _M. amieti, M. etsatsaensis_ has a very slender spore body that tapers to a sharper degree, forming a narrow anterior end. Furthermore, the polar capsules of _M. etsatsaensis_ are more elongated and almost parallel to one another. _Myxobolus etsatsaensis_ differs from _M. beninensis_, found in the gills of _Sarotherodon melanotheron_ by Sakiti et al. (1991), in having a more slender and elongated spore body with a more pointed anterior end. The polar capsules of _M. beninensis_ also take up more than half of the spore body, but are slightly more voluminous and are equal in size.

_Myxobolus etsatsaensis_ is very similar to _M. kriebiensis_ which was found in various organs of _Brycinus longipinnus_ by Fomena and Bouix (1994), also having very elongated spores. The anterior end of _M. etsatsaensis_ is, however, much narrower and tapers to a sharper degree to form a blunt point. The anterior end of _M. kriebiensis_ is also narrow, but does not taper to the same degree. The polar capsules of _M. kriebiensis_ are very voluminous and fill just about the entire spore body and have between 19 and 28 coils in the polar filament. The spores of _M. kriebiensis_ are also much larger than _M. etsatsaensis_. _Myxobolus etsatsaensis_ is very similar to a _Myxobolus_ sp. described by Obiekezie and Okaeme (1990) from the kidneys and spleen of various cichlid species, but there are differences in the spore sizes as well as the shell valve thickness.

_Myxobolus paludinosus_ sp. n. Figs. 7, 14

_Description of vegetative stages:_ Sporogonic plasmodia found within secondary gill lamellae. Poly-sporous plasmodia, small, rounded, whitish, 0.3 mm in diameter.
Description of spores (based on 10 spores from fully mature plasmodia): In valvular view, spore body pyriform to ovoid with anterior end tapering to blunt point and posterior end rounded, 11.2-13.7 (12.0 ± 0.87) in length. Widest region of spore observed towards posterior ends of polar capsules, 7.5-10.0 (8.6 ± 0.75) in width. Two smooth shell valves visible with sutural ridge along edge of spore, becoming broader posteriorly. Two polar capsules of equal size situated in anterior end of spore, 5.0-6.8 (5.7 ± 0.88) long × 2.0-2.5 (2.4 ± 0.21) wide. Polar filaments have six to seven coils within polar capsules. Sporoplasm situated in posterior half of spore.

Type host: Barbus paludinosus Peters, 1852.

Site of infection: Secondary gill lamellae.

Type material: Holotype, slide 1999/07/05-11 (NMBP 24) and paratypes, spores in 10% neutral buffered formalin, 1999/07/03-06A (NMBP 25), 1999/07/03-06B (NMBP 220) in the collection of the National Museum, Bloemfontein, South Africa.

Remarks: Myxobolus paludinosus does not conform to the description of any other Myxobolus species described in Africa. When compared to those found parasitising Barbus hosts in Africa the following differences can be found. Myxobolus paludinosus is distinct from M. njinei described by Fomena et al. (1985), in having an anterior end that tapers to a blunt point and polar capsules that are completely spherical. Myxobolus paludinosus differs from M. nkolyaensis in that the latter species also has an almost spherical shape, with sub-spherical polar capsules. The spore dimensions of M. nkolyaensis are smaller than that of M. paludinosus. Myxobolus nyongana is similar to M. paludinosus in having a spore body that tapers anteriorly to a blunt point with a rounded posterior end, but the spores of M. paludinosus are not as slender as those of M. nyongana. The polar capsules of M. paludinosus do not lie parallel to one another, as in the case of M. nyongana. Myxobolus paludinosus is distinct from M. oloi as the latter species has an almost entirely spherical body with two unequal polar capsules.

Myxobolus paludinosus is overall similar to M. amieti described by Fomena et al. (1985), but differs, since the latter has a more slender, pyriform spore, with slender polar capsules that take up two thirds of the spore body. Although having a similar spore shape, M. paludinosus is distinct from M. beninensis in that the latter species has two polar capsules that take up two thirds of the spore body. The spores of M. paludinosus are also slightly wider than those of M. beninensis. Myxobolus paludinosus is very similar to M. israelensis Landsberg, 1985, in having similar spore dimensions, but the anterior end of the latter species is more rounded than the anterior end of the former species. The polar capsules of M. israelensis also take up more space in the spore body, leaving little place for the sporoplasm (Landsberg 1985). Myxobolus paludinosus appears to conform to the description of Myxobolus sp. 2 (Fomena et al. 1985), but there are differences in spore sizes.

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