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Myxosporean infection of Grey Mullet in the Ebro Delta: Identification and Ultrastructure of *Myxobolus ichkeulensis* Bahri & Marques, 1996 Infecting the Gills of *Mugil cephalus* L.

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Summary. The ultrastructural characteristics of the sporogenesis of *Myxobolus,* which infect the brachial arches of *Mugil cephalus,* is presented. The prevalence of infection was 52.7%. The ultrastructural features of the *Myxobolus* species studied in general comply with known features of this genus. Based on the ultrastructural morphology and specificity to the host organ, we conclude that this species is *Myxobolus ichkeulensis* (Bahri and Marques 1996).

Key words: fish parasite, aquaculture, Myxozoa, ultrastructure, *Myxobolus ichkeulensis*, *Mugil cephalus.*

INTRODUCTION

The *Mugilidae* are widely distributed, particularly so *Mugil cephalus*. They are euryhaline fish that are found around the globe. They have been used for centuries as a source of food for humans in different parts of the world. They are intensively and semi-intensively farmed throughout the Mediterranean, from the Spanish coast as far as Israel (Merella and Garippa 2001). *M. cephalus* ovaries produces botargo (salted and dried grey mullet roes), a product of great economic value, known as the 'caviar of the Mediterranean.' This justifies the considerable interest in farming this species. The importance of mullet for aquaculture and the pathologic potential of some parasites, in particular Myxosporea, motivates their detailed study.

Fish constitute a favourite biotope of a large number of organisms, including Myxosporea (Lom and Dyková 1992, J. Kent *et al*. 2001). Myxosporean epizootics are believed to be a direct cause of fish mortality (Lom and Dyková 1995, Rigos *et al*. 1999, Brown and Bruno 2006).

Site selection and the specific location of Myxozoa infecting the gills have been studied in detail by Molnár (2002). Apparently, the gills are the primary and most

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common site of infection for most parasites, though they can be found at other sites: gill arches, kidneys, fins, gall bladder, eyes, swimbladder and in various other tissues.

The genus *Myxobolus* is the largest group of Myxosporea, and its members are important pathogens of freshwater and marine fish in several geographical areas (Eiras *et al*. 2005).

Studies of the ultrastructure of Myxosporea allow the different species to be precisely identified. Studies of the ultrastructure of *Myxobolus* genus include, among others: Mitchell *et al.* (1985), Bahri and Marques (1996), Fall *et al*. (1997), Ali *et al.* (2003), Abdel-Ghaffar *et al*. (2005), Ali *et al.* (2007), Adriano *et al.* (2009), Azevedo *et al.* (2010).

Here, for the first time, we present both, light and electron microscope data, concerning a myxosporean species found infecting the gills of the teleostean fish, *M. cephalus*. Some ultrastructural aspects of the plasmodium, developmental stages and capsulogenic processes are described and discussed. This work forms part of ongoing research into parasites from fish of interest for aquaculture in NE Spain.

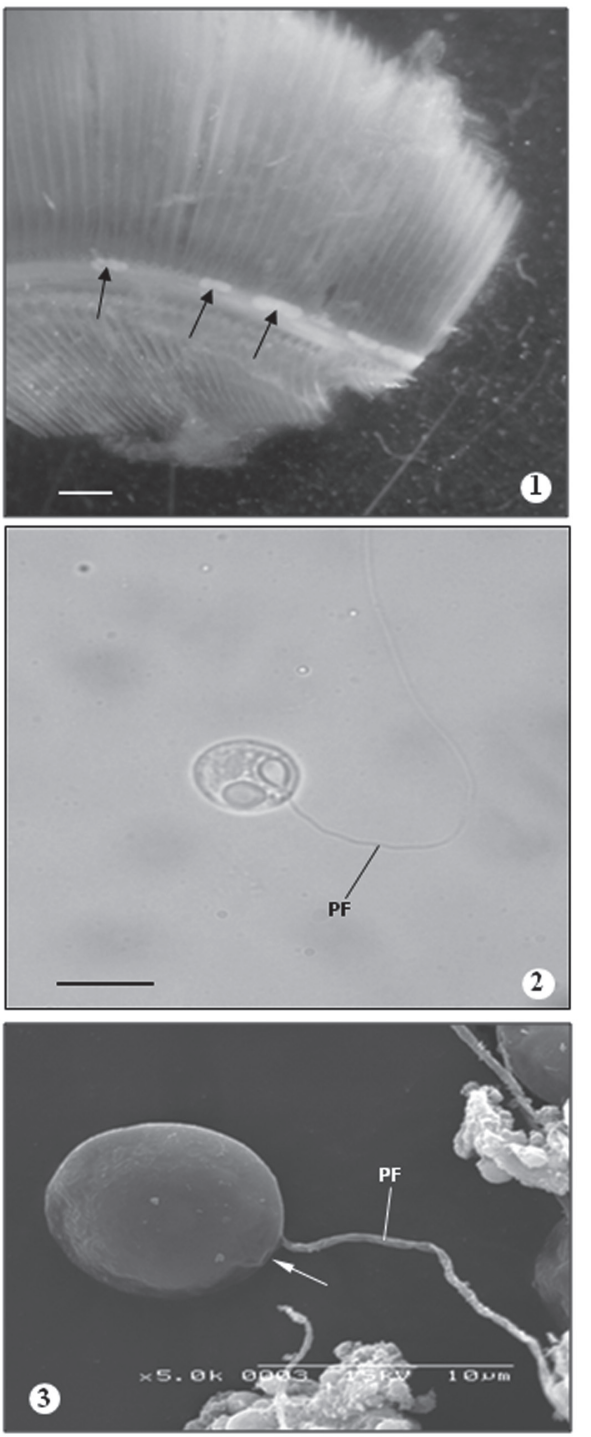
MATERIAL AND METHODS

A total of 192 specimens of grey mullet (mean length: 17.4 ± 1.3 cm; mean weight: 50.6 ± 11.0 g) were collected periodically from a brackish coastal lagoon known as *l'Encanyissada*, within the Ebro Delta River (this lagoon was selected as representative of coastal lagoons in NE Spain). Each hemibranch was excised and checked for myxozoan plasmodia under a stereo microscope. Plasmodia infecting the gill arches (gill arch type, Molnar 2002) were carefully removed from the tissues and studied in fresh smears under a compound microscope, or processed for ultrastructural studies (both transmission – TEM – and scanning – SEM – electron microscopy) following Gracia *et al.* (1997). Spore measurements were taken as described in Lom and Arthur (1989). Prevalence was used according to Bush *et al.* (1997).

RESULTS

Gill infection by a species of Myxosporea was found in 52.78% of the examined fish. Parasite formed elongate whitish cyst masses (1–4 mm in length) distributed at the base of the gill arches (Fig. 1). The number of cysts found on each fish varied from 3 to 11.

Asynchronous histozoic plasmodia containing several developmental stages of the parasite in the endo-



Figs 1–3. *Myxobolus ichkeulensis*. Life cycle stages of the parasite from the gill arches of *Mugil cephalus*. **1** – Plasmodia of *Myxobolus ichkeulensis* developed on the surface of the gill arch (arrow). Fresh preparation. Scale bar: 3 mm; **2** – fresh mature spore of *Myxobolus ichkeulensis* released from a plasmodium. Frontal view. Note the discharged polar filament (PF). Scale bar: 10 µm; **3** – scanning electron micrograph of the spore. Note the elongated shape of the discharging orifice of the polar filament (arrow) and a discharged polar filament (PF). SEM. Scale bar: 10 µm.

plasm were observed (generative cells, immature and mature spores) (Fig. 4). TEM revealed that polysporous plasmodia present an external layer of connective tissue (host tissue) in contact with a single plasmodial membrane (Fig. 4).

Spores were typical of the genus *Myxobolus* Bütschli, 1882, as they were rounded, almost spherical in valvular view and biconvex in sutural view, and the shell valves were smooth with no projections (Figs 2–3). They measured 10.5–11.3 µm in length and 9.0–11.0 µm in width. No mucus envelope was observed at the surface of the spore. The spores consisted of two equally dense valves adhering together along the suture line forming the wall (Figs 5–9). Spores had from nine to eleven sutural marks along the sutural edge. Internally, two capsulogenic cells, located side by side, contained prominent, oval, equally smooth polar capsules, located symmetrically about the spore axis, and measuring about 3.28 µm × 2.09 µm. Inside the polar capsules, a polar filament displayed 4 or 5 coils. Polar capsules did not extend past the mid-length of the spore. No intercapsular appendix was present. At the posterior pole of the spore, a binucleated sporoplasm contained numerous electron-dense vesicles.

Based on the ultrastructural morphology and specificity to the host organ we concluded that this species is *Myxobolus ichkeulensis* Bahri and Marques, 1996.

DISCUSSION

The *Myxobolus* species studied is histozoic, and colonises the cartilage of the gill arch of *M. cephalus*. The genus *Myxobolus* Bütschli, 1882 is mainly represented by gill histozoic Myxosporea (Sakiti *et al*., 1991), which can cause different pathogenic effects, due to weakening of the cartilaginous tissues of the gill arches. It is important to specify the location within the gill precisely, as first pointed out by Paperna (1973).

Myxobolus species belonging to the group of fish gill parasites, are characterised by strict tissue specificity (Molnár 2002), so that each of them can always be collected from its characteristic location. Variation in the host response is related to the site of infection (Egusa *et al*. 1989). Similar spores can occur in the same tissues of different hosts.

There are 744 nominal species of *Myxobolus* listed (Eiras *et al*. 2005). Of these, 21 species have been described as infecting different organs of *M. cephalus* (Eiras and D'Souza 2004). The species of the genus *Myxobolus* described account for about 55.11% of the Myxosporea. There have been numerous synonymies within the genus *Myxobolus* because of the morphological similarities between many species (Lom and Arthur 1989).

The specimens studied were first compared with all the *Myxobolus* species described for *M. cephalus.* The identification of *Myxobolus* species is principally based on spore morphology. The material present was also compared with the full characteristics of species of *Myxobolus* representing nearly all the species described to date (Eiras *et al*. 2005).

Comparison of the plasmodium characteristics and sporogenesis of the life-cycle stages of this species to those of other *Myxobolus* spp. also revealed morphologic and ultrastructural similarities. The mature spores obtained from gill arches of *M. cephalus* revealed morphological similarities to those of the genus *Myxobolus* Bütschli, 1882 (family Myxobolidae).

Myxobolus species spores originate from uninuclear generative cells and have valve cells, capsule cells and sporoplasm (Lom and de Puytorac 1965, Fomena 1995, Bahri and Marques 1996). We compared our species with descriptions of other *Myxobolus* species paying particular attention to spore size and number of coils of the polar filament. Comparing these results with those obtained for different *Myxobolus* spp. particularly with the twenty one species described in *M. cephalus*, we observed several morphological similarities with *M. ichkeulensis*: based on these similarities, and the ultrastructural morphology and specificity to the host organ, we concluded that this species is *Myxobolus ichkeulensis* Bahri and Marques, 1996.

The histozoic species of Myxosporea are the most pathogenic and susceptible to cause important lesions that can be fatal (Shariff 1982, Lom and Dyková 1992). The parasites found in this study may represent a potential risk for fish farms. *Myxobolus* sp. can cause lesions that serve as a gateway to secondary viral and bacterial infections. Most Myxosporea are tolerated by their hosts, but some species can be responsible of epizootic diseases in wild and cultured fish.

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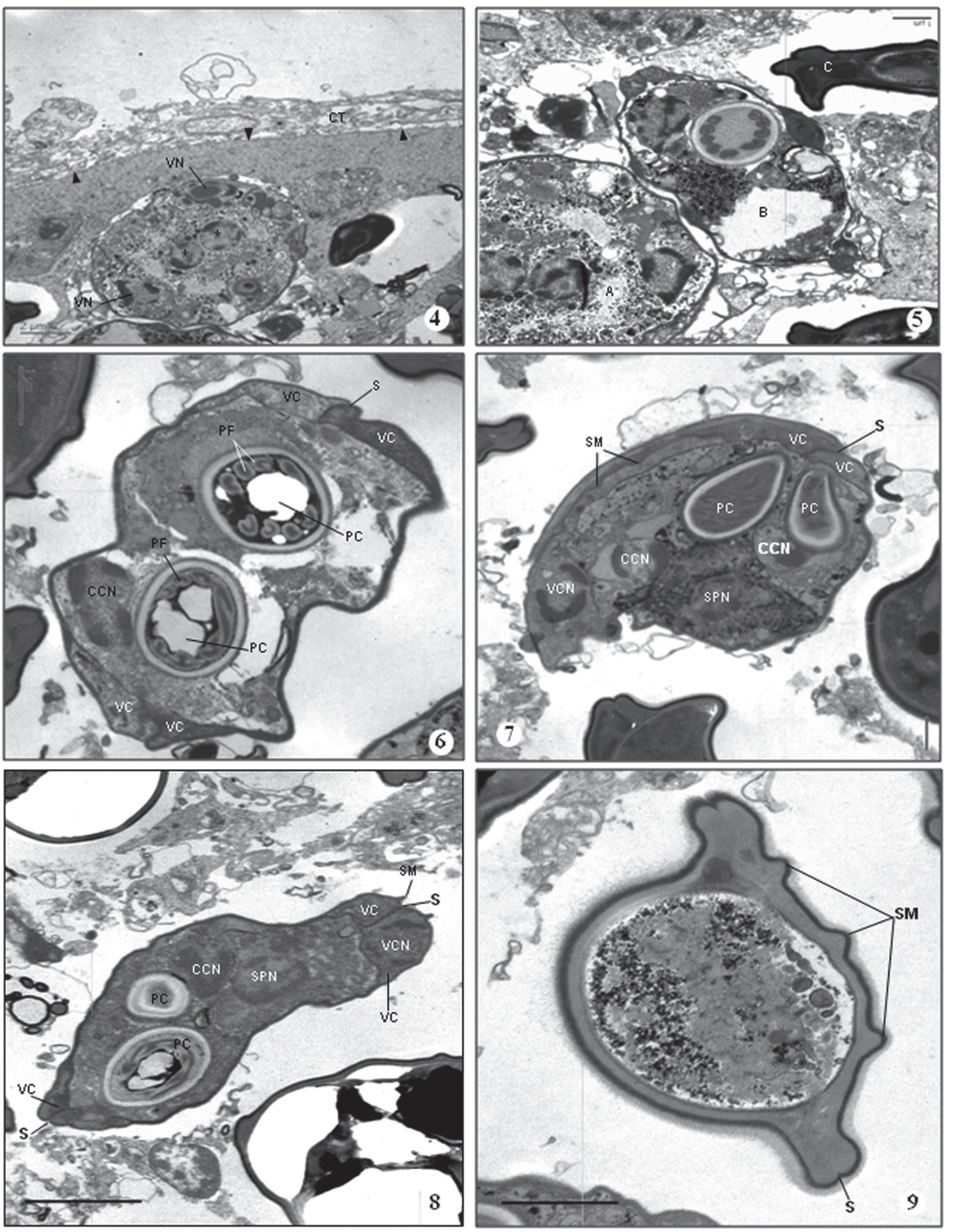

Figs 4–9. *Myxobolus ichkeulensis*. Life cycle stages of the parasite from the gill arches of *Mugil cephalus*. **4** – ultrathin section of the periphery of a plasmodium (arrowheads) showing a surrounding connective tissue layer (CT) and internally a spore in formation. Note two sporoplasm nucleus (*) and two valvogenic nucleus (VN). TEM. Scale bar: 2 μm; **5** – ultrathin section of polysporous plasmodium with spores at different stages of development. Spores in formation (A, B), mature spore (C). TEM. Scale bar: 1 μm; **6** – ultrathin transverse section of a spore. Valvogenic cells (VC), suture (S), capsulogenic cell nucleus (CCN), polar capsule (PC), polar filament (PF). TEM. Scale bar: 1 μm; **7** – ultrathin section of a mature spore with two capsulogenic cells showing valvogenic cells (VC), valvogenic cell nucleus (VCN), suture (S), capsulogenic cell nucleus (CCN), polar capsule (PC), sporoplasm nucleus (SPN) and sutural marks (SM). TEM. Scale bar: 1 μm; **8** – Ultrathin section of a mature spore. Valvogenic cells (VC), valvogenic cell nucleus (VCN), suture (S), capsulogenic cell nucleus (CCN), polar capsule (PC), sporoplasm nucleus (SPN) and sutural marks (SM). TEM. Scale bar: 2 μm; **9** – ultrathin transverse section through a mature spore. TEM. Scale bar: 2 μm.

REFERENCES

Abdel-Ghaffar F., Abdel-Baki A.-A., El-Garhy M. (2005) Ultrastructural characteristics of the sporogenesis of genus *Myxobolus* infecting some Nile fishes in Egypt. *Parasitol. Res.* **95:** 167–171

Adriano E. A., Arana S., Carriero M. M., Naldoni J., Ceccarelli P. S., Maia A. A. M. (2009) Light, electron microscopy and histopathology of *Myxobolus salminus* n. sp., a parasite of *Salminus brasiliensi* from the Brazilian Pantanal. *Vet. Parasitol*. **165:** 25–29

Ali M. A., Abdel-Baki A. S., Sakran T., Entzeroth R., Abdel-Ghaffar F. (2003) Light and electron microscopic studies of *Myxobolus stomum* n. sp. (Myxosporea: Myxobolidae) infecting the blackspotted grunt *Plectorhynicus gaterinus* (Forsskal, 1775) in the Red Sea, Egypt. *Parasitol. Res.* **91:** 390–397

Ali M. A., Abdel-Baki A. S., Sakran T., Entzeroth R., Abdel-Ghaffar F. (2007) *Myxobolus lubati* n. sp. (Myxosporea: Myxobolidae), a new parasite of haffara seabream *Rhabdosargus haffara* (Forsskal, 1775), Red Sea, Egypt: a light and transmission electron microscopy. *Parasitol. Res*. **100:** 819–827

Azevedo C., Casal G., Mendonça I., Carvalho E., Matos P., Matos E. (2010) Light and electron microscopy of *Myxobolus sciades* n. sp. (Myxozoa), a parasite of the gills of the Brazilian fish *Sciades herzbergii* (Block, 1794) (Teleostei: Ariidae). *Mem Inst Oswaldo Cruz*. **105:** 203–207

Bahri S., Marques A. (1996) Myxosporean parasites of the genus *Myxobolus* from *Mugil cephalus* in Ichkeul lagoon, Tunisia: Description of two new species. *Dis. Aquat. Org.* **27:** 115–122

Brown L. L., Bruno D. W. (2006) Infectiuos diseases of coldwater fish in fresh water. In: Diseases and disorders on finfish in cage culture, (Eds. P. T. K. Woo, D. W. Bruno, L. H. S. Lim). CAB International, Wallingford, 107–170

Bush A. O., Lafferty K. D., Lotz J. M., Shostak A. W. (1997) Parasitology meets ecology on its owns terms: Margolis *et al*. revisited. *J. Parasitol*. **83:** 575–583

Cuadrado M., Marques A., Diamant A., Sitjà-bobadilla A., Palenzuela O., Alvarez-pellitero P., Padrós F., Crespo S. (2008) Ultrastructure of *Enteromyxum leei* (Diamant, Lom, & Dyková, 1994) (Myxozoa), an Enteric Parasite Infecting Gilthead Sea Bream (*Sparus aurata*) and Sharpsnout Sea Bream (*Diplodus puntazzo*) *J. Euk. Microbiol*, **55:** 178–184

Egusa S., Jyo Y.; Oka H., et al. (1989) Skin disease of *Mugil cephalus* due to *Myxobolus* spp Myxozoa Myxobolidae. *Fish Pathol*. **24:** 59–60

Eiras J. C., D'Souza J. (2004) *Myxobolus goensis* N. Sp. (Myxozoa, Myxosporea, Myxobolidae), a parasite of the gills of *Mugil cephalus* (Osteichthyes, Mugilidae) from Goa, India. *Parasite* **11:** 243–248

Eiras J. C.; Molnár K.; Lu Y. S. (2005) Synopsis of the species of *Myxobolus* Butschli, 1882 (Myxozoa : Myxosporea : Myxobolidae). *Systematic Parasitol.* **61:** 1–46

Fall M., Kpatcha K. P., Diebakate C., Faye N., Toguebaye B. S. (1997) Observations sur des Myxosporidies (Myxozoa) du genre *Myxobolus* parasites de *Mugil cephalus* (Poissons, Téléostén) du Senegal. *Parasite* **2:** 173–180

Fomena A. (1995). Les Myxosporidies et Microsporidies des Poissons d'eau douce du Sud-Cameroun. Etude faunistique, ultra structurale et biologie. Thèse d'Etat, Univ. de Yaoundé I, Cameroun, 397 pp.

Gracia M. P., Maíllo P. A.; Amigó J. M., Salvadó H. (1997) Ultrastructural study of *Sphaeromyxa balbianii*, Thelohan 1892 (Myxozoa, Myxosporea: Bivalvulida), a parasite of *Cepola macrophthalma*, Linnaeus 1758. *Acta Protozool.* **36:** 171–179

Kent M. L., Andree K. B., Bartholomew J. L., El-Matbouli M., Desser S. S., Delvin R. H., Feist S.W., Hedrick R. P., Hoffmann R. W., Khattra J., Hallett S. L., Lester R. J. G., Longshaw M., Palenzuela O., Siddall M. E., Xizo C. X. (2001) Recent advances in our knowledge of the Myxozoa. *J. Euk. Microbiol.* **48:** 395–413

Lom J., Arthur R. (1989) A guideline for the preparation of species descriptions in Myxosporea. *J. Fish Dis.* **12:** 151–156

Lom J., de Puytorac P. (1965) Studies on the myxosporidian ultrastructure and polar capsule development. *Protistologica* **1:** 53–65

Lom J., Dyková I. (1992) Myxosporidia (Phylum Myxozoa). In: Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science, Vol. 26, Elsevier Science Publishers, Amsterdam, 159–235

Lom J., Dyková I. (1995) Myxosporea (Phylum Myxozoa). In: Fish Diseases and Disorders, Vol. 1, Protozoan and Metazoan Infections, (Ed. P. T. K. Woo). CAB International, Wallingford, 97–148

Merella P., Garippa G. (2001) Metazoan parasites of grey mullets (Teleostea: Mugilidae) from the Mistras Lagoon (Sardinia, western Mediterranean). *Scientia Marina* **65:** 201–206

Mitchell L. G., Seymour C. L., Gamble J. M. (1985) Light and electron microscopy of *Myxobolus hendricksoni* sp. nov. (Myxozoa: Myxobolidae) infecting the brain of the fathead minnow, *Pimephales promelas* Rafinesque. *J. Fish Dis.* **8:** 75–89

Molnár K. (2002) Site preference of fish myxosporeans in the gill. *Diseases of Aquatic Organisms*. **48:** 197–207

Paperna I. (1973) Occurrence of Cnidospora infections in freshwater fishes in Africa. *Bull. Inst. Fondam. Afr. Noire*, **A35:** 509–521

Rigos G., Christophilogiannis P., Yiagnisi M., Andriopoulou A., Koutsodimou M., Nengas I., Alexis M. (1999) Myxosporean Infections in Greek Mariculture. *Aquacult. Int*. **7:** 361–364

Sakiti N., Blanc E., Marques A., Bouix G. (1991) Myxosporidies (Myxozoa, Myxosporea) du genre *Myxobolus* Bütschli 1882 parasites de poissons Cichlidae du lac Nokoué au Benin (Afrique de l'Ouest). *J. Afr. Zool*. **105:** 173–186

Shariff M. (1982) *Henneguya shaharini* new species protozoa myxozoa a parasite of marble goby *Oxyeleotris Marmoratus. J. Fish Dis*. **5**: 37–46