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Development of *Myxobolus hungaricus* (Myxosporea: Myxobolidae) in oligochaete alternate hosts

Amina El-Mansy*, Kálmán Molnár

Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, PO Box 18, Hungary

ABSTRACT: The extrapiscine development of *Myxobolus hungaricus* Jaczó, 1940, a myxosporean parasite of the gill of bream *Abramis brama*, was studied in experimentally infected oligochaetes *Tubifex tubifex* and *Limnodrilus hoffmeisteri*. Uninfected tubificids were infected with mature spores of *M. hungaricus*, and development of actinosporean stages was first observed by light microscopy 21 d later. In histological sections, early pansporocysts were located in the gut epithelium of the experimental oligochaetes. Mature pansporocysts, each containing 8 triactinospores, appeared 89 d after infection. After rupture of the epithelial cell and the pansporocyst, free actinosporean stages were found in the gut lumen of the oligochaete. Actinospores emerged from the oligochaetes and appeared in water 102 d after infection. They were floating in water and showed a typical triactinomyxon form. Each triactinospore had 3 pyriform polar capsules, a sporoplasm with 18 secondary cells inside the spore body, a moderately long style and 3 slightly bent, conically ending tails. The total length of the triactinospore was approximately 315 µm. The prevalence of infection in 67 oligochaetes proved to be 43.3%. No infection was found in control oligochaetes.

KEY WORDS: *Myxobolus hungaricus* · Myxozoa · Triactinomyxon stage · Development in alternate host · *Tubifex tubifex* · *Limnodrilus hoffmeisteri*

INTRODUCTION

Wolf & Markiw (1984) proved that the extrapiscine development of *Myxobolus cerebralis* took place in oligochaete alternate hosts (*Tubifex tubifex*) in which triactinomyxon stages, classified earlier into the class Actinosporea, Myxozoa, developed. Since then, several experiments have demonstrated that actinosporean spores developing in oligochaetes infect fishes and cause myxosporean infections, while myxosporean spores start their development only in oligochaetes. The life cycles of the following parasites belonging to the *Myxobolus* genus have been studied: *M. cotti*, a parasite of the bullhead *Cottus gobio* (El-Matbouli & Hoffmann 1989), *M. pavlovskii*, a parasite of the silver carp *Hypophthalmichthys molitrix* (Ruidisch et al. 1991), *M. carassii*, a parasite of the crucian carp *Carassius carassius* (El-Matbouli & Hoffmann 1993), *M. arcticus*, a parasite of the sockeye salmon *Oncorhynchus nerca* (Kent et al. 1993), and *M. cultus*, a parasite of the goldfish *Carassius auratus* (Yokoyama et al. 1995). Of other genera of myxosporeans, successful life-cycle studies have been done on *Hoferellus*, *Ceratomyxa*, *Zschokkella*, *Myxidium*, *Thelohanellus* spp. and on the causative agent of proliferative gill disease of channel catfish (Styer et al. 1991, Yokoyama et al. 1991, 1993, Bartholomew et al. 1992, El-Matbouli et al. 1992, Grossheider & Körting 1992, Benajiba & Marques 1993, Uspenskaya 1995, Trouillier et al. 1996, Yokoyama 1997). In each case various Oligochaeta spp. proved to be alternate hosts.

Myxobolus hungaricus was described by Jaczó (1940) from Lake Balaton (Hungary). Since its original description, this parasite has been detected again only

*On leave from the National Institute of Oceonography and Fisheries, Cairo, Egypt. Student of the doctoral program in Zootaxonomy, Synbiology and Hydrobiology at the University of Eötvös Loránd, Budapest, Hungary.
E-mail: kalman@novell.vmri.hu

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quite recently by Molnár (unpubl.). In both cases, cysts containing spores were recorded in the gills of the common bream *Abramis brama*.

The present paper reports on experiments in which the oligochaetes *Tubifex tubifex* and *Limnodrilus hoffmeisteri* were experimentally infected with *Myxobolus hungaricus* spores, and actinosporean stages of triactinomyxon type were found to develop in them.

MATERIALS AND METHODS

Spores of *Myxobolus hungaricus* were collected from mature cysts from the gills of the bream *Abramis brama* (L.), where they were found in the central part or close to the base of the primary filaments.

Oligochaetes *Tubifex tubifex* (Müller) and *Limnodrilus hoffmeisteri* (Claparéde), identified according to Brinkhurst (1963), were collected from a muddy pool near the top of a hill where no fish are to be found. They were transferred into sterilised mud and propagated in the laboratory in aerated aquaria. The oligochaetes were fed some drops of granulated fish food, and pieces of chicken faeces were added to increase the organic matter content of the mud. Normal tapwater was used throughout the experiment. The temperature of the room varied between 18 and 22°C.

Two types of dishes were used. Some of the oligochaetes were placed into small aquaria containing 5 l of water, while others were transferred into small plastic cups. All dishes were permanently aerated and supplied regularly with fresh water to compensate for evaporation and to refresh the water. The large tanks contained about 100 to 300 specimens and the smaller ones 10 to 30 specimens. The oligochaetes were infected by mixing spores into the dishes. An additional dish containing *Tubifex tubifex* and *Limnodrilus hoffmeisteri* specimens from the same stock was used as control. From the infected stocks, 57 *T. tubifex* and 10 *L. hoffmeisteri* specimens were examined for the presence of developmental stages during the survey. The same number of oligochaetes from the control stock was examined.

The development of the actinosporean stages of *Myxobolus hungaricus* was followed by the methods: (1) Twice a week some of the oligochaetes were placed carefully under a coverslip and examined under microscope at 200× magnification for the presence of developmental stages. (2) From the third week after infection, 10 oligochaetes were placed into 2 ml/cell well plates (Yokoyama et al. 1991) 3 times a week, and after incubation for 1 d they were checked for the release of actinosporean spores under a microscope. (3) Every 2 d, water from the aquaria and the small dishes was filtered through a fine mesh of 10 µm pore size. The filtrates were taken up in a small amount of water and examined for the presence of actinosporean stages. (4) Every week 2 oligochaetes were sacrificed. For histological examination, 17 infected *Tubifex tubifex* and 10 *Limnodrilus hoffmeisteri* specimens were fixed in Bouin's solution, embedded in paraffin wax, cut into 5 µm thick sections, and stained with haematoxylin and eosin.

Triactinomyxon stages released by oligochaetes were examined under a coverslip. Images were recorded with the help of a video image program on videotapes. Photos and drawings were made, and measurements were noted. All measurements are given in µm. The description of the actinosporean stage of *Myxobolus hungaricus* has been given using the terminology of Janiszewska (1957) as modified by Lom et al. (1997).

RESULTS

Light microscopy

Myxobolus hungaricus was detected developing in both *Tubifex tubifex* and *Limnodrilus hoffmeisteri*. During a 3.5 mo period, 26 *T. tubifex* (45.6%) and 3 *L. hoffmeisteri* specimens (30%) proved to be infected by actinosporean developmental stages. The first sign of infection in living *T. tubifex* was recorded 21 d after infection. Developmental stages were seen in the gut epithelium of some segments of the worms. Subsequently these stages grew in number and size. From crushed specimens of *T. tubifex*, pansporocysts each containing 8 developing triactinomyxons were obtained (Fig. 1). Actinospores were first released from living oligochaetes into the water 102 d after infection and their presence in the water was recorded for about 1 mo after the start of release. Infection was recorded in both *T. tubifex* and *L. hoffmeisteri*. The released actinospores proved to be typical triactinomyxon forms.

Histological evidence

The first developing stages observed in the gut epithelium were young pansporocysts located in epithelial cells (Fig. 2). They were first recorded 3 wk after infection in *Tubifex tubifex* (Fig. 2). Four weeks after infection with *Myxobolus hungaricus*, it was clearly seen in a transverse section made from *Limnodrilus hoffmeisteri* that large numbers of more developed, round pansporocysts with a dark cytoplasm infected the epithelium of the midgut region. Mature pansporocysts in the midgut of the tubificid were formed 89 d after infection. These cysts were oval or round, and each contained 8 sporoblast cells of irreg-

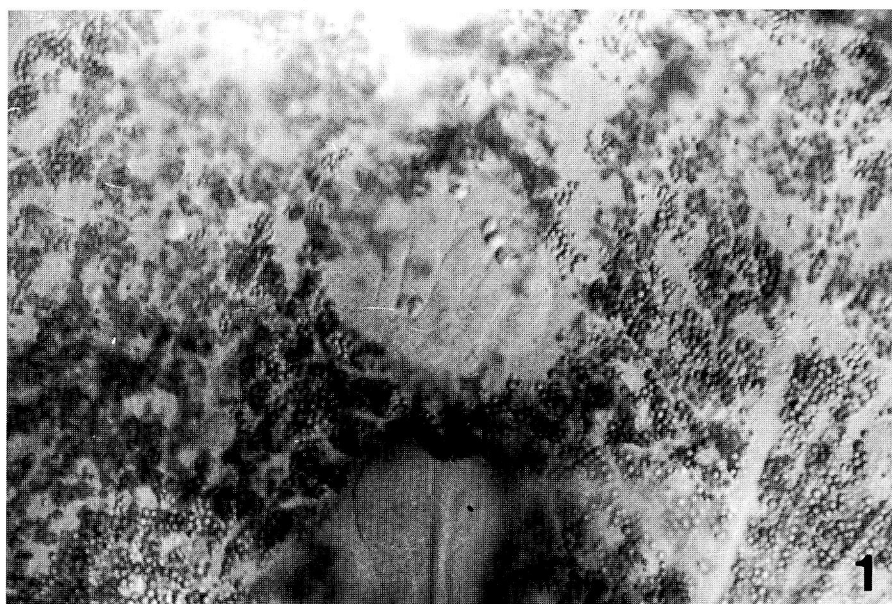

Fig. 1. *Tubifex tubifex.* Squash preparation from an infected tubifex. Inside a pansporocyst several triactinospores with polar capsules are seen. ×375

ular shape. In some more developed pansporocysts the spore body and the folded projections of the future triactinospore were visible. In some of our histological preparations it could be clearly seen that some mature triactinomyxon stages inside the pansporocyst were released from the epithelial cells into the gut lumen. In these advanced stages the 3 polar capsules (Fig. 3), the sporoplasm with the secondary cells, and the folded projections of the future style and caudal processes were easily detectable.

Description of triactinospores

Triactinospores released from the tubificid body and floating in the water were characterised by 3 pyriform polar capsules, a sporoplasm with 18 secondary cells, a moderately long style and 3 slightly bent caudal processes (Figs. 4, 5 & 6). Polar capsules were pyriform in shape, 7.7 (7–8) in length and 3 (2.5–3.5) in width. Sporoplasm, which was elliptical, 38.9 (30–45) long and 9.5 (9–10) wide, contained approximately 18 secondary cells. A conical structure of 13 µm in length containing a spherical, dark body was attached to the caudal end of the sporoplasm; length 80.8 (80–84), width at its anterior part about 6. The length of the caudal processes was 196.7 (160–240). Caudal processes, slightly bent, tapered evenly toward the end and finished in a sharp point. The whole length of the triactinospore was about 315. The prevalence of infection in experimental oligochaetes was 43.3%.

No actinosporean infection was found in control *Tubifex tubifex* and *Limnodrilus* spp. examined simultaneously by the same method and in the same number.

Differential diagnosis

The triactinospores of *Myxobolus hungaricus* differ from the known triactinospores in the following features. The number of sporoplasm nuclei in the triactinospore of *M. hungaricus* was 18 while in that of *M. cerebralis* it was 32 to 50; for mature *M. cotti* 8 sporoplasm nuclei (El-Matbouli et al. 1992) and for *M. carassii* about 150 secondary cells (sporozoites) (El-Matbouli & Hoffmann 1993) were recorded. The total length of *M. cerebralis* and *M. cotti* (135 µm and 88 µm, respectively) was shorter than that of *M. hungaricus.* The length of the *M. carassii* triactinospore seems to be greater, as the caudal processes alone measure 277 µm.

DISCUSSION

Myxobolus hungaricus is a little known parasite species of the common bream, for which a redescription and details on its intrapiscine development will be published in the nearest future (Molnár unpubl.). Data obtained on the extrapiscine development of this parasite show that this species follows the same developmental pattern as was described by Wolf & Markiw (1984), El-Matbouli & Hoffmann (1989, 1993) and Ruidisch et al. (1991). *M. hungaricus* develops in tubificid alternate hosts, and its development was successfully reproduced in both *Tubifex tubifex* and *Limnodrilus hoffmeisteri.* Within these alternate host oligochaetes typical triactinospores developed which, however, differed from the known triactinospores in their shape and size. The majority of *Myxobolus* spe-

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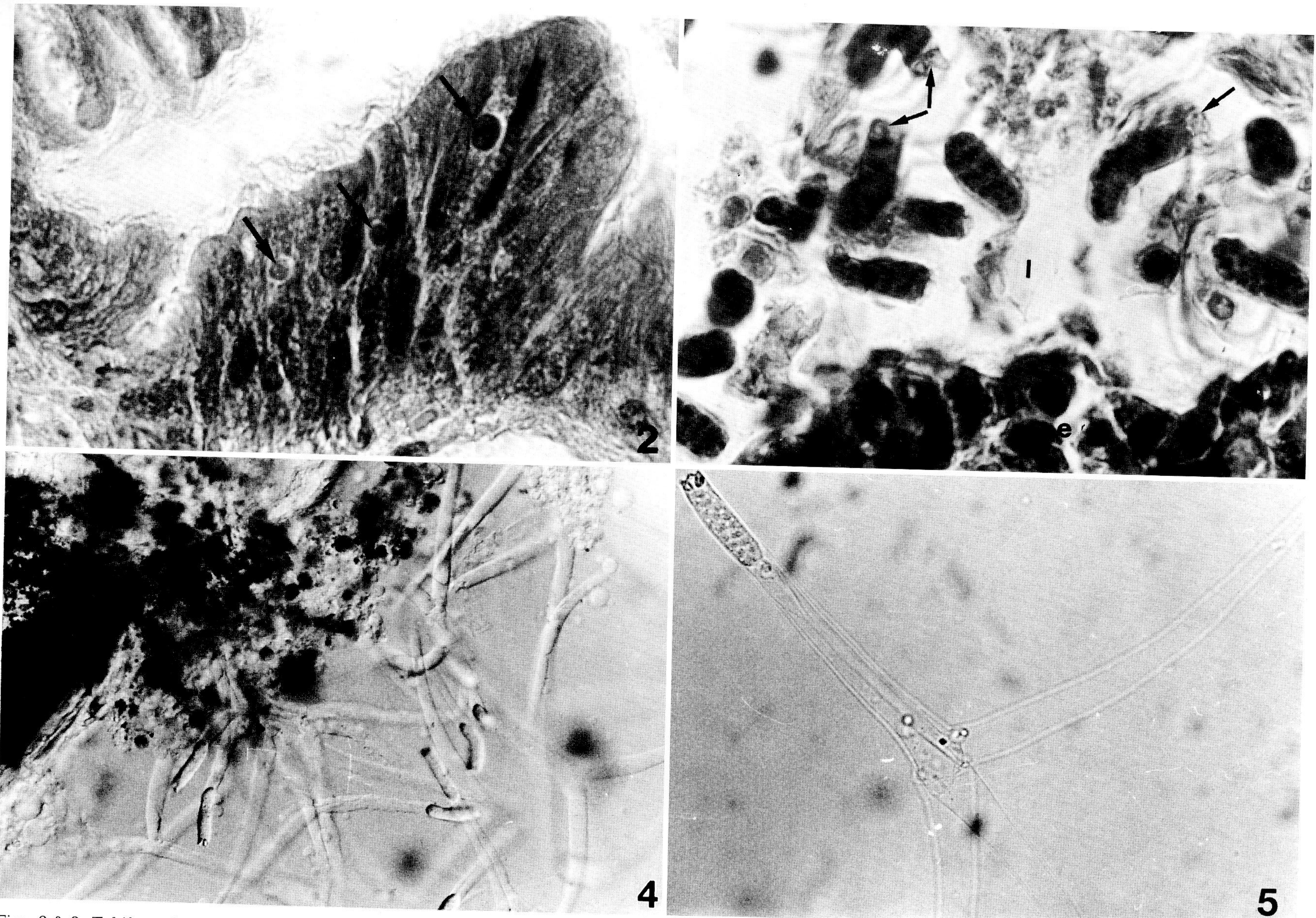

Figs. 2 & 3. *Tubifex tubifex.* H&E stained histological preparations of transversally sectioned oligochaetes. Fig. 2. Gut epithelium showing early stages of pansporocysts (arrows) inside cells 21 d after infection with *Myxobolus hungaricus.* ×400. Fig. 3. Triactinospores just released from the intestinal epithelium (e) inside the lumen (l) of the gut. In the spore body of cross-sectioned specimens the secondary cells of the sporoplasm and the polar capsule (arrows) are well discernible. ×1000

Figs. 4 & 5. *Myxobolus hungaricus.* Fig. 4. Release of triactinospores from the body of a *Tubifex tubifex* in a cell well plate.

cies (*M. cerebralis*, *M. cotti*, *M. hungaricus*) seem to form triactinospores in the alternate host, but Ruidish et al. (1991) reported that *M. pavlovskii* developed in *T. tubifex* into a hexactinomyxon, while Yokoyama et al. (1995) found that *Myxobolus cultus* developed in *Branchiura sowerbyi* into a raabeia-type actinospore. At an average temperature of 20°C the development was completed and the first triactinospores appeared 102 d after infection, which roughly corresponds to results of El-Matbouli et al. (1992), who, in their studies on *M. cerebralis*, *M. cotti* and *M. çarassii*, found that intraoligochaete development took 80 to 120 d.

Unfortunately the whole developmental cycle could not be followed in our experiment, because the intrapiscine development could only have been followed after successful infection of laboratory-cultured uninfected breams. Lacking this stock of fish, we can only infer the possible development by utilising field observations made on Lake Balaton bream in this study. These speculations allow us to outline the possible development of *Myxobolus hungaricus* (Fig. 7). According to this pattern, after infection of the bream with triactinospores, intrapiscine development takes place in the gills, while intraoligochaete development starts when these alternate hosts become infected with myxosporean spores of *M. hungaricus*.

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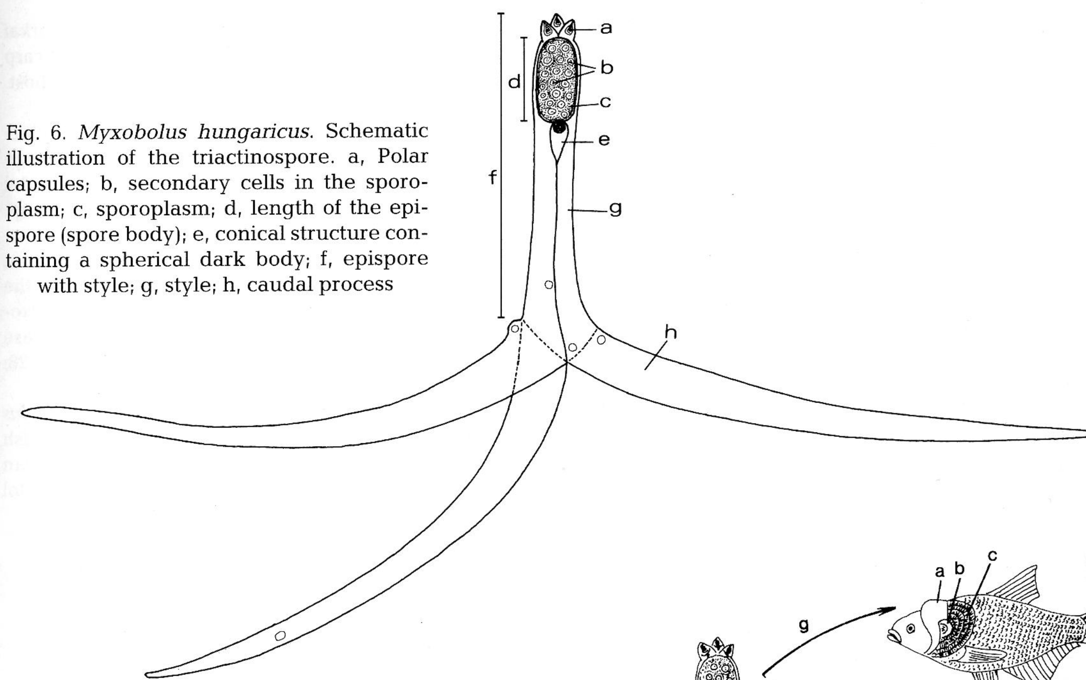

Fig. 6. *Myxobolus hungaricus.* Schematic illustration of the triactinospore. a, Polar capsules; b, secondary cells in the sporoplasm; c, sporoplasm; d, length of the epispore (spore body); e, conical structure containing a spherical dark body; f, epispore with style; g, style; h, caudal process

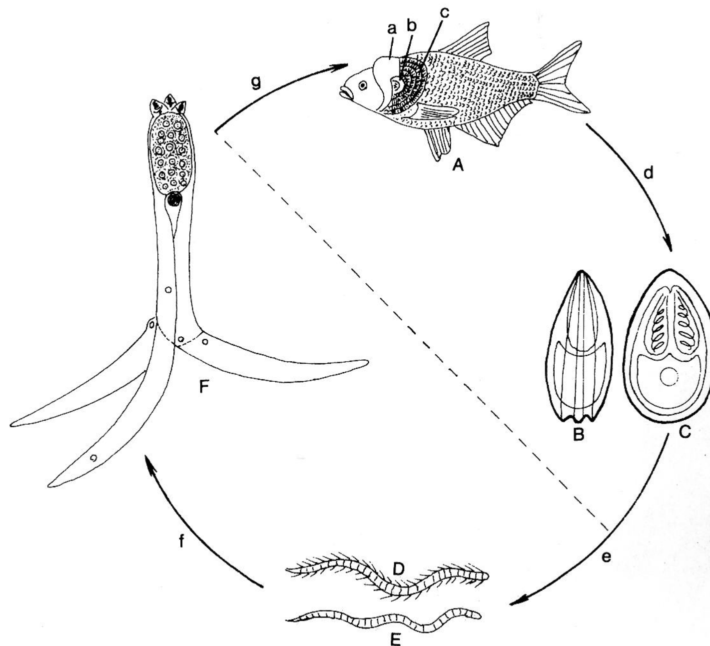

Fig. 7. Schematic diagram of the life-cycle of *Myxobolus hungaricus.* (A) Intrapiscine development in the gills of *Abramis brama.* a, Operculum; b, cartilaginous gill arch; c, plasmodia in the gill filament; d, mature spores sink to the bottom of the lake. (B) Lateral view of a *M. hungaricus* spore. (C) Frontal view of a *M. hungaricus* spore. e, Ingestion of mature spores by oligochaetes within the mud. (D) and (E) Intraoligochaete development in the alternate hosts *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, respectively. f, Within the midgut epithelia of oligochaetes pansporocysts develop. Inside pansporocysts first sporoblasts, then triactinospores develop. Triactinospores released from epithelial cells are shed from the gut of the oligochaetes into the water. (F) Triactinospores float freely in the water. g, Infection of fish takes place by contact with triactinospores

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