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

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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

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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.

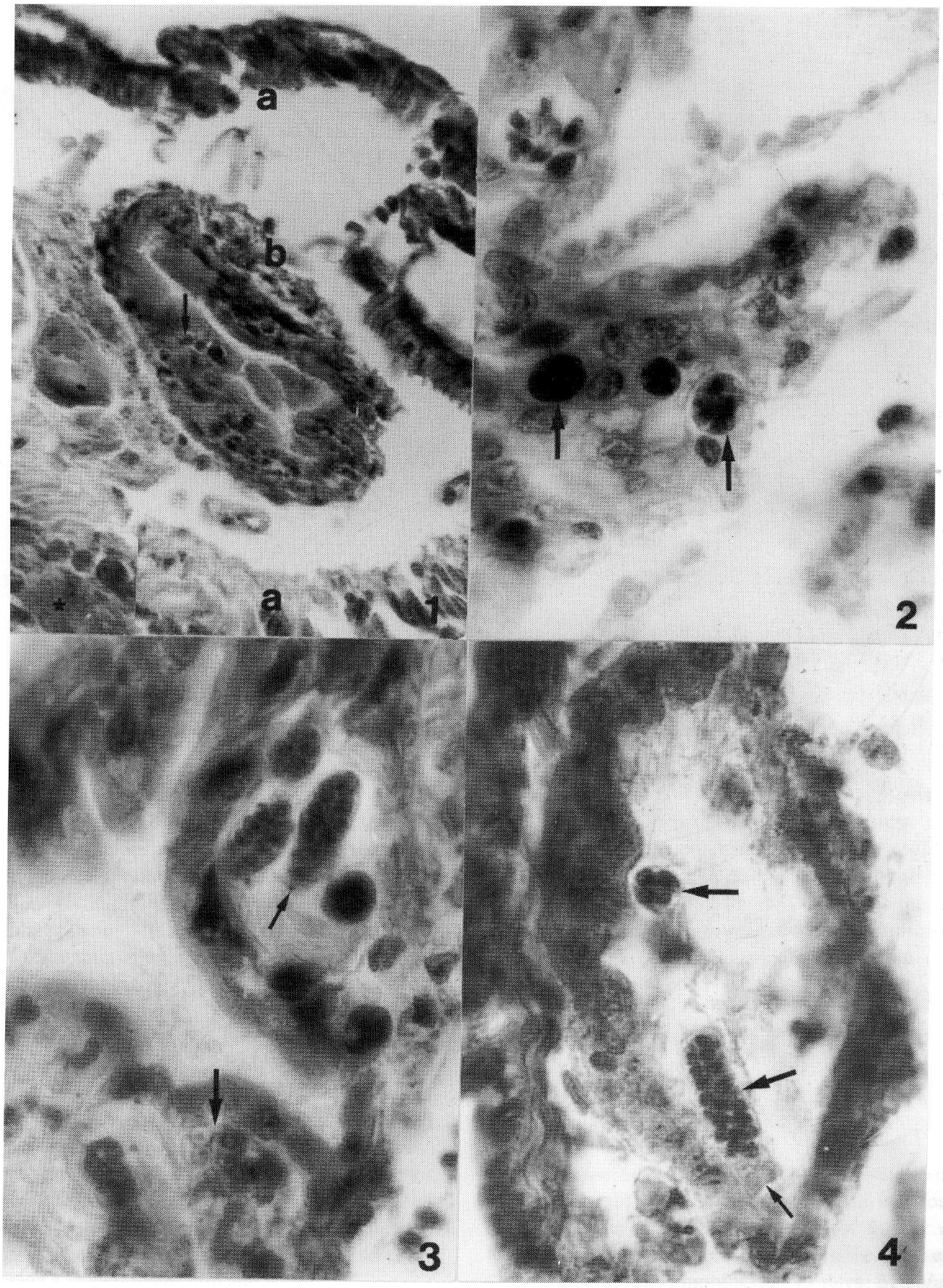
Oligochaetes *Tubifex tubifex* (Müller) and *Limnodrilus hoffmeisteri* (Claparede), identified according to Brinkurst (1963), were collected from a muddy pool on the top of a hill where no fishes live. They were transferred into autoclaved mud and propagated in the laboratory in aerated aquaria. The 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.

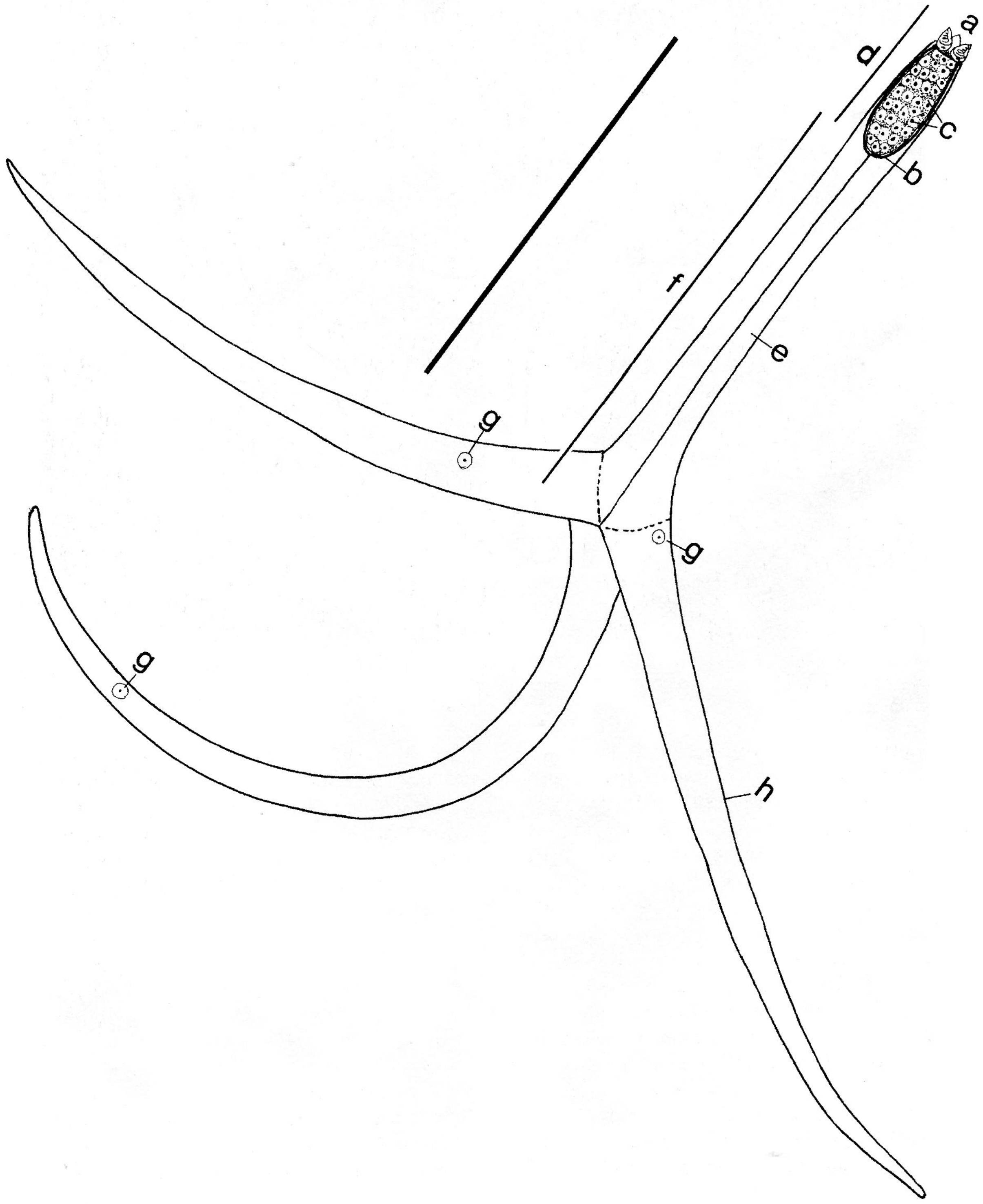
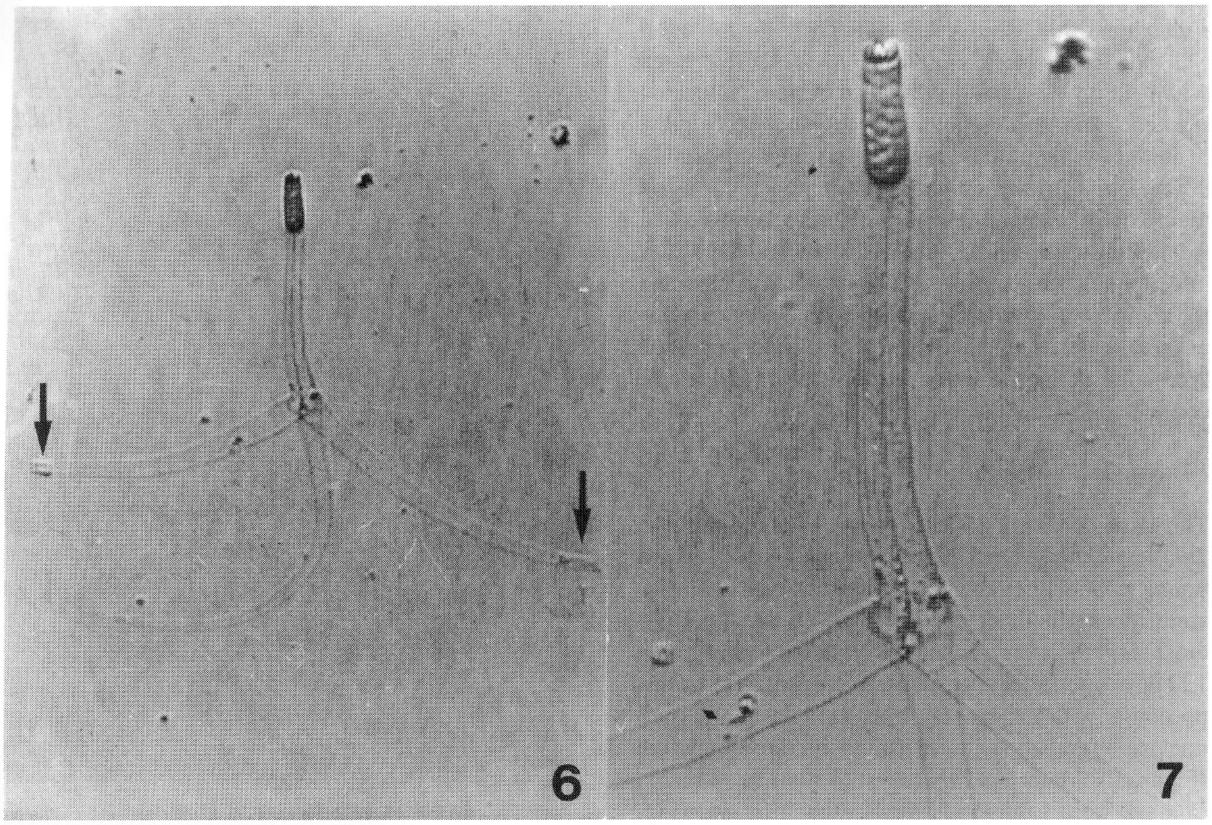


Figure 5. Schematic illustration of the triactinospore of *Myxobolus portucalensis.* (a) polar capsules; (b) sporoplasm; (c) secondary cells in th sporoplasm; (d) length of the spore body; (e) style; (f) length of the style; (g) nuclei of the valvogenic cells; (h) caudal process. *Scale-bar*: 10 μm.



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 cauda processes are slightly curved, tapering toward th end and terminating in a sharp point. The length o the caudal processes is 190.7 (156–225) and thei width is 15.3 (14.0–16.5). Among triactinospore with fully-developed caudal processes there wer specimens with less erect tails bearing a fold at th end of the caudal processes (Figure 6). The lengt from the apical point of the polar capsules to the en of the sporoplasm (epispore or spore body) measure 36.6 (36.5–37.0). The distance from the polar capsule to the end of style (spore body plus style) measure 143 (142–143.5).

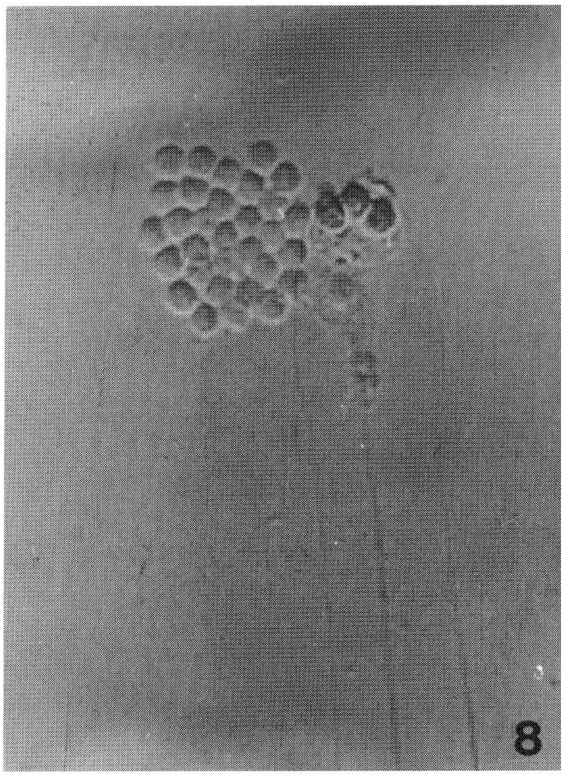


Figure 8. Waterborne triactinospore of *Myxobolus portucalensi* releasing secondary cells from the sporoplasm × 1,000.

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

Differential diagnosis

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

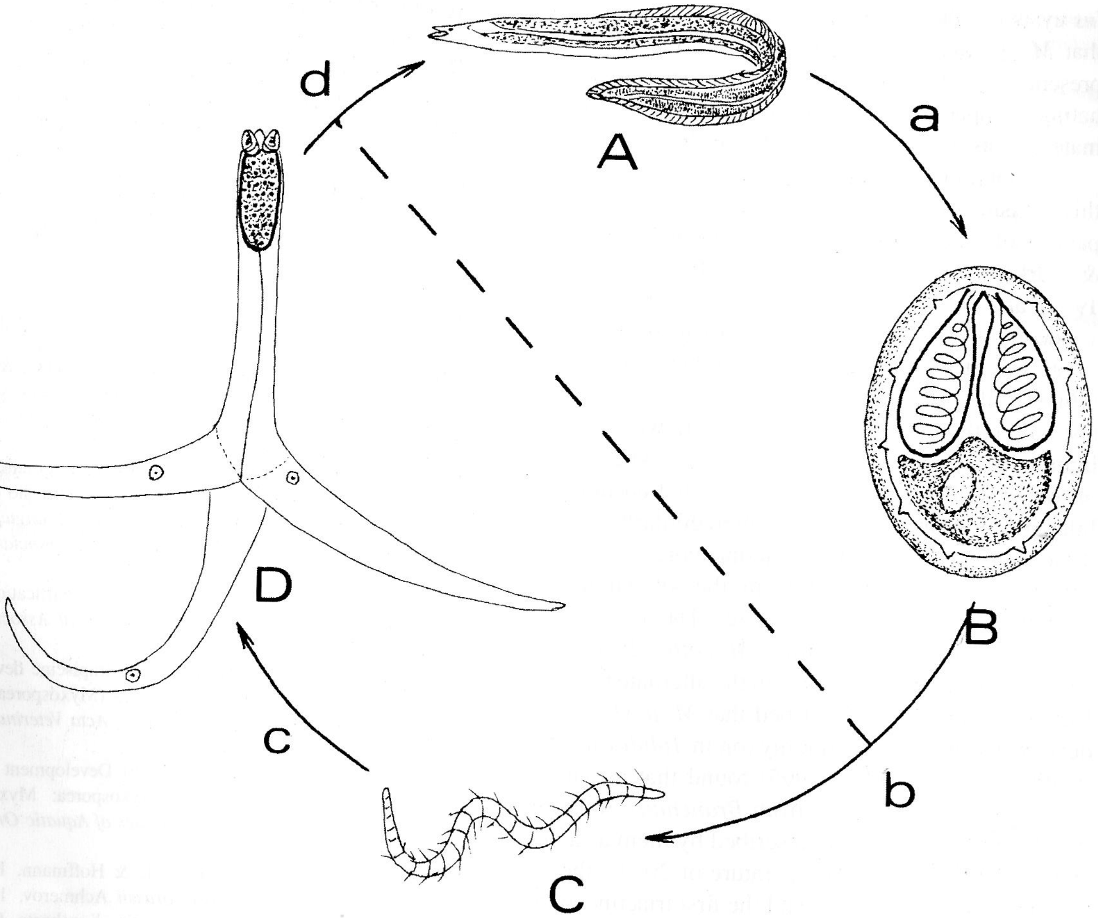


Figure 9. Schematic diagram of the life cycle of *Myxobolus portucalensis*: (A) intrapiscine development; (B) myxospore; (C) intraoligochaete development; (D) actinospore; (a) mature myxospores leave the fish; (b) ingestion of myxospores from the mud by the oligochaete; (c) triactinospores developed 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

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STUDIES ON THE OCCURRENCE OF ACTINOSPOREAN STAGES OF FISH MYXOSPOREANS IN A FISH FARM OF HUNGARY, WITH THE DESCRIPTION OF TRIACTINOMYXON, RAABEIA, AURANTIACTINOMYXON AND NEOACTINOMYXON TYPES

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Actinosporean infection of the oligochaete fauna living in the mud and on the vegetation of fish ponds used for rearing common carp in polyculture was studied during a one-year survey at a fish farm in Hungary, located south of Budapest. Twenty-eight actinospore types were isolated from the oligochaetes *Tubifex tubifex, Branchiura sowerbyi, Limnodrilus hoffmeisteri, Nais elinguis,* and *Stylaria lacustris* collected during the survey, which could be classified into the triactinomyxon, raabeia, aurantiactinomyxon and neoactinomyxon groups. Drawings depicting individual actinospore types are presented on plates and their characteristic dimensions have been summarised in tables. The prevalence and seasonality of actinosporean infections observed in the various oligochaete species have been illustrated graphically. Infection by actinospores showed a pronounced seasonality. In the spring, summer and autumn the prevalence of raabeia infection in *Branchiura* exceeded 90%, while in the winter it dropped to 42%. A similar phenomenon could be observed for aurantiactinomyxon infection, while neoactinomyxon infection reached its peak in the autumn. In *Tubifex, Limnodrilus, Nais* and *Stylaria* species the peak of actinosporean infection occurred, with minor differences, in the spring and summer. Actinosporean infection in the individual Oligochaeta species showed much higher prevalence values than had been reported in the literature, which can be explained by the novelty of the examination technique used. It cannot be decided with absolute certainty which myxosporean developmental stage the different actinospore types described during this survey represent of the species of Myxosporea parasitic in the given ponds. This would require an experimental study for which the data presented here may serve as a basis.

Key words: Actinospore, myxosporeans, Myxozoa, survey, triactinomyxon, raabeia, aurantiactinomyxon, neoactinomyxon

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The first report on actinosporeans was published by Stolc (1899), who described these organisms found in Bohemia as parasites related to myxosporeans. Although nearly one hundred years have elapsed since their first description, until quite recently only few researchers had studied actinosporeans. Of them, Ikeda (1912) and Mackinnon and Adam (1924) detected tetractinomyxon and triactinomyxon forms in England, while in Poland Janiszewska (1955, 1957) performed detailed studies on the morphology, ecology and systematics of actinosporeans. The ultrastructure and biology of these parasites have been studied most intensively by Marques (1984), Lom and Dykova (1997), and Lom et al. (1997*a*). Research on actinosporeans gathered momentum after Wolf and Markiw (1984) had demonstrated that actinosporeans did not constitute an independent taxonomic unit but corresponded to fish-parasitic myxosporean stages developing in oligochaetes. After Wolf and Markiw (1984), other researchers also produced experimental evidence that in the developmental cycle of a given species of Myxosporea the intra-piscine myxosporean and the intra-oligochaete actinosporean stages alternated (El-Matbouli and Hoffmann, 1989; Ruidisch et al., 1991; El-Matbouli et al., 1992; Grossheider and Körting, 1992; El-Matbouli and Hoffmann, 1993; Kent et al., 1993; Yokoyama et al., 1993*a*; El-Matbouli et al., 1995; Yokoyama et al., 1995; Uspenskaya, 1995; Trouillier et al., 1996; Yokoyama, 1997; El-Mansy and Molnár, 1997; El-Mansy and Molnár, in press). Relying on these studies, Kent et al. (1995) proposed that the class Actinosporea should be merged into the class Myxosporea as a synonym of the latter, and that the names of actinosporean genera should in the future be used only for typing actinospores developing in oligochaetes. The major taxonomic changes of recent years are reflected in the works of Smothers et al. (1994), Kent et al. (1995), Siddal et al. (1995), and Schlegel et al. (1996), who reassigned phylum Myxozoa, earlier regarded by many authors as protozoa, from protozoan to metazoan parasites.

The actinosporean infection of oligochaetes in fish farms and natural waters in relation to the developmental cycle of myxosporeans has so far been studied among others by Hamilton and Canning (1987), Burtle et al. (1991), Székely (1991), Styer et al. (1992), Pote and Waterstrat (1993), Koller (1994), Pallós (1995), and McGeorge et al. (1997).

The objective of the present study was to gain a better understanding of the actinosporean stages of species of Myxosporea occurring in Hungary. A one-year survey was carried out to determine the occurrence of actinosporean stages in water samples taken from the ponds of a fish farm as well as in oligochaetes collected from the ponds.

Material and methods

The survey was carried out at a fish farm situated south of Budapest, between March 1996 and March 1997. From three selected ponds of the farm and from their drain channels oligochaete-containing water-weed and mud samples as well as water samples were taken at biweekly intervals. In the period of study, and also previously, polycultural fish breeding was conducted in the given ponds; however, fish species other than those reared in the ponds could also enter the farm with the inflow water. The water-weed, mud and water samples collected from the ponds were transported to the laboratory for the isolation of oligochaetes and then actinospores.

Preparation of oligochaetes for examination for actinospores

Oligochaetes of small size (3–30 mm) were isolated from the plants and from particles floating in the water under stereomicroscope in Petri dishes, identified to the genus or species level, then placed into small (0.5–5 l) aerated aquaria, using a separate aquarium for each species. Oligochaetes of large body size (40–150 mm) were washed out from the mud, assorted under stereomicroscope, then placed into small aerated aquaria in groups separated by species. Before filling the aquaria with tap-water, mud sterilised by boiling was layered onto the bottom of the aquaria to give a 2–3 cm thick layer. The oligochaetes placed into the aquaria were fed a few granules of fish food twice a week. Chicken faeces was added to the water to increase the organic matter content of the mud.

In the course of the one-year survey the following oligochaete species were examined for the occurrence of actinospores: *Branchiura sowerbyi* (Beddard), *Tubifex tubifex* (Müller), *Limnodrilus hoffmeisteri* (Claparede), *Nais elinguis* (Müller) and *Sylaria lacustris* (L.) [identified according to Brinkhurst (1963)].

Examination of oligochaetes for actinosporean infection. Morphology and differentiation of the actinospore types

After the identification of oligochaetes to the species or genus level, they were examined for the presence of actinospores by the cell-well plate method of Yokoyama et al. (1991). A 24-well plate was used, with wells having a volume of 2 ml and containing tap-water. The worms were placed one by one into the wells which then were covered with a plastic sheet to prevent worms from climbing from one well into another. The plates were kept in thermostat at a temperature of 15 °C and examined under compound microscope for the possible presence of actinospores released from the worms and floating in the water. Any floating actinospores found were examined under an Olympus research microscope at a higher magnification and then recorded on videotape with the help of a microscope video attachment. Subsequently still images of the spores were taken from the video recordings with the help of a video image program (Imago®). In many cases, simultaneously with the video recordings, photographs were also taken of the actinospores with the help of a conventional photographic attachment. Subsequently drawings were made of the actinospores and their measurements were taken.

Some of the oligochaetes were lifted out of the wells, carefully placed onto slides and examined under a coverslip, in live state, for the presence of actinosporean developmental stages by microscopy. When such stages were found, the live material was first recorded on videotape. Subsequently the infected worms were fixed in Bouin's solution for 4 h, embedded in paraffin, and 3–4 µm thick sections were prepared from them. The sections were stained with haematoxylin and eosin, then examined under an Olympus research microscope to determine the location of spore development.

If the oligochaetes were negative on first examination, the plates containing them were placed back into the thermostat and then re-examined every two days until they released actinospores. Those worms were considered negative which did not release spores from their body even after 3 months. The majority of large oligochaetes (*Branchiura, Tubifex, Limnodrilus*) survived in the thermostat for 3–4 months even without feeding; in their case only water had to be replenished at each examination. The prevalence of infection of the worms was recorded at each examination and the data were summarised by season.

The characteristic dimensions of actinospores recorded on videotape (polar capsules, spore body, style, caudal processes, whole length) were measured with the help of the Imago program. In addition, these measurements were also taken subsequently with the help of a scale recorded on the videotape together with the actinospores separately for each magnification (Székely, 1997). The actinospore types were compared to the actinospore forms reported in the literature earlier (Janiszewska, 1955, 1957; Marques, 1984; Yokoyama et al. 1993*a,b*, 1995). Measurements were always taken on the basis of the average values measured for 10–50 mature spores. The measurements of actinospore types have been summarized in a table according to the guidance given by El-Matbouli (1988) and Lom et al. (1997*b*).

Filtration of pond-water

Due to technical difficulties (high floating alga count, etc.), direct filtration of water from the ponds was attempted in a few cases only. In the majority of cases, especially when examining oligochaetes of small body size, mud and water-weed samples were brought to the laboratory, together with particles floating in the water which often contained specimens of *Nais elinguis* and *Sty-*

laria lacustris. In the laboratory these samples were placed into aquaria. On every second day one litre of water was taken from each of these aquaria and filtered through a mesh of 10 µm pore size for examination for actinospores. Using a small volume of water, the filtrate was then washed off the filtering material onto a slide, and examined for the presence of actinospores at low magnification under a microscope.

Myxosporeans occurring in the fish ponds surveyed

Since the fish farm studied is conducting primarily common carp breeding in polyculture, the commonest farmed fish species are the common carp and the Japanese coloured carp (*Cyprinus carpio*), the silver carp (*Hypophthalmichthys molitrix*), the bighead (*Aristichthys nobilis*), the grasscarp (*Ctenopharyngodon idella*), the goldfish (*Carassius auratus auratus*), and, as complementary predators, first of all the pikeperch (*Stizostedion lucioperca*), the pike (*Esox lucius*), the European catfish (*Silurus glanis*), and the sterlet (*Acipenser ruthenus*). Naturally, non-farmed fish species other than those listed above also gain entry to the fish ponds with the inflow waters in low numbers.

The fish species bred at the farm have been subjected to regular health checks for several years. These checks have detected the following main species of Myxosporea in the fish farm concerned (unpublished):

Common carp (*Cyprinus carpio*): *Myxobolus cyprini, M. dispar, M. basilamellaris, M. encephalicus, Thelohanellus nikolskii, T. hovorkai, Hoferellus cyprini, Sphaerospora renicola, S. molnari.*

Goldfish (*Carassius auratus*): *Hoferellus carassii, Sphaerospora renicola.*

Silver carp (*Hypophthalmichthys molitrix*): *Myxobolus pavlovskii, M. drjagini, Myxobilatus* sp., *Sphaerospora* sp., *Chloromyxum* sp.

Bighead (*Aristichthys nobilis*): *M. pavlovskii, Myxobilatus* sp., *Sphaerospora* sp., *Chloromyxum* sp.

Grasscarp (*Ctenopharyngodon idella*): *Chloromyxum* sp., *Myxidium* sp.

Pike (*Esox lucius*): *Myxobolus anurus, Henneguya psorospermica, Myxidium lieberkuehni.*

Sterlet (*Acipenser ruthenus*): *Sphaerospora colomani, Chloromyxum inexpectatum*

Pikeperch (*Stizostedion lucioperca*): no data are available.

Results

Occurrence of actinospore types in oligochaetes collected from the fish ponds

A total of 28 actinospore types (Figs 1–4 and 12–13) were isolated from the 889 oligochaete specimens collected from the ponds, representing 5 species (*Branchiura sowerbyi, Tubifex tubifex, Limnodrilus hoffmeisteri, Nais elinguis* and *Stylaria lacustris*). The actinospores can be classified into four main groups (triactinomyxon, raabeia, aurantiactinomyxon and neoactinomyxon). The specimens of *Branchiura sowerbyi* exhibited the most prevalent infection, and were in many cases infected by several actinospore types at the same time. Of the 253 *Branchiura* specimens examined, 215 (85%) were infected by raabeia, 149 (59%) by aurantiactinomyxon, and 50 (20%) by neoactinomyxon type actinospores (Fig. 5). Triactinomyxon type infection was not detected in *Branchiura.* Of the 338 *Tubifex* specimens examined, 108 (32%) were infected by triactinomyxon, 4 (1.2%) by raabeia, and 3 (0.9%) by aurantiactinomyxon (Fig. 6). Of the 193 *Limnodrilus* specimens, 65 (34%) were infected by aurantiactinomyxon and 49 (25%) by triactinomyxon (Fig. 7). Neither raabeia nor neoactinomyxon type infection was found in the *Limnodrilus* specimens. Four out of the 31 *Nais elinguis* specimens examined (13%) were infected exclusively by triactinomyxon (Fig. 7), while 3 out of the 74 *Stylaria* specimens examined (4%) was also found to have exclusively triactinomyxon infection (Fig. 7).

Infection by actinospores exhibited a pronounced seasonality. In the spring, summer and autumn the prevalence of raabeia infection in *Branchiura* exceeded 90% (Fig. 8), while in the winter it dropped to 42%. A similar phenomenon was observed for aurantiactinomyxon infection, with the difference that in this case the spring and summer peaks were around 80% and the prevalence of infection dropped to around 40% by the autumn and to a level as low as 14% by the winter. It was interesting to note that neoactinomyxon infection was at a low level in the spring and summer, reaching a high (over 90%) peak in the autumn, followed by a marked decrease during the winter. For the species *Tubifex, Limnodrilus, Nais* and *Stylaria* the highest prevalence of actinospore infection occurred, with minor differences, in the spring and summer (Figs 9–11).

2. Filtration of pond-water

After filtration of water samples from the ponds triactinomyxon, raabeia, aurantiactinomyxon and neoactinomyxon type actinospores were detected (Figs 1–4, Tables 1–4), which were often identical with the actinospore types directly obtained from the worms.

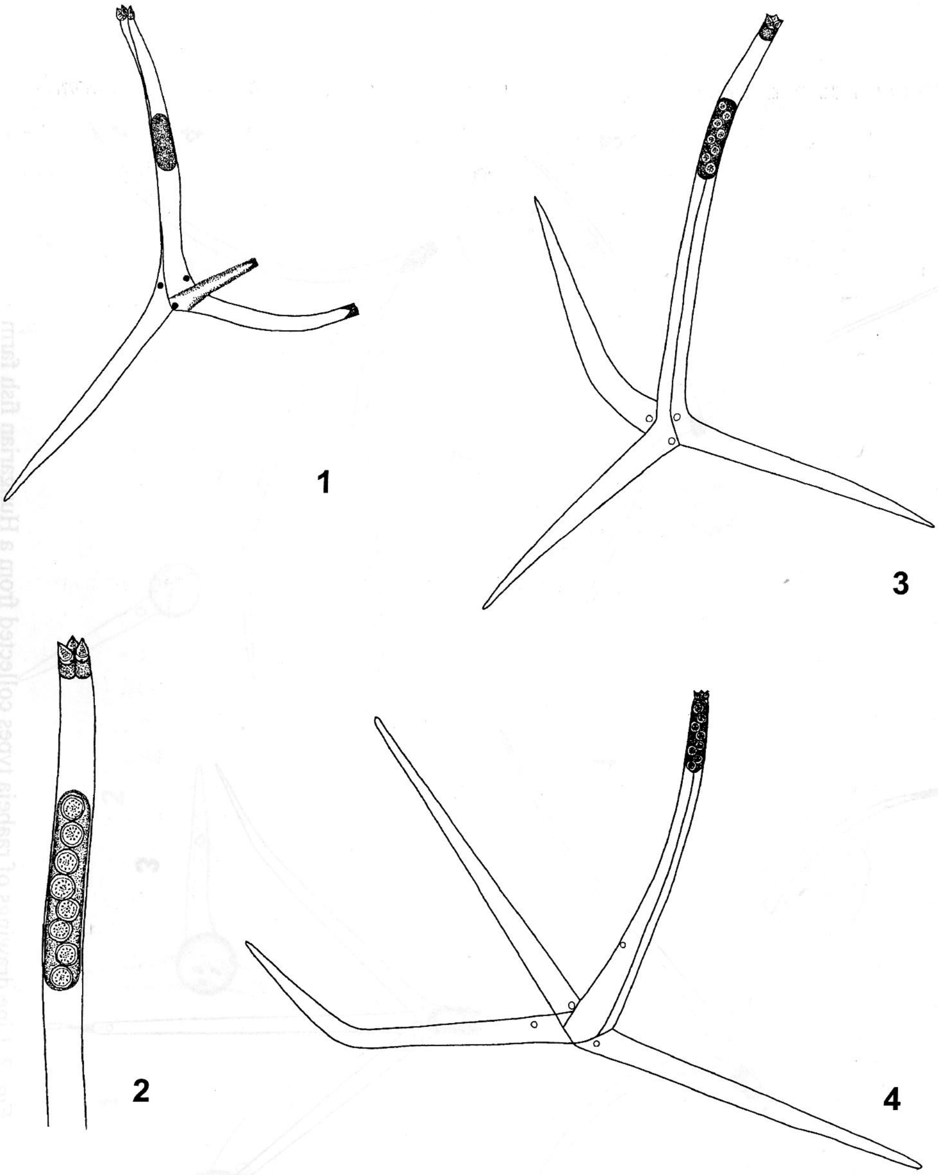


Fig. 1. Line drawings of triactinomyxon types collected from a Hungarian fish farm

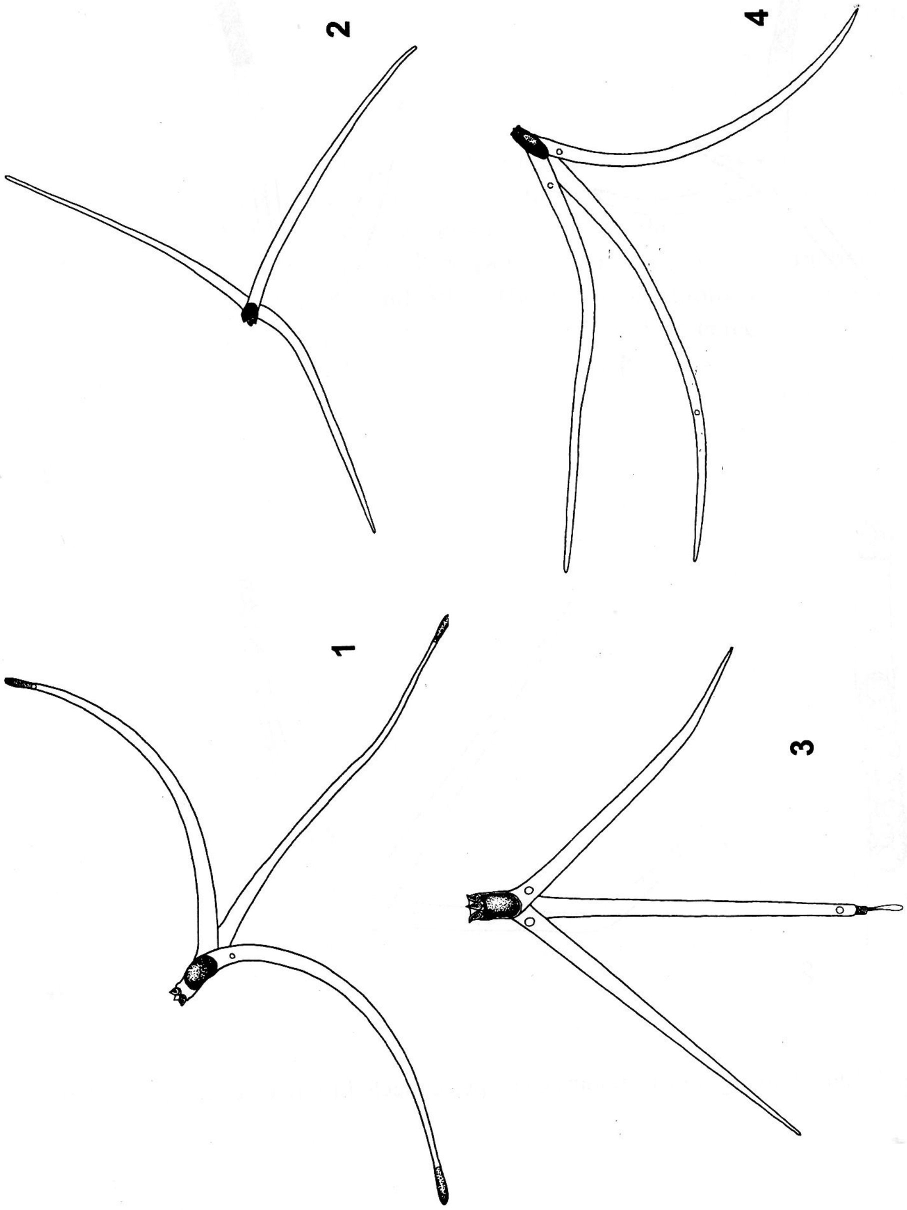


Fig. 2. Line drawings of raabeia types collected from a Hungarian fish farm

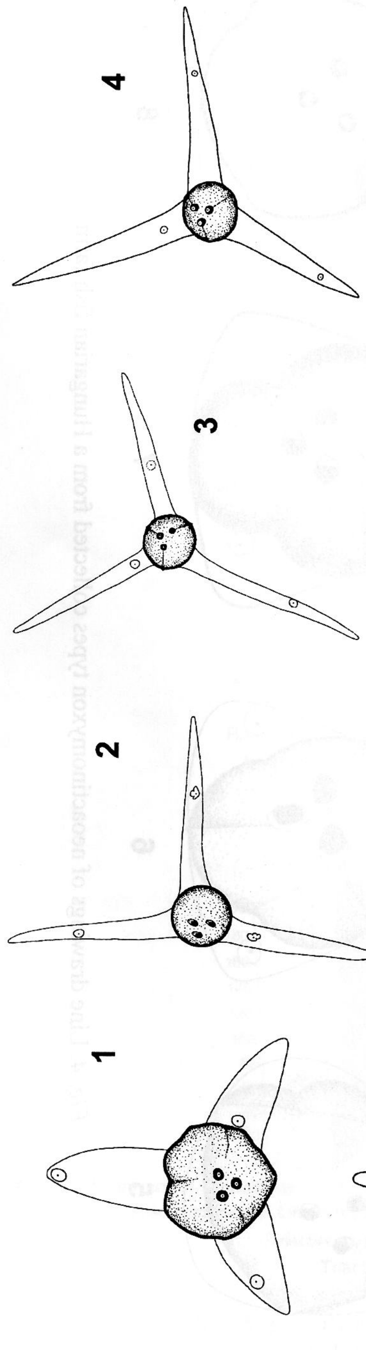


Fig. 2. Line drawings of raabeia types collected from a Hungarian fish farm

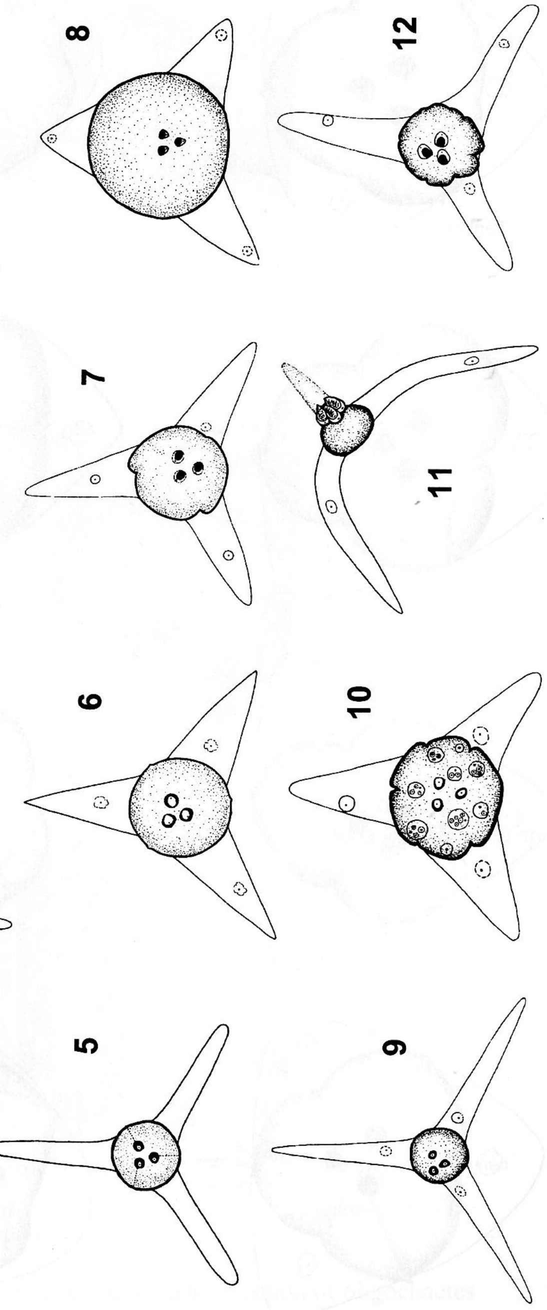


Fig. 3. Line drawings of aurantiactinomyxon types collected from a Hungarian fish farm

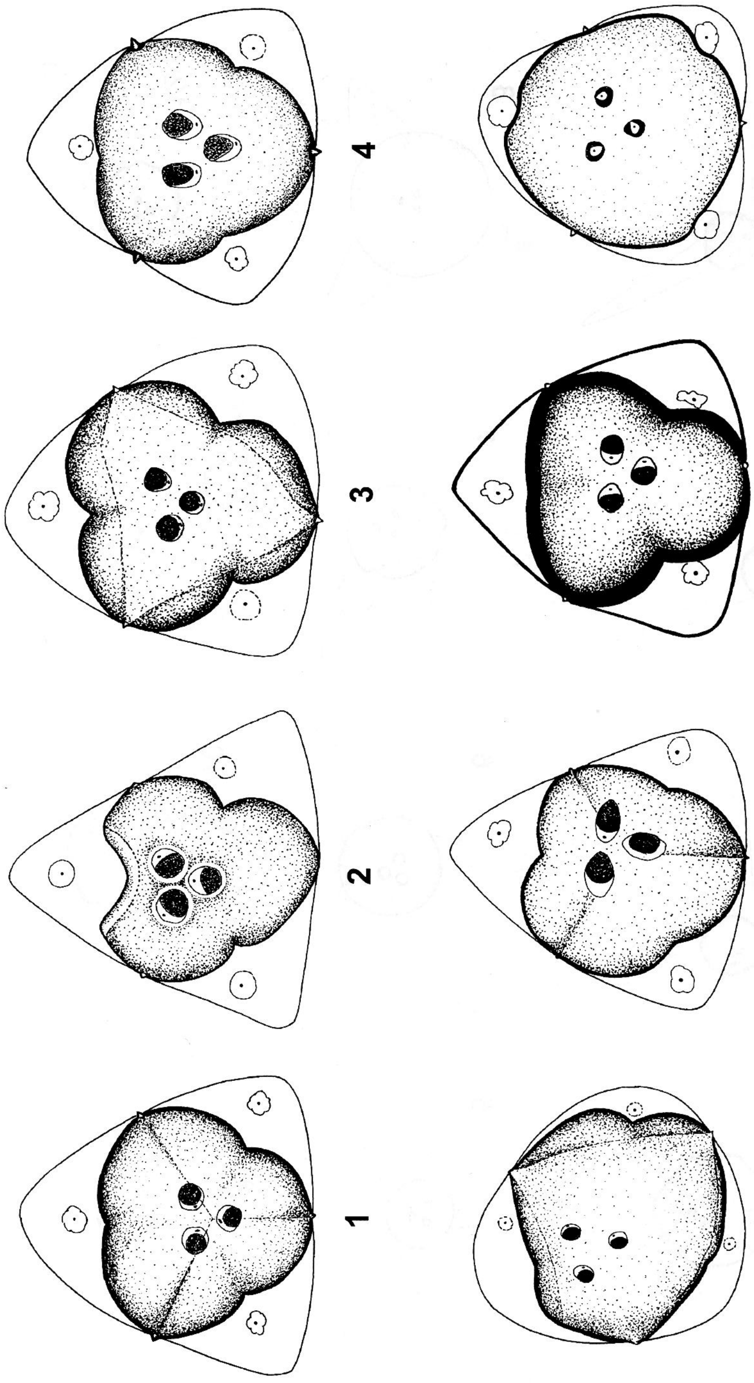
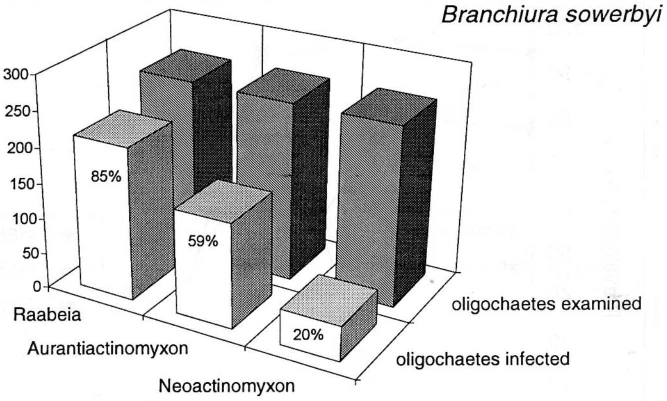
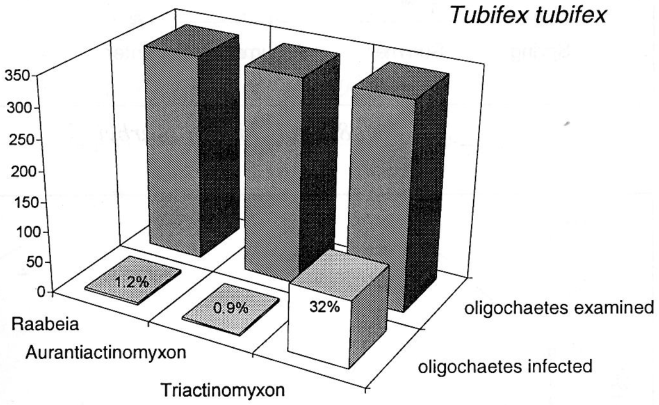
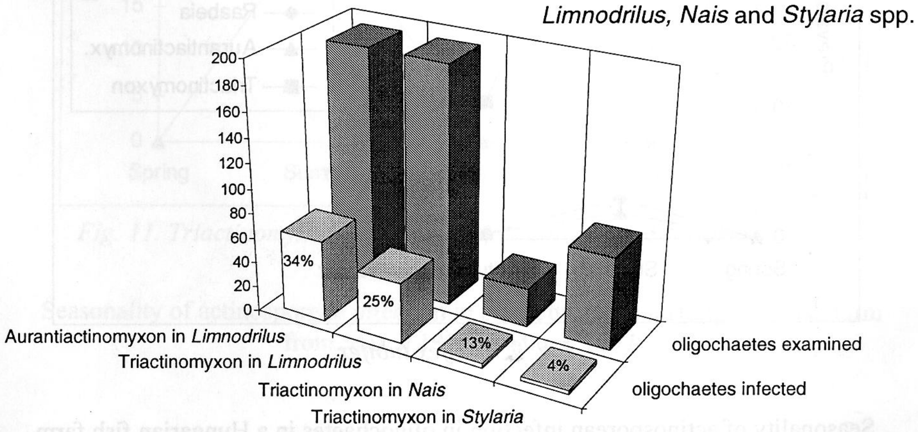


Fig. 4. Line drawings of neoactinomyxon types collected from a Hungarian fish farm







Figs 5–7. All-year prevalence of actinosporean infection of oligochaetes in a Hungarian fish farm from March 1996 to March 1997

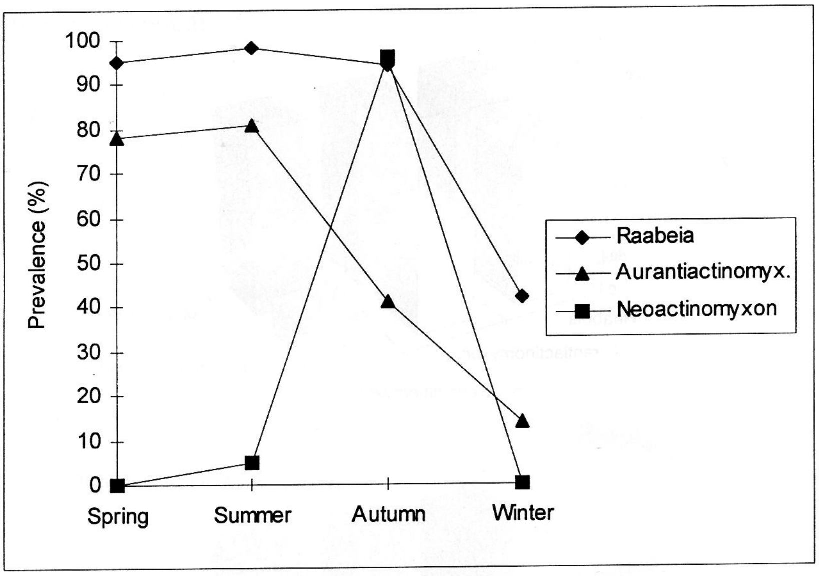


Fig. 8. Branchiura sowerbyi

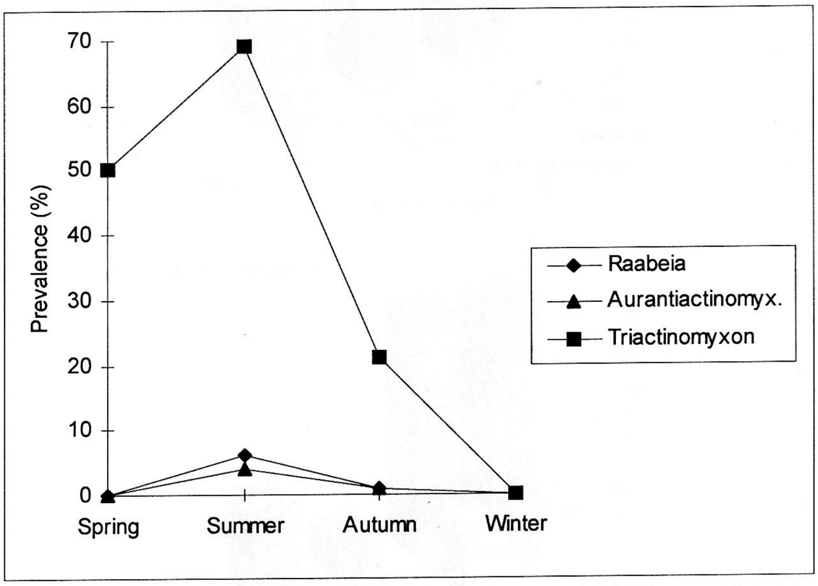


Fig. 9. Tubifex tubifex

Seasonality of actinosporean infection in oligochaetes in a Hungarian fish farm from March 1996 to March 1997

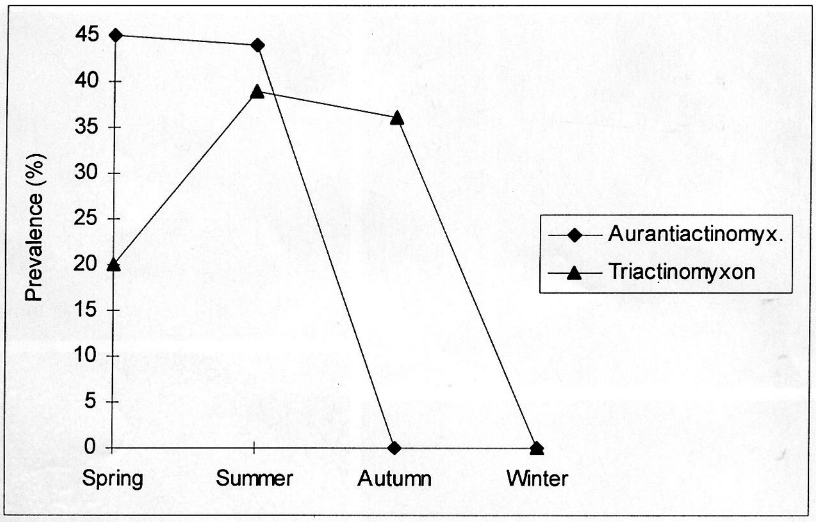


Fig. 10: Limnodrilus hoffmeisteri

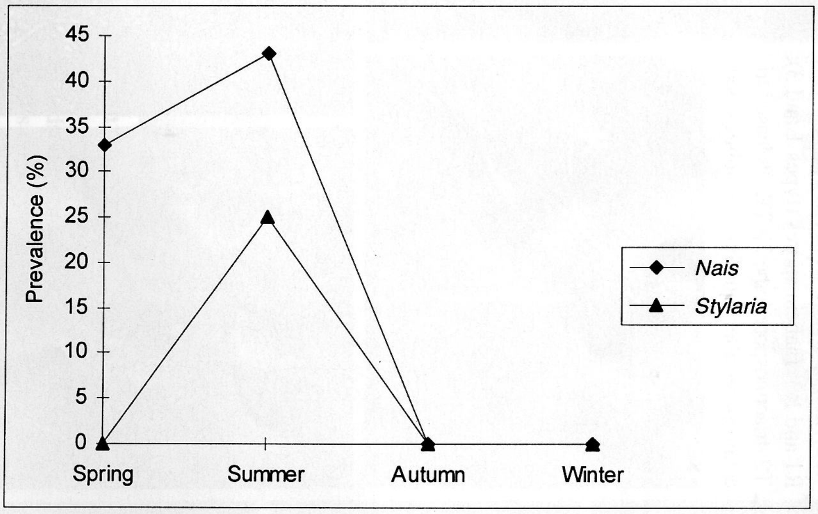


Fig. 11. Triactinomyxon infection in *Nais elinguis* and *Stylaria lacustris*

Seasonality of actinosporean infection in oligochaetes in a Hungarian fish farm from March 1996 to March 1997

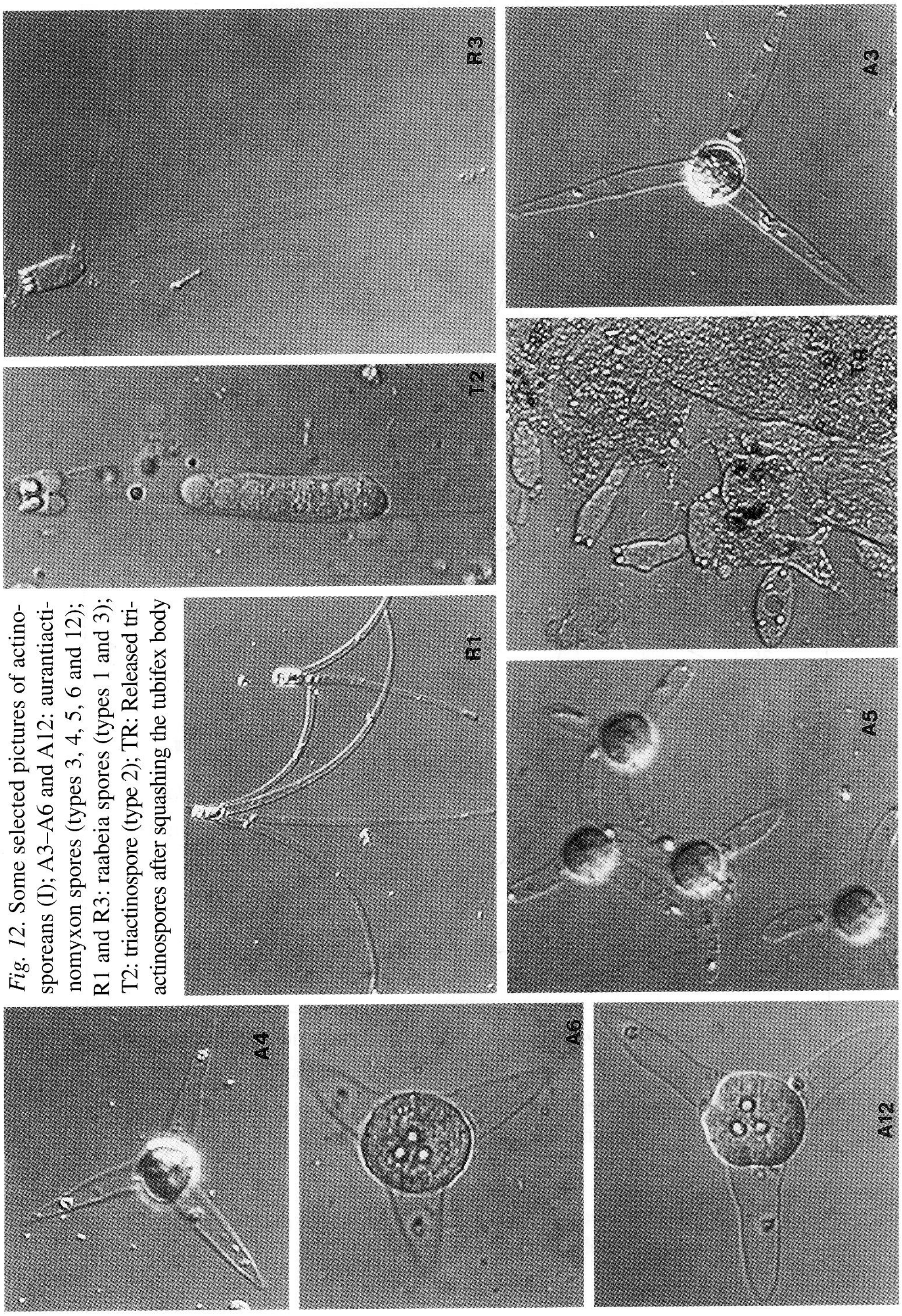


Fig. 12. Some selected pictures of actinosporeans (I); A3–A6 and A12: aurantiactinomyxon spores (types 3, 4, 5, 6 and 12); R1 and R3: raabeia spores (types 1 and 3); T2: triactinospore (type 2); TR: Released triactinospores after squashing the tubifex body

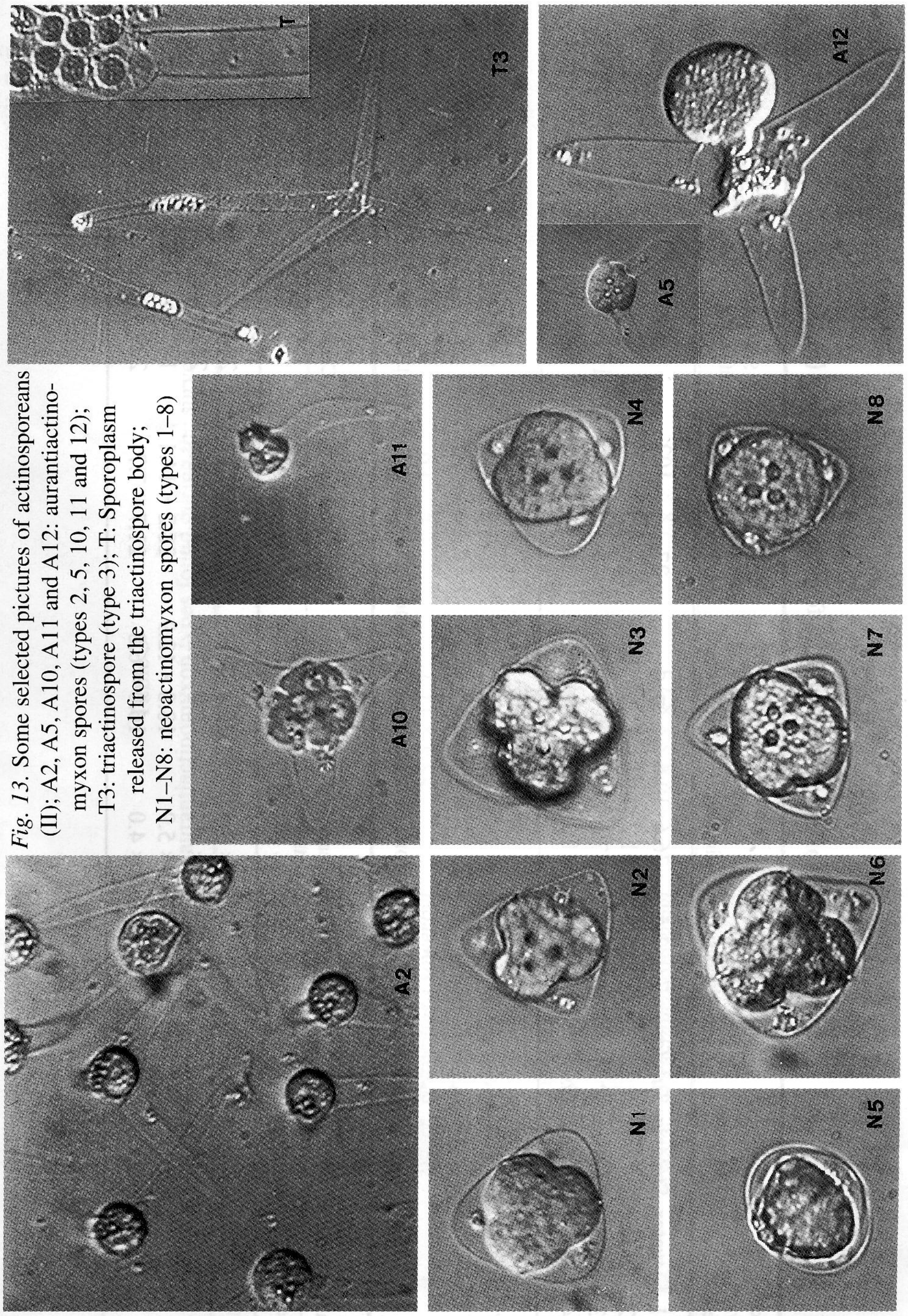


Fig. 13. Some selected pictures of actinosporeans (II); A2, A5, A10, A11 and A12: aurantiactinomyxon spores (types 2, 5, 10, 11 and 12); T3: triactinospore (type 3); T: Sporoplasm released from the triactinospore body; N1–N8: neoactinomyxon spores (types 1–8)

Table 1

Average dimensions of triactinomyxon types found during the survey (in µm)

Triactino-myxon type No.	Origin of spores	Polar capsule dimensions	Spore body dimensions	Secondary cell dimensions	No. of secondary cells	Style length	Style width at start/at end	Caudal process dimensions	Whole length
1	water, *Stylaria, Tubifex*	7.0×3.5	36.6×10.6	n. d.	≈ 27	102	9.4/16.5	128×10.6	236.3
2	water	11.8×5.9	101.2×14.1	11.8×10.6	n. d.	n. d.	n. d.	n. d.	n. d.
3	*Nais, Tubifex*	5.9×4.5	47.1×10.6	6.0×4.7	8	150	15.3/10.6	127.5×14.5	399.4
4	water, *Limnodrilus*	4.7×2.4	41.2×8.8	5.9×4.7	8	137.7	9.4/20.0	173.4/14.3	352.3

n. d. = not determined

Table 2

Average dimensions of raabeia types found during the survey (in µm)

Raabeia type No.	Origin of spores	Polar capsule dimensions	Sporoplasm dimensions	Spore body dimensions	Caudal process dimensions	Whole length (from polar capsules to the end of caudal process)
1	water, *Branchiura, Tubifex*	5.9 × 3.5	20 × 11.8	25.9 × 11.8	294 × 9	320
2	water, *Branchiura*	5.9 × 4.7	8.2 × 12.4	14.1 × 12.4	202.8 × 8.2	216.9
3	*Tubifex*	7.5 × 5.9	18.8 × 12.9	28.2 × 14.1	183.6 × 10.6	211.8
4	water	5.7 × 4.0	16.0 × 6.8	21.7 × 7.7	209.4 × 6.6	231.1

Table 3

Average dimensions of aurantiactinomyxon types found during the survey (in µm)

Aurantiactino-myxon type No.	Origin of spores	Caudal process length and width near the sporoplasm	Polar capsule dimensions	Spore cavity diameter	Largest span
1	*Tubifex tubifex*	17.5 × 9.9	2 × 2	18.3	45.4
2	water,*Branchiura sowerbyi*	65.7 × 10.5	4 × 1.7	22.8	142.5
3	water, *Branchiura sowerbyi*	70.3 × 8.0	2.9 × 2.9	22.8	149.3
4	water, *Branchiura sowerbyi*	55.7 × 11.2	2.9 × 2.9	19.4	122
5	water, *Branchiura sowerbyi*	17.2 × 3.9	1.4 × 1.4	9.9	39.5
6	*Limnodrilus* sp.	24.2 × 11.2	2.8 × 2.8	19.7	55.6
7	water	24.4 × 9.5	2.8 × 2.5	18.9	58.4
8	*Limnodrilus* sp.	12.2 × 9.0	1.4 × 1.4	22.6	39.8
9	water, *Branchiura sowerbyi*	51.3 × 9.5	2.3 × 2.3	18.8	103.2
10	water, *Branchiura sowerbyi*	16.7 × 8.8	1.7 × 1.7	15.5	39.5
11	water	31.9 × 3.7	3.4 × 2.0	8.5	46.5
12	water, *Branchiura sowerbyi*	26.5 × 8.7	2.8 × 3.1	12.1	59.2

Table 4

Average dimensions of neoactinomyxon types found during the survey (in μm)

Neoactinomyxon type No.	Origin of spores	Polar capsule dimensions (length × width)	Spore body width (angle to base)	Caudal process dimensions (length × width)	Span between two caudal processes
1	*Branchiura sowerbyi*	2.5 × 2.8	21.2	8.5 × 16.4	29.3
2	*Branchiura sowerbyi*	3.1 × 2.8	18.3	10.8 × 14.4	31
3	*Branchiura sowerbyi*	2.8 × 2.3	22	8.5 × 16	30.2
4	*Branchiura sowerbyi*	3.7 × 2.8	22.3	7 × 16	29
5	*Branchiura sowerbyi*	1.7 × 1.7	21.2	4.4 × 13.6	23.1
6	*Branchiura sowerbyi*	4.2 × 2.8	20.3	7.8 × 12.7	29.3
7	*Branchiura sowerbyi*	2.8 × 3.1	22.6	8.5 × 16.4	31.5
8	*Branchiura sowerbyi*	2 × 2	22.8	4.2 × 11.3	26.8

Light microscopy

Actinosporeans representing different stages of development (primarily the stages showing advanced spore formation) could be recognised even if live worms were examined microscopically, gently pressed down under a coverslip (Fig. 12, TR).

Description of the detected actinospore types

Most of the detected actinospore types were measured as described by Lom et al. (1997*b*). The main parameters of the actinospores found are presented in Tables 1–4.

Histological evidences

After fixation, histological processing and examination of infected worms selected in this way, actinospore parasites were found to occur first of all in the worms' gut epithelium and less frequently in their body cavity (Fig. 14).

Discussion

Primarily in recent years, actinosporean infection of oligochaetes has been studied successfully by numerous authors, among others by Mackinnon and Adam (1924), Markiw (1986), El-Matbouli and Hoffmann (1989), Yokoyama et al. (1991), El-Matbouli and Hoffmann (1993), Kent et al. (1993), El-Matbouli et al. (1995), Kent et al. (1995), Uspenskaya (1995), Yokoyama et al. (1995), and Trouillier et al. (1996). Most of the above-listed authors reported a relatively low (around 1%) prevalence of infection in worm populations. Yokoyama et al. (1993*a,b*) were the only investigators who detected > 4% actinosporean infection in *Branchiura sowerbyi* in a goldfish-culturing pond. In a survey of the actinosporean infection of oligochaetes in natural waters of Hungary, Székely (1989) and Pallós (1995) also found similarly low levels of infection. In contrast, in the present survey e.g. raabeia infection of *Branchiura sowerbyi* showed a prevalence of 98% in certain periods of the year, and in the warm months a triactinomyxon infection of 3% and 4% prevalence was recorded even in the *Stylaria* and *Nais* specimens, respectively, which were found to have the lowest level of infection in this survey. These values are markedly higher than those reported earlier by other authors. The outstandingly high values recorded by us, however, only partially mean that the level of actinospore infection is so much higher in the fish ponds surveyed than in other habitats. The observed difference can be attributed primarily to the different examination technique used by us. Today, it is already well known that a given oligochaete may be infected by

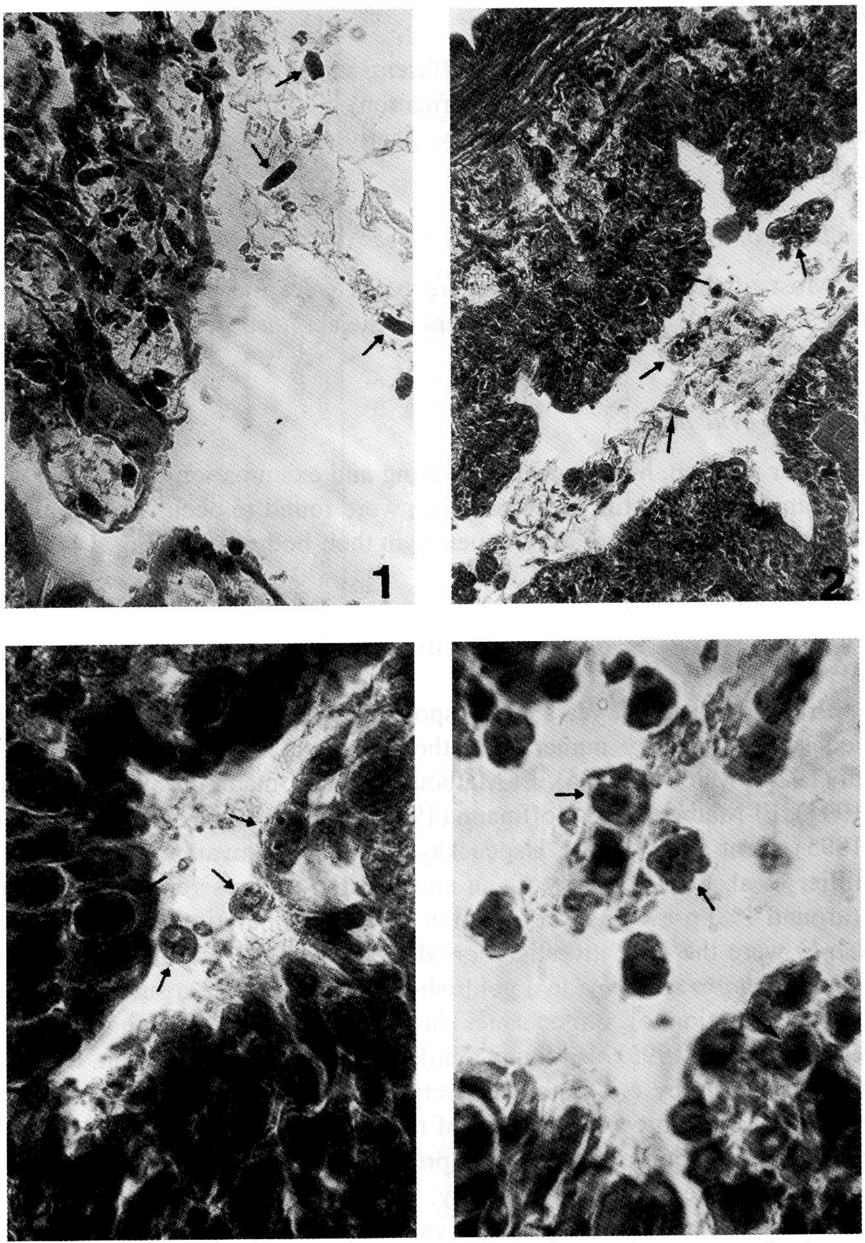


Fig. 14. Histological sections of actinosporean-infected oligochaetes; 1. Triactinomyxon infection in the gut of *Tubifex tubifex* (× 210); 2. Raabeia infection in the gut of *Branchiura sowerbyi* (× 250); 3. Aurantiactinomyxon infection in the gut of *Branchiura sowerbyi* (× 960); 4. Neoactinomyxon infection in the gut of *Branchiura sowerbyi* (× 1750); arrows: spores in the gut lumen and in the gut epithelium

actinospores representing different development stages while the mature spores will be excreted only after an about 3-month period of intra-oligochaete development. The fact that in this study individual oligochaete specimens were regularly examined over a period of about 3 months, greatly elevated the ratio of positive individuals and revealed that a single examination of any given worm cannot give reliable data on the prevalence of actinosporean infection, as it shows exclusively the ratio of worms that contain mature spores and spores being excreted at the given point of time.

The data obtained in this study indicate that actinospore infection of oligochaetes persists throughout the year and is characterised by seasonality manifesting itself primarily in the appearance of worms containing mature spores, which reaches its peak in the warmer months.

A further explanation of prevalence values far exceeding those reported in the literature may be that in the present survey the worms were collected from ponds in which several fish species were reared simultaneously in an intensive manner. As a result, the number of myxosporeans (and thus the actinospore forms) occurring there can be far higher as compared to the level of actinosporean infection of oligochaetes derived from ponds in which other fish species (salmonids, goldfish, catfish) are reared in monoculture (Pote and Waterstrat, 1993; Yokoyama et al., 1993*a,b*; McGeorge et al., 1997).

The different actinospore types could be found in both the smaller and the larger oligochaetes. Thus, triactinomyxons were detected in *Tubifex tubifex* of large body size just like in the smaller *Stylaria* and *Nais* species. It should be mentioned, however, that triactinomyxon types, which are so common in other oligochaetes, could not be detected in *Branchiura sowerbyi* showing the highest prevalence of infection. The actinospores found during the present survey were defined on the basis of the main taxonomic units (triactinomyxon, raabeia, aurantiactinomyxon and neoactinomyxon) described by Janiszewska (1955, 1957) and Marques (1984); however, we agree with the view of El-Matbouli et al. (1992), Kent et al. (1993), Yokoyama et al. (1993*a*), Kent et al. (1995), Yokoyama et al. (1995), Trouillier et al. (1996), and McGeorge et al. (1997), i.e. that these cannot be regarded as independent taxonomic units. The accepted names are used only for denoting the type of developmental stage of a given myxosporean species. The number of the 28 actinospore types detected by us roughly corresponds to that of myxosporean species hitherto found in the fish farm. It is likely, however, that some of the actinospores found during this survey had come from the inflow water, rather than being a developmental stage of myxosporeans parasitising fish reared in the ponds. While we do not venture to identify any of the detected actinospore types as a developmental stage of any of the myxosporean species parasitic in the ponds in question, based upon the experimentally proven developmental cycles reported in the literature (Wolf and

Markiw, 1984; El-Matbouli and Hoffmann, 1989; El-Matbouli et al., 1992; Benajiba and Marques, 1993; El-Matbouli and Hoffmann, 1993; Kent et al., 1993; Uspenskaya, 1995; Yokoyama et al., 1995; El-Mansy and Molnár, 1997) as well as our own work currently under publication (El-Mansy and Molnár, 1998) it can be assumed that triactinomyxons and raabeias represent the developmental stages of species belonging to the *Myxobolus* genus while neoactinomyxons and aurantiactinomyxons may be the developmental stages of species included in the genera *Myxidium, Zschokkella, Hoferellus* and *Thelohanellus.*

Differential diagnosis

On the basis of their dimensions presented in Tables 1–4 and their schematic drawings shown in Figs 1–4, the 4 triactinomyxon, 4 raabeia, 12 aurantiactinomyxon and 8 neoactinomyxon types detected during the survey relate to the types hitherto described in the literature as follows.

As this survey provided a picture of the actinosporean infection of oligochaetes derived from a fish farm culturing cyprinids, it is not surprising that the 4 triactinomyxon types found by us differ from the triactinomyxons detected by other authors (Hamilton and Canning, 1987; El-Matbouli and Hoffmann, 1993; McGeorge et al., 1997) in waters inhabited by trouts. Nor are these four triactinomyxon types identical with the triactinospore of *M. drjagini* and *M. hungaricus* described by El-Mansy and Molnár (1997, 1998) from a fish farm and from Lake Balaton, respectively. Although some of the dimensions (e.g. those of the style or the caudal processes) may be identical with the data reported by other authors, in these cases differences are found e.g. in the number of secondary cells located in the triactinospores.

As with the triactinomyxons, the 4 raabeia types found by us did not prove to be identical with the forms hitherto described in the literature (Janiszewska, 1957; Janiszewska and Krzton, 1973; Yokoyama et al., 1995; McGeorge et al., 1997). Although three out of the 4 raabeia types found by us (types 1, 2 and 4) have caudal processes similar in size to that described by Yokoyama et al. (1995) from *Branchiura sowerbyi* and, moreover, type 4 even has spore body parameters identical with those of the latter, type 4 described by us has much larger polar capsules than that reported by the above-cited Japanese authors. The other raabeia types included in our survey differ from those described by other authors in several parameters.

By their dimensions, none of the 12 aurantiactinomyxon types can be identified with the forms described by Marques (1984), Styer et al. (1992) and Troullier et al. (1996). Nor can the forms found by us be compared with the aurantiactinospores described by El-Matbouli et al. (1992), Grossheider and Körting (1992), and Benajiba and Marques (1993), as the authors did not give spore dimensions in these works. Although the aurantiactinomyxon type presented by McGeorge et al. (1997) resembles the 12 aurantiactinomyxon types found by us in many of its parameters (spore cavity diameter, polar capsule dimensions and length of caudal processes), the caudal processes of the Scottish form widen out much more than those of the forms found by us. These two forms also differ in their habitat and alternative host. The form described by Yokoyama (1997) as the aurantiactinospore of *Thelohanellus hovorkai* closely resembles our type 12 also found in *Branchiura sowerbyi*, with only slight differences.

The eight neoactinomyxon types collected by us differed from the forms described in the literature by Janiszewska (1955), Marques (1984) and Yokoyama et al. (1993*a*) in several of their dimensions.

A paper providing general and consistent guidelines on the description of actinospores on their own or as the alternative form of myxosporeans has been published only quite recently (Lom et al., 1997*b*). This is why it is often difficult to compare the forms found by us with those described in earlier works. It is to be hoped that in the future all descriptions will conform to the general guidelines, which would make it easier to compare the results obtained by different researchers.

The main objective of the present work was to complement our knowledge of the myxosporeoses of fish farms with data on those myxosporeans' alternative forms living in oligochaetes. Our future goal is to assign the highest possible number of the detected actinosporeans to individual species of myxosporeans, with the help of experimental work.

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