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# Occurrence of actinosporean stages of myxosporeans in an inflow brook of a salmon hatchery in the Mena River System, Hokkaido, Japan

**Csaba Székely[1,*], Shigehiko Urawa[2], Hiroshi Yokoyama[3]**

[1]Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary
[2]National Salmon Resources Center, Fisheries Agency of Japan, Sapporo, Japan
[3]Department of Aquatic Bioscience, The University of Tokyo, Japan

ABSTRACT: Actinospore infection of oligochaetes living in the mud and on the roots of vegetation in an inflow brook of a Hokkaido salmon hatchery was studied within the framework of a Japanese-Hungarian research program. Two triactinomyxon types, 1 echinactinomyxon, and 1 neoactinomyxum type were isolated from the oligochaete *Rhyacodrilus komarovi* Timm, 1990 collected during the survey. The aurantiactinomyxons were recorded over a period of 3 mo starting from the day after oligochaete collection. The oligochaetes released actinospores for several weeks from the first day of the study. Spore excretion of individual oligochaetes was not synchronous. Of the oligochaetes examined, 0.7, 7, 3 and 3%, were infected with the echinactinomyxon, neoactinomyxum and the 2 types of triactinomyxon spores, respectively. Actinospore infection was intense in the positive oligochaetes in all 4 types. Of the 4 actinospore types presented here, 3 are described for the first time.

KEY WORDS: Actinosporean · Myxosporean · Oligochaete · Salmon hatchery · Hokkaido · Japan



## INTRODUCTION

The first report on actinospores described these organisms (found in Bohemia) as parasites related to myxosporeans (Stolc 1899). Until quite recently this group of parasites was believed to represent an independent taxonomic entity. Research on actinosporea became more intensive after Wolf & Markiw (1984) proved that, instead of representing a distinct taxonomic category, actinosporeans corresponded to the intraoligochaete developmental stages of fish-parasitic myxosporeans (Kent et al. 2001). The relevant research includes earlier surveys (Ormieres & Frezil 1969), as well as studies of actinospore infection of oligochaetes in natural waters and fish farms in connection with the life cycle of myxosporeans (Hamilton & Canning 1987, Székely 1989, Burtle et al. 1991, Styer et al. 1992, Pote & Waterstrat 1993, Yokoyama et al. 1993, Pallós 1995, McGeorge et al. 1997, El-Mansy et al. 1998a,b, Xiao & Desser 1998a,b,c, Özer & Wootten 2000, Székely et al. 2000, Negredo & Mulcahy 2001). Although some actinospore types have been detected in salmon waters on Hokkaido previously (Urawa pers. comm.), their descriptions have not been published. The main objective of this work was to describe the actinospore stages of myxosporeans from a salmon biotope on Hokkaido, and thus to facilitate research on the developmental cycle of myxosporean species causing disease in salmonids. In this survey we attempted to detect the actinospore stages of myxosporean parasites of salmon in oligochaete alternate hosts in an inflow brook of a salmon hatchery of the Mena River System on the island of Hokkaido, Japan.

## MATERIALS AND METHODS

The survey was conducted in an inflow brook of a salmon hatchery in the Mena River System on Hokkaido in April 2000 at the time of the melting of

*E-mail: szekely@vmri.hu

snow, at a water temperature of 4°C. The brook and the Mena River belong to the Shiribetsu River system. The Masu salmon *Onchorhynchus masou* is infected by several myxosporean species, the intraoligochaete spore types of which, however, have not yet been identified. To detect these intraoligochaete actinospore types, the small oligochaetes were washed free of the mud and organic matter accumulated on the bottom of the brook with a close-meshed net, put in a small amount of brook-water in a plastic collection dish, and transported to the Sapporo Laboratory of the National Salmon Resources Center; 5 h after collection the oligochaetes were put individually into the wells of cell-well plates (Yokoyama et al. 1991). Before replacing the lids, the plates were covered with self-adhesive plastic foil to prevent the worms from crawling out. Starting from the subsequent day, the water layer above each oligochaete in each well was regularly examined for the presence of released actinospores. The plates were kept in refrigerator at 4°C throughout the study, and the water in the wells was changed once or twice a week, as needed. On the day after collection the oligochaetes were transported to the University of Tokyo, where they were examined for a further 10 d before transportation to Hungary, where they were monitored for actinospore release for an additional 3 mo, up to the death of the last oligochaete.

If stereomicroscopic examination indicated spore release, the actinospores floating in or adhering to the wells of the cell-well plates were examined on a slide by light microscopy at higher magnification, and microphotographs were taken of the spores using a conventional microscope photographic attachment and a digital microscope camera. Subsequently, drawings were made of the actinospores and their measurements were taken. Some of the oligochaetes observed on the cell-well plates and found to be infected were fixed in 5% glutaraldehyde, washed in sodium cacodylate solution, post-fixed in 2% osmium tetroxide, and again washed in sodium cacodylate solution. Finally the worms were embedded in Durcupan resin and cut into 0.5 to 1 µm thick sections with a Reichert Jung 2040 microtome, using a glass knife. The semithin sections were stained with toluidine blue, examined in an Olympus research microscope, and digital photographs were taken of them to determine the site of spore development. The characteristic dimensions of actinospores (polar capsules, spore body, caudal processes, number of infective cells) were recorded by measuring newly released spores according to Lom et al. (1997). To determine the dimensions of actinospores, the measurements of 6 to 30 mature spores were averaged. Some of the infected oligochaetes were fixed in 80% ethanol and identified in Estonia as described by Timm (1997).

## RESULTS

The several hundred oligochaete specimens collected from the inflow brook of the salmon hatchery represented species belonging to the genera *Rhyacodrylus*, *Nais*, *Lumbriculus*, and *Enchytraeus*. During the period of study, only the small, white oligochaete *Rhyacodrylus komarovi* Timm 1990 (Fig. 1), the most common oligochaete in the brook, was found to be infected by actinospores. The oligochaetes kept individually in the wells of the cell-well plates released actinospores as early as 24 h after collection. However, spore release from the individual oligochaetes was not synchronous, as some oligochaetes released mature spores only 7 to 10 h after collection (i.e. in Tokyo), while others did so only after further transportation (after their arrival in Hungary) over a period of 2 wk and 3 mo after collection.

### Description of detected actinospore types

Of the detected actinospores, 1 proved to be a neoactinomyxum (Figs. 2 to 5 & 22), 2 were triactinomyxons (Figs. 6 to 8, 9 to 11 & 23 to 24), and 1 was an echinoactinomyxon (Figs. 12 to 15 & 25). The dimensions of the individual actinospore types were determined as described by Lom et al. (1997). The most important parameters of the 4 detected actinospore types are presented in Tables 1 to 3.

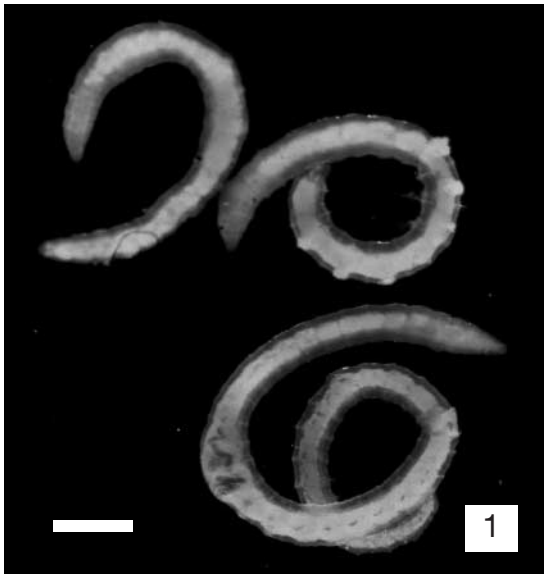
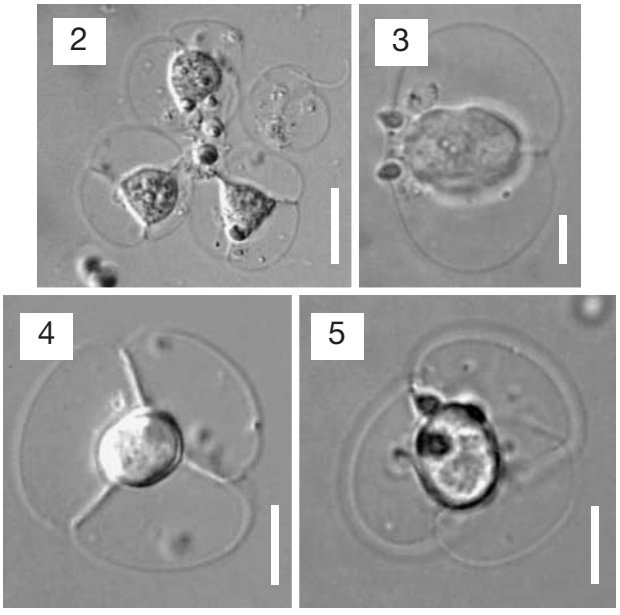



Fig. 1. *Rhyacodrilus komarovi* Timm 1990, a freshwater oligochaete alternate host. Scale bar = 1 mm




Figs. 2 to 5. Neoactinomyxum-type actinospore released from an infected *Rhyacodrilus komarovi*, under a coverslip. Fig. 2. Side view (scale bar = 10 µm). Fig. 3. Side view (scale bar = 5 µm). Fig. 4. Apical view (scale bar = 5 µm). Fig. 5. Semi-apical view (scale bar = 5 µm). Note the 3 polar capsules and the short caudal processes

**Processing by semi-thin technique**

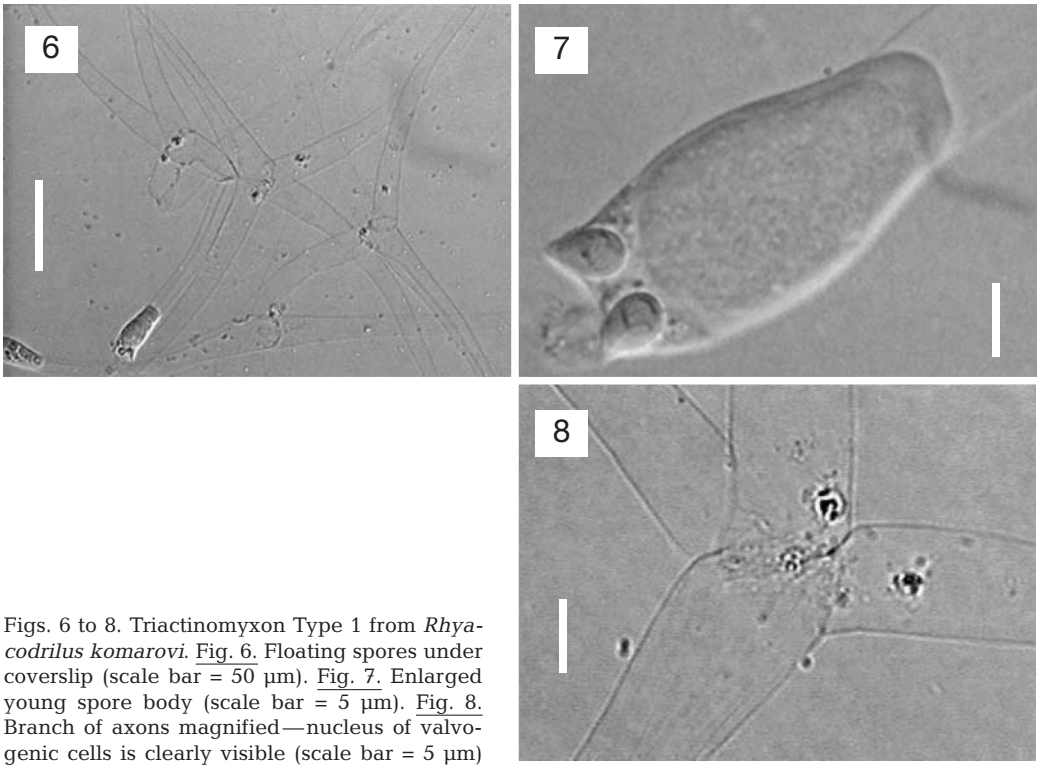
Oligochaetes releasing neoactinomyxum spores and triactinospore Type 1 on the cell-well plates were also processed by the semi-thin technique. Examination revealed the parasites in the gut epithelium of the worms or, in the case of neoactinomyxum, large masses of mature spores infesting the gut lumen. Oligochaetes found positive for actinospores were consistently extremely highly infected (Figs. 16 to 21).

**Differential diagnosis**

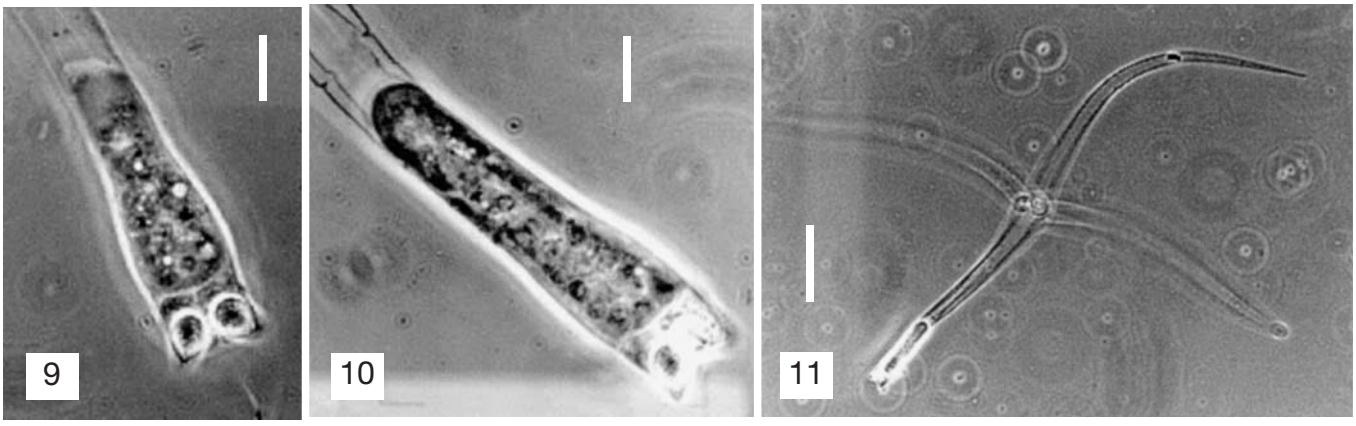
The dimensions (Tables 1 to 3) of the actinomyxon types found during the survey were compared with the types described in the literature so far:

Neoactinomyxum

The neoactinomyxum is not identical to the types described previously in the literature, although its general shape and dimensions bear great resemblance to the actinospore described as *Aurantiactinomyxon eiseniellae* by Ormieres & Frezil (1969) from the oligo-

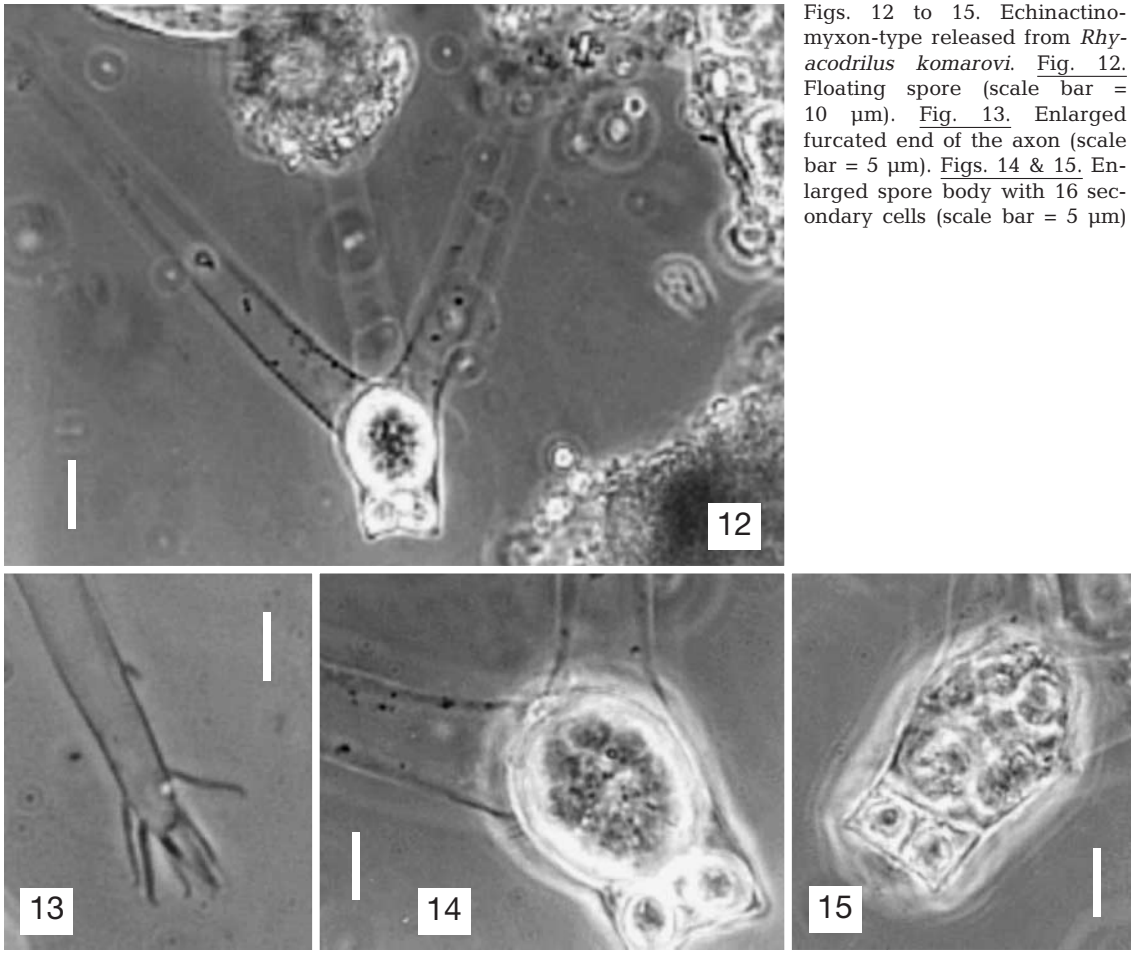



Figs. 6 to 8. Triactinomyxon Type 1 from *Rhyacodrilus komarovi*. Fig. 6. Floating spores under coverslip (scale bar = 50 µm). Fig. 7. Enlarged young spore body (scale bar = 5 µm). Fig. 8. Branch of axons magnified—nucleus of valvogenic cells is clearly visible (scale bar = 5 µm)




Figs. 9 to 11. Triactinomyxon Type 2 infecting *Rhyacodrilus komarovi*. Figs. 9 & 10. Enlarged spore body (scale bar = 10 µm). Fig. 11. Floating triactinospore under coverslip (scale bar = 50 µm)

chaete *Eiseniella tetraedra* (Lumbricida). However, the processes of the neoactinomyxum described in this paper are much closer together than those of the species described by Ormieres & Frezil (1969). The neoactinomyxum type isolated by Negredo & Mulcahy (2001) from *Lumbriculus variegatus* from a river in Ireland is morphologically very similar to the type reported here; however, all dimensions of the presently described neoactinomyxum spores are much smaller, while the number of secondary cells in the




Figs. 12 to 15. Echinactinomyxon-type released from *Rhyacodrilus komarovi*. Fig. 12. Floating spore (scale bar = 10 µm). Fig. 13. Enlarged furcated end of the axon (scale bar = 5 µm). Figs. 14 & 15. Enlarged spore body with 16 secondary cells (scale bar = 5 µm)

Table 1. Neoactinomyxum-type actinospore detected during the survey. Dimensions (µm); values are means of 30 spores, with range in parentheses

| Parameter | Mean (range) |
|---|---|
| Spore body | |
| Length, side | 13.2 (13.0–13.5) |
| Width, apical | 11.5 (10.8–12.1) |
| Caudal process | |
| Length, apical | 10.8 (9.2–11.0) |
| Width, apical | 21.1 (19.2–23) |
| Polar capsule | |
| Length, side | 2.1 (2.8–3.0) |
| Width, side | 2.9 (2.0–2.2) |
| No. sporoplasm cells (approx.) | 32 |

sporoplasm is double that in the Irish isolate. The neoactinomyxum detected in this work greatly differs in shape (and size) from all other neoactinomyxum types described in the literature (Janiszewska 1955, Yokoyama et al. 1993, El-Mansy et al. 1998a).

### Triactinomyxon Types 1 and 2

The smaller of the 2 detected triactinospores (Type 1) is not identical with any of the triactinomyxon types described in the literature so far, differing either in its dimensions or in the number of secondary cells located in the sporoplasm (Janiszewska 1957, El-Matbouli & Hoffmann 1989, 1993, 1998, Kent et al. 1993, Urawa 1994, El-Mansy & Molnár 1997a,b, El-Mansy et al. 1998a,b,c, Xiao & Desser 1998a, Székely et al. 1999,

Table 2. Triactinomyxon Types 1 and 2. Measurements (µm); values are means of 20 triactinospores, with range in parentheses

| Parameter | Type 1 | Type 2 |
|---|---|---|
| Polar capsules | | |
| Length | 6.0 (5.5–6.2) | 5.6 (5–5.9) |
| Width | 3.5 (3.2–3.8) | 3.7 (3.5–4.2) |
| No. infective cells (approx.) | 32 | 64 |
| Sporoplasm | | |
| Length | 35 (28.9–30.8) | 62 (57.5–75) |
| Width | 12.5 (10–14) | 9.1 (8.75–12.2) |
| Style | | |
| Length | 125 (112–150) | 138 (122–142) |
| Caudal processes | | |
| Width | 10 (7–14) | 13.8 (13–14.2) |
| Length | 178 (162–200) | 187 (175–205) |
| Largest span | 354 (330–390) | 375 (350–401) |

Table 3. Echinactinomyxon actinospore. Measurements (µm); values are means of 6 spores, with range in parentheses

| Parameter | Mean (range) |
|---|---|
| Polar capsules | |
| Length | 6 (5–7) |
| Width | 4.4 (4–5) |
| Sporoplasm | |
| Length | 24.3 (21.1–27.5) |
| Width | 18.5 (17.6–20.2) |
| No. infective cells (approx.) | 16 |
| Caudal processes (furcated portion) | |
| Length | 7.8 (5.8–9.6) |
| Caudal processes | |
| Length | 99.1 (87.5–109.8) |
| Width (at the base) | 8.9 (8.2–10.3) |

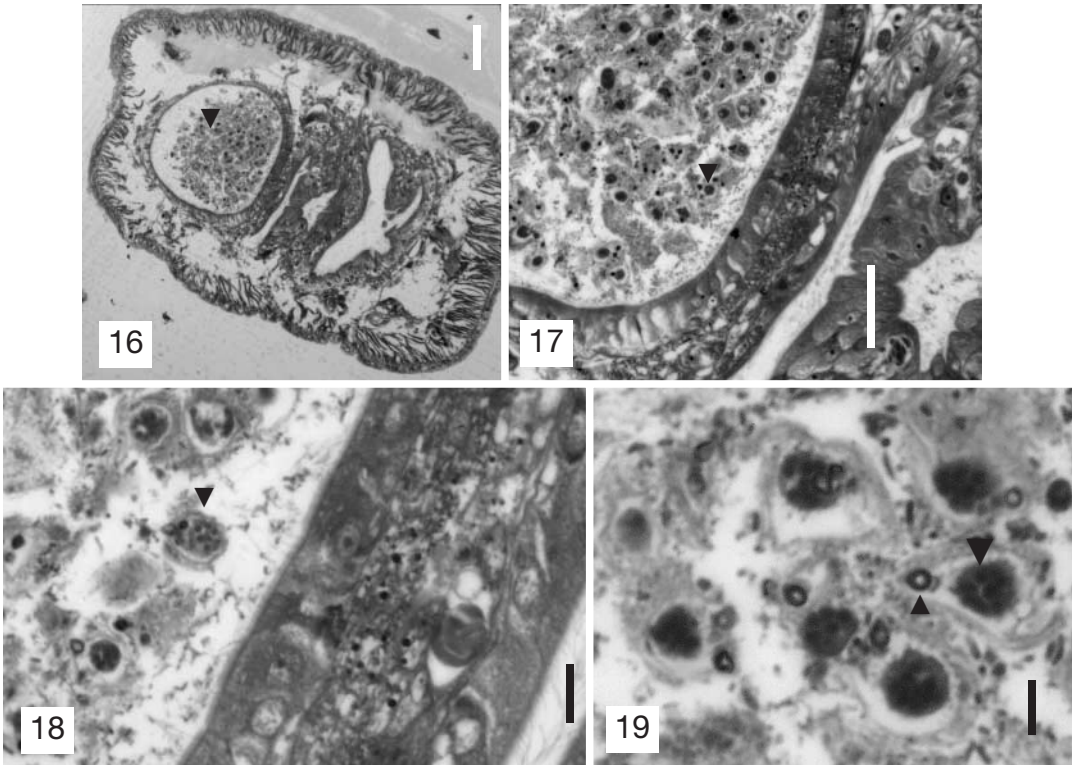
Eszterbauer et al. 2000). The larger spore (Type 2) also differs from the triactinomyxon types described by the above-cited authors, but shows similarity, both in its dimensions and in the number of secondary cells in the sporoplasm (>60), to the triactinomyxon type isolated by McGeorge et al. (1997) from an unidentified oligochaete collected from a salmon hatchery in Scotland.

### Echinactinomyxon

Unlike echinactinomyxons described in the literature so far, which all have a non-branched style (Janiszewska 1957, Xiao & Desser 1998a,b, Özer & Wootten 2000), the axon ends of the echinactinomyxon spore detected in this study are furcated. The new type of echinactinomyxon shows similarity only with the actinospore described by Janiszewska & Krzton (1973) as *Raabeia furciligera* and with the *Myxobolus dispar* actinospore obtained experimentally by Molnár et al. (1999); both of these have furcated axon ends. The echinactinomyxon reported in this paper differs from the latter actinospores in several morphological features, however; e.g. it has 16 secondary cells in its sporoplasm, while the other 2 types have 24 and 32 secondary cells, respectively.

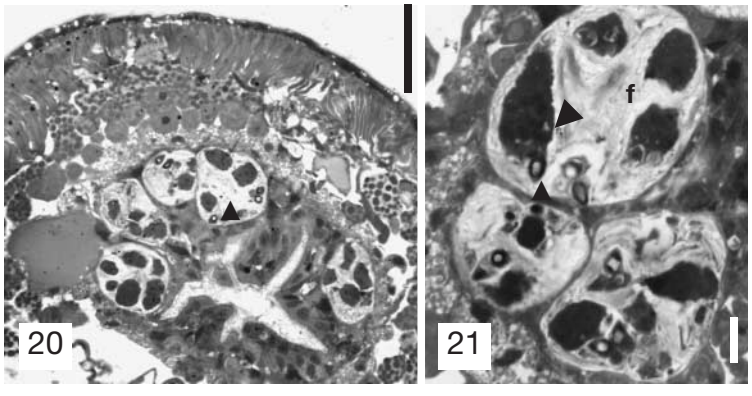
## DISCUSSION

While Ikeda (1912), Mackinnon & Adam (1924), Janiszewska (1955, 1957) and Ormieres & Frezil (1969) regarded actinospores exclusively as parasites of oligochaetes, the natural-water surveys conducted after the pioneering work of Wolf & Markiw (1984) recorded these parasites as actinospore forms of fish-parasitic myxosporeans, developing in oligochaetes as

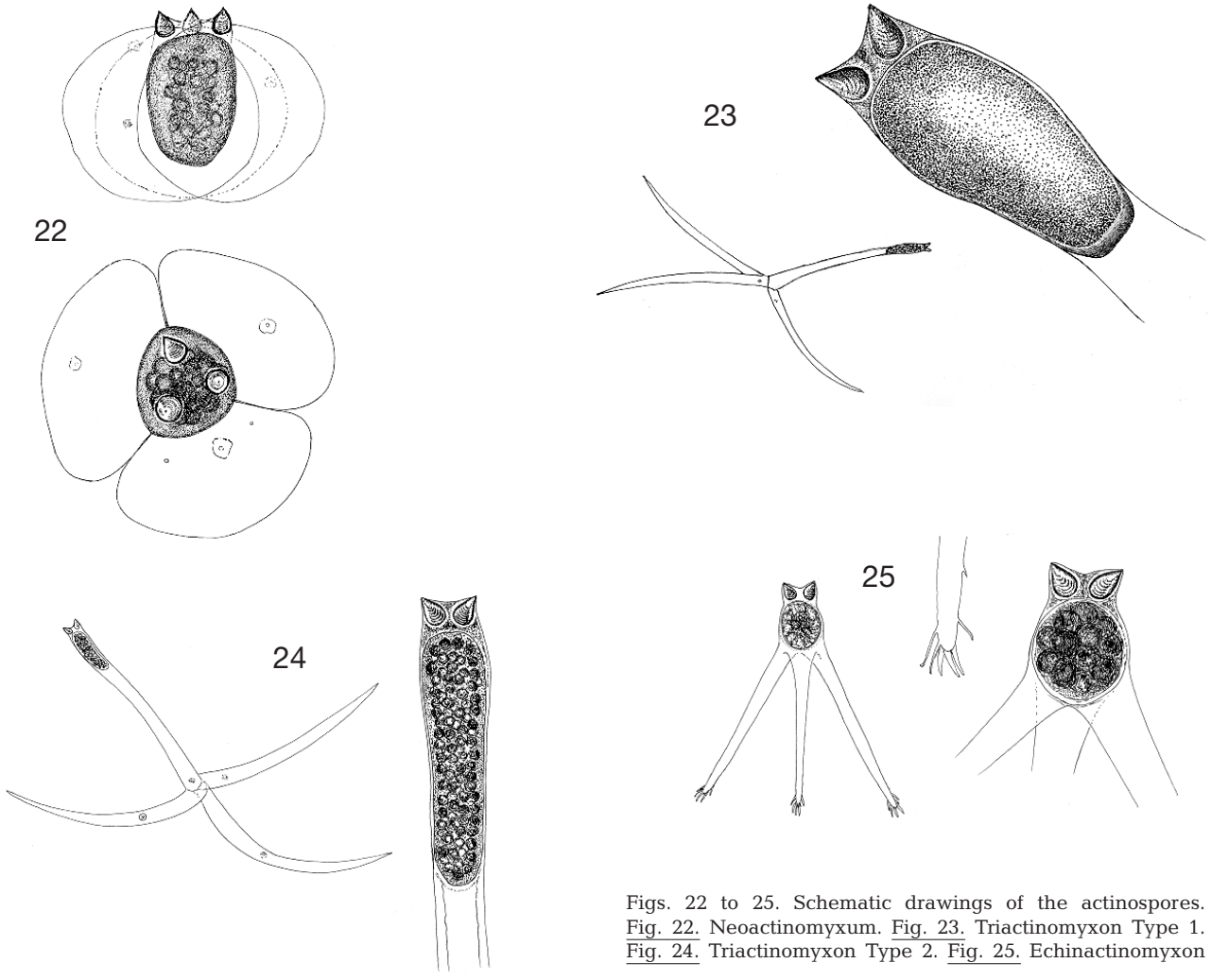



Figs. 16 to 19. Neoactinomyxum-infected *Rhyacodrilus komarovi* oligochaete: semi-thin sections stained with toluidine blue. Fig. 16. Low-magnification (scale bar = 200 µm) of a cross-section of the infected worm, numerous neoactinospores can be seen in the gut-lumen (arrowhead). Fig. 17 (scale bar = 100 µm) & Fig. 18 (scale bar = 20 µm). Intestinal epithelium of the worm infected with mature pansporocysts, but the majority of the spores are released into the gut lumen (arrowheads). Fig. 19. Enlarged spores from the gut-lumen (scale bar = 10 µm), secondary cells (large arrowhead) of the spores, and polar capsule (small arrowhead)

alternate hosts (Hamilton & Canning 1987, Székely 1989, Burtle et al. 1991, Styer et al. 1992, Pote & Waterstrat 1993, Yokoyama et al. 1993, Koller 1994, Pallós 1995, McGeorge et al. 1997, El-Mansy et al. 1998a,b, Xiao & Desser 1998a,b,c, Negredo & Mulcahy 2001). Most authors reported relatively low (<1%) prevalence of infection, and only Yokoyama et al. (1993), El-Mansy et al. (1998a,b) and Negredo & Mulcahy (2001) detected infections of a prevalence much higher than that in oligochaetes in natural waters. However, the method used by the later authors substantially differed from that used in the earlier studies; namely, while the earlier authors determined the infection of an oligochaete specimen at a given moment in time, El-Mansy et al. (1998a,b), Székely et al. (2000), Negredo & Mulcahy (2001), and the present authors monitored the infection status of individual oligochaetes on cell-well plates by daily checks over a long period of time. This possibly explains the much higher prevalence values in the later studies. However, with low-level infection




Figs. 20 & 21. Triactinomyxon Type 1-infected *Rhyacodrilus komarovi*; semi-thin section (stained with toluidine blue). Fig. 20. Heavy infection of the gut epithelium with pansporocysts (scale bar = 50 µm). Fig. 21. Enlarged pansporocysts (scale bar = 10 µm); the polar capsules (small arrowhead), the infectious cells (large arrowhead) and the folded axons (f) are well differentiated




Figs. 22 to 25. Schematic drawings of the actinospores. Fig. 22. Neoactinomyxum. Fig. 23. Triactinomyxon Type 1. Fig. 24. Triactinomyxon Type 2. Fig. 25. Echinactinomyxon

in the biotope, even long-term surveys would reveal a low prevalence of actinospore infection of oligochaetes (Xiao & Desser 1998a,b,c).

Based upon the features described in the subsection 'Differential diagnosis', 3 of the 4 actinospore types detected in this study appear to be new forms hitherto not described in the literature. However, triactinomyxon Type 2 is likely to be identical with the type detected by McGeorge et al. (1997) in an oligochaete collected from the sediment of a salmon hatchery.

The possible relationship of these actinospore forms to other myxosporean species needs to be estimated by infection experiments or by the use of molecular biological techniques.

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