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Myxobolus nodulointestinalis sp. n. (Myxosporea, Myxobolidae), a parasite of the intestine of *Barbus sharpeyi*

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ABSTRACT: A new myxosporean, *Myxobolus nodulointestinalis* sp. n., has been found in the intestinal walls of 2 Mesopotamian fishes, *Barbus sharpeyi* (Günther, 1874) and *B. luteus* (Heckel, 1843), in rivers of southwest Iran. Large cysts about 5 mm in diameter containing mature spores were located in the muscular layer separating the intestinal serosa from the mucosa. The cysts were branched and separated by septa. They bulged deep into the lumen of the gut and the abdominal cavity. Plasmodia showed an affinity to smooth muscle cells and were covered by a degenerated layer of muscular elements. The spores, which had an elongated oval or trapezoid shape, differed in size and morphology from the known *Myxobolus* species of other barbels.

KEY WORDS: New species · Myxozoa · *Myxobolus* · Iranian barbels · Histopathology

INTRODUCTION

The genus *Myxobolus* comprises several hundred species. Landsberg & Lom (1991) recorded 444 valid *Myxobolus* spp., the majority of which were reported from Eurasia and North America. Since that time further species of the genus have been described. Little is known about the occurrence of myxosporeans in Iran. The territory of Iran consists of 3 different faunal regions (the Aralo-Ponto-Caspian subregion of the Palaearctics, the Mesopotamian intermediate region, and the Indian great faunal region). From the northern territory of Iran, which belongs to the Aralo-Ponto-Caspian faunal region, only a single species (*Myxobolus lobatus*) has been reported from *Barbus brachicephalus* (Mokhayer 1981). From the neighbouring Central Asian territories of that region belonging to the former Soviet Union, however, numerous *Myxobolus* spp. have been described. Donec & Shulman (1984) recorded 37 *Myxobolus* spp. from barboid fishes of the Aralo-Ponto-Caspian region. Similarly, a large number of *Myxobolus* spp. are known from the Indian faunal region (Tripathi 1952, Lalithakumari 1969, Hagargi & Amoji 1981). The fauna of southeast Iran is influenced by the latter region. The southwestern region of Iran (the water basin of the Tigris River) belongs to the Mesopotamian intermediate faunal region, which is populated mostly by endemic fishes including a wide variety of *Barbus* species. Several barboid fishes, among them *Barbus sharpeyi*, are economically important species in that region. The information available about the myxosporean fauna of these fishes is scarce. The first report regarding the Tigris River basin came from Herzog (1969) who described the occurrence of *Myxobolus muelleri* and *M. oviformis*. Subsequently, Al-Salim (1986) and Rashid et al. (1989) reported the occurrence of *Myxobolus pfeifferi* in different *Barbus* species. In the neighbouring territories of Iran, Ebrahimzadeh & Kaylani (1976) and Moghainemi & Abasi (1992) recorded some *Myxobolus* spp. from the internal organs of fishes in the Karun River. A detailed description of 2 species, *Myxobolus karuni* and *M. persicus*, from the gills of *Barbus grypus* was given by Masoumian et al. (1994).

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The majority of the currently known species were described on the basis of the resistant spores without detailed information on the vegetative stages and the site of plasmodium development. It is supposed that even after the revision made by Landsberg & Lom (1991) several synonymous species have remained, while other species, presently assigned to some well-known species, might turn out to be distinct, separate species. To prevent inadequate descriptions, Lom & Arthur (1989) presented recommendations on the requirements of a proper publication. In a recent paper Molnár (1994) pointed out that the majority of myxosporeans are host-, tissue- and organ-specific parasites and that, in the future, descriptions should contain an accurate designation of the location of development.

The present paper describes a new species designated *Myxobolus nodulointestinalis* from the intestinal wall of *Barbus sharpeyi*, along with histological evidence on the location of the parasite and the pathologic effects caused by it.

MATERIALS AND METHODS

Fishes used in this study included 59 specimens of *Barbus luteus* (Heckel, 1843) 14 to 30 cm in length and 83 specimens of *B. sharpeyi* (Günther, 1874) 14 and 31 cm in length. They were collected between June and October 1993 and between May and October 1994 from Hoor-Elazim, Shadgan Marsh and 6 different stations of the Karun River in Khuzestan province of southwest Iran.

Immediately after collection, the live fish were transported to the laboratory where they were weighed and measured before being killed by transection of the spinal cord. They were then examined for myxosporean parasites macroscopically and under stereo and light microscope. Spores were obtained from mature cysts. On the average, 30 spores were measured using the dimensions recommended by Lom & Arthur (1989). Permanent preparations were made by placing a portion of the spores in glycerol-gelatin and mounting them under coverslip. The structure of the polar capsules and the iodophilous vacuole were studied by Nomarski interference microscopy.

For histological examinations, infected organs were fixed in 10% buffered formalin, then embedded in paraffin wax, cut in 5 µm thick sections, and stained with H&E and Krutsay's (1980) trichrome stain.

RESULTS

Eight specimens of *Barbus sharpeyi* (9.6%) and 9 specimens of *Barbus luteus* (15.3%) were infected by large white-yellowish *Myxobolus* cysts in the intestinal wall (Fig. 1). In 2 *Barbus sharpeyi*, 14 to 16 cysts around 5 mm in diameter were recorded. These cysts showed a branch-like structure (see Figs. 1 & 6).

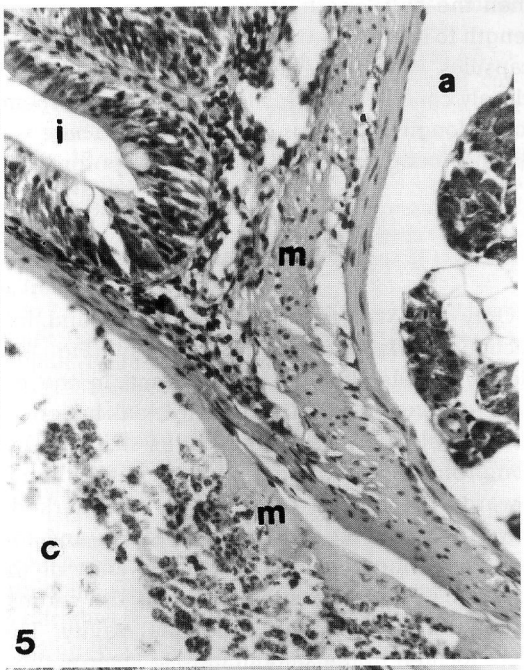
Description of the species (based on spores collected from *Barbus sharpeyi*)

Myxosporea Bütschli, 1881
Bivalvulida Schulman, 1959
Myxobolidae Thelohan, 1892
Myxobolus Bütschli, 1882
Myxobolus nodulointestinalis sp. n.
Type host: *Barbus sharpeyi* (Günther, 1874)
Additional host: *Barbus luteus* (Heckel, 1843)
Type locality: Hoor-Elazim, Shadgan Marsh and Karun River in southwest Iran.
Site of infection: Smooth muscle layer of the intestinal wall in different segments of the intestinal tract.

Description of the spores (Figs. 2 & 3)

The shape of the spore is relatively constant. It is a comparatively large, elongated ovoid, trapezoid in character, narrower at the posterior end in frontal view, lemon shaped in lateral view, with a protruding sutural edge, a distinct sutural line and small indistinct intercapsular appendix. Spore valves are symmetrical and smooth. Wall of the spore seems to be very thick, but this thickness comes from the emerging sutural edge. Spores 12.6 (11.7 to 13) µm long, 8.1 (7.8 to 9.1) µm wide and 6.3 (5.2 to 7.2) µm thick. Two polar capsules, pyriform in shape, equal in size, 3.6 (2.6 to 3.9) µm long, 2.4 (2.2 to 2.6) µm wide. Polar capsules smaller

Figs. 1 to 7. *Myxobolus nodulointestinalis* sp. n. Fig. 1. Large, branched cysts in the intestinal wall of *Barbus sharpeyi*. ×1.5. Fig. 2. Schematic illustration of the spores (a) frontal view (b) lateral view. Fig. 3. Unfixed spores. ×2000. Fig. 4. Cross section of the infected part of the gut of *B. sharpeyi*. The cyst bulging into the intestinal lumen and into the abdominal cavity contains a mass of spores. Krutsay's trichrome stain, ×30. Fig. 5. Cyst in the smooth muscle layer of the intestinal wall of *B. sharpeyi*. Intestinal lumen (i), abdominal cavity (a), cyst (c), muscle layer (m). H&E, ×120. Fig. 6. Closely associated, 'branched' cysts in the intestinal wall of *B. sharpeyi*. H&E, ×30. Fig. 7. Part of the intestinal wall of *B. sharpeyi* covering the plasmodium. Intestinal mucosa (m), propria (p), degenerated muscle layer (mu). H&E, ×500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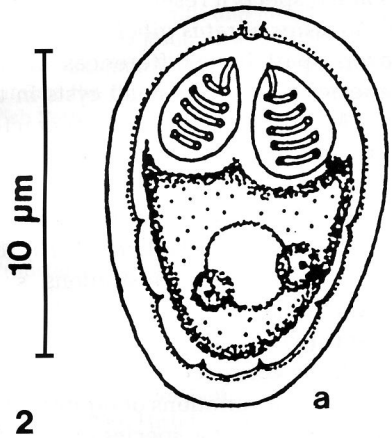
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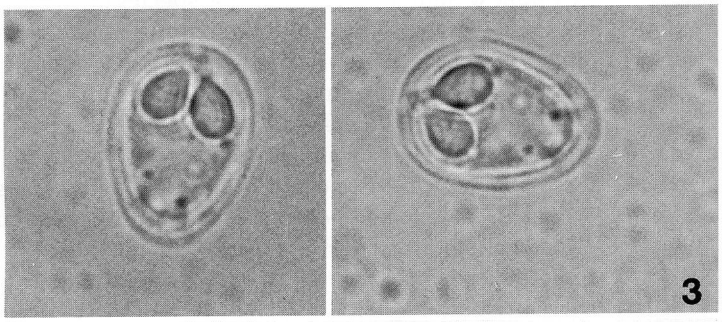

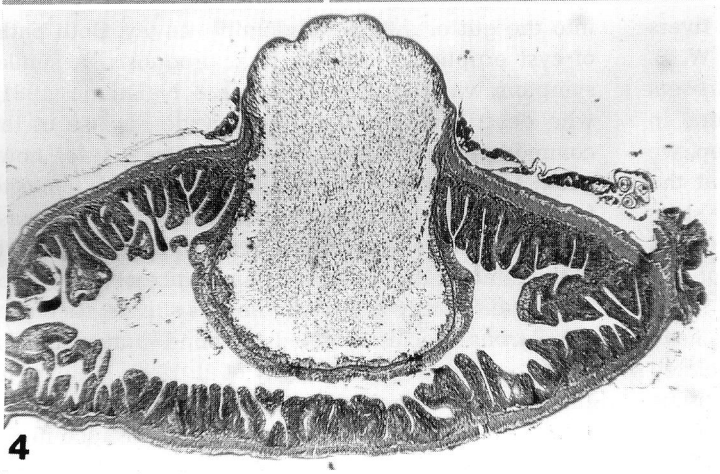

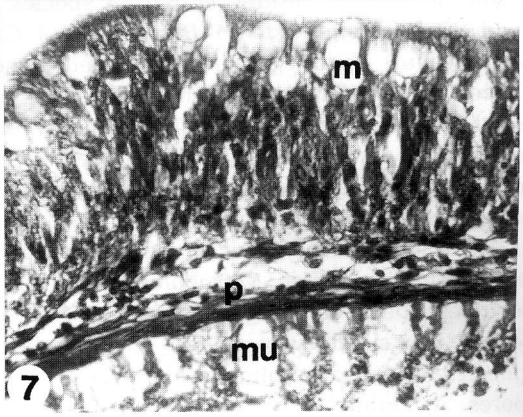
m
p
mu

than the half length of the spores. Ratio of capsular length to spore length 2 to 7. Anterior ends of the polar capsules set apart from each other. Polar filaments closely coiled with 4 to 5 turns, situated perpendicular to the longitudinal axis of the capsule. There is a large distinct iodophilous vacuole in each sporoplasm.

Histological findings

Only cysts with mature spores were found. In a cross section of the infected gut segment (Fig. 4) it was clearly visible that the cyst formed in the gut wall bulged deep into both the intestinal lumen and the abdominal cavity on account of its size. The cyst portion protruding into the gut lumen pushed the mucosa towards the lumen; the mucosa had markedly narrowed, as had the underlying propria and muscular layer covering the cyst. The other part of the cyst, covered by the tela subserosa and the serous membrane, bulged into the abdominal cavity. The cysts were located in the smooth muscle layer of the intestinal wall and separated the bundles of muscle cells into 2 parts (Fig. 5). In some segments of the intestine, branched cysts closely associated with each other and separated by a thin cyst wall were found (Fig. 6). The plasmodium was covered by a degenerated layer of smooth muscle (Fig. 7). The ectoplasm of the plasmodium consisted of a layer with dark-staining nuclei of generative cells, while the centre of the plasmodium was filled with spores (Figs. 4, 5 & 6). From this advanced stage of plasmodial development, one cannot conclude whether the plasmodium finished its development inside an extremely enlarged host cell or was located intercellularly surrounded by several muscle cells.

DISCUSSION

Barboid fishes are common inhabitants of the rivers of southwest Iran. Besides 11 *Barbus* species, W. B. Coad (pers. comm.) listed 1 *Barillus* and 4 *Capoeta* (*Varicorhinus*) species from the related genera in Khuzestan. In identification of the *Myxobolus* spp. we found, we accepted Molnár's (1994) theory that the majority of *Myxobolus* species have a relatively strict host specificity which is restricted to closely related fishes. Therefore, during our studies, the *Myxobolus* sp. found in *B. sharpeyi* was compared with and separated from the known *Myxobolus* spp. which have been described from the genus *Barbus* or from related genera. In a similar way, priority was given to tissue specificity over the morphological characteristics of the spores. According to Molnár (1994), there might be several morphologically similar spores but a given species always develops in a single type of host cell. The type of host cell determines even the location within the body; therefore, organ specificity can be neglected only in cases when the parasite develops in tissues that are common in several organs. In the present case, the results clearly show that *M. nodulointestinalis* is a typical parasite of the intestine and starts its development in the smooth muscle layer, where it forms large cysts. Some of the cysts showing a branch-like character were possibly formed by the fusion of several neighbouring cysts. By the shape and size of the spores, *Myxobolus nodulointestinalis* distinctly differs from other *Myxobolus* species known from the intestine of barboid fishes (*M. ellipsoides*, *M. impressus*, *M. obpyriformis*). In some respects it resembles *M. kubanicus*, a parasite of *Carassius auratus gibelio* and *Cyprinus carpio*; however, apart from differences in host range, the latter species forms only small cysts in the intestinal wall while *M. nodulointestinalis* is characterized by large cysts which can easily be recognized even with the unaided eye. The spores of *M. nodulointestinalis* also resemble the spores of *M. ellipsoides* as they were redescribed by Ergens & Lom (1970), but they distinctly differ from other illustrations summarised by Donec & Shulman (1984). Most of the well-known *Myxobolus* spp. (*M. exiguus*, *M. ellipsoides*, *M. muelleri*, etc.) seem to be conspecies comprising several spp. The morphological variations of figures given by Donec & Shulman (1984) for this species are so variable that, applying the suggestions of Lom & Arthur (1989) for description of Myxosporea, they could be separated into several species without hesitation.

Myxobolus nodulointestinalis appears to be a pathogenic species. The morphological changes produced in the intestinal wall are accompanied by functional disorders. In the infected portions of the intestine, the gut lumen had become constricted and the wall thickened. From these symptoms it could be determined that the spores released from the matured cysts broke either into the gut or into the abdominal cavity. Both paths of cyst eruption suggest a fatal prognosis. A similar symptom was reported by Egusa & Nakajima (1981), who described intestinal giant cystic disease of the common carp caused by *Thelohanellus kitauei*; however, according to Rhee et al. (1990) this parasite forms cysts in the intestinal mucosa. While *T. kitauei* is a disease-causing agent characterized by high pathogenicity, the economic importance of *M. nodulointestinalis* is only supposed. *Barbus sharpeyi* is one of the *Barbus* species selected for pond culture in Iran (Yazdipour et al. 1991, Nikpay et al. 1992, Jamili et al. 1993). On that basis, it cannot be excluded that the new species will acquire economic importance in the near future.

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LITERATURE CITED

Al-Salim NK (1986) *Myxobolus pfeifferi*, 1984 a new record from *Carasobarbus luteus* (Heckel, 1843) (Family: Cyprinidae) from Shatt Al-Arab River, Basrah, Iraq. Dirasat Nat Sci 13:163–166

Donec ZS, Shulman SS (1984) Cnidosporidia. In: Bauer ON (ed) Key to the parasites of freshwater fishes of USSR fauna, Vol 1. Nauka, Leningrad, p 88–251 (in Russian)

Ebrahimzadeh A, Kaylani R (1976) Survey of the parasites of digestive organs, gills and muscles of Karoon River fishes. Chamran Univ Publ, Ahwaz (in Persian)

Egusa S, Nakajima K (1981) A new Myxozoa, *Thelohanellus kitauei*, the cause of intestinal giant-cystic disease of carp. Fish Pathol 15:213–218

Ergens R, Lom J (1970) Puvodci parazitárnich nemoci ryb. Academia, Prague

Hagargi SS, Amoji SD (1981) *Myxosoma karnakatae* n. sp. (Protozoa: Myxosporidia) from the caudal muscles of freshwater teleost fish *Barbus chola* (Ham. et Buch). Arch Protistenk 124:385–390

Herzog PH (1969) Untersuchungen über die Parasiten der Süsswasserfische des Irak. Arch FischWiss 20:132–147

Jamili Sh, Oryan Sh, Sheifabadi J (1993) Influence of salinity upon growth rate and tolerance of *B. sharpeyi*. Iran Fish Bul 2:45–55 (in Persian)

Krutsay M (1980) Histologic techniques. Medicina, Budapest (in Hungarian)

Lalithakumari PS (1969) Studies on parasitic protozoa (Myxosporidia) of freshwater fishes of Andhra Pradesh, India. Riv Parassit 30:153–226

Landsberg JH, Lom J (1991) Taxonomy of the genera *Myxobolus/Myxosoma* group (Myxobolidae: Myxosporea), current listing of the species and revision of synonyms. Syst Parasitol 18:165–186

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

Masoumian M, Baska F, Molnár K (1994) Description of *Myxobolus karuni* sp. n. and *Myxobolus persicus* sp. n. (Myxosporea, Myxozoa) from *Barbus grypus* of the River Karun, Iran. Parasitol Hung 27:21–26

Moghainemi RS, Abasi S (1992) Parasitic fauna of economically important fishes from Hoor-Elazim Marsh. IFRTO Publications, Tehran (in Persian)

Mokhayer B (1981) Survey on the fish parasites of Sefid-Roud basin. Faculty of Vet Sci Publ 36:61–75 (in Persian)

Molnár K (1994) Comments on the host, organ and tissue specificity of fish myxosporeans and the types of their intrapiscine development. Parasitol Hung 27:5–20

Nikpay M, Marashi SZ, Moazedi J (1992) Survey on the biological features of the *Barbus sharpeyi* and *B. grypus*. IFRTO Publications, Tehran (in Persian)

Rashid ARA, Othman H, Nsayf ZM (1989) Preliminary study on some freshwater fish parasites from Little Zab, northeast of Iraq. J Biol Sci Res 20:107–114

Rhee KJ, Kim JO, Kim PG, Park BK (1990) Prophylactic and therapeutic studies on intestinal giant cystic disease of the Israel carp caused by *Thelohanellus kitauei*. I. Course of formation and vanishment of the cyst. Korean J Parasitol 28:183–194

Tripathi YR (1952) Studies on parasites of Indian fishes. I. Protozoa: Myxosporidia, together with a checklist of parasitic Protozoa described from Indian fishes. Rec Indian Mus 50: 63–88

Yazdipour A, Marashi SZ, Moazedi J (1991) Biotechnological report on artificial propagation of *Barbus sharpeyi*. IFRTO Publications, Tehran (in Persian)

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