Journal of Fish Diseases 1996, 19, 15–21

Description of *Myxobolus bulbocordis* sp. nov. (Myxosporea: Myxobolidae) from the heart of *Barbus sharpeyi* (Günther) and histopathological changes produced by the parasite

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Abstract

A new myxosporean, *Myxobolus bulbocordis* sp. nov., has been found in the heart of a Mesopotamian fish, *Barbus sharpeyi* (Günther), in rivers in Southwest Iran. Cysts containing matured spores were located on the serosa of the atrium cordis, bulbus arteriosus, larger gill arteries and inside the wall of the bulbus. The species showed an affinity to connective tissue cells and was never associated with the muscles. Mature cysts were surrounded by a connective tissue capsule composed of two to three layers. Spores in disrupted cysts were infiltrated by epithelioid cells and macrophages. The spores found in *Barbus sharpeyi* differed in size and morphology from species known from other barbels.

Introduction

In a recent paper, Molnár (1994) has pointed out that myxosporeans are host-, tissue- and organspecific parasites. Most of them have a relatively strict host specificity and show affinity to a certain tissue of the host fish. Because of this specificity, most myxosporeans only develop in a given organ, and they are ubiquitous in location only if they start their development in a tissue which occurs in different parts of the fish body (loose connective tissue, endothelium or muscle cells). The majority of the known *Myxobolus* spp. have a well-defined site of development, and there are species specific to the gills, skin, kidney, intestine and so on. Unfortunately, data on vegetative development is only available for a few of the known *Myxobolus* spp., and the majority of species have been described by the morphology of spores disseminated in the host after the disruption of cysts. In these cases, the site of infection cannot be designated properly, which makes it difficult to evaluate organ specificity.

Occurrence in the heart is commonly recorded for *Myxobolus* spp., but only a few species like *M. cordis* and *M. dogieli* develop plasmodia and cause pathological changes in this organ (Keysselitz 1908; Bauer, Voronin & Yunchis 1991). In Iran, the occurrence of a *Myxobolus* sp. in the heart of *Barbus sharpeyi* had already been reported by Moghainemi & Abasi (1992); however, these latter authors failed to give detailed information on the species found.

This paper reports the occurrence of a new *Myxobolus* species in *Barbus sharpeyi*, a fish endemic in Mesopotamian rivers and in Southwest Iran. The new species is described under the name *Myxobolus bulbocordis* and the pathological changes caused by it are presented.

Materials and methods

The fish surveyed in this study were composed of 83 specimens of *B. sharpeyi* (Günther), 14–31 cm in length. They were collected in the period between June and October 1993 as well as May and October 1994 from Hoor-Elazim, Shadgan Marsh and six different stations of the Karun River in Khuzestan Province in Southwest Iran.

Immediately after collection, the fish were transported alive to the laboratory where they were

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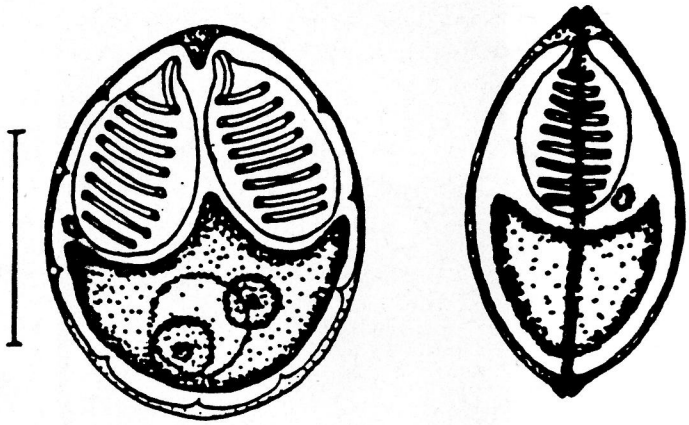

Figure 1 Drawing representing the spore of *M. bulbocordis* in frontal and side view (bar = 10 μm).

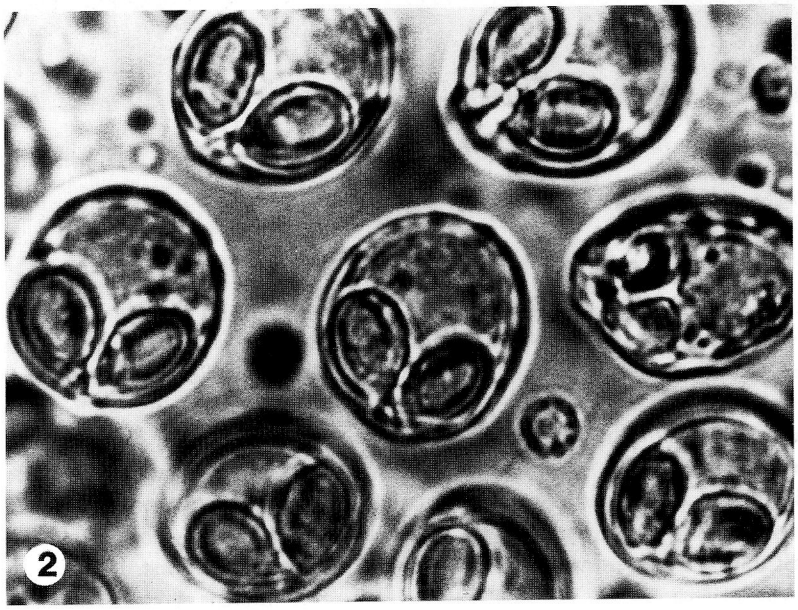

Figure 2 Microphotograph of *M. bulbocordis* spores examined in fresh preparation (× 1690).

observed on the surface of the serosa (Fig. 8).

The tissues containing cells with fusiform, elongated nuclei and which surrounded the cysts were easily discernible by Farkas-Mallory's method, in which the connective tissue elements stained blue, the muscle red, while the spores assumed a conspicuous yellow colour.

Discussion

The number of known *Myxobolus* species is extremely large. In a synopsis of the genus *Myxobolus*, Landsberg & Lom (1991) listed 444 valid species, indicating their type hosts. The majority of species known to date have been described on the basis of spore morphology. Although some of the species can be well characterized by the shape and size of spores, morphological variance is still limited. The similarity of spores has resulted in the misidentification of numerous species, and even species occurring in systematically distinct hosts and in dissimilar locations have been identified with some already-known species. Thus, Donec & Shulman (1984) recorded as many as 40 hosts for

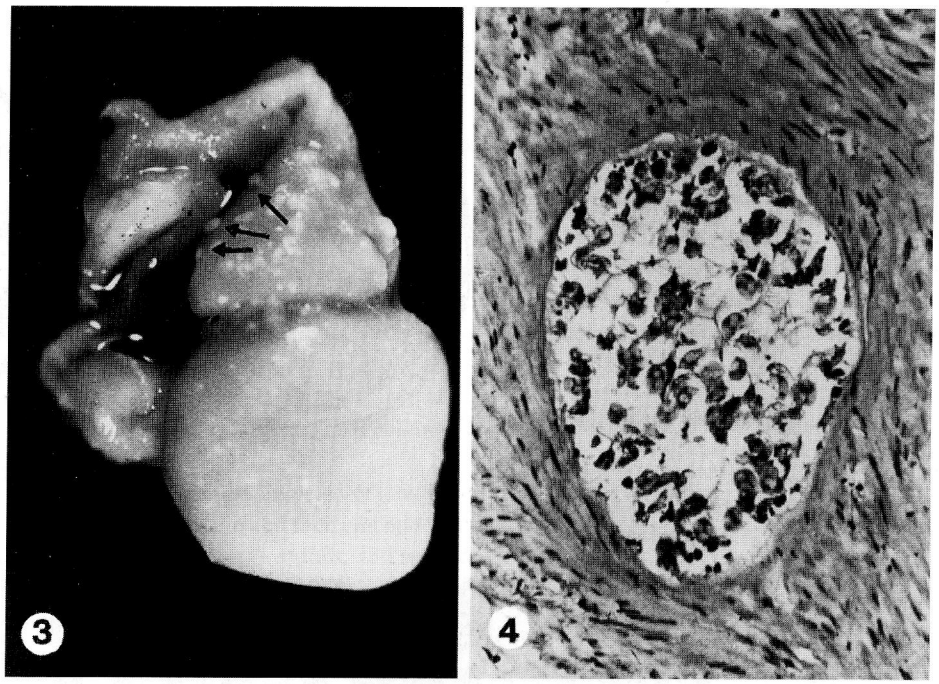

Figure 3 *Myxobolus bulbocordis* cysts (arrows) in the bulbus arteriosus and atrium cordis of the heart (× 5·5).

Figure 4 *Myxobolus bulbocordis* cyst containing mature spores among the connective tissue cells forming the wall of the bulbus arteriosus (Farkas-Mallory's stain, × 190).

some species; these are obviously conspecies. To enable a more accurate identification of parasites, Lom & Arthur (1989) suggested morphological characteristics which should be taken into consideration besides spore size and shape, while Molnár (1994) has called attention to the fact that host, organ and tissue specificity represent a feature which must not be neglected when describing new species of Myxosporea.

Thus far, six *Myxobolus* species *(Myxobolus dogieli, M. muelleri, M. bramae, M. musculi, M. cordis* and *M. ellipsoides)* have been recorded from the heart of cyprinid fishes (Donec & Shulman 1984). *Myxobolus muelleri, M. bramae, M. musculi* and *M. ellipsoides* differ from *M. bulbocordis* by the size and morphology of spores. The size of *M. bulbocordis* spores is much larger than that of *M. muelleri, M. bramae* and *M. musculi*. The spores of *M. ellipsoides* are about the same size, but they distinctly differ from the spores of *M. bulbocordis* by having an elongated ellipsoidal shape. In addition to morphological differences, *M. bramae* differs from *M. bulbocordis* by being a specific gill parasite of the bream, while *M. musculi* is known to occur only in striated muscle cells.

Myxobolus cordis, a well-known parasite of the European *Barbus* spp., morphologically resembles *M. bulbocordis*, but its spores are significantly smaller than those of the new species.

The spores of *M. dogieli* are also similar to *M. bulbocordis* but somewhat smaller, varying between 9 and 16 μm in spore length. Bykhovskaya & Bykhovsky (1940), who first described this species, designated six different cyprinid fish as hosts for *M. dogieli*. The wide range of spore measurements characterizing infections of different cyprinids suggests that *M. dogieli* is a conspecies comprising several undifferentiated species. Bauer *et al.* (1991) also remarked that only the species infecting the common carp should be regarded as *M. dogieli*.

Two other *Myxobolus* species, *M. karuni* and *M. persicus* have been described from *Barbus sharpeyi* (Masoumian, Baska & Molnár 1994), but these species are specific parasites of the gills and they also differ from *M. bulbocordis* in spore morphology. *Myxobolus karuni* differs from *M. bulbocordis* by its large, elongated ellipsoidal polar capsules in which the polar filaments turn at least ten times, and by its prominent intercapsular appendix. The spores

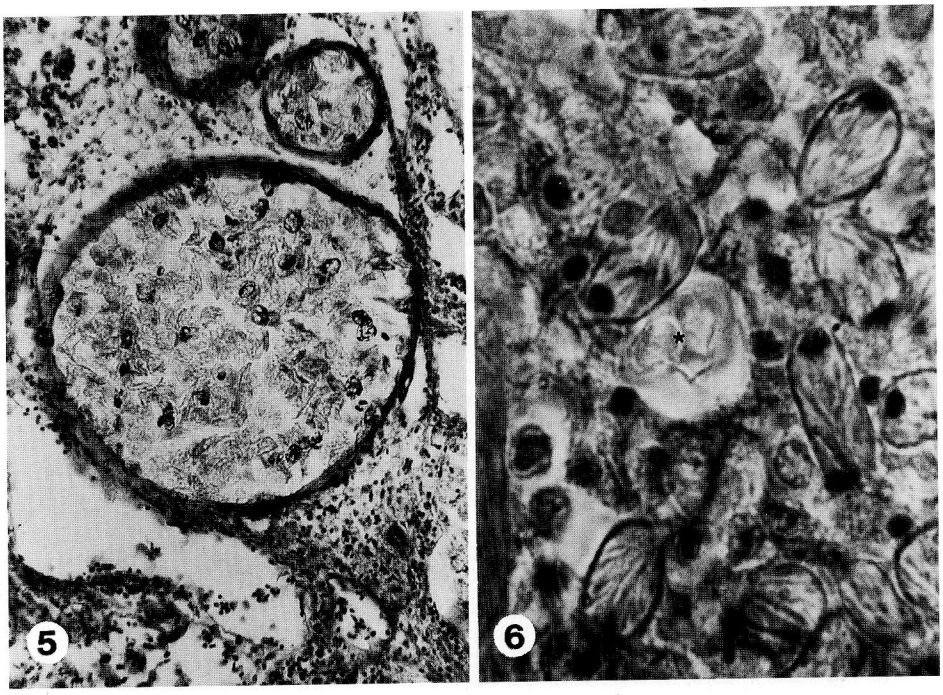

Figure 5 Granulation tissue penetrating into, and proliferating in, older cysts amongst *M. bulbocordis* spores. Spores stuck in the cysts are immobilized by a connective tissue reaction and undergo gradual necrosis. Among the mass of damaged spores, some dark staining, still-viable spores are seen. The cyst is surrounded by a thick, multilayered connective tissue capsule (H&E, × 140).

Figure 6 The endocardium of the infected fish contains rodlet cells and solitary spores (*) of *M. bulbocordis* (H&E, × 960).

M. persicus are significantly smaller than those of *M. bulbocordis* and have only a small intercapsular appendix.

In *M. bulbocordis* infection of *B. sharpeyi*, the location of the cysts was always restricted to the heart, but in more severe cases, cysts attached to the serosa of efferent arteries of the heart were also recorded. Although *M. dogieli* also has a predilection for the heart, Bauer *et al.* (1991) remarked that, in severe cases, the 'muscles of the gill vessels' also harboured cysts. While studying *M. tucunarensis* infection of a South American fish, Molnár & Békési (1993) found that the heart was consistently infected but other organs were also involved.

Myxobolus bulbocordis typically has a connective tissue affinity and never affects the musculature of the ventricle. Striated muscles of the fish body are often infected by several species of *Myxobolus*. Molnár & Kovács-Gayer (1985) and Baska (1987) described that location for *M. cyprini* and *M. pseudodispar*, respectively, and it is also known that the barbel parasites *M. pfeifferi* and *M. musculi* occur exclusively in that intracellular location. However, there is no evidence to show that any of the species designated as heart parasites would develop in or among the heart muscle cells.

The histological findings of this study show severe changes in the infected heart tissues, but no functional disorders were recorded in *Barbus sharpeyi* living in natural waters. In *M. dogieli* infection, Bykhovskaya & Bykhovsky (1940) observed mortality among infected fish with the development of pericarditis and endocarditis. Bauer *et al.* (1991) also recorded deaths in stocks of carp infected by *M. dogieli* and severe pathological changes caused by the sausage-like cysts of *M. dogieli* penetrating into the muscle of the heart's precardium. This latter statement seems to be incorrect as the precardium does not contain muscular elements. In the present study, cysts were only occasionally found in the precardium, but a relatively heavy infection of the bulbus arteriosus was established. The cysts had a rounded shape, even inside the connective tissue of the bulbus, and distinctly differed from the sausage-like cysts of *M. dogieli*.

From histological examinations, no data

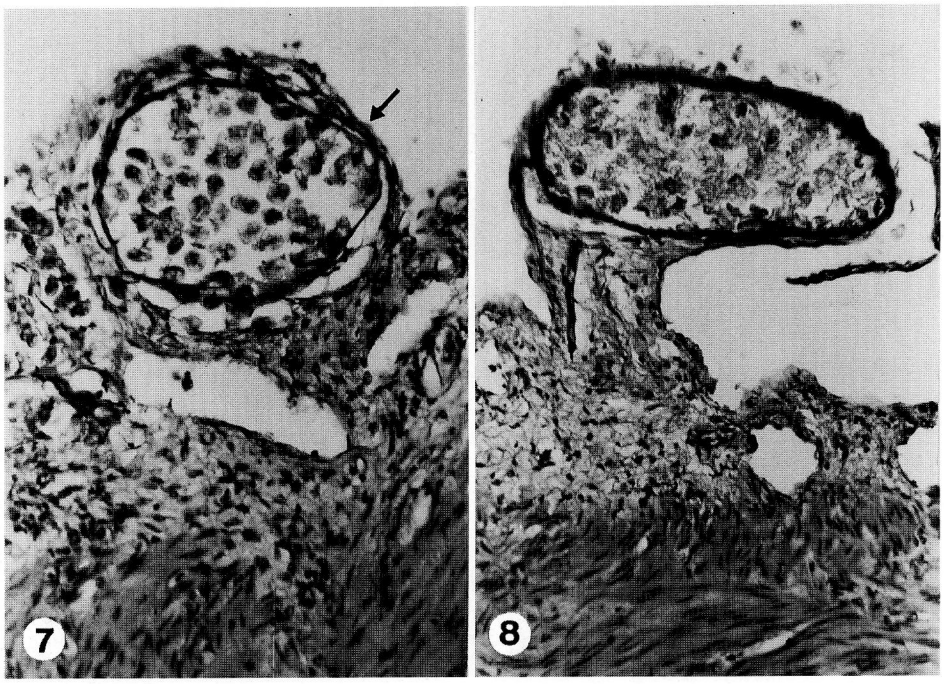

Figure 7. An *M. bulbocordis* cyst containing mature spores (arrowhead) located in the serosa covering the wall of the bulbus arteriosus (H&E, × 190).

Figure 8. Cysts located on the serous membrane of the heart connected to the surface of the bulbus by a small peduncle (Farkas-Mallory's stain, × 190).

could be collected on the stage of plasmodium development. However, the local pathological changes caused by mature and disrupted cysts were well visible. The most elementary form of host reaction was the formation of a connective tissue capsule around the cyst. Disrupted cysts consistently became penetrated by granulation tissue containing epithelioid cells and macrophages; however, direct spore phagocytosis was not seen. At the same time, gradual necrosis of the spores and the cells surrounding them gave rise to a tissue debris which became absorbed by the granulation tissue. The mechanism of granulation, the penetration of the tissue containing epithelioid cells, the appearance of melanomacrophages and the accumulation of yellowish pigment occurred in a manner analogous to that described by Kovács-Gayer & Molnár (1983) for the gill parasite of the common carp, *Myxobolus basilamellaris.*

The differentiation of spore-containing mature plasmodia surrounded by connective tissue from encapsulated spore masses remaining from earlier infections often poses a problem in histopathological examinations. In such cases, the type of capsule may provide guidance, as the wall of the cyst is usually thin while encapsulated spores are surrounded by a thick connective tissue capsule consisting of split fibres.

The pathological importance of *M. bulbocordis* cannot be assessed properly as only natural infections have been recorded. From the intensity of infection, however, it may be concluded that in the future during intensive pond culture of the host species similar pathological problems to those seen in *M. dogieli* infection of the common carp might appear. The host fish, *Barbus sharpeyi*, is one of the most important fishes selected for artificial propagation in Iran, and successful efforts have already been made to culture this species in ponds (Yazdipour, Marashi & Moazedi 1991; Jamili, Oryan & Seifabadi 1993).

Acknowledgments

The authors thank Dr S. R. Moghainemi for his help in collecting material in Iran. The studies of the senior author were funded by the Iranian Fisheries Research and Training Organization. The work of the Hungarian authors was supported by the US-Hungarian Joint Fund, J. F. No. 326.

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