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SURVEY ON *MYXOBOLUS* INFECTION OF THE BLEAK (*ALBURNUS ALBURNUS* L.) IN THE RIVER DANUBE AND IN LAKE BALATON

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In a three-year survey of myxosporean infections of the bleak *(Alburnus alburnus*), involving the examination of 205 fish specimens from the River Danube and 50 from Lake Balaton, four *Myxobolus* species (two gill parasites, one fin parasite and a species parasitising the skeletal muscles) were detected. Two of the species could be identified as *M. alburni* and *M. obesus.* Of the other two species, the gill parasite proved to be a hitherto undescribed species which is described here as a new species by the name of *M. margitae*. One of the two gill-parasitic species, *M. obesus,* formed plasmodia in the respiratory lamellae of the gill filaments, while the plasmodia of *M. margitae* n. sp. were formed in the afferent artery of the primary gill filaments. The plasmodia containing spores morphologically identifiable with the species *M. alburni* were located in the connective tissue between the fin rays. The less frequently found muscle-parasitic *Myxobolus* species has not been identified precisely. The plasmodia of *M. obesus* were found in the fish in May and June, while those of *M. alburni* and *M. margitae* n. sp. in July and August. The prevalence of infection in fish examined in these periods was 15.5% for *M. obesus*, 11.5% for *M. margitae* and 14.0% for *M. alburni*.

Key words: *Myxobolus*, bleak, new species, histology

The bleak (*Alburnus alburnus* L.) is a fish species commonly occurring in the waters of Central Europe, including the River Danube and Lake Balaton. Because of its small size, its economic importance is negligible. Numerous papers have been published on the myxosporean fauna of this fish species. On the basis of data published in these faunistic works, the monographs of Shulman (1966) and Donec and Shulman (1984) recorded the occurrence of 20 *Myxobolus* species from this fish *(M. alburni, M. carassii, M. dispar, M. dogieli, M. dujardini, M. ellipsoides, M. ergensi, M. exiguus, M. gigas, M. macrocapsularis, M. musculi, M. muelleri, M. nemachili, M. nemeczeki, M. obesus, M. oviformis, M. pseudodispar, M. rotundatus, M. saidovi, M. schulmani*). According to Landsberg and Lom (1991), only 4 of the above (*M. alburni* Donec, 1984, *M. ergensi*

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Lom, 1969, *M. obesus* Gurley, 1893, *M. saidovi* Gasimagomedov, 1970) can be considered species described from the bleak as typical host. Only a single species, *M. ergensi* is known to occur in bleak in Hungary, the solitary spores of which species were found by Lom (1969) in the kidney of bleak from the River Tisza.

This paper describes four *Myxobolus* species from the gills, fins and muscles of bleak from the River Danube and Lake Balaton. Two of the four (*M. alburni, M. obesus*) have proved to be known species, while one of the other two is described as a new species by the name of *M. margitae* sp. n.

Materials and methods

The studies were done continuously from 1996 to 1998 in the framework of a survey on the parasite fauna of fishes of the River Danube and Lake Balaton. Only the 205 bleak specimens originating from the River Danube were examined in detail, while the 50 bleaks collected from Lake Balaton served for comparison. The fish were collected in the fishing period between April and October. During the three years of the survey, the following numbers of bleaks were collected from the River Danube for dissection: 10 in April, 45 in May, 100 in June, 20 in July, 17 in August, and 13 in September. The majority of fish measuring 7–14 cm in length were collected with our own seine. The fish were always transported to the laboratory alive, in plastic bags supplied with oxygen. In the laboratory they were kept in aerated or flow-through aquaria and processed within one week. The fish were killed by decapitation and then subjected to full parasitological examination extending to all organs. If *Myxobolus* plasmodia were found, the spores were released from a few mature plasmodia and attempts were made to identify them in live condition. Sometimes the spores were also recorded on videotape. A certain proportion of the spores were placed into distilled water and stored in refrigerator for further examination. The dimensions of the spores were determined with the help of the IMAGO® computer program. Using the same program, digitised still pictures were also made of spores previously recorded on videotape according to the method of Székely (1997). Parallel to these operations, from a few hundred spores permanent preparations were made under a coverslip, in glycerol-gelatin or in ammonium picrate.

For histological examination, some of the infected gills were fixed in Bouin's solution, embedded in paraffin, cut into 4 μm sections, and the sections were stained with haematoxylin and eosin. The histological preparations were photographed with an Olympus Dp 10 digital camera mounted on an Olympus BH-2 microscope.

Results

Two known, a hitherto unknown and a yet precisely not identified *Myxobolus* species were detected in bleak from the River Danube during the survey. Two of the detected species formed plasmodia on the gills, one occurred on the fins while the fourth one in the muscles. In addition to the above, solitary spores also often occurred in the kidneys, gills, intestine and skin, but only a certain proportion of them were identified to the species level. Based upon the morphological characteristics of the spores, the species found on the fins was identified with *M. alburni* Donec, 1984. Of the species developing in the gills, the plasmodia of the species identified as *M. obesus* Gurley, 1893 appeared in the respiratory lamellae in the first days of May, produced mature spores by the end of May, and could not be detected in the remaining part of the year. Six out of the 45 fish examined in May (15.5%) were infected. The early plasmodia of a hitherto unknown species, described below by the name of *M. margitae* n. sp., were first detectable in the gill filaments at the end of May. The spore-containing plasmodia of that parasite were observed in June and its disintegrating cysts at the beginning of July. The prevalence of infection was 2/45 (4.4%) in May, 15/100 (15%) in June and 2/20 (10%) in July; overall prevalence in the above three months was 11.5%. On the fins the formation of *M. alburni* plasmodia was observed only in June, when the prevalence of infection was 14/100 (14%). No *Myxobolus* plasmodia were found in the fish dissected in August and September. Nor could plasmodia be recorded on the gills or fins of bleaks collected from Lake Balaton.

In addition to the above *Myxobolus* species, *Myxobolus* infection was often found in the musculature and in the kidneys of bleaks from both the River Danube and Lake Balaton. The spores of the species developing intracellularly in the muscle cells, resembling *M. pseudodispar*, were not studied in detail. The solitary and sometimes deformed spores stuck in the macrophage centres of the kidney were not studied either.

In addition to description of the new species and redescription of the species *M. alburni* and *M. obesus*, this paper presents data on the seasonal occurrence of gill- and fin-parasitic species and on their location within the host on the basis of histological examinations.

Description of *Myxobolus margitae* n. sp.

Type host: *Alburnus alburnus* L.
Locality: River Danube near Budapest.
Site of infection: Gill filaments, solitary spores in the melano-macrophage centres of the kidney.
Type material: Spores have been deposited in the protozoological collection of the Hungarian Natural History Museum, Budapest.

Etymology: The species was named in honour of Margit Székely, DTP Editor of this journal.
Description of vegetative stages: Young plasmodia with a globule shape and a diameter of 0.3 to 0.5 mm were found lined up inside the afferent arteries of the primary gill filaments. More developed plasmodia in the same location had a globule to ellipsoidal shape and measured 0.5–0.7 by 0.7–1.0 mm.
Description of spores: Spores (Figs 1 and 2) are ellipsoidal in frontal view and lemon shaped in lateral view. Spore valves are relatively thin, symmetrical and smooth. Sutural line indistinct, sutural edge less protruding. Spores measure 13.7 (13–14) µm in length, 9.7 (9.5–10) µm in width and 5.7 (5.5–6) µm in thickness. Two polar capsules are pyriform in shape, equal in size and measure 5 (4.5–5.5) µm in length and 3 (2.8–3.2) µm in width. Polar capsules are located in the spores almost parallel and open at the base of the intercapsular appendix. Polar filaments are closely coiled with 7 or 8 turns in the polar capsule, situated perpendicularly to the longitudinal axis of the capsule. The extruded polar filament measures 70 (68–74) µm in length. A large, triangular intercapsular appendix is located between the polar capsules and the anterior end of the spore. A large iodinophilous vacuole and the two nuclei of the sporoplasm were well discernible in spores. Mucous or membranaceous envelope was not found around spores.

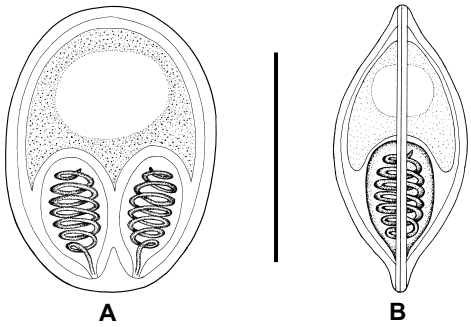

Fig. 1. Schematic representation of the spore of *Myxobolus margitae* n. sp. (A) in frontal view and (B) in lateral view. Bar = 10 µm

Remarks: Four species (*Myxobolus alburni* Donec, 1962; *M. ergensi* Lom, 1969; *M. obesus* Gurley, 1893 and *M. saidovi* Gazimagomedov, 1970) have been described from *Alburnus alburnus*. All of these species differ from *M. margitae* n. sp. in their morphology. *M. alburni* and *M. saidovi* differ from *M. margitae* n. sp. by their spherical spores, while the spores of *M. ergensi* have an oval shape and no vacuoles in the sporoplasm. There is also a significant difference between the present species and *M. obesus*, as the latter is much smaller and has a knoblike structure on the anterior end of the spores. *M. margitae* n. sp. somewhat re-

sembles *M. cycloides* Gurley 1893 and *M. schulmani* Donec, 1962, but differs from them by its less elongated and dorsoventrally flattened spores.

Histology: The young plasmodia of *M. margitae* started their development in the afferent artery of the primary gill filaments, in the immediate vicinity of the cartilaginous structure of the filaments (Fig. 3). In less intensive infection the solitary or fused, spore-containing plasmodia located in the lumen of the artery substantially increased in size and resulted in a well-visible thickening of the affected segments of the gill filament (Fig. 4). In intensive infection the relatively small plasmodia gathered in the afferent artery like a string of pearls markedly dilated the artery and completely deformed the structure of the gill filaments. In some cases they caused generalised gill changes involving multiple hemibranchia (Fig. 5).

Redescription of *Myxobolus alburni* Donec, 1962

Host: Alburnus alburnus.

Locality: River Danube near Budapest.

Site of infection: Fins, solitary spores in the melano-macrophage centres of the kidney.

Type material: Spores have been deposited in the protozoological collection of the Hungarian Natural History Museum, Budapest.

Description of vegetative stages: Plasmodia containing developing and mature spores were found in the fins, first of all in the caudal ones. They were located in the connective tissue connecting the skin duplicates between two neighbouring fin rays. Ellipsoidal plasmodia, flattened from one side, measured 0.7–1.4 mm in length, 0.6–1.0 mm in width and 0.3–0.4 mm in thickness.

Description of spores: Spores (Figs 6 and 7) are ellipsoidal in frontal view and lemon shaped in lateral view. Spore valves are relatively thin, symmetrical and smooth. Sutural line indistinct, sutural edge less protruding. Spores measure 13.7 (13–14) µm in length, 11 (9.5–12) µm in width and approximately 8 µm in thickness. Two polar capsules are pyriform or drop-like in shape, equal in size and measure 5.3 (5–5.5) µm in length and 3.3 (3–3.5) µm in width. Polar capsules diverge toward the posterior end of the spore and open very close to each other at the base. No intercapsular appendix was found but a small thickening of the anterior end of the spore exists. Polar filaments are loosely coiled with 4 turns in the polar capsule, situated obliquely to the longitudinal axis of the capsule. A large iodinophilous vacuole and the two nuclei of the sporoplasm are well discernible in spores. The spores are surrounded by a relatively thick mucous envelope (Fig. 7).

Remarks: *Myxobolus alburni* Donec, 1962 was described only briefly by Donec and Shulman (1984). The spores found in this study conform to the original description. The only difference is their typical location in the fins.

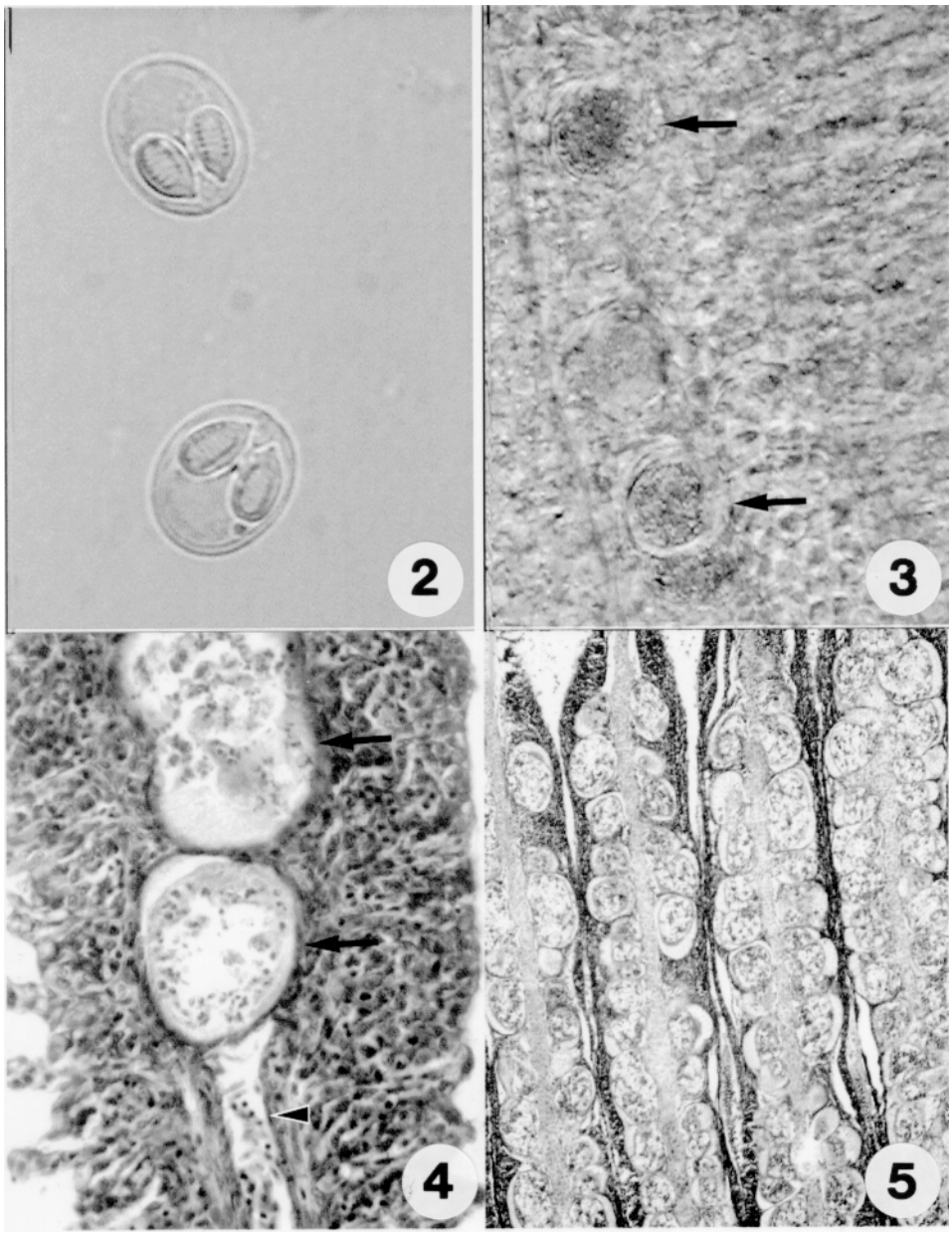

Fig. 2. Spores of *Myxobolus margitae* n. sp., released from mature plasmodia. × 1500
Fig. 3. Young plasmodia (arrows) of *Myxobolus margitae* n. sp. within the afferent artery of the gills of bleak. Fresh examination, × 1000
Fig. 4. Spore-containing plasmodia (arrows) of *Myxobolus margitae* n. sp. within the afferent artery (arrowhead) of the gills of bleak. Haematoxylin and eosin (H.-E.), × 400
Fig. 5. Intensive infection of the gills with *Myxobolus margitae* n. sp. plasmodia in bleak. H.-E., × 100

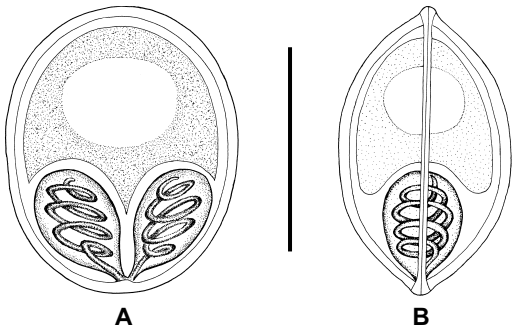

Fig. 6. Schematic representation of the spore of *Myxobolus alburni* (A) in frontal view, (B) in lateral view. Bar = 10 μm

Histology: In histological sections *M. alburni* was detectable from the connective tissue between the fin rays of the fins, first of all the caudal fins (Fig. 8), where the mostly elongated plasmodia were covered from two sides by the epithelial layer of the fins. In the semi-mature plasmodia the ectoplasm and the spore-containing endoplasm were distinctly separated.

Redescription of *Myxobolus obesus* Gurley, 1893

Host: Alburnus alburnus.
Locality: River Danube near Budapest.
Site of infection: Gill filaments, solitary spores in the melano-macrophage centres of the kidney.
Type material: Spores have been deposited in the protozoological collection of the Hungarian Natural History Museum, Budapest.
Description of vegetative stages: Young plasmodia with a globule shape and a diameter of 0.3 to 0.5 mm were found inside the capillary network of the secondary gill lamellae. More developed plasmodia in the same location had a globule to ellipsoidal shape and measured 0.5–0.7 by 0.7–1.0 mm.
Description of spores: Spores (Figs 9 and 10) are ellipsoidal in frontal view with a small protrusion at the anterior end and lemon shaped in lateral view. Spores are covered by a mucous envelope. Spore valves are relatively thin, symmetrical and smooth. Sutural line indistinct, sutural edge protruding. Spores measure 10.5 (10–11) μm in length, 8.5 (8.2–9.0) μm in width and 6.8 (6.0–7.0) μm in thickness. Two polar capsules are pyriform in shape, equal in size and measure 5.1 (5.0–5.3) μm in length and 3.4 (3.0–3.5) μm in width. Polar capsules diverge slightly toward the posterior end of the spore and open close to each other at the base. Intercapsular appendix is a small drop-like thickening at the anterior end of the spore. Polar filaments are loosely coiled with 4 turns in the polar capsule, situated in most cases obliquely to the longitudinal axis of the capsule. A small io-

dinophilous vacuole and the two nuclei of the sporoplasm were discernible in spores. The spores are surrounded by a relatively thick mucous envelope (Fig. 10).
Remarks: In frontal view the spores collected in this study were the same as those of *Myxobolus obesus* Gurley, 1893 depicted by Lom (1961) and Kashkovskii (1965; cit. by Donec and Shulman, 1984). In lateral view, Kashkovskii (1965) claimed to see a trilobate structure at the posterior end of the spores; in this study, however, the spores had a typical lemon shape in that view. Since Kashkovskii (1965) claimed to have found *M. obesus* in three other cyprinids besides the bleak, the lobated spores must have represented developmental stages of some other species.
Histology: In histological sections *M. obesus* was located in the respiratory lamellae of the gill filaments (Fig. 11), where the mostly globule-shaped plasmodia were covered by the endothelium of the respiratory lamellae from the two sides. The large plasmodia often protruded over the respiratory lamellae and compressed the neighbouring lamellae.

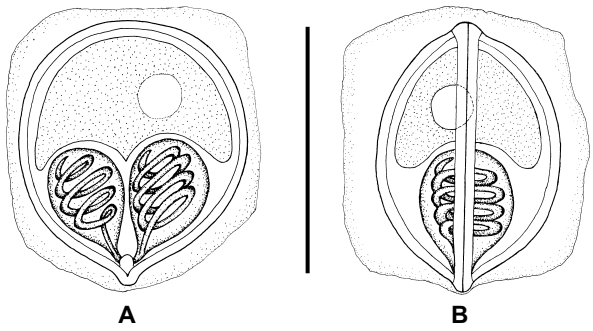

Fig. 9. Schematic representation of the spore of *Myxobolus obesus* (A) in frontal view and (B) in lateral view. Bar = 10 μm

Discussion

The myxosporean fauna of the bleak (*Alburnus alburnus*) is well studied. Thorough studies on the occurrence of these parasites have been done especially in the republics of the former Soviet Union, where the monograph of Donec and Shulman (1984) mentioned the occurrence of 20 *Myxobolus* species in the bleak on the basis of domestic findings and data collected from other publications. However, since Donec and Shulman (1984) adopted data into their monograph without strict critical evaluation, their list may obviously contain numerous erroneously identified species.

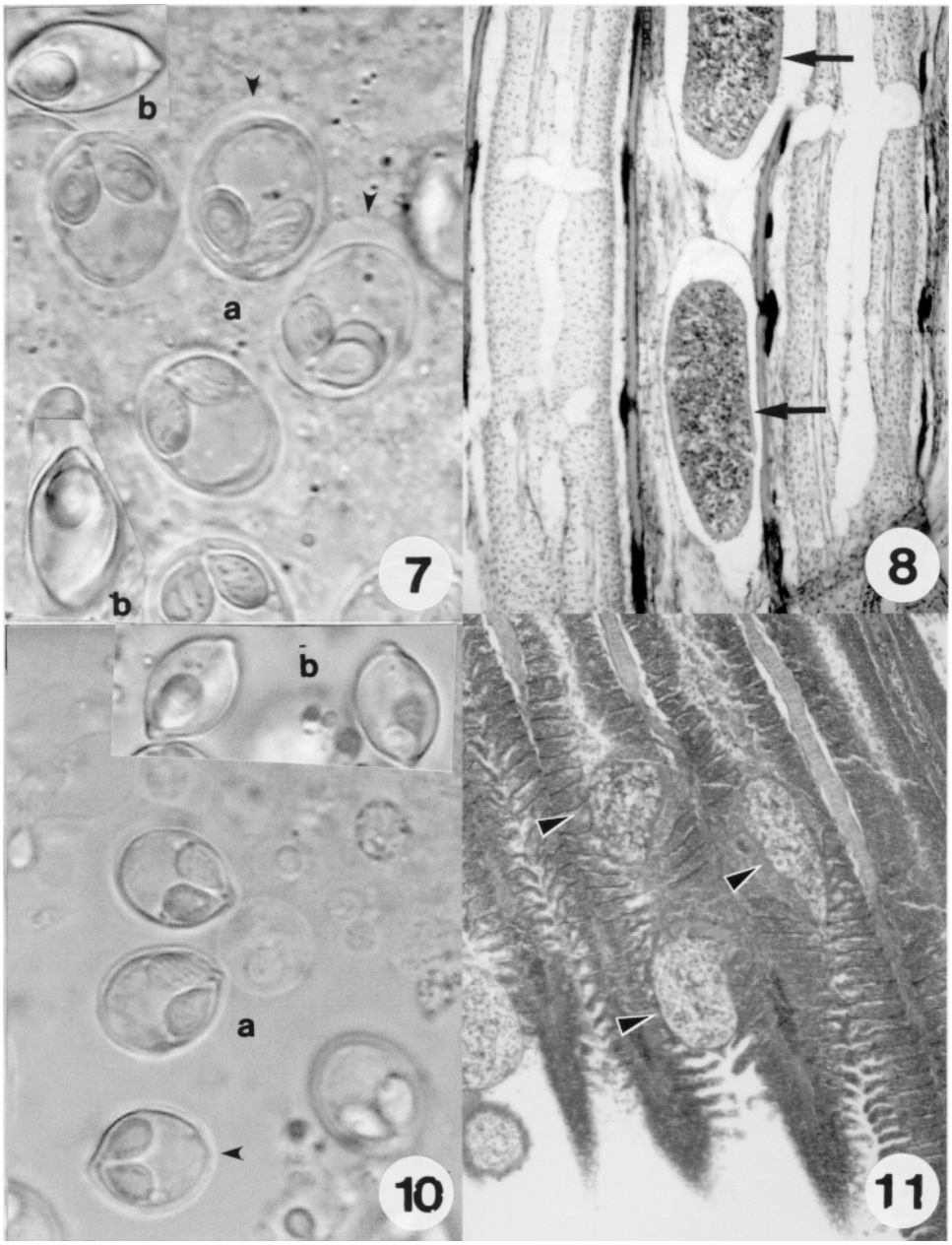

Fig. 7. Spores of *Myxobolus alburni* released from mature plasmodia (a) in frontal view and (b) in lateral view. See the mucous envelope (arrowheads) around spores. × 1500
Fig. 8. Plasmodia of *Myxobolus alburni* (arrows) among the fin rays of the caudal fin of bleak. H.-E., × 100; *Fig. 10.* Spores of *Myxobolus obesus* released from mature plasmodia (a) in frontal view and (b) in lateral view. See the mucous envelope (arrowhead) around spores. × 1500
Fig. 11. Plasmodia of *Myxobolus obesus* (arrowheads) in the gill lamellae. H.-E., × 100

Two out of the three *Myxobolus* species found on the gills and fins of Danubian bleaks (*M. alburni, M. obesus*) could be identified with known species, while the third one of relatively common occurrence is described as a new species. Identification of *Myxobolus* species is an extremely difficult task. Accurate species identification is hampered by the uncertainty of identification based merely on spore morphology, as nearly 500 species of similar spore structure have been described until now. As a consequence, the same parasite species have been described also from hosts taxonomically distant from their type host. A further factor that adds to the uncertainty of species identification is that only observations exist on the host specificity of these parasites, and no experimental studies have been done because of the complex life cycle of myxosporeans. From the observations it is unquestionable that there are species having a relatively broad host range, such as *M. cerebralis* which can colonise most salmonids, while other species are characterised by expressed host specificity and can produce infection only in their type host and in the fish species most closely related to it. Examples of the latter include two parasites of the silver carp (*Hypophthalmichthys molitrix*), *M. drjagini* and *M. pavlovskii*: the former infects exclusively the silver carp, while the latter produces infection in both the silver carp and the bighead (*Aristichthysis nobilis*) (Molnár, 1979). The commonest and longest-known species, such as *M. muelleri* Bütschli, 1882, *M. ellipsoides* Thélohan, 1892, *M. cycloides* Gurley, 1894, *M. dispar* Thélohan, 1895, *M. pfeifferi* Thélohan, 1895, *M. cyprini* Doflein, 1898 and *M. bramae* Reuss, 1906 have been identified from more than 40 fish species by different authors, presumably on the basis of an erroneous diagnosis (Donec and Shulman, 1984). It is especially disturbing that the kidney, the spleen and the gills are simultaneously indicated as the location of spores and plasmodia. This raises the suspicion that the spores detected in these organs originate from plasmodia disintegrating in other tissues, and e.g. the plasmodia described from the kidney are nothing else than deformed spores transported there by the blood and stuck in the macrophage centres. In order to eliminate the above errors, Lom and Arthur (1989) proposed that during the preparation of species descriptions the presence of accessory elements such as the membranaceous envelope and mucous envelope should be observed in addition to the spore characteristics and, as far as possible, the form of vegetative stages and their location within the host should also be determined. Assuming the existence of numerous erroneously identified species, Landsberg and Lom (1991) drew up a list of the type hosts on the basis of the original descriptions. For accurate species identification, Molnár (1994) considers it indispensable to determine the organ and tissue specificity of a given parasite and to determine the site of plasmodium development. However, safe species differentiation is rendered possible only by molecular biological procedures (Andree et al., 1997; Andree et al., 1999). This latter procedure requires the processing of a well-defined, pure spore material, i.e. determination of the host species and intrapis-

cine location of the collected parasite, and the morphological characterisation of vegetative and spore stages. Collecting a pure material is by no means an easy task, as according to Molnár and Székely (1999) spores of different species may occur even within the same 'cyst' as a result of the fusion of plasmodia.

The parasite described as a new species by the name of *M. margitae* can be characterised as a typical *Myxobolus* species on the basis of the shape and internal morphology of the spores, and it resembles several *Myxobolus* species considered common (*M. cycloides, M. schulmani, M. muelleri*). These latter are, however, regarded as collective species which probably contain several hitherto undifferentiated *Myxobolus* species. In contrast to the uncertain organ specificity of the collective species described earlier, the intrapiscine location of *M. margitae* is easy to characterise and corresponds to the interlamellar type of location. The plasmodia start their development exclusively in the afferent artery of the gill filaments, where they rarely reach a large size and, in the majority of cases, line up in the lumen of the artery as strings of small plasmodia. The intralamellar form typical of the species *M. obesus* was not observed in the case of *M. margitae*; thus, the two species are well distinguishable also by histological examination.

The spores of *M. obesus* correspond to the characteristics described by Gurley (1893) and to the figure published by Lom (1961). At the same time, the lateral spore view depicted by Kashkovskii (1965, cit: Donec és Shulman, 1984) differed from that found in this study. As Kashkovskii (1965) collected the spores identified by him as *M. obesus* from several fish species, it cannot be excluded that he depicted mixed species. In the Danubian bleaks this parasite occurred in May, and its plasmodia developed typically in intralamellar location. In June *M. obesus* could no longer be detected.

The *Myxobolus* species found in the fins is identified with the species *M. alburni* Donec, 1984. In this study the spores proved to have a slightly more elongated shape than those depicted by Donec and Shulman (1984). No major difference was found in the size of the polar capsules either. Regarding their dimensions and their expressly divergent polar capsules, the spores found in this study show a high degree of similarity to the description given by Donec and Shulman (1984). On the basis of the short original description it is likely that Donec and Shulman (1984) saw only the spores scattered in the organism and had no data on the site of spore development. The *Myxobolus* species seen in this study was a typical fin parasite showing affinity to connective tissue; however, its spores were found throughout the body, primarily in the macrophage centres of the kidney. This is surprising as it would be easy for the spores to get to the outworld directly through the thin epithelial layer of the fins. The parasite was typically found in June.

Gill myxobolosis of the bleak is well distinguishable from *M. bramae* and *M. macrocapsularis* infection of the bream, characterised by the simultaneous presence of interlamellar and intralamellar forms. *Myxobolus margitae* typically

occurs in the summer months, first of all in June, and the appearance of the first *M. margitae* plasmodia in bleak can be expected after *M. obesus* infection has passed off.

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