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Redescription and Histopathology of *Myxobolus cyprinicola* Reuss, 1906, an Intestinal Parasite of the Common Carp (*Cyprinus carpio* L.)

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Summary. Although *Myxobolus* spores can often be detected from the gut of fish, from the mucus covering the intestinal wall and from the intestinal content, the number of species actually developing in the gut wall is rather low. *Myxobolus cyprinicola* can be considered a parasite rarely occurring in Europe. This parasite was found to cause infection in the gut of common carp caught from Lake Balaton. Its pinhead-sized plasmodia were located in the lamina propria at the tip of the mucosal folds of the intestine, immediately below the basement membrane. The plasmodia were closely connected with the capillaries of the lamina propria. The large number of red blood cells found between the basement membrane and the plasmodium wall suggests that they started their development in the lumen of a capillary. By their morphological characteristics, the spores of *M. cyprinicola* are well distinguishable from those of *M. cyprini,* which more commonly occur in the gut within necrotic macrophages (yellow bodies) after having reached that site as a result of a secondary process. Because of the small size of the plasmodia, the subepithelial location, and the low prevalence and intensity, *M. cyprinicola* can be considered a less pathogenic species.

Key words: carp, histology, intestine, *Myxobolus*, Myxosporea, Pisces.

INTRODUCTION

Of the approx. 500 *Myxobolus* species known at present, various authors described a large number of species from the common carp (*Cyprinus carpio*). In their monograph Donec and Shulman (1984) recorded 25 *Myxobolus* species in common carp, while Chen and Ma (1998) have reported the occurrence of 50 carp-parasitic *Myxobolus* species in China. Landsberg and Lom (1991) regard 19 of these species as species originally described from the common carp. To date, four species (*Myxobolus basilamellaris, M. cyprini, M. dispar, M. intrachondrealis*) have been known to occur in Hungary (Molnár 1979, 2000; Lom and Molnár 1983; Molnár and Kovács-Gayer 1985). All species recorded in Hungary were characterised by distinct tissue and organ specificity: *M. cyprini* proved to be a typical muscle cell parasite (Molnár and Kovács-Gayer 1985), *M. dispar* formed plasmodia at the apical end and *M. basilamellaris* at the base of the gill filaments (Lom and Molnár 1983), while the plasmodia of *M. intrachondrealis* developed in the cartilaginous substance of the gill arch (Molnár 2000). In addition to developing and mature spores of plasmodia in the wall of the intestine, different organs (skin, gills, kidney, gut,

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etc.) often contained solitary spores that corresponded to *M. cyprini* spores dispersed throughout the body by the blood stream (Molnár and Kovács-Gayer 1985). According to the above authors, such spores were particularly often detectable in the mucus drawn off the gut wall, in which they occurred as solitary spores or as small clusters of spores embedded in yellow bodies.

This paper reports the occurrence and specific location of *M. cyprinicola* Reuss, 1906, a species forming plasmodia in the gut wall, in common carp from Lake Balaton, and it also presents a redescription of this rare species.

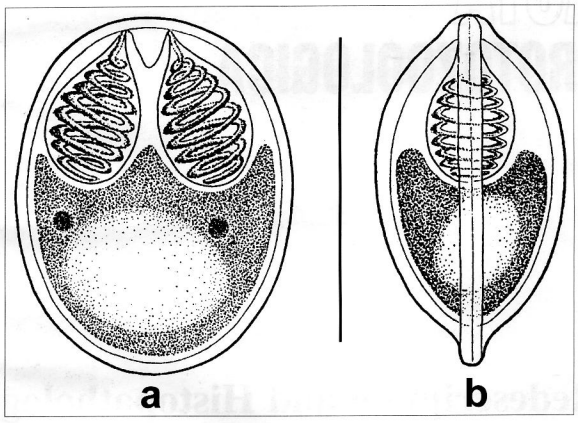

Fig. 1. Schematic representation of *Myxobolus cyprinicola* spores in frontal view (a) and in lateral view (b). Scale bar - 10 μm

MATERIALS AND METHODS

The studies were conducted from September 1999 to July 2001 in the framework of a survey of the parasite fauna of Lake Balaton fishes. The samples originated from four different biotopes of the lake, from the area of Siófok, Balatonszemes, Tihany and Keszthely. A total of 48 two- to four-year-old common carp measuring 18 to 48 cm (average: 33 cm) in length were examined. The carp were subjected to complete parasitological examination that included studying *Myxobolus* infection of the gut. To remove the intestinal content, the fish were kept in flow-through water in the laboratory for 1-2 days, then killed by exposure to MS-222 (3-aminobenzoic acid ethyl ester) solution, and dissected. The gut was cut open in its entire length, the remaining gut content was carefully removed, and gut segments were examined thoroughly under stereomicroscope at tenfold magnification. To avoid shining, 0.65% saline solution was sprayed onto the intestinal mucosa in a thin layer, and top lighting was applied to search for nodules that contrasted with the gut mucosa by their whitish colour. From the plasmodia located within the nodules the spores were sucked out with a pipette. Unfixed spores were studied by an Olympus BH2 microscope using Nomarski differential interference contrast. The spores were recorded on video, digitised images were obtained according to the method of Székely (1997), and the measurements of the spores were taken. For histological examination, small samples were excised from the infected gut segments and fixed in Bouin's solution for 4 h. From the paraplast-embedded blocks 4 μm thick sections were made by cutting through the intestine, and the sections were stained with haematoxylin and eosin.

RESULTS

Seven out of the 48 common carp examined from Lake Balaton (14.6%) proved to be infected. Infection occurred at all sampling sites and in all periods, and was found in the smallest (18 cm) and bigger (40 cm) fish specimens alike. The intensity of infection was low, 1-11 (4) plasmodia. Only mature plasmodia containing spores (Fig. 1) were found during the survey. The spherical plasmodia 0.3 to 1.5 mm in diameter were located in the gut wall. In 9 fish, solitary spores located outside plasmodia, in yellow bodies, were also found in the mucus covering the enterocytes; these, however, proved to be spores of the species *Myxobolus cyprini* by their shape and size (Fig. 2). In the framework of other projects and routine diagnostic examinations several hundred common carp of similar age and size were examined from fish farms, but *M. cyprinicola* infection could not be detected in any of them.

On the basis of 50 spores released from the plasmodia the redescription of the species *M. cyprinicola* Reuss, 1906 is given as follows: spores (Figs 1, 3) are ellipsoidal in frontal view and lemon shaped in lateral view. Spore valves are relatively thick, symmetrical and smooth. Sutural line distinct, sutural edge protruding, forming a longer process in the posterior and a shorter protrusion on the anterior end in lateral view. Spores measure 11.8 (11-12.2) μm in length, 9.0 (8.1-9.4) μm in width and 6.2 (6.0-6.4) μm in thickness. Two polar capsules are pyriform in shape, equal in size and measure 5.0 (4.8-5.2) μm in length and 3.2 (3.0-3.4) μm in width and thickness. Polar capsules are slightly converging toward the posterior end of the spores and open at the base of the intercapsular appendix. Polar filaments are closely coiled with 8 turns in the polar capsule, situated perpendicularly to the longitudinal axis of the capsule. The extruded polar filament measures 41 (38-47) μm in length. A large, triangular intercapsular appendix is located between the polar capsules at the anterior end of the spore. A large iodinophilous vacuole and two nuclei of the sporoplasm were well discernible in spores.

Histopathological findings. In histological sections, the spores of *M. cyprinicola* were found in the lamina

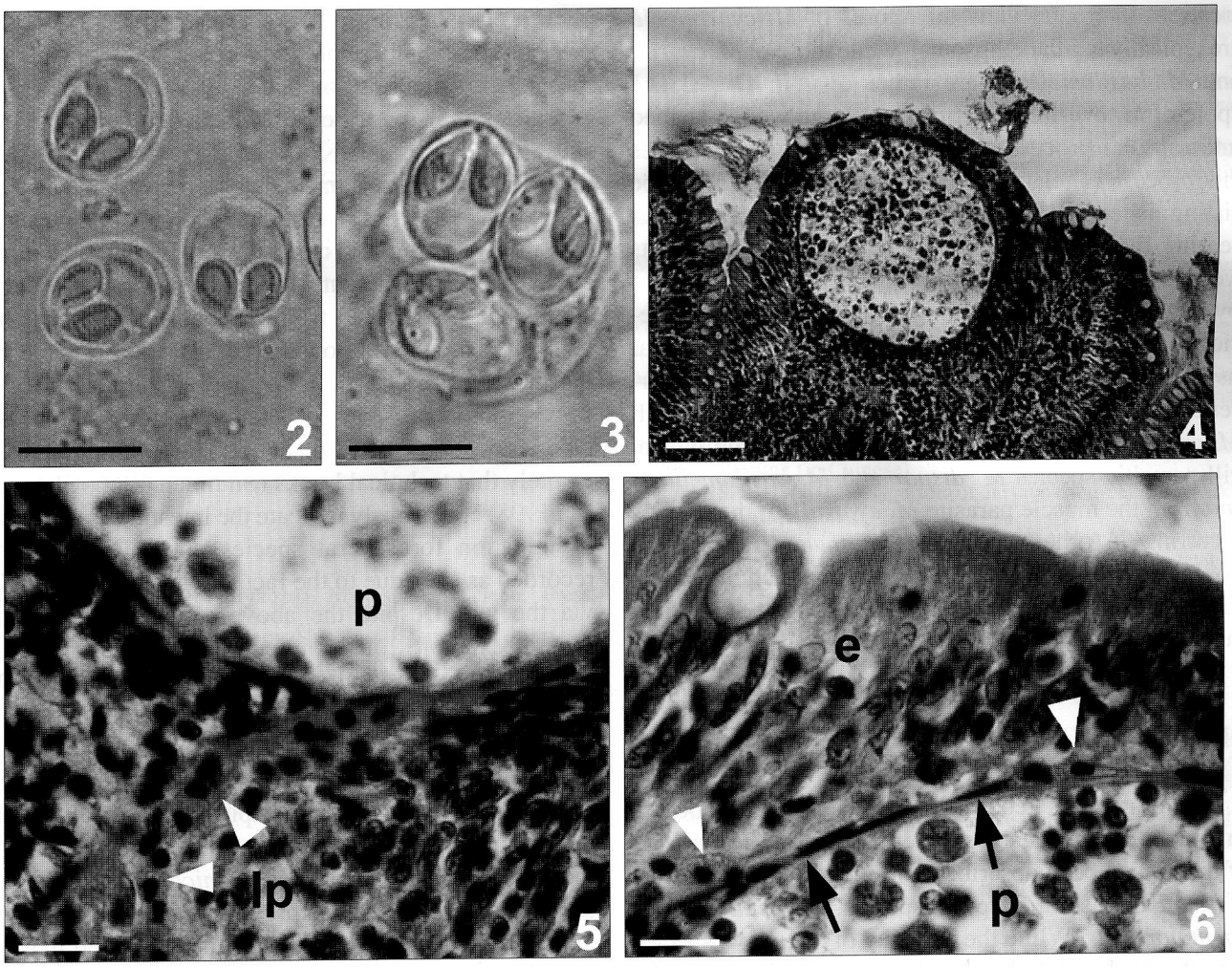

Fig. 2. *Myxobolus cyprinicola* spores released from a mature plasmodium. x 1800
Fig. 3. *Myxobolus cyprini* spores embedded in a yellow body from the gut of common carp. x 1800
Fig. 4. *Myxobolus cyprinicola* plasmodium at the tip of the mucosal folds of the gut in common carp. Histological section. Haematoxylin and eosin (H & E.), x 200
Fig. 5. *Myxobolus cyprinicola* plasmodium (p) containing spores in the lamina propria of the intestinal wall (lp). A capillary (arrowheads) is seen to be connected with the wall of the plasmodium. H & E, x 1200
Fig. 6. The very thin ectoplasm of the *M. cyprinicola* plasmodium (p) located under the epithelial layer (e) of the gut is bordered by flattened cells (arrowheads) which contain red blood cells (arrows) in some places. H & E, x 1200

propria of the gut, in spherical, relatively small plasmodia 0.3-1.5 mm in diameter (Fig. 4). The plasmodia were usually located at the tip of the mucosal folds of the intestine, closely adhering to the basement membrane of the epithelium. The spore-filled endoplasm of the plasmodium was surrounded only by a very thin ectoplasm, and the latter was bordered by a very thin capsule of host origin, consisting of a cell row presumably formed from the pillar cells. From the propria, fine capillaries (Fig. 5) led to the capsule, and the red blood cells seen scattered around the capsule were also indicative of the remnants of a capillary covering the ectoplasm of the plasmodium (Fig. 6). Although we could not observe young plasmodia, the close connection with the capillaries suggests that the formation of plasmodia may commence inside a capillary. Infection caused by the relatively small plasmodia was accompanied by negligible local histological changes appearing as flattening of the epithelium above the plasmodium.

Remarks. The *Myxobolus cyprinicola* species detected from the lamina propria of the intestine differs from the four species (*Myxobolus cyprini, M. dispar, M. basilamellaris* and *M. intrachondrealis*) reported from Hungary from common carp by its shape and tissue

location. In frontal view the spores of *M. cyprinicola* are typically regular ellipsoidal, while those of *M. cyprini* and *M. basilamellaris* are rather oval in shape. The spore of *M. cyprinicola* contains a large wedge-shaped intercapsular process, while in the other three species this process is relatively small. *M. cyprinicola* can be distinguished from the species *M. intrachondrealis* bearing the greatest resemblance to the former by its well-developed iodinophilous vacuole in frontal view and by its thick sutures protruding at the anterior and posterior ends of the much wider spores in lateral view. As far as the intrapiscine location is concerned, by its occurrence in the lamina propria of the intestinal wall *M. cyprinicola* is well distinguishable from the typical gill-parasitic species *M. dispar* and *M. basilamellaris* and from the muscle parasite *M. cyprini*. Of the species occurring in other cyprinids, *M. muelleri* bears the closest resemblance to *M. cyprinicola* which, however, differs from the former by its more elongated elliptical shape.

DISCUSSION

The number of recorded *Myxobolus* species occurring in the common carp is extremely large. The majority of them have been described and identified as parasites of the Far-Eastern carp subspecies, *Cyprinus carpio haematopterus*. While the majority of the above species are obviously included in the parasite fauna of the common carp as a result of erroneous identification, it is unquestionable that even the accurately identified *Myxobolus* parasites of the Far-Eastern carp subspecies exceed in number those parasitising the European carp subspecies.

The common occurrence of three *Myxobolus* species, *M. cyprini, M. dispar* and *M. basilamellaris*, in Hungarian carp farms has long been known (Molnár 1979, Molnár and Kovács-Gayer 1985). Recently *M. intrachondrealis* has been added to these species (Molnár 2000). Unpublished data also suggest that the above parasites occur also in common carp living in natural waters of Hungary. Of the numerous species recorded from common carp, in Czechoslovakia Dyková and Lom (1988) detected *M. cyprini, M. cyprinicola, M. dispar* and *M. basilamellaris,* and additionally the species *M. encephalicus* (Mulsow, 1911), *M. muelleri* Bütschli, 1882 and *M. oviformis* Thélohan, 1892. The species *M. cyprinicola*, only recently found in Hungary but known to occur in Europe for a long time, appears to be a rarely occurring parasite causing infection of low prevalence, which explains why it has escaped the researchers' attention so far. Obviously, many researchers mistake this parasite for *M cyprini,* which until the study of Molnár and Kovács-Gayer (1985) had been regarded as species parasitising also the gut. After the disruption of muscle cells the spores of *M. cyprini* become scattered all over the body and are often excreted through the gut. At such times large masses of spores can be detected from the intestinal mucus, embedded in so-called yellow bodies. It seems probable that the much less frequent *M. cyprinicola* infection often passes unnoticed. The gut relatively rarely serves as a typical location for the different *Myxobolus* species. It is likely that technical books (Shulman 1966, Chen and Ma 1998) erroneously indicate the intestine as a location of infection for several species, and has only been recorded as such due to the fact that spores are excreted via the gut. Despite the above probability there are several examples of the infection of *Myxobolus*-plasmodia in the intestinal wall. Masoumian *et al.* (1996) detected rather large plasmodia of *M. nodulointestinalis* from the muscular layer of the gut of *Barbus sharpeyi*, while in the book edited by Pan *et al.* (1990) the plasmodia of *M. artus* were depicted in a location fully resembling that of *M. cyprinicola* described here. Although the plasmodia of *M. cyprinicola* were indisputably located in the lamina propria of the intestinal wall, i.e. in the connective tissue, it cannot be stated that this parasite shows affinity to the connective tissue. Rather, the capillaries found around the plasmodia indicate that the parasite started developing within a capillary of the lamina propria. In this regard, despite its unquestionable gut wall location *M. cyprinicola* more closely resembles the better studied species that commence their development within the capillaries of the gill lamellae (Dyková and Lom 1978, Molnár 2002). In view of its low prevalence and negligible intensity of infection, *M. cyprinicola* can be considered a rare species of low pathogenicity, which, however, may have diagnostic importance.

Major changes in the identification of Myxosporea species can be anticipated in the near future. Species descriptions based merely on the morphological characteristics of spores are inadequate, and besides the determination of the typical host, host groups and intrapiscine locations, molecular biological identification will also become indispensable. For instance, for *M. cyprinicola* Donec and Shulman (1984) listed, besides the typical host common carp, 10 other cyprinids and also a *Nemacheilus* species as hosts. It seems likely that in the

future, following accurate redescription of the different *Myxobolus* species, determination of their organ and tissue specificity, and DNA analysis, many parasites currently regarded as valid species will prove to be synonyms. At the same time, it is also probable that species erroneously identified as synonyms from taxonomically distant hosts based upon the morphological similarity of the spores will prove to represent separate species and add to the number of existing *Myxobolus* species.

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