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

Cecilé C. Reed, Linda Basson and Liesl L. Van As

Department of Zoology and Entomology, University of the Free State, P.O. Box 339, Bloemfontein, 9300, South Africa

Key words: Myxozoa, Myxobolus, new species, taxonomy, fish parasites, Botswana

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 thamalakanensis Fowler, 1935 and M. paludinosus sp. n. from Barbus thamalakanensis Fowler, 1935 and M. paludinosus Peters, 1852 are described. Myxobolus africanus Fomena, Bouix et Birgi, 1985, M. camerounensis Fomena, Marquès et Bouix, 1993, M. hydrocyni Kostóíngue et Toguébaye, 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 Boulenger (1911) who published a brief note on an anabantid showing a mouth-brooding habit from the Okavango River. According to Peters (1971), Boulenger 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 plasmidia 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 plasmidia 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 plasmidia in the present study were very small, spores from a number of plasmidia were measured. However, in all cases the plasmidia were obtained from the same host specimen. The fixed spores were photographed using a Zeiss Axiophot 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 sporogonic 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 ± 0.5) in length. Widest region of spore observed behind polar capsules, 11.2-12.5 (11.6 ± 0.60) in width. Two smooth shell valves visible with narrow sutural ridge passing along edge of spore. Two rounded pyriform polar capsules of equal size situated in anterior part of spore measuring 6.8-7.5 (7.3 ± 0.32) long × 3-3.8 (3.6 ± 0.42) wide. Five to six coils visible in polar filament.

Hosts: Oreochromis niloticus (Castelnau, 1861), Tilapia ruweti (Poll et Thys van den Audenaerde, 1965).

Site of infection: Gill arch and buccal cavity.

Locality: Xaro and Etsatsa Mainstreams in the Okavango River and Delta (Botswana).

Material examined: 1998/08/09-04 (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 et 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 camerounensis Fomena, Marquès et Bouix, 1993  Figs. 2, 9

Description of vegetative stages: Sporogonic plasmodia found in epithelium of buccal cavity and gill arch of host. Polysporous plasmodia, spherical to elongate, whitish, 1-3 mm in length.

Description of spores (based on 10 spores from 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 ± 0.5) in length. Widest region of spore observed behind polar capsules, 11.2-12.5 (11.6 ± 0.60) in width. Two smooth shell valves visible with narrow sutural ridge passing along edge of spore. Two rounded pyriform polar capsules of equal size situated in anterior part of spore measuring 6.8-7.5 (7.3 ± 0.32) long × 3-3.8 (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 Audenaerde, 1965).

Site of infection: Gill arch and buccal cavity.

Locality: Xaro and Etsatsa Mainstreams in the Okavango River and Delta (Botswana).

Material examined: 1998/08/09-04 (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 et 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 hydrocyni Kostoïngue et Toguebaye, 1994  Figs. 3, 10, 16

Description of vegetative stages: Sporogonic plasmodia found within epithelial cells of gill operculum as well as in gill arch cartilage. Polysporous plasmodia spherical, whitish, 1 mm in diameter.
Reed et al.: *Myxobolus* species from Botswana

Figs. 1-7. *Myxobolus* Bütschli, 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 hydrocyni* 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 thamalakanensis* 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 Hydrocyamus forskalii in Chad by Kostinougue and Toguebaye (1994). Myxobolus hydrocyni is similar in shape to M. amieti Fomena, Bouix et Birgi, 1985 which was found in various organs of Ctenopoma namum by Fomena et al. (1985). These species both have two polar capsules that do not lie parallel to each other, but only converge in the anterior half of the spore. The spores of M. hydrocyni differ in being more spherical than the slender, more elongated spores of M. amieti. The polar capsules of M. amieti extend through more than half of the spore body, which differs from those of M. hydrocyni that reach only the centre of the spore. Having a similar spore body shape to M. noukouensis Sakiti, Blanc, Marquès et Bouix, 1991, M. hydrocyni is distinct in having polar capsules that extend to the centre of the spore and are neither short nor pyriform.

Myxobolus hydrocyni is recorded for the first time in Botswana.

Myxobolus nyongana (Fomena, Bouix et Birgi, 1985) Fomena et Bouix, 1997

Host: Pseudoplatystoma squamiferum (Boulenger, 1896).

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.

Myxobolus cf. tilapiae Abolarin, 1974 Figs. 5, 12, 18

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, spore 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: 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
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 hydrocyni Kostoïngué 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 poechii 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 Polycentropis 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.

**Type host:** Barbus thamalakanensis Fowler, 1935.

**Site of infection:** Secondary gill filaments.

**Type locality:** Etsatsa Mainstream (18°51′47.0″S; 22°25.5′06″E) Okavango River and Delta (Botswana).

**Etymology:** Named after the original collection locality of the type host.

**Type material:** 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.

**Remarks:** As already mentioned, four Myxobolus species, namely *M. niinei* Fomena, Bouix et Birgi, 1985, *M. nkolyaensis*, *M. nyongana* and *M. oloi*, 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 thamalakanensis* Fowler, 1935. Scale bars = 10 µm.

**Myxobolus etsatsaensis** sp. n. from the gills of *Barbus thamalakanensis* Fowler, 1935. Scale bars = 10 µm.

**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 locality:** Etsatsa Mainstream (18°51′47″S; 22°25′5″06″E), Okavango River and Delta, (Botswana).

**Etymology:** Named after the type host.

**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 in the latter species there is a more slender, almost entirely 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.

**Acknowledgements.** This study was funded by the Debswana Diamond Company, Botswana as well as the National Research Foundation (NRF) of South Africa. Sincere gratitude to Prof. Iva Dyková from the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, for her assistance during the preparation of the draft copy.

**REFERENCES**


Received 9 July 2001  Accepted 14 December 2001