Acta Veterinaria Hungarica 45 (4), pp. 427–438 (1997)

EXTRAPISCINE DEVELOPMENT OF *MYXOBOLUS DRJAGINI* AKHMEROV, 1954 (MYXOSPOREA: MYXOBOLIDAE) IN OLIGOCHAETE ALTERNATIVE HOSTS

Amina EL-MANSY*+ and K. MOLNÁR#

Veterinary Medical Research Institute, Hungarian Academy of Sciences, H–1581 Budapest, P.O. Box 18, Hungary

(Received May 14, 1997; accepted June 9, 1997)

The extrapiscine development of *Myxobolus drjagini*, a myxosporean parasite of the head, operculum and buccal cavity of the silver carp (*Hypophthalmichthys molitrix*), was studied in experimentally infected oligochaetes, *Tubifex tubifex.* After infection of uninfected tubificids with mature spores of *M. drjagini,* development of actinosporean stages was first observed by light microscopy 27 days after infection. Triactinomyxon stages of *M. drjagini* emerged from the worms after 91 days of intraoligochaete development. In histological sections, early pansporocysts were found in the gut epithelium of the experimental oligochaetes 42 days after infection. Mature pansporocysts, each containing 8 triactinomyxons, appeared 79 days after infection. After rupture of the epithelial cell and the pansporocyst, free actinosporean stages were found in the gut lumen of the oligochaete. Actinosporean stages released from oligochaetes appeared in the water 91 days after infection. They were floating in the water and showed a typical triactinomyxon form. Each triactinomyxon had three pyriform polar capsules, a sporoplasm with 14 secondary cells inside the spore body, a moderately long style and slightly bent, trifurcated, conically ending tails. The total length of the triactinomyxon measured approximately 198 μm. The prevalence of infection in 51 oligochaetes proved to be 9.8%. No infection was found in the control oligochaetes.

Key words: *Myxobolus drjagini,* Myxozoa, triactinomyxon stage, development in alternative host, *Tubifex tubifex*

Since the two-host life cycle of *Myxobolus cerebralis*, the causative agent of whirling disease in salmonid fish, was first described in the pioneering studies of Wolf and Markiw (1984), several papers have been published to prove that all myxosporean species have an obligatory actinosporean developmental stage in various oligochaete species. Successful experimental studies have been per-

* On leave from the National Institute of Oceanography and Fisheries, Cairo, Egypt;
+ Student of the Doctoral Program in Zootaxonomy, Synbiology and Hydrobiology at "Loránd Eötvös" University, Budapest, Hungary;
# To whom reprint requests should be addressed

formed on several myxosporeans. The life cycles of the following parasites belonging to the *Myxobolus* genus have been studied: El-Matbouli and Hoffmann (1989) studied the life cycle of *M. cotti*, a parasite of the bullhead *Cottus gobio*, Ruidisch et al. (1991) that of *M. pavlovskii*, a parasite of the silver carp *Hypophthalmichthys molitrix*, El-Matbouli and Hoffmann (1993) that of *M. carassii,* a parasite of the golden orfe *Leuciscus idus*, Kent et al. (1993) that of *M. arcticus,* a parasite of the sockeye salmon *Oncorhynchus nerca,* and Yokoyama et al. (1995) that of *M. cultus,* a parasite of the goldfish *Carassius auratus.* Of other genera of myxosporeans, successful life-cycle studies have been performed on *Hoferellus, Ceratomyxa, Zschokkella, Myxidium* spp. and on the causative agent of the proliferative gill disease of the channel catfish (Styer et al., 1991; Bartholomew et al., 1992; Grossheider and Körting, 1992; Benajiba and Marques, 1993; El-Matbouli et al., 1992; Yokoyama et al., 1993; Uspenskaya, 1995; Trouillier et al., 1996). In each case various Oligochaeta spp. proved to be alternative hosts.

Myxobolus drjagini was described by Akhmerov (1954) from silver carp of the Amur River. During the colonisation of the host fishes (Molnár, 1971) it was introduced into Hungary where it has caused severe infection in one-year-old silver carp populations.

The present paper reports on experiments in which the oligochaete *Tubifex tubifex* was experimentally infected with *Myxobolus drjagini* spores and the actinosporean stages of triactinomyxon types developed.

Materials and methods

Spores of *Myxobolus drjagini* were collected from mature cysts from the skin of the head, operculum and buccal cavity of the silver carp *Hypophthalmichthys molitrix* (Cuvier and Valenciennes, 1844), where they formed 1 to 2 mm large, white plasmodia (Fig. 1).

Oligochaetes, *Tubifex tubifex* (Müller) identified according to Brinkhurst (1963), were collected from a muddy pool on the top of a hill where no fishes live. They were transferred into sterilised mud and propagated in the laboratory in aerated aquaria. The oligochaetes were fed on some 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 dishes served for experiments. A certain part of the oligochaetes was placed into small aquaria containing 5 litres of water, while the other part was placed into small plastic cups. All dishes were permanently aerated, and supplied regularly with fresh water to prevent evaporation and to regenerate water in order to refresh the oligochaetes. The large tanks contained about 100 to 300 specimens while the smaller ones 10 to 30 specimens. Oligochaetes were infected by adding spores to the content of the dishes. A third dish containing *Tubifex* specimens from the same stock was used as control and regularly checked in the same number as the infected ones.

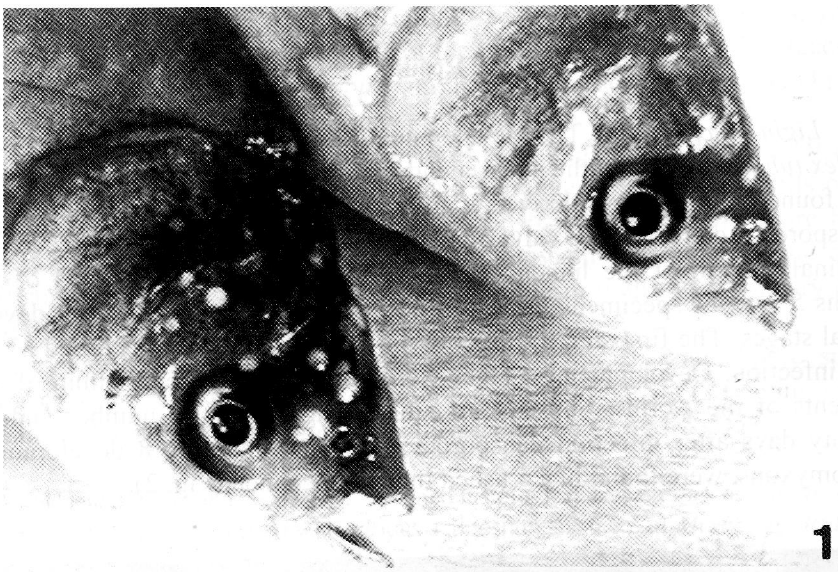

Fig. 1. Myxobolus drjagini plasmodia in the head of a naturally infected silver carp. × 2.5

From the infected stocks 51 *Tubifex* specimens were examined for the presence of developmental stages during the survey.

The development of actinosporean stages of *M. drjagini* was monitored by the following methods: (1) Twice a week some of the oligochaetes were carefully placed under coverslip and examined at 200-fold magnification of the microscope for the presence of developmental stages. (2) After the third week of infection, 10 oligochaetes were placed into 2-ml cell-well plates three times a week (Yokoyama et al., 1991) and after a day of incubation these plates were checked for the release of actinosporean spores under a microscope. (3) Every two days water from the aquaria and from the small dishes was filtered through a fine mesh of 10 µm pore size. The filtrates were taken up in a small volume of water and examined for the presence of actinosporean stages. (4) Two oligochaetes were sacrificed every week. For histological examination, 5 infected *Tubifex* specimens were fixed in Bouin's solution, embedded into paraffin wax, cut into 5 µm thick sections, and stained with haematoxylin and eosin.

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

Results

Light microscopy. The development of *M. drjagini* was followed in *Tubifex tubifex* from both the aquaria and the small dishes. Infected oligochaetes were found only in worms derived from the small dishes. Some days after infection, spores and empty shell valves of *M. drjagini* were regularly recorded in the intestinal content of the lumen of the worm's gut. During a period of three months 5 *Tubifex* specimens proved to be infected with actinosporean developmental stages. The first sign of infection in living *Tubifex* was recorded 27 days after infection. Developmental stages were seen in the gut epithelium of some segments of the worms. Subsequently these stages grew in number and size. Seventy days after infection pansporocysts, each containing 8 developing triactinomyxons, were found in a crushed tubifex specimen (Fig. 2).

Fig. 2. Squash preparation of an infected tubifex. Polar capsules of some triactinomyxons are seen inside a pansporocyst. × 1000

Actinosporean stages were first released from living oligochaetes into the water 91 days after infection and their presence in the water was recorded for about three days after the start. The released actinosporean stages proved to be typically triactinomyxon types.

Histological evidence. Twenty-seven days after infection only intact spores and spores with empty shells of *M. drjagini* (Fig. 3) were found in the intestinal content of the tubifex in histological sections. The first developing stages, young pansporocysts located in epithelial cells of the gut epithelium, were first recorded 42 days after infection in *Tubifex tubifex*. In that stage of development round pansporocysts with dark-staining cytoplasm were found (Fig. 4).

Mature pansporocysts within the tubificid midgut were formed 79 days after infection (Figs 5 and 6). Each of them contained 8 sporoblast cells of irregular shape (Fig. 5). In some more developed pansporocysts the spore body and the folded projections of the future triactinomyxon were seen. In these advanced stages the three polar capsules, the sporoplasm with the secondary cells and the future style were well detectable (Fig. 6a).

Description of triactinomyxon stages (based on 20 water-borne specimens). Actinosporean stages released from the tubificid body and floating in the water showed a typical triactinomyxon form (Figs 6b, 7 and 8). Length of the

Figs 3–6. Haematoxylin and eosin (H.-E.) stained histological preparations of transversally sectioned *Tubifex tubifex* infected with various actinosporean stages of *Myxobolus drjagini*

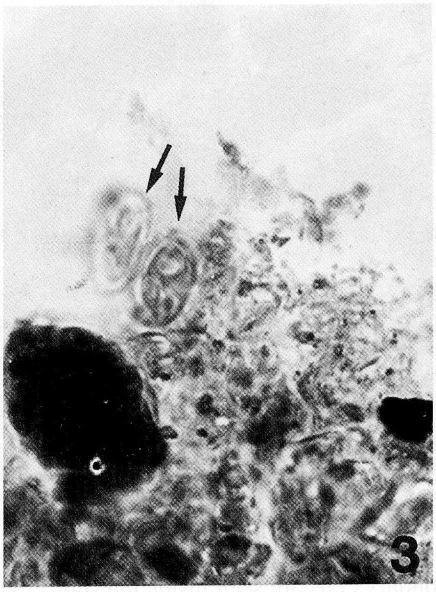

Fig. 3. M. drjagini spores in the content of the gut lumen of a tubifex. × 1000

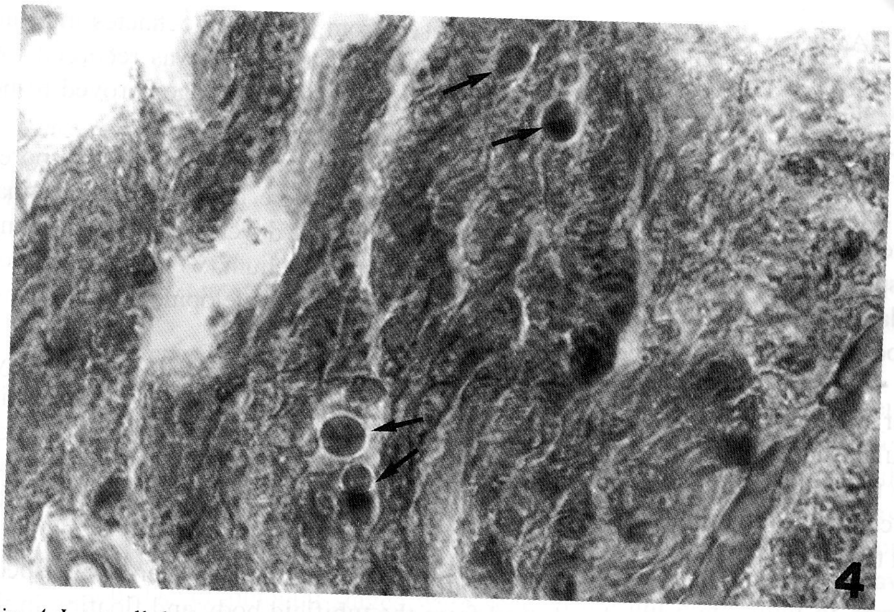

Fig. 4. Intracellularly located early pansporocysts (arrows) in the gut epithelium 42 days after infection. × 1000

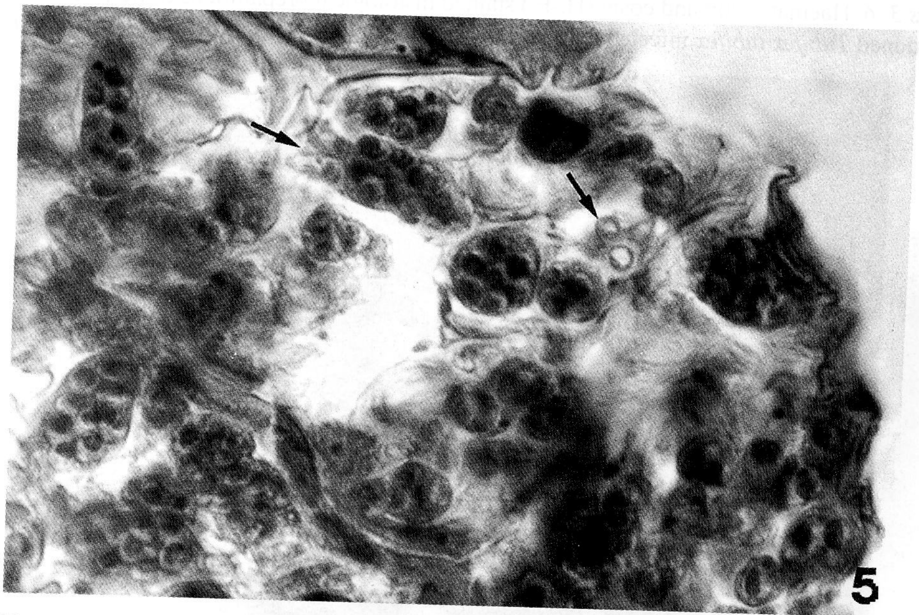

Fig. 5. A part of the gut epithelium heavily infected with pansporocysts harbouring triactinomyxon stages. Secondary cells are seen inside the sporoplasm. Note also the 3 polar capsules (arrows). × 1000

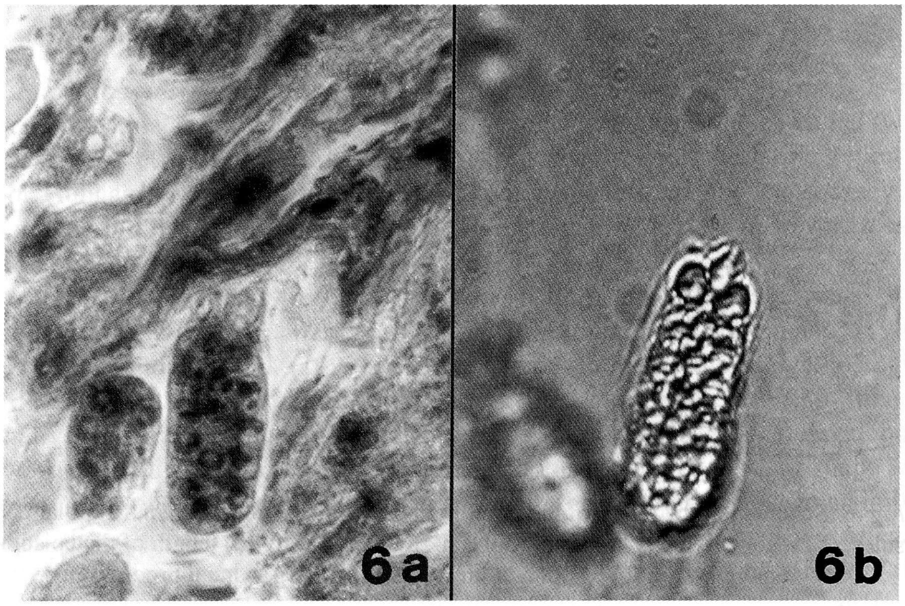

Fig. 6. Spore bodies of *M. drjagini* triactinomyxons. (a) mature triactinomyxon in the epithelium of the tubifex; (b) water-borne triactinomyxon (unstained). × 1000

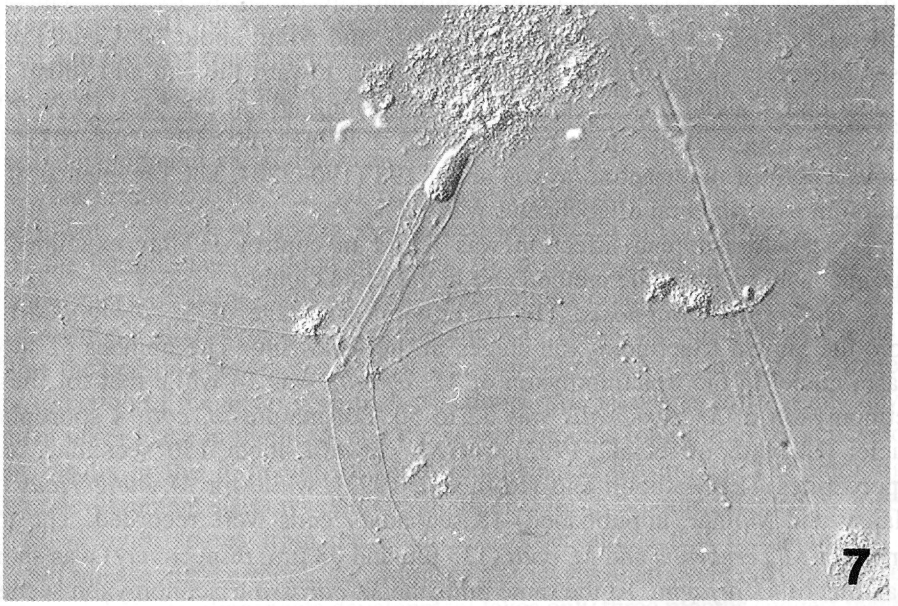

Fig. 7. Water-borne triactinomyxon of *M. drjagini* with relatively short style and thick caudal processes. The region of the polar capsules is damaged. × 350

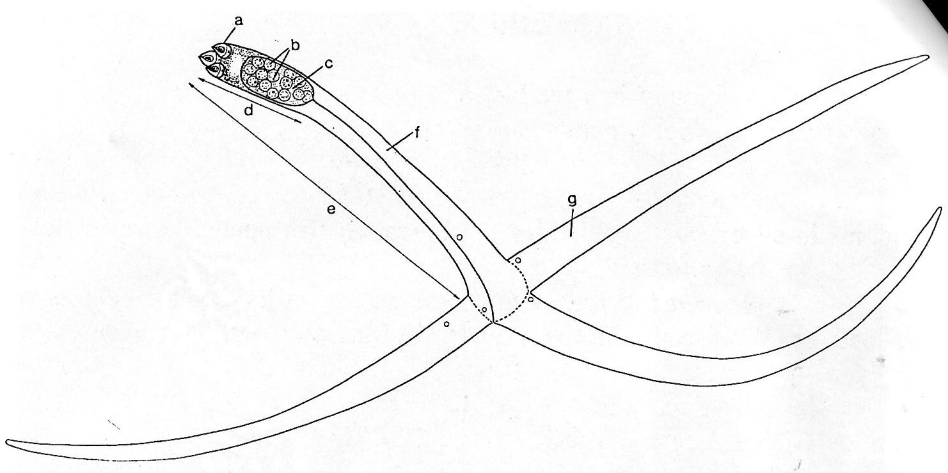

Fig. 8. Schematic illustration of the triactinomyxon stage of *M. drjagini.* (a) polar capsules, (b) secondary cells in the sporoplasm, (c) sporoplasm, (d) length of the epispore (spore body), (e) epispore with style, (f) style, (g) caudal process

epispore (spore body) 39 (37–42). Polar capsules pyriform in shape, 7.0 (6.5–7.5) in length and 4.0 (3.5–4.5) in width. Sporoplasm elliptical, 32 (30–35) long and 10.5 (10–11.5) wide, containing approximately 14 secondary cells. The style moderately long, its length about 66 (60–70), and width about 10. Caudal processes relatively stout, slightly bent, evenly taper toward the end and finish in a conical end. Caudal processes 126 (120–130) long and at the base 10 (9–10.5) wide. The length of the spore body and the style measures 105 (100–108). The whole length of the triactinomyxon about 198 (190–220). The prevalence of infection in experimental oligochaetes was 9.8%.

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

Differential diagnosis. The triactinomyxon stage of *M. drjagini* differs from the known triactinomyxon stages in the following: The number of sporoplasm nuclei in the triactinomyxon of *M. drjagini* was 14 while that for the triactinomyxon of *M. cerebralis* was 32 to 50. For mature *M. cotti* 8 sporoplasm nuclei (El-Matbouli et al., 1992), for *M. carassii* about 150 secondary cells (sporozoites) (El-Matbouli and Hoffmann, 1993), while for *M. hungaricus* (El-Mansy and Molnár, unpublished) 18 secondary cells were recorded. The total length of *M. cerebralis* and *M. cotti* (135 μm and 88 μm, respectively) was shorter than that of *M. drjagini.* At the same time, the *M. carassii* triactinomyxon seems to be longer, as the length of the caudal processes alone measures 277 μm.

Discussion

Myxobolus drjagini is a well-known parasite of the silver carp. It is regarded in China as a highly pathogenic parasite, which causes the so-called twist disease (Wu et al., 1975). Since this myxosporean species was first reported from Hungary (Molnár, 1971), it has commonly occurred and caused severe symptoms in silver carp populations of Hungarian fish ponds; however, losses have not been attributed to it yet.

M. drjagini seems to follow the same pattern in its development as was described by Wolf and Markiw (1984), El-Matbouli and Hoffmann (1989, 1993), and Ruidisch et al. (1991). It develops in a tubificid alternative host, and its development was successfully reproduced in *Tubifex tubifex*. Within this alternative host oligochaete species typical triactinomyxon stages developed; however, they differed from the known triactinomyxon forms by their shape and size. *M. drjagini* forms triactinomyxon spores in the alternative host in a similar way as the majority of other *Myxobolus species (M. cerebralis, M. cotti, M. carassii*). It seems, however, that besides the triactinomyxon forms members of the genus *Myxobolus* might develop through other actinosporean forms as well. Ruidish et al. (1991) e.g. described that *M. pavlovskii* developed in *Tubifex tubifex* into a hexactinomyxon, while Yokoyama et al. (1995) found that *Myxobolus cultus* developed in *Branchiura sowerbyi* into a raabeia-type actinosporean spore. At the average temperature of 20 °C, the development was completed and the first triactinomyxons were recorded 91 days after infection, which roughly corresponds to that of El-Matbouli et al. (1992) 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.

Unfortunately, the whole developmental cycle could not be followed in our experiment. For lack of laboratory-cultured uninfected silver carp, we could not study the intrapiscine development. Thus, the possible development in fish could only be guessed on the basis of field observations obtained in this study at the Warm-water Fish Hatchery of Százhalombatta. These speculations allow us to outline the possible development of *M. drjagini* (Fig. 9). According to this pattern, after infection of the silver carp with triactinomyxon stages an intrapiscine development takes place in the head, and the several months long intrapiscine development results in the formation of large lentil-shaped plasmodia. The intraoligochaete development starts when these alternative hosts become infected by ingesting *M. drjagini* spores from the mud. The observations that plasmodia of *M. drjagini* release mature spores according to a strict seasonal cycle only in the early spring and that intraoligochaete development takes only three months suggest that the intrapiscine development of *M. drjagini* is rather long, lasting at least 8 to 9 months.

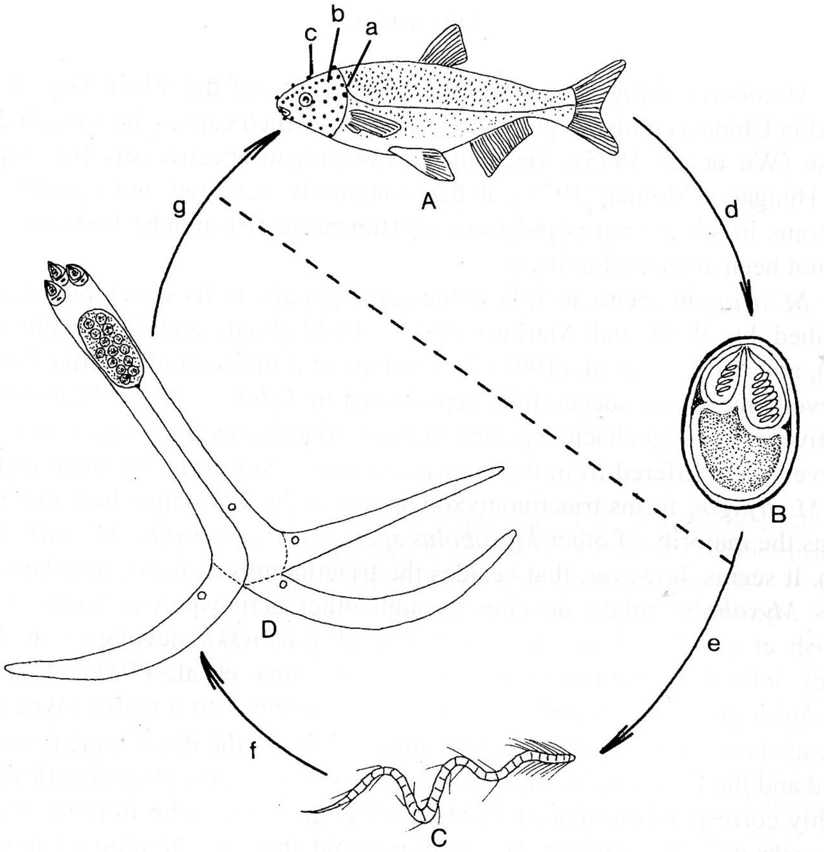

Fig. 9. Schematic diagram of the life cycle of *M. drjagini.* (A) Intrapiscine development in the head of silver carp: (a) operculum, (b) head, (c) plasmodium of *M. drjagini,* (d) mature spores sink down to the bottom of the pond. (B) Frontal view of a *M. drjagini* spore. (e) Mature spores from the mud are ingested by oligochaetes. (C) Intraoligochaete development in the alternative host *Tubifex tubifex.* (f) Pansporocysts develop within epithelial cells of the midgut. Inside the pansporocysts sporoblasts and triactinomyxon spores are formed. Triactinomyxon stages released from epithelial cells are shed from the gut of the oligochaetes into the water. (D) Triactinomyxon stages float freely in the water. (g) Infection of fish takes place by contact with triactinomyxon stages

Acknowledgements

The study was supported by the National Research Fund of Hungary (OTKA, project no. T014648) and by the US–Hungarian Research Fund, J. F. No.326. The authors thank Dr. Cs. Székely and Ms. Emese Papp for their help in collecting and culturing tubificids.

References

Akhmerov, A. Kh. (1954): On closely related species of a new myxosporean genus (in Russian). Dokl. Akad. Nauk. SSSR. **97,** 1101–1103.

Bartholomew, J. L., Fryer, J. L. and Rohovec, J. S. (1992): *Ceratomyxa shasta* infections of salmonid fish. Proceedings of the Oji International Symposium of Salmonid Diseases, Sapporo, Japan, 21–25 October, pp. 267–275.

Benajiba, M. H. and Marques, A. (1993): The alternation of actinomyxidian and myxosporidian sporal forms in the development of *Myxidium giardi* (parasite of *Anguilla anguilla*) through oligochaetes. Bull. Eur. Ass. Fish Pathol. **13,** 100–103.

Brinkhurst, R. O. (1963): A Guide for the Identification of British Aquatic Oligochaeta. Freshwater Biological Association, Scientific Publication No. 22, 50 pp.

El-Matbouli, M., Fischer-Scherl, T. and Hoffmann, R. W. (1992): Transmission of *Hoferellus carassii* Achmerov, 1960 to goldfish *Carassius auratus* via an aquatic oligochaete. Bull. Eur. Ass. Fish Pathol. **12,** 54–56.

El-Matbouli, M. and Hoffmann, R. W. (1989): Experimental transmission of two *Myxobolus* spp. developing bisporogeny via tubificid worms. Parasitol. Res. **75,** 461–464.

El-Matbouli, M. and Hoffmann, R. W. (1993): *Myxobolus carassii* Klokaceva, 1914 also requires an aquatic oligochaete, *Tubifex tubifex* as intermediate host in its life cycle. Bull. Eur. Ass. Fish Pathol. **13,** 189–192.

Grossheider, G. and Körting, W. (1992): First evidence that *Hoferellus cyprini* (Doflein, 1898) is transmitted by *Nais* sp. Bull. Eur. Ass. Fish Pathol. **2,** 17–20.

aniszewska, J. (1957): Actinomyxidia II. New systematics, sexual cycles, description of new genera and species. Zoologia Poloniae **8,** 3–34.

Kent, M. L., Whitaker, D. J. and Margolis, L. (1993): Transmission of *Myxobolus arcticus* Pugachev and Khokhlov, 1979, a myxosporean parasite of Pacific salmon, via a triactinomyxon from the aquatic oligochaete *Stilodrilus heringianus* (Lumbriculidae). Can. J. Zool. **72,** 932–937.

om, J., McGeorge, J., Feist, S. W., Morris, D. and Adams, A. (1997): Guidelines for the uniform characterisation of the actinosporean stages of parasites of the phylum Myxozoa. Dis. aquat. Org. (in press).

lolnár, K. (1971): Protozoan diseases of the fry of herbivorous fishes. Acta Vet. Hung. **21,** 1–14.

uidisch, S., El-Matbouli, M. and Hoffmann, R. W. (1991): The role of tubificid worms as an intermediate host in the life cycle of *Myxobolus pavlovskii* Achmerov, 1954. Parasitol. Res. **77,** 663–667.

yer, E. L., Harrison, L. R. and Burtle, G. J. (1991): Experimental production of proliferative gill disease in channel catfish exposed to a myxozoan-infected oligochaete, *Dero digitata*. J. Aquat. Anim. Health **3,** 288–291.

ouillier, A., El-Matbouli, M. and Hoffmann, R. (1996): A new look at the life-cycle of *Hoferellus carassii* in the goldfish (*Carassius auratus auratus*) and its relation to "kidney enlargement disease" (KED). Folia Paras. **43,** 173–187.

penskaya, A. V. (1995): Alternation of actinosporean and myxosporean phases in the life cycle of *Zschokella nova* (Myxozoa). J. Euk. Microbiol. **42,** 665–668.

olf, K. and Markiw, M. E. (1984): Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. Science **225,** 1449–1452.

ı, B. H., Wang, S. X., Chen, Y. S., Lu, C. C. and Huang, L. F. (1975): On the pathogenic agent of the twist disease of the silver carp in Hangzhou region of the Jiang province. Acta Zool. Sin. **21,** p. 57. (Abstract in English).

Yokoyama, H., Ogawa, K. and Wakabayashi, H. (1991): A new collection method of actinosporeans. A probable infective stage of myxosporeans to fish from tubificid and experimental infection of goldfish with the actinosporean, *Raabeia* sp. Fish Pathol. **26,** 133–138.

Yokoyama, H., Ogawa, K. and Wakabayashi, H. (1993): Involvement of *Branchiura sowerbyi* (Oligochaeta: Annelida) in the transmission of *Hoferellus carassii* (Myxosporea: Myxozoa), the causative agent of kidney enlargement disease (KED) of goldfish *Carassius auratus*. Fish Pathol. **28,** 135–139.

Yokoyama, H., Ogawa, K. and Wakabayashi, H. (1995): *Myxobolus cultus* n. sp. (Myxosporea: Myxobolidae) in the goldfish *Carassius auratus* transformed from the actinosporean stage in the oligochaete *Branchiura sowerbyi*. J. Parasitol. **81,** 446–451.