

Four New *Myxobolus* spp. (Myxosporea: Myxobolidae) from Iranian Barboid Fishes

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Summary: Four new *Myxobolus* species are described from cyprinid fishes of the Mesopotamian Fauna Region in Iran. Each species infects a specific site of their fish hosts. *Myxobolus iranicus* sp. n. forms large plasmodia in the spleen of *Barbus sharpeyi* and *B. luteus*. The plasmodia of *M. mesopotamiae* sp. n. are located in the fins of *B. grypus*, *B. luteus* and *B. rajanorum*. The plasmodia of *M. shadgani* sp. n. develop on the cartilagenous rays of the gill filament in *B. rajanorum*, while *M. sharpeyi* sp. n. forms plasmodia inside the cartilagenous gill arches of *B. sharpeyi*.

Key Words: New species, *Myxobolus iranicus*, *M. mesopotamiae*, *M. shadgani*, *M. sharpeyi*, Iranian barbels, histology.

Introduction

Myxosporeans are common parasites of freshwater and marine fishes. The myxosporean fauna of fishes is relatively well studied in the Palaearctic, Amuro-Sino and Indian Great Fauna Regions, but relatively little is known about other parts of the world. The territory of Iran belongs to three fauna regions. The Ponto-Caspian territory of Northern Iran is part of the Palaearctic, Southeast Iran is greatly influenced by the Indian Fauna Region, while Southwest Iran belongs to the Mesopotamian Intermediate Fauna Region. According to BERG (1940), the composition of the fish fauna reflects these territorial differences. While the waters of Iran's Ponto-Caspian region are populated by fishes common also in Europe, the fish fauna of the Mesopotamian region is composed of endemic fish species including several barbels (COAD 1979). The parasite fauna of these fishes is little studied. More is known about monogeneans, of which several new species have been described recently (JALALI & MOLNÁR 1990; GUSSEV et al. 1993a, b, c). Of the known Myxosporea of the world the genus *Myxobolus* contains

the highest number of species. In a recent review LANDSBERG & LOM (1991) recorded 444 species. *Myxobolus* spp. are especially numerous in barbels. From the Ponto-Caspian territory of the former Soviet Union alone, DONEC & SHULMAN (1984) reported 37 *Myxobolus* spp. from different *Barbus* species. Besides the rich myxosporean fauna of the Palaearctic, several *Myxobolus* spp. are known from the Indian Fauna Region (TRIPATHI 1952; LALITHAKUMARI 1969; HALDAR et al. 1983). Before the investigations conducted by the present authors, *Myxobolus lobatus* had been the only species known in Iran from *Barbus brachicephalus* in the Ponto-Caspian territory (MOKHAYER 1981). Little is known about parasites of fishes in the Mesopotamian Intermediate Region of Iran. From that part of the country, EBRAHIMZADEH & NABAWI (1975), EBRAHIMZADEH & KAYLANI (1976), MOGHAINEMI & ABASI (1992) recorded some unidentified *Myxobolus* spp. from fishes of Karun river and recently MASOUMIAN et al. (1994) described two new species, *Myxobolus karuni* and *M. persicus*, from the gills of *Barbus luteus* and *B.*

sharppei. More data are available on the myxosporean fauna of the Mesopotamian fishes in Iraq, where HERZOG (1969), AL-SALIM (1986), and RASHID et al. (1989) studied that parasite group in the River Tigris and its tributaries. The present paper describes four new *Myxobolus* species from *Barbus sharppei*, *B. grypus*, *B. luteus* and *B. rajanorum*. Besides spore morphology, histological data are presented on the vegetative development of the new species.

Materials and Methods

The fishes included in this study consisted of 59 specimens of *Barbus luteus* (HECKEL 1843), 15–30 cm in length, 50 specimens of *Barbus grypus* (HECKEL 1843), 17–42 cm in length, 83 specimens of *Barbus sharppei* (GÜNTHER 1874), 11–31 cm in length, and 18 specimens of *Barbus rajanorum* (HECKEL 1843), 14–31 cm in length.

They were collected in the periods of June to November 1993 and May to October 1994 from Hoor-Elazim, Shadgan Marsh and six different stations of River Karun in the southwestern part of Iran.

Immediately after collection, the fish were transported alive 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 under dissecting and compound microscope.

Spores were obtained from mature plasmodia in each organ sample. An average of 30 spores were measured with an ocular micrometer using the dimensions recommended by LOM & ARTHUR (1989). Permanent preparations were made by placing a portion of the spores into glycerine-gelatine and mounting them under a coverslip. The structure of the polar capsules and the iodophilous vacuole was studied under Nomarski interference microscope.

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 haematoxylin-eosin and by Farkas-Mallory's method.

Results

Each of the four *Barbus* species was infected by *Myxobolus* plasmodia. Four different, undescribed *Myxobolus* spp. were found in these fishes. Spleen infection was found in four *Barbus luteus* (7%), three *B. grypus* (6%) and four *B. sharppei* (5%). Fin infection was found in eight *B. grypus* (16%), eight *B. luteus* (14%), and one *B. rajanorum* (6%). Infection of the gill filaments was found in six *B. rajanorum* (33%). *Myxobolus* plasmodia in the cartilagenous gill arches were recorded in eight *B. sharppei* (10%).

Descriptions

Myxosporea, BÜTSCHLI 1881

Bivalvulida, SHULMAN 1959

Myxobolidae, THÉLOHAN 1892

Myxobolus, BÜTSCHLI 1882

- *Myxobolus iranicus* n. sp.

Type host: *Barbus luteus* (HECKEL, 1843)

Additional hosts: *Barbus sharppei* (GÜNTHER 1874), *Barbus grypus* (HECKEL 1843)

Locality: Hoor-Elazim and Shadgan Marsh, River Karun in Southwest Iran

Site of infection: Spleen (Fig. 1)

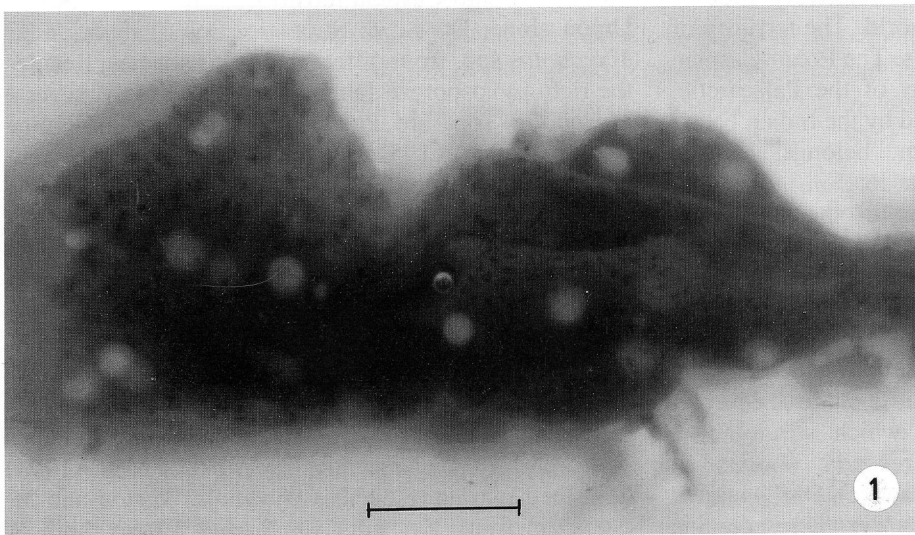


Fig. 1. *Myxobolus iranicus* n. sp. Infection of the spleen of *B. luteus* with plasmodia. (Bar = 2 mm).

Type material: Holotype deposited in the protozoan collection of the Zoological Department, Hungarian Natural History Museum, Budapest, Coll. No. 67149.

Description of the species (based on spores collected from *Barbus luteus*): All plasmodia found contained mature spores. The large plasmodia had an ellipsoidal shape and measured up to 580–660×350–420 µm in diameter.

Spores (Figs. 2 and 6) relatively large, ovoid, with narrow and rounded anterior pole in frontal view, lemon shape in side view, with a protruding sutural edge in the anterior pole, and with a distinct sutural line. Spore valves symmetrical, smooth, with several sutural edge markings. They are relatively thin. The wall seems to be enlarged at the anterior end, but the thickness of this part comes from the emerging sutural edge. Spores are 13.6 (13.2–14.0) µm long, 8.9 (7.5–9.2) µm wide, and 6.0 (5.6–6.3) µm thick. Two polar capsules, elongated ellipsoidal in shape, tapering only at the discharging canals of the polar filaments. They are unequal (or occasionally equal) in size. The larger 7.3 (6.9–7.5) µm long, 3.3 (2.9–3.5) µm wide, the smaller 7.0 (6.6–7.2) µm long. The larger polar capsule is slightly longer than the half length of the spore. The anterior ends of the polar capsules are set apart to each other. The spore has a distinct intercapsular appendix. Polar filaments closely coiled with 7 turns in the larger and 6 in the smaller polar capsule, situated perpendicularly to the longitudinal axis of the capsule. A large and distinct

iodinophilous vacuole was found in the sporoplasm. No projection or membranaceous envelope was found.

In histological sections the species was found in the spleen where it formed large plasmodia in the parenchyma (Fig. 10). Plasmodia were surrounded by a thin connective tissue layer and were filled with mature spores.

Comments: This species resembles *M. oviformis* THÉLOHAN but we regard it specific for *Gobio* spp. and a parasite of the gill filaments. *M. iranicus* also resembles *M. valdogeli* (DOGIEL) LANDSBERG et LOM commonly known from *Barbus* spp., but differs from the latter by the larger dimensions of the spores and by its typical location in the spleen. It is also the organ specificity by which *M. iranicus* differs from *M. macrocapsularis*, a conspecific described from at least thirty species of cyprinids.

• *Myxobolus mesopotamiae* n. sp.

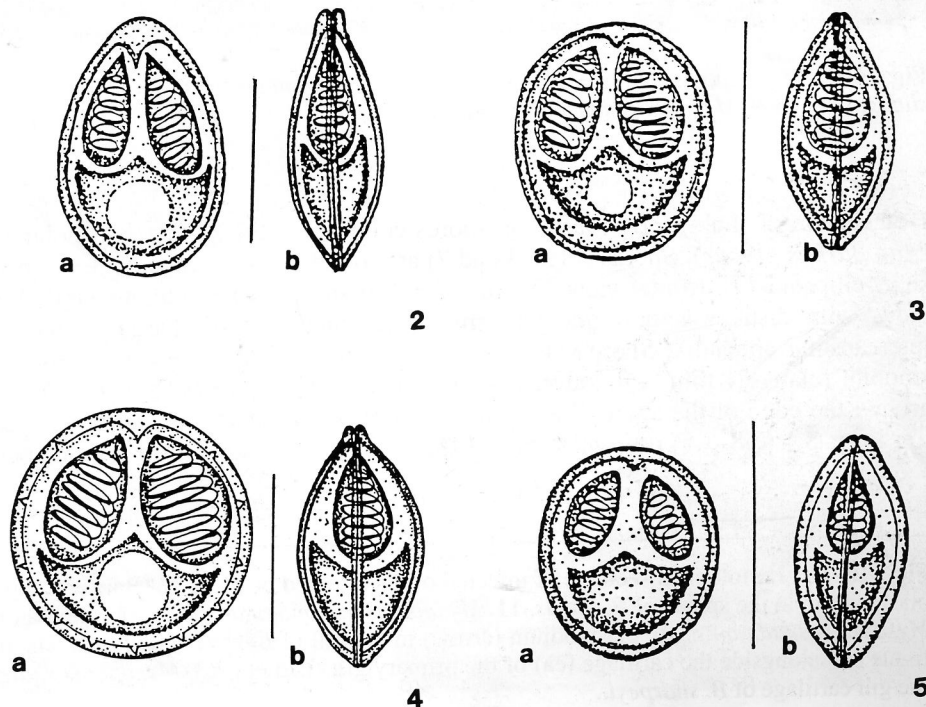
Type host: *Barbus grypus* (HECKEL 1843)

Additional hosts: *Barbus luteus* (HECKEL 1843), *Barbus rajanorum* (HECKEL 1843)

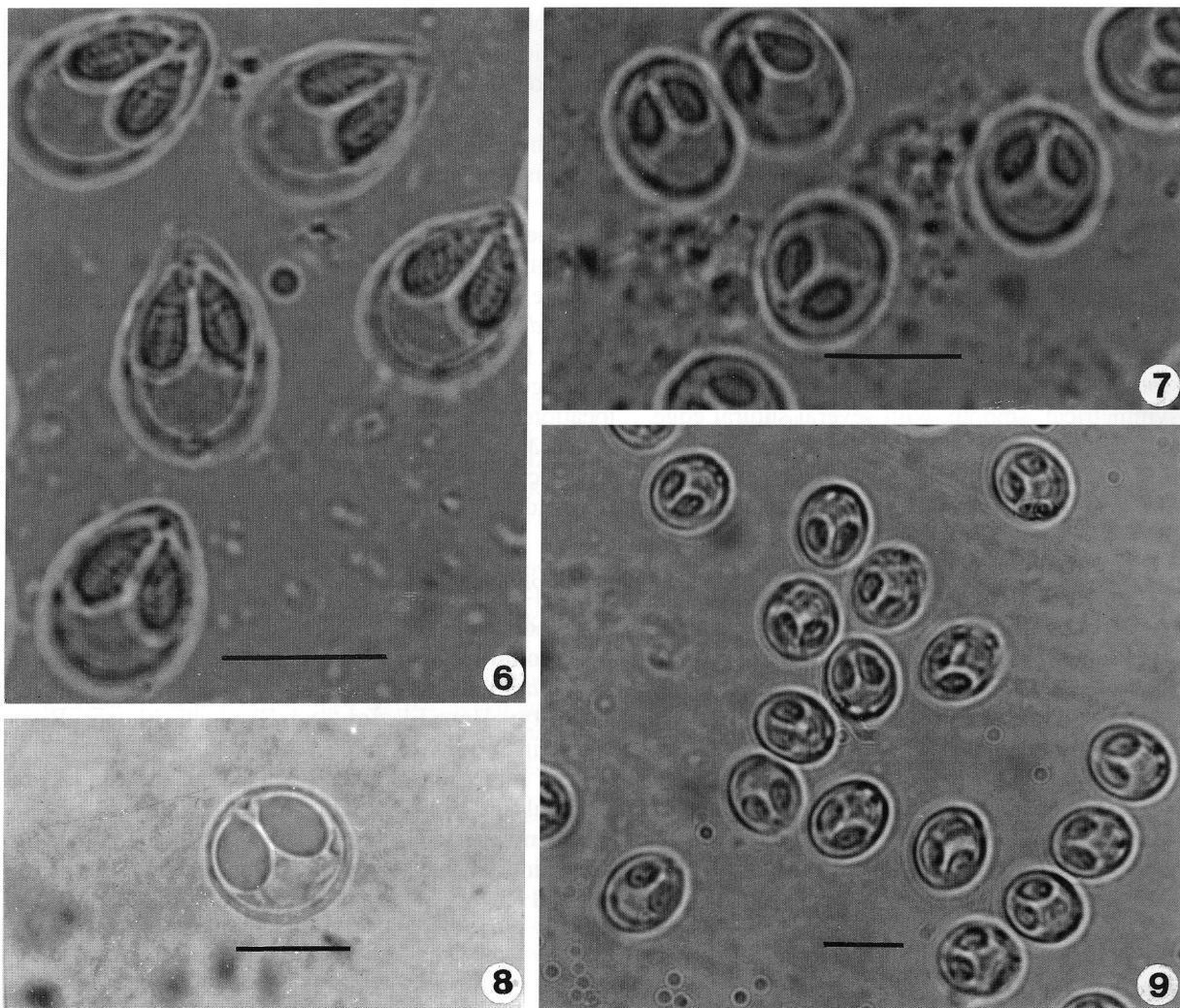
Locality: Hoor-Elazim and Shadgan Marsh, River Karun in Southwest Iran

Site of infection: Connective tissue of the caudal and pectoral fins

Type material: Holotype deposited in the protozoan collection of the Zoological Department, Hungarian Natural History Museum, Budapest. Coll. No. 67150.



Figs. 2–5. Line drawings of the spores. (a) Frontal view; (b) Side view. (Bars = 10 µm). 2. *Myxobolus iranicus* n. sp. 3. *Myxobolus mesopotamiae* n. sp. 4. *Myxobolus shadgani* n. sp. 5. *Myxobolus sharpeyi* n. sp.

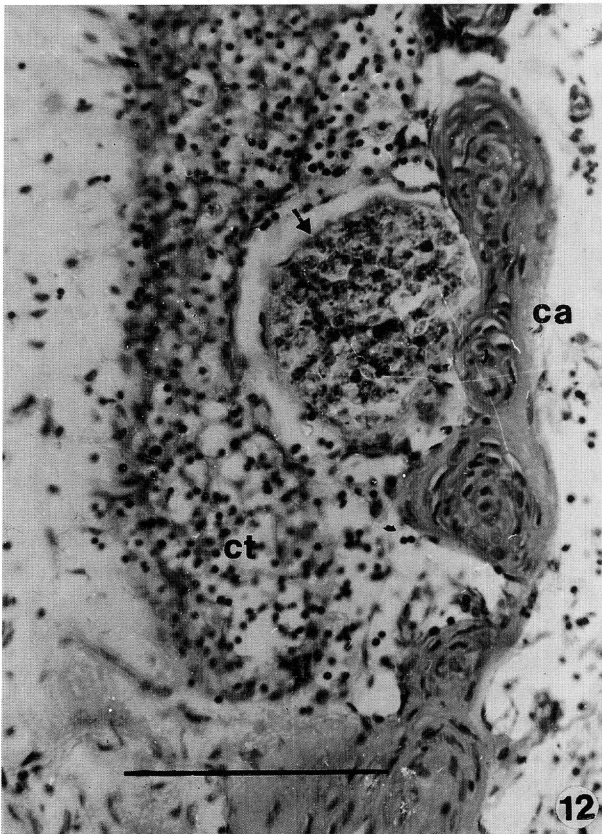
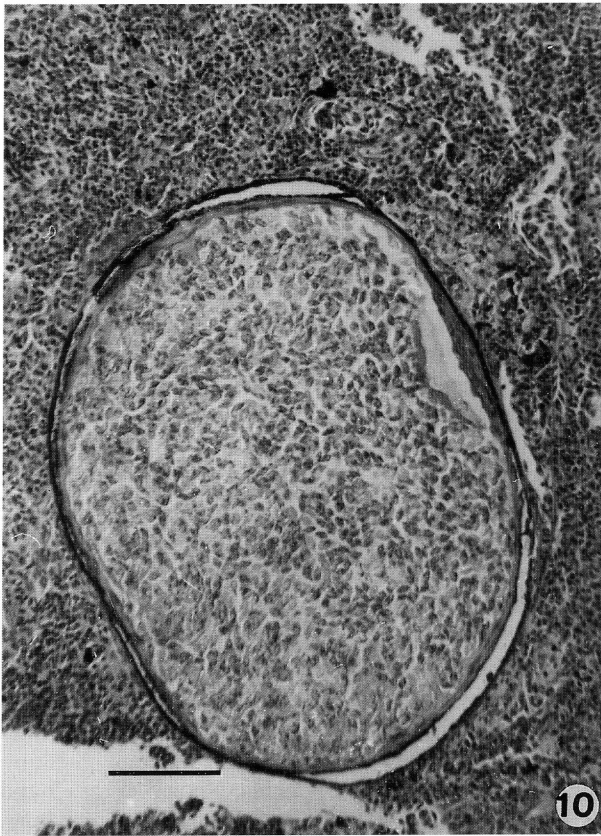


Figs. 6–9. Fresh spores. (Bars = 10 μm). **6.** *Myxobolus iranicus* n. sp. **7.** *Myxobolus mesopotamiae* n. sp. **8.** *Myxobolus shadgani* n. sp. **9.** *Myxobolus sharpeyi* n. sp.

Description of the species (based on spores collected from *Barbus grypus*): Spores (Figs. 3 and 7) are round, short ellipsoidal in frontal view, lemon shaped in side view, with distinct sutural line and small indistinct intercapsular appendix. Spore valves are symmetrical, smooth, relatively thin, with several sutural markings around the edge of the spore. Spores are 9.2 (8.9–9.4) μm long, 8.1 (7.8–8.5) μm wide and 5.8 (5.2–6.0) μm

thick. The two polar capsules are pyriform in shape, equal in size, 3.8 (3.6–4.2) μm long, 2.5 (2.4–2.7) μm wide, running parallel to each other, tapering slightly at the discharging canals of the polar filaments. Polar capsules are smaller (or equal) than the half length of the spores. The anterior ends of the polar capsules are set apart to each other. Polar filaments are closely coiled with 7 turns situated perpendicularly to the longitudinal

Figs. 10–13. Histological sections of infected organs stained by H. & E. (Bars = 100 μm). **10.** *Myxobolus iranicus* n. sp. plasmodium in the spleen of *B. luteus*. **11.** *Myxobolus mesopotamiae* n. sp. plasmodia (arrows) in the fin of *B. grypus*. **12.** *Myxobolus shadgani* n. sp. plasmodium (arrow) in the gill of *Barbus rajanorum*. The plasmodium lies in the connective tissue (ct) alongside the cartilage (ca) of the primary gill filament. **13.** *Myxobolus sharpeyi* n. sp. plasmodium (arrow) in the gill cartilage of *B. sharpeyi*.



axis of the capsules. In 10% of spores an indistinct iodophilous vacuole was found in the sporoplasm which was, however, seen only by Nomarski interference microscopy. There is no mucous envelope or membranaceous envelope on the spores.

In histological sections, plasmodia containing spores were found in the fins inside the connective tissue layer beneath the epithelium (Fig. 11).

Comments: *M. mesopotamiae* seems to be a specific parasite of the fin of barbels. Morphologically it resembles *M. musajevi* KANDILOV a parasite of Central Asian barboid fishes, but it differs from the latter species by its less expressed sutural line and its typical location in the fins. *M. mesopotamiae* sp. n. also resembles *M. squamae* KEYSSELITZ, a common parasite of *Barbus barbuis*, but differs from it by the more elongated shape of its polar capsules. The name of the species derives from the region.

• *Myxobolus shadgani* n. sp.

Host: *Barbus rajanorum* (HECKEL 1843)

Locality: Hoor-Elazim and Shadgan Marsh, River Karun in Southwest Iran

Site of infection: Gills

Type material: Holotype deposited in the protozoan collection of the Zoological Department, Hungarian Natural History Museum, Budapest. Coll. No. 67152.

Description of the species: Spores (Figs. 4 and 8) are relatively large, of round shape in frontal view, lemon shaped in side view, with distinct sutural line, and small intercapsular appendix. Spore valves are symmetrical, with several sutural edge markings. Spores are 13.9 (13.3–14.1) μm long, 13.7 (13.3–14.1) μm wide, and 8.4 (8.3–8.6) μm thick. The two polar capsules are pyriform in shape but relatively wide, unequal (or occasionally equal) in size. The larger is 8.2 (7.9–8.3) μm long, 5.3 (4.9–5.5) μm wide. The smaller is 7.9 (7.6–8.1) μm long and 5.2 (4.6–5.4) μm wide. The polar capsules are longer than the half length of the spore. The anterior ends of the polar capsules are close to each other. The polar filament is closely coiled with 8 turns in the larger and 7 turns in the smaller one, situated perpendicularly to the longitudinal axis of the capsules. There is a small, round and indistinct iodophilous vacuole in the sporoplasm which can be seen by Nomarski interference microscopy. There is no mucous envelope or membranaceous envelope on the spores.

In histological sections plasmodia were found in the primary gill filaments surrounded by a single layer of connective tissue cells. The plasmodia lay with one side to the gill cartilage while the other side was covered by the multilayered epithelium of the non-lamellated part of the filament (Fig. 12).

Comments: By its roundish shape *M. shadgani* sp. n. resembles *M. rotundus* NEMECZEK and *M. rotundatus* DOGIEL et ACHMEROV, but differs from them by the larger size of the spores and by its large polar capsules. The size and shape of *M. amurensis* ACHMEROV from Amur wild carp are about the same as those of *M. shadgani* sp. n., but the measurements of its polar capsules are smaller. The size and shape of the spores of *M. sprostonae* SHULMAN and *M. krokhini* KONOVALOV et SHULMAN are about the same as those of *M. shadgani* sp. n., but these species were found in systematically different fishes and infect organs other than the gills. The name of the species was given after the region of the collecting place.

• *Myxobolus sharpeyi* n. sp.

Host: *Barbus sharpeyi* (GÜNTHER 1874)

Locality: Hoor-Elazim and Shadgan Marsh, River Karun in Southwest Iran

Site of infection: Gill cartilage

Type material: Holotype deposited in the protozoan collection of the Zoological Department, Hungarian Natural History Museum, Budapest. Coll. No. 67153.

Description of the species: Spores (Figs. 5 and 9) are relatively small. The majority of the spores are short ellipsoidal or ellipsoidal in frontal view and lemon shaped in side view. The spores have an indistinct sutural line, a protruded sutural edge at the anterior end, and a small intercapsular appendix. Spore valves are symmetrical and smooth. Wall of the spore seems to be thick, but this thickness comes from the emerging sutural edge. Short ellipsoidal spores are 9.6 (9.2–9.8) μm long, 8.1 (8.6–7.5) μm wide, and 4.8 (5.3–4.4) μm thick. Ellipsoidal spores are slightly longer, 9.9 (9.5–10.2) μm in length. The two polar capsules are equal in size, 3.6 (3.3–4.0) μm long, 2.8 (2.2–2.4) μm wide, ellipsoidal in shape, tapering only at the discharging canals of the polar filaments. Polar capsules are equal with (or slightly bigger than) the half length of the spore. The anterior ends of the polar capsules are close to each other. Polar filaments are closely coiled with 5 turns, perpendicular to the longitudinal axis of the capsules. There is a small indistinct iodophilous vacuole in the sporoplasm. There is no mucous envelope or membranaceous envelope on the spores.

In histological slides plasmodia were found in the cartilage tissues of the gill arches directly surrounded by cartilageous cells (Fig. 13).

Comments: *M. sharpeyi* sp. n. resembles *M. branchialis* (MARKEWITSCH) and *M. circulus* (ACHMEROV) from Amur wild carp by the shape and size of the spores but differs from them by its shorter polar capsules. *M. persicus* MASOUMIAN et al. and *M. karuni* MASOUMIAN

et al. infect the gills of the same host, but these species differ from *M. sharpeyi* sp. n. both in morphology and in size. As regards the site of infection, a North American species, *M. cartilaginis* (HOFFMAN et al.) is the most similar to *M. sharpeyi* sp. n.; however, besides the larger dimensions of its spores, *M. cartilaginis* is a specific parasite of centrarchid fishes. The species was named after the host fish.

Discussion

Among the large number of myxosporean genera the *Myxobolus/Myxosoma* group is the best known and the most numerous in species. The first representatives of this group became known already at the end of the nineteenth century and in the first years of the twentieth century when BÜTSCHLI (1882), GURLEY (1893), THÉLOHAN (1895), DOFLEIN (1898), HOFER (1903), REUSS (1906), AUERBACH (1906), KEYSSELITZ (1908), and NEMECZEK (1911) described several *Myxobolus* spp. The description of species by the above authors concerned mainly the resistant spores and only scarce information was given on the vegetative stages. Some of these descriptions provided data only on the size and shape of the spores and the polar capsules. Although *Myxobolus* spores differ in size and shape, their morphological variability is limited. If only these data were used for differential diagnosis, most of the species known so far could be identified with one or another species of these early authors. This is why LOM & ARTHUR (1989) suggested a more precise description of the new species and gave a key to the proper characterisation of spores. For a long time THÉLOHAN'S (1892) classification was accepted, which differentiated the genus *Myxobolus* from *Myxosoma* by the presence of the iodophilous vacuole in the sporoplasm and, moreover, created for the latter genus the family Myxosomatidae. WALLIKER (1968), however, concluded that the iodophilous vacuole, which consists mostly of glycogen, was not a stable mark and he synonymized the two genera. Although Russian authors, among them DONEC & SHULMAN (1984) still make a distinction between the two genera, other authors (MITCHELL 1977; LOM & NOBLE 1984; LANDSBERG & LOM 1991; LOM & DYKOVÁ 1992) regard them as synonyms, and use the presence or absence of the vacuole only as one of the marks for characterizing the spore.

Artificial infection with myxosporeans became possible only recently when MARKIW & WOLF (1983) proved that myxosporeans develop by oligochaete alternative hosts in which the so-called actinosporean stages develop. In the absence of artificial infection, for a long time there were no reliable data on the host specificity of myxosporeans, and spores found in genetically differ-

ent host fishes were in most cases identified with some well-known species or were described as new ones. As a result, in the review written on myxosporeans by DONEC & SHULMAN (1984) some *Myxobolus* species were recorded from more than 40 hosts while others were mentioned from a single host only. In his review, MOLNÁR (1994) stated that the majority of myxosporeans are host-, organ- and tissue-specific organisms which develop only in closely related fishes and have a strict affinity to a certain tissue type.

Based on the supposed organ specificity, from the great number of *Myxobolus* spores found in Iranian barboids, only those were studied in details where there was supportive histological evidence to localize the site of vegetative development and tissue affinity of the given species. At a similar way, we supposed a certain host specificity and for differential diagnosis we compared them mostly with species described from barbels. The number of the latter is relatively high, and DONEC & SHULMAN (1984) recorded 37 species from different *Barbus* species of the Palaearctic. This number is increased by further species known from India (TRIPATHI 1952; LALITHAKUMARI 1969; HAGARGI & AMOJI 1981).

Our survey on *Myxobolus* species of barbels from Southwest Iran shows that these Mesopotamian barbels are infected by other *Myxobolus* spp. than barbels of the Palaearctic and Indian Great Fauna Regions. This finding is consistent with data reported by GUSSEV et al. (1993a, b, c) who found similar differences between the Monogenea fauna of barbels of the Mesopotamian and Palaearctic Regions. At the same time, the occurrence of new *Myxobolus* spp. points to the formation of the fish fauna, e.g. that during evolution the divergence of *Barbus* species was accompanied by a divergence of *Myxobolus* spp.

The pathological effects of *Myxobolus* spp. are well known. *Myxobolus cerebralis*, causing whirling disease of salmonids, is regarded as the most important pathogen, but *M. cyprini*, the agent of pernicious anaemia of the common carp, and *M. pfeifferi*, the causative agent of nodular disease of the European barbel, are also considered major pathogens. Little is known about the economic importance of the *Myxobolus* spp. described in this study; however, in intensive cultures of the host their pathogenic effect cannot be excluded. Two of the host fishes examined in this study, *Barbus sharpeyi* and *B. grypus*, are potential pond-cultured species, whose artificial propagation and culture have already been started (NIKPAY et al. 1992; YAZDIPOUR et al. 1991; JAMILI et al. 1993) in Iran.

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