Systematic Parasitology **41:** 95–103, 1998.

Development of *Myxobolus portucalensis* Saraiva & Molnár, 1990 (Myxosporea: Myxobolidae) in the oligochaete *Tubifex tubifex* (Müller)

A. El-Mansy*, K. Molnár & Cs. Székely
Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, PO Box 18, Hungary

Accepted for publication 27th January, 1998

Abstract

The extrapiscine development of *Myxobolus portucalensis*, a myxosporean parasite of the subcutaneous connective tissue of eel *Anguilla anguilla* L. was studied in the experimentally infected oligochaete *Tubifex tubifex*. After infecting parasite-free tubificids with mature spores of *M. portucalensis*, development of actinosporean stages was first observed with a light microscope 26 days after infection. Triactinospores of *M. portucalensis* emerged from the worms after 160 days of intra-oligochaete development. It was observed in histological sections that early pansporocysts were located in the gut epithelium of the experimental oligochaetes. Mature pansporocysts, each containing eight triactinospores, appeared 149 days after infection. After the rupture of pansporocysts, free actinospores were found in the lumen of the oligochaete gut. Released actinospores were floating in the water and showed a typical triactinomyxon form. Each triactinospore had three pyriform polar capsules, a sporoplasm with 32 secondary cells, a moderately long style and three slightly curved, sharply pointed caudal processes. The prevalence of infection in *T. tubifex* proved to be 52.5% (n = 40). No infection was found in *Limnodrilus hoffmeisteri* or in control *Tubifex* specimens.

Introduction

Wolf & Markiw (1984) proved that the extrapiscine development of *Myxobolus cerebralis* Hofer takes place in oligochaete alternate hosts (*Tubifex tubifex*) which act as the site of development for the triactinomyxon stages previously attributed to the Class Actinosporea of the Myxozoa. Since then, several experiments have demonstrated that actinosporean spores developing in oligochaetes infect fishes which, after a complicated intrapiscine development, produce myxosporean spores capable of infecting oligochaetes. In this way, successful experimental studies have been done on several myxosporeans. The following parasites of the genus *Myxobolus* Bütschli have been studied so far as to their life-cycle: *M. cotti* El-Matbouli & Hoffmann, a parasite of the bullhead *Cottus gobio* by El-Matbouli & Hoffmann (1989), *M. pavlovskii* Akhmerov, a parasite of the silver carp *Hypophthalmichthys molitrix* by Ruidisch et al. (1991), *M. carassii* Klokacheva, a parasite of the golden orfe *Leuciscus idus* by El-Matbouli & Hoffmann (1993), *M. arcticus* Pugachev & Khokhlov, a parasite of the sockeye salmon *Oncorhynchus nerka* by Kent et al. (1993), and *M. cultus* Yokoyama, Ogawa & Wakabayashi, a parasite of the goldfish *Carassius auratus* by Yokoyama et al. (1995). Of other genera of myxosporeans, successful life-cycle studies have been performed on *Hoferellus* Berg, *Ceratomyxa* Thélohan, *Zschokkella* Auerbach, *Myxidium* Bütschli and on the causative agent of proliferative gill disease of channel catfish (Styer et al., 1991; Bartholomew et al., 1997; Grossheider & Körting, 1992; El-Matbouli et al., 1992a; Benajiba & Marques, 1993; Yokoyama et al., 1993; Uspenskaya, 1995; Trouillier et al., 1996; Yokoyama, 1997). In each case various Oligochaeta spp. proved to be alternate hosts.

More recently the intraoligochaete development of two more myxosporidians, *Myxobolus drjagini* Akhmerov and *M. hungaricus* Jaczó, has been studied

* On leave from the National Institute of Oceanography and Fisheries, Cairo, Egypt.
Student of the doctoral programme in Zootaxonomy, Synbiology and Hydrobiology at “Loránd Eötvös” University, Budapest, Hungary.

experimentally in our laboratory (El-Mansy & Molnár, 1997a, 1997b).

Myxobolus portucalensis was originally described by Saraiva & Molnár (1990) from rivers of Portugal. Plasmodia containing spores were recorded inside the fins in the subcutaneous connective tissue of eel *Anguilla anguilla.*

The present paper reports on experimental infection of the oligochaete *Tubifex tubifex* with *M. portucalensis* spores, followed by the development of actinosporean stages of the triactinomyxon type in that worm.

Materials and methods

Spores of *Myxobolus portucalensis* were collected from mature plasmodia in the fin of European eel *Anguilla anguilla* (L.) from Lake Balaton, Hungary.

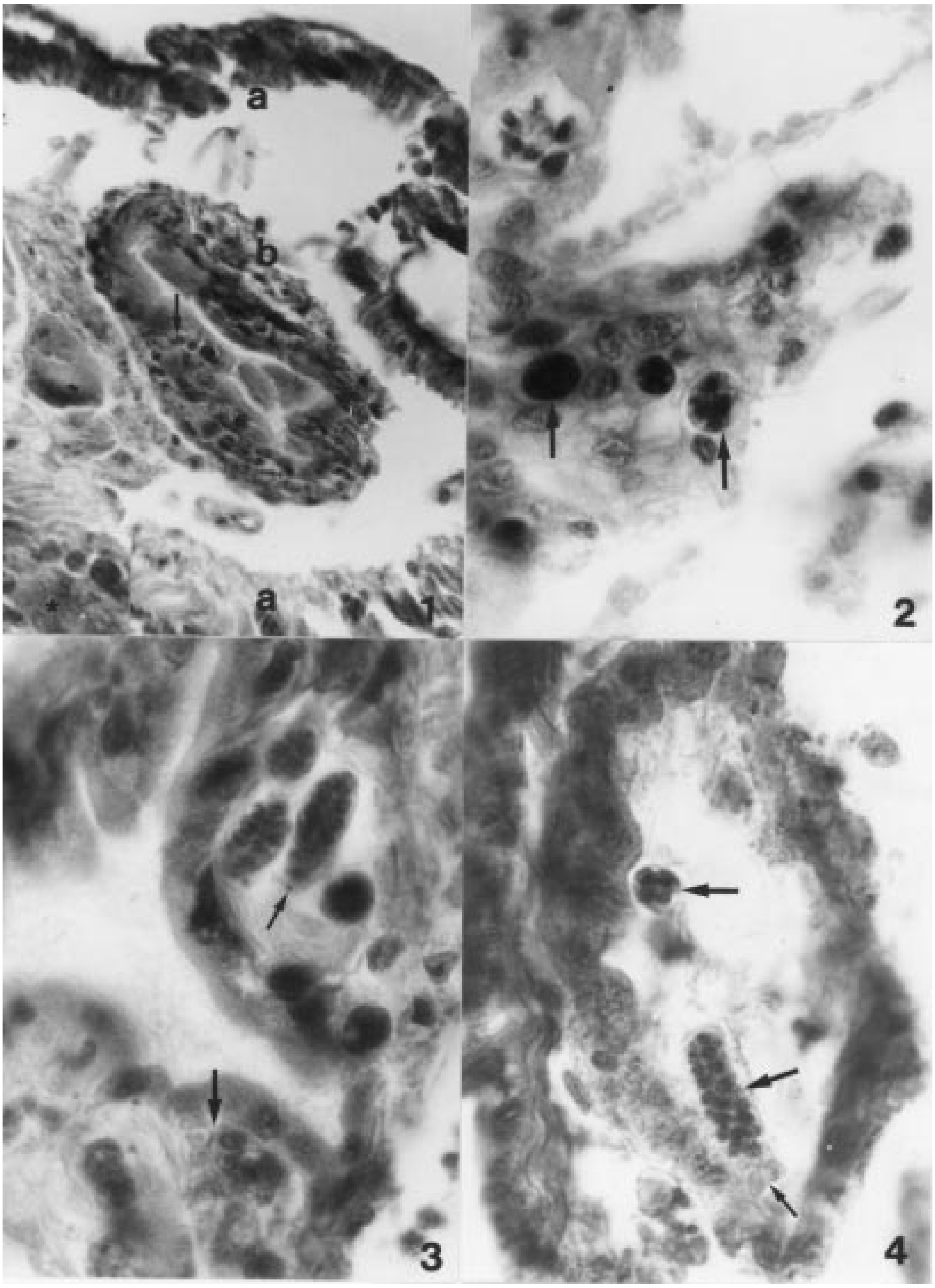
Oligochaetes *Tubifex tubifex* (Müller) and *Limnodrilus hoffmeisteri* (Claparede), identified according to Brinkurst (1963), were collected from a muddy pool on the top of a hill where no fishes live. They were transferred into autoclaved mud and propagated in the laboratory in aerated aquaria. The worms were fed on drops of granulated fish food, and pieces of chicken faeces were added to increase the organic matter content of the mud. Normal tap-water was used throughout the experiment. The temperature of the room varied between 18 and 22 °C.

Two types of containers were used for the experiments. A proportion of the oligochaetes was placed into an aquarium containing 5 litres of water, while others were placed into a small plastic cup of 500 ml volume. A third dish (a plastic cup) containing *Tubifex* and *Limnodrilus* specimens from the same stock was used as a control. All containers were permanently aerated and regularly supplied with fresh water to prevent evaporation and to refresh the water for the oligochaetes. The aquarium contained about 100–300 specimens (*Tubifex* and *Limnodrilus* in about the same number), while the plastic cup contained about 100 specimens. Oligochaetes in both the aquarium and the plastic cup were exposed to infection by adding 700,000 myxospores. From the exposed stocks, 40 *Tubifex* and 40 *Limnodrilus* specimens were examined for the presence of development stages during the experiment. The same number of oligochaetes were checked from the control group.

The development of actinosporean stages of *M. portucalensis* was checked regularly by the follow-

Table I. Measurements of the triactinospore (n = 25) of *Myxobolus portucalensis* released from experimentally infected *Tubifex tubifex* (means and ranges in micrometres)

Entire length	Style length	Style width at start/at end	Spore body dimensions	Caudal process dimensions	Polar capsule dimensions	No. of secondary cells
328 (326–330)	101 (97–106)	10.2 (8.5–12)/19.6 (19–20)	36.6 (36–37) × 10.6 (9.5–12)	190.7 (156–225) × 15.3 (14–16.5)	5.2 (4.5–5.5) × 3.2 (3.0–3.5)	32



Figures 1–4. Histological sections of *Tubifex tubifex* infected by *Myxobolus portucalensis* actinosporean stages (H & E) 1. Cross-section of a tubifex. Cuticle of the worm (a), intestine of the worm (b), early developmental stages, presumably young pansporocysts (arrow) in the epithelium 26 days after infection with myxospores × 400. Inset: Enlarged part of the pansporocyst-infected area (asterisk) × 800. 2. Gut epithelium of a tubificid harbouring progressed stages of pansporocysts 122 days after infection × 1,000. 3. Mature pansporocysts in the gut epithelium containing triactinospores 150 days after infection. Polar capsules of triactinospores (small arrow). Sporoplasm of triactinospores containing secondary cells (large arrow) × 1,000. 4. Triactinospores released into the lumen of the oligochaete's gut 155 days after infection. Polar capsules of triactinospores (small arrow). Secondary cells of the sporoplasms (large arrow) × 1000.

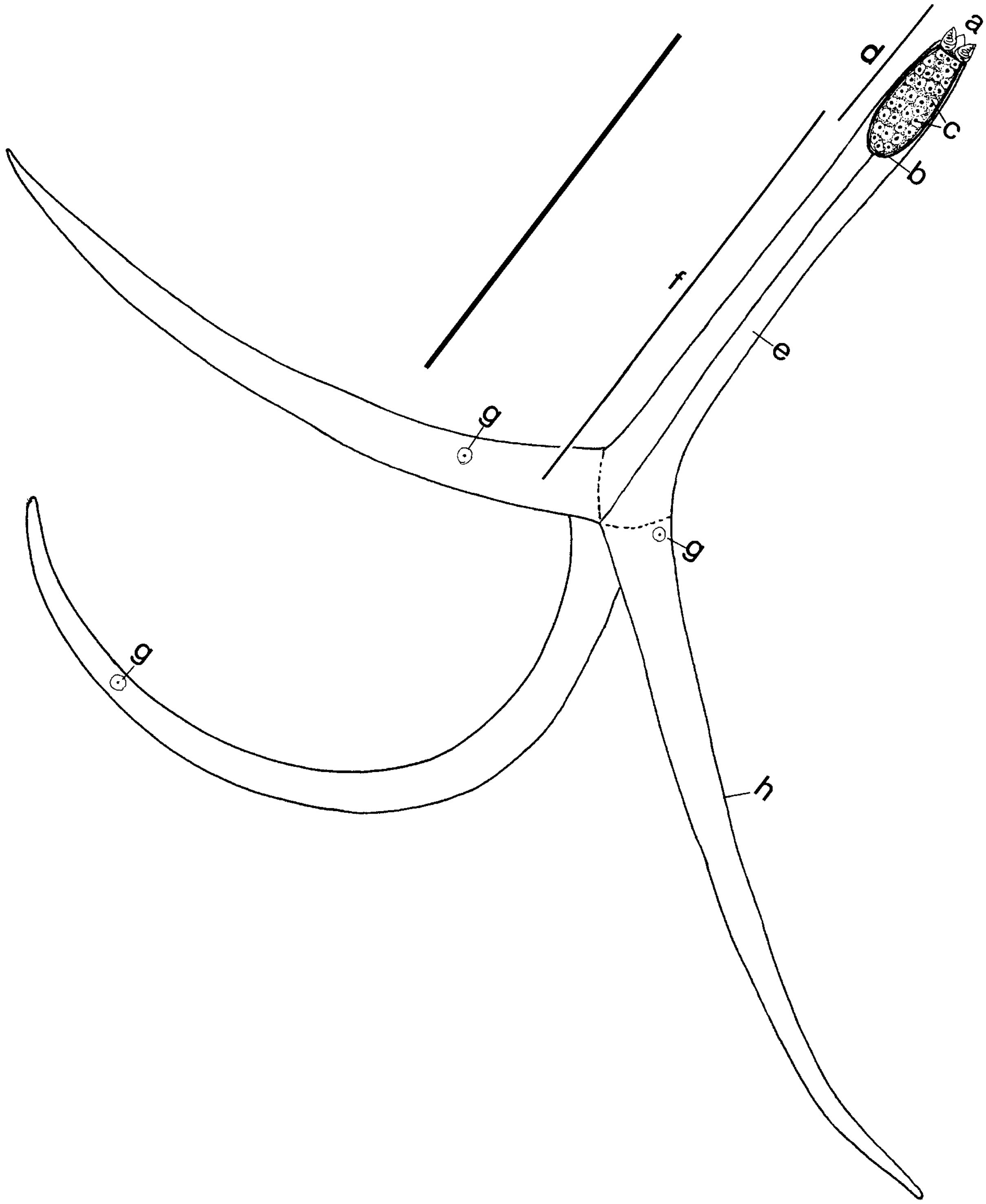
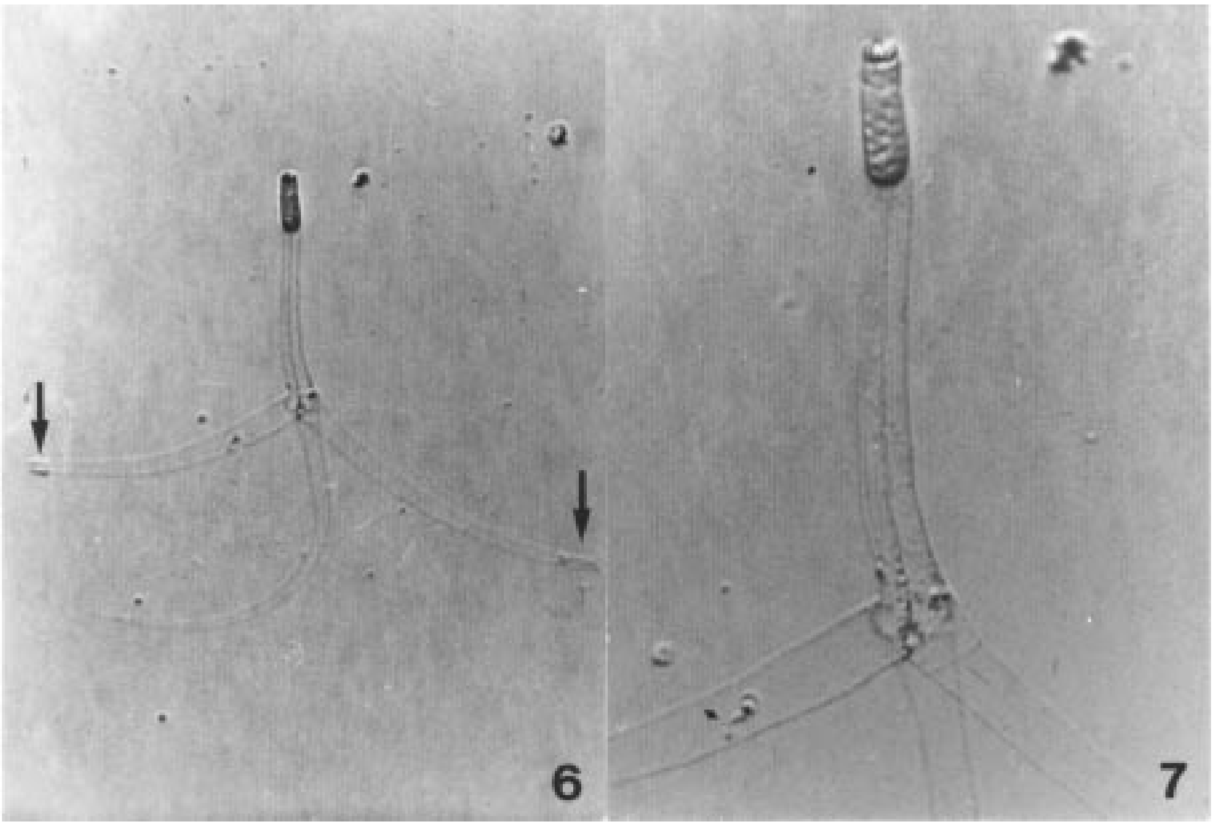


Figure 5. Schematic illustration of the triactinospore of *Myxobolus portucalensis*. (a) polar capsules; (b) sporoplasm; (c) secondary cells in the sporoplasm; (d) length of the spore body; (e) style; (f) length of the style; (g) nuclei of the valvogenic cells; (h) caudal process. *Scale-bar*: 100 μm.



Figures 6-7. Waterborne triactinospore of *Myxobolus portucalensis*. Invaginations at the tip of the caudal processes (arrows) × 220. 7. Enlarged view of triactinospore × 400.

ing methods: (1) Twice a week the oligochaetes were placed carefully under a coverslip and examined under 200× magnification for the presence of developmental stages. (2) Starting from the third week after infection, seven oligochaetes were placed into 2 ml cell-well plates (Yokoyama et al., 1991) three times a week, and after a day of incubation the water of the wells was checked for the presence of actinosporean spores (subsequently called actinospores) under a compound microscope. (3) Every two days, water from the aquarium and from the small dishes was filtered through a fine mesh of 10 μm pore size. The filtrates were added to a small amount of water and examined for the presence of actinospores. (4) Every week two oligochaetes were sacrificed for histological examination, a total of 15 infected *Tubifex* being fixed in Bouin's solution, embedded in paraffin wax, cut into 3 μm thick sections and stained with haematoxylin and eosin.

Triactinospores released by the oligochaetes were examined under a coverslip. They were recorded with the help of a video image program on videotapes (Székely, 1997). Photos and drawings were made and measurements recorded. All measurements in the description are given in micrometres on the basis of 25 triactinospores. In the description of the actinosporean stages of *M. portucalensis* the terminology of Janiszewska (1957), as modified by Lom et al. (1997), is used.

Results

Light microscopy

Only *Tubifex tubifex* proved to be a good alternate host for *M. portucalensis* and no infection was found in *Limnodrilus hoffmeisteri*. Infection of *Tubifex* was found only in specimens collected from the small cup. During a period of 5 months and 10 days, 21 *Tubifex* specimens proved to be infected with actinosporean developmental stages. The first sign of infection in living specimens was recorded 26 days after infection. At that time, developmental stages were located in the gut epithelium of the worms. In severe cases the infection was found in most of the segments. In less severe cases the infection was found only in the centrally located segments. In the subsequent period these stages grew in number and size and pansporocysts, each containing 8 developing triactinomyxons, were observed inside the body of *Tubifex*. Actinospores were first released from living oligochaetes into the water 160 days after infection and their presence in the water was recorded for about 3 weeks after first release. The released actinospores proved to be characteristic triactinomyxon types. No actinosporean infection was found in the control tubifex specimens.

Histological evidence

The first developing stages were first recorded in *Tubifex tubifex* 26 and 27 days after infection. These young trophozoites and early stages of pansporocysts were located both in the intestinal epithelium (Figure 1) and the ovary. In a transverse section prepared 122 days after infection, more developed, round pansporocysts with a relatively light cytoplasm and dark nuclear aggregations were found in the epithelium (Figure 2). Mature pansporocysts within the tubificid mid-gut were formed 150 days after infection (Figure 3). These pansporoblasts were oval or rounded and each contained 8 sporoblast cells of irregular shape. At that stage of development the spore body and the folded projection of the future triactinospore could already be seen. It was very apparent that some mature triactinospores were released from the pansporocysts into the gut lumen 155 days after infection. In these advanced stages the 3 polar capsules, the sporoplasm with the secondary cells, the folded projection of the future style and the caudal processes were detected (Figure 4).

Description of triactinospores

Triactinospores (Table I, Figures 5–7) released from the tubificid body and floating in the water were characterised by 3 pyriform polar capsules, a sporoplasm, a moderately long style and 3 slightly curved, sharply pointed caudal processes. The whole length of the triactinospore was 328 (326–330). The polar capsules are pyriform in shape, 5.2 (4.5–5.5) in length and 3.2 (3.0–3.5) in width. The sporoplasm is elliptical, 31.9 (31.5–32.0) in length, 10.6 (9.5–12.0) in width and contains approximately 32 spherical secondary cells which are 4.4 (4.0–4.5) in diameter (Figure 8). The style is moderately long, being 101.4 (97–106) in length. Its width measures 10.2 (8.5–12.0) anteriorly and 19.6 (19–20) posteriorly. The caudal processes are slightly curved, tapering toward the end and terminating in a sharp point. The length of the caudal processes is 190.7 (156–225) and their width is 15.3 (14.0–16.5). Among triactinospores with fully-developed caudal processes there were specimens with less erect tails bearing a fold at the end of the caudal processes (Figure 6). The length from the apical point of the polar capsules to the end of the sporoplasm (epispore or spore body) measures 36.6 (36.5–37.0). The distance from the polar capsules to the end of style (spore body plus style) measures 143 (142–143.5).

The prevalence of infection in experimental *Tubifex* specimens was 52.5% (n = 40).

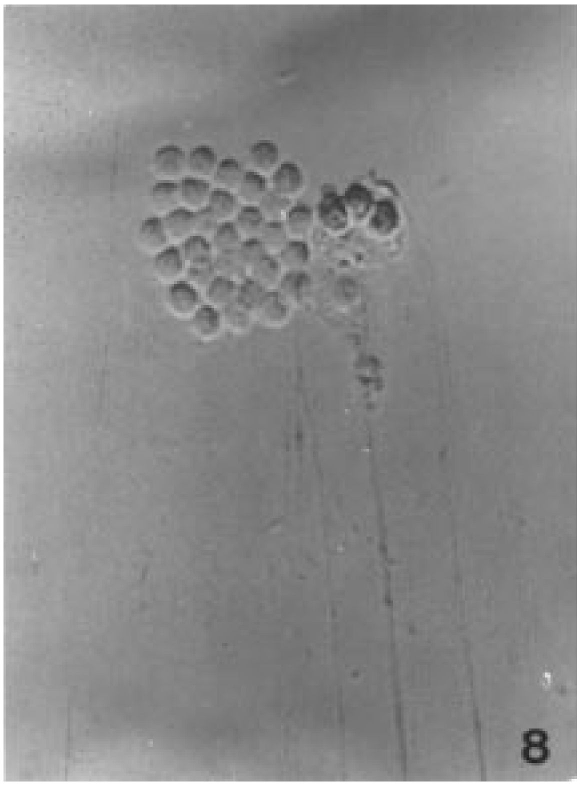


Figure 8. Waterborne triactinospore of *Myxobolus portucalensis* releasing secondary cells from the sporoplasm × 1,000.

Differential diagnosis

The triactinospores of *M. portucalensis* differ from the known triactinospores in the following character-

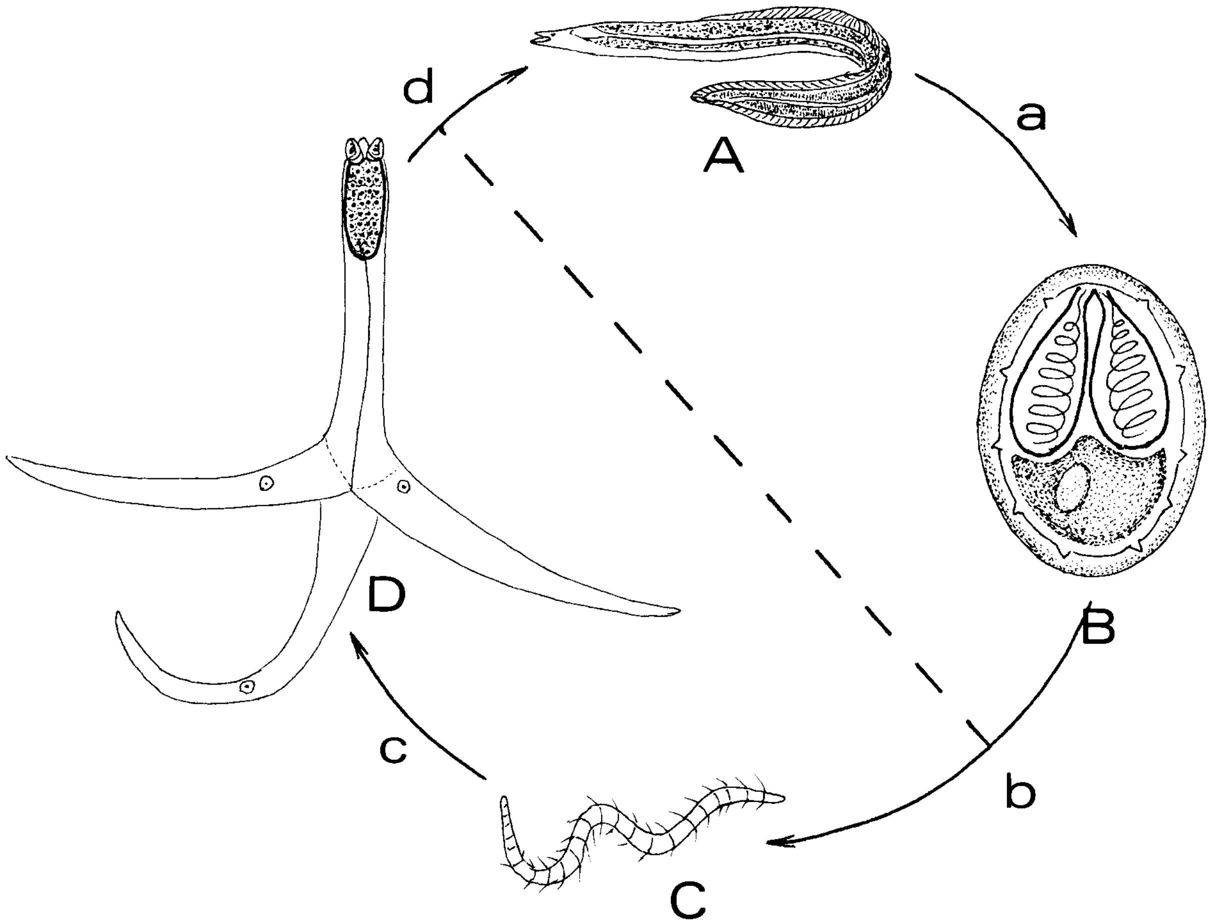


Figure 9. Schematic diagram of the life cycle of *Myxobolus portucalensis*: (A) intrapiscine development; (B) myxospore; (C) intraoligochaete development; (D) actinospore; (a) mature myxospores leave the fish; (b) ingestion of myxospores from the mud by the oligochaete; (c) triactinospores developed in the tubifex are released into the water; (d) waterborne triactinospores infect the eel.

istics. The number of secondary cells of sporoplasm in the triactinospore of *M. portucalensis* was constant (32) while in the case of triactinospores of *M. cerebralis* it varied from 32 to 50 (Wolf & Markiw, 1984). For mature *M. cotti* and *M. hungaricus* 18 secondary cells (El-Matbouli et al., 1989; El-Mansy & Molnár, 1997b), for *M. drjagini* 14 (El-Mansy & Molnár, 1997a) and for *M. carassii* about 150 secondary cells (sporozoites) (El-Matbouli & Hoffmann, 1993) were recorded.

The total length of *M. cerebralis*, *M. cotti* and *M. drjagini* (135, 88 and 198 μm, respectively) was less than that of *M. portucalensis* (326–329 μm). On the other hand, the length of *M. carassii* appears to exceed that of *M. portucalensis,* as in *M. carassii* the long caudal processes alone measure 277 μm. The total length of *M. hungaricus* is about the same, but the style of the latter species (80 μm) is shorter than that of *M. portucalensis.*

Discussion

Two *Myxobolus* spp. (*M. kotlani* Molnár, Lom & Malik, 1986 and *M. portucalensis* Saraiva & Molnár, 1990) are known from the subdermal tissues of the European eel *Anguilla anguilla*. The spores show only slight differences in their measurements. Saraiva & Molnár (1990) distinguished their species first of all on the basis of its different location. New data suggest that the two species might be synonymous. Although *M. kotlani* has been described from the skin and *M. portucalensis* from the fins, the first species was found in very small fishes where the fins are underdeveloped and unsuitable to support the development of *Myxobo-*

lus cysts of a relatively large size. Despite our fears that *M. portucalensis* might be a synonym, for the present, we will continue to use this name for designating the species studied in our experiments, as our material was obtained from the fins of large eels.

Data obtained on the extrapiscine development of this parasite show that this species follows the same pattern of development as was described by Wolf & Markiw (1984), El-Matbouli & Hoffmann (1989, 1993) and Ruidisch et al. (1991). *M. portucalensis* developed in the tubificid alternate host and its development was successfully reproduced in *Tubifex tubifex*. The development took place both in the intestinal epithelium and in the ovary; however, using light microscopy we could not decide whether the observed developmental stages occupied an intracellular position or were located intercellularly. Within these alternate hosts typical triactinospores developed, which, however, differed from the known triactinospores in their shape and size. The majority of *Myxobolus* spp. (*M. cerebralis*, *M. cotti*, *M. carassii*) appear to form triactinospores in the alternate host, but Ruidish et al. (1991) described that *M. pavlovskii* as developing into a hexactinomyxon in *Tubifex tubifex*, while Yokoyama et al. (1995) found that a raabeia-type actinospore obtained from *Branchiura sowerbyi* developed into *M. cultus*, described by them as a new species. At an average temperature of 20 °C, the development was completed and the first triactinospores released 160 days after infection, which is a somewhat longer time than that reported by El-Matbouli et al. (1992b), who, in their studies on *M. cerebralis*, *M. cotti* and *M. carassii*, found that the duration of intraoligochaete development varied between 80 and 120 days.

The entire developmental cycle could not be followed in our experiment, because intrapiscine development could have been followed only after successful infection of laboratory-cultured uninfected eels. Lacking a pathogen-free stock of these fishes, we can only estimate the possible development by utilising field observations made on Lake Balaton eels during this study. This hypothesis allows us to suggest the possible development of *M. portucalensis* (Figure 9). According to this pattern, after infection of the eel with triactinomyxon stages an intrapiscine development takes place in the subcutaneous connective tissue of the fin, and intraoligochaete development commences when this alternate host becomes infected with the myxosporean spores of *M. portucalensis*.

Acknowledgements

The study was supported by the National Research Fund of Hungary (OTKA, project no. T02 0044) and by a grant given by the Balaton Secretariat of The Prime Minister's Office. The authors thank Dr F. Baska and Ms Emese Papp for their help in collecting and culturing tubificids.

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