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Morphological and genetic differences among actinosporean stages of fish-parasitic myxosporeans (Myxozoa): difficulties of species identification

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Abstract

The occurrence and morphology of actinosporean stages of myxosporeans were studied at a fish farm and in the River Tisza in Hungary. The 43 samples sequenced belonged to 10 'genotypes', from which six were determined as new actinosporean types. Based on DNA sequence analysis, the assumed actinosporean developmental stages of four myxozoan species were identified. The raabeia type collected from the worm *Branchiura sowerbyi* was identified as *Myxobolus cultus* Yokoyama, Ogawa & Wakabyashi, 1995. This was the first occurrence of this parasite in Hungary. Aurantiactinomyxon type 'A' was assigned as a developmental stage of *Thelohanellus hovorkai* Achmerov, 1964, triactinomyxon type 'D' was identified as *Myxobolus* sp. from the fins of roach *Rutilus rutilus*, while the DNA sequence of the guyenotia type actinosporean was 99.9–100% identical with *Sphaerospora* sp. from the kidney tubules of goldfish *Carassius auratus auratus*. Partial 18S rDNA sequences of the myxosporeans *Thelohanellus hovorkai, T. nikolskii* Achmerov, 1955 and *Myxobolus* sp. from *Rutilus rutilus* were new additions to GenBank. The DNA sequence analysis revealed that, in the case of actinosporeans, different 'morphotypes' can belong to the same 'genotype'. This study confirmed that actinospore classification based solely on traditional morphological features may lead to false conclusions, thus sequence analysis of the 18S rDNA and/or other genes is recommended in species and type descriptions.

Introduction

Actinosporean research became an important field of fish parasitology after Wolf & Markiw (1984) demonstrated that actinosporeans do not constitute an independent taxon but represent developmental stages of fish-parasitic myxosporeans. Following this pioneering study, other researchers also obtained experimental evidence that actinosporean stages exist in the life-cycle of various Myxozoa (see Kent et al., 2001; Székely et al., 2002a; Canning & Okamura, 2004; Kallert et al., 2005a, b).

Actinosporean infections of oligochaetes collected from natural waters and fish farms has been studied by numerous authors, including Burtle et al. (1991), Pote & Waterstrat (1993), Yokoyama et al. (1993b), McGeorge et al. (1997), Roubal et al. (1997), El-Mansy et al. (1998a, b), Xiao & Desser (1998a, b, c), Hallett et al. (1999, 2002), Székely et al. (2000, 2002b, 2003, 2005), El-Mansy (2001), Negredo & Mulcahy (2001), Rácz & Timm (2002), Özer et al. (2002), Oumouna et al. (2003) and Rácz et al. (2005). These studies were predominantly morphological examinations, whereas, recently, several papers have been published on the comparative molecular and morphological studies of different actinospores and myxospores (Holzer et al., 2004; Hallett et al., 2004, 2005). Holzer et al. (2004) studied the community of myxosporeans and actinosporeans in a Scottish highland stream, submitting 20 new partial 18S rDNA sequences of different Myxosporea species. Using molecular methods, they also assigned the actinosporean counterparts of two *Chloromyxum* Mingazzini, 1890 and a *Myxidium* Bütschli, 1882 species. Hallett et al. (2004) applied a combined

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PCR-RFLP method to investigate the morphometric variations within a triactinomyxon type actinosporean. Their results indicated that the high morphological variability within the given triactinomyxon type may cause difficulties in identification. Therefore, the use of molecular data in combination with phenotypic data aids the correct identification of actinosporeans.

The objective of the present study was the morphological and molecular characterisation of actinospores collected from oligochaetes in two different habitats in Hungary. We aimed to identify the myxospore counterparts of these actinospores and analyse the phylogenetic relationship between the actinosporean types examined.

Materials and methods

Collection and identification of oligochaetes

The survey of actinosporean infections of oligochaetes was conducted at the Temperate Water Fish Farm (henceforth referred to as the 'fish farm'), located at Százhalombatta (near Budapest) and in the River Tisza close to Tiszafüred, Hungary. Sampling at the fish farm was carried out between April and June, 2002 and from April to September 2003, while samples were collected in the River Tisza from September to November, 2002 and March to July, 2003. The farm conducts polycultural fish-breeding based on carp culture (common carp *Cyprinus carpio*, goldfish *Carassius auratus*, grass carp *Ctenopharyngodon idella*, silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*), but in the spawning period predacious fishes (sheatfish, pike and pikeperch) are also found in the ponds. Mud samples were withdrawn monthly from the fish beds, which collect the draining water of the neighbouring ponds.

Oligochaetes were identified morphologically following the guidelines of Brinkhurst (1963). As most tubificid specimens were immature, species identification was based on chaetae only. Oligochaete hosts were not identified at a DNA level.

Isolation and morphological characterisation of actinospores

The worms from the mud samples were transferred to cell-well-plates and the release of actinospores was checked daily for 1 month, as described by Yokoyama et al. (1991). The plates were stored at 4°C. Photomicrographs were taken from fresh material of 30–50 actinospores per sample, using a DH-10 digital camera mounted on an Olympus BH-2 microscope and subsequently measured using IMAGO® software. For the morphological characterisation of actinospores, the guidelines provided by Lom et al. (1997) were followed. Photomicrographs of the actinospores examined are available on request from the authors.

Molecular methods

Actinospore samples collected were stored in Eppendorf tubes at −20°C until further use. For DNA extraction, samples were centrifuged at 5,000 *g* for 5 min. Spore pellets were suspended in 500 µl lysis buffer (100 mM NaCl, 10 mM Tris, 10 mM EDTA, 0.2% SDS and 0.4 mg/ml Proteinase K) and incubated at 55°C for 3–4 h. DNA was then purified using the Miniprep Express Matrix (BIO 101, USA) (Eszterbauer, 2004). A nested PCR system was used for amplification. DNA was amplified with the 18e-18g' universal primer pair (Hillis & Dixon, 1991; modified by Andree et al., 1999). This was followed by a second round PCR with the MX5-MX3 primer pair (Andree et al., 1999) (Table 1). In both steps of the nested PCR, the total volume of PCR reactions was 50 µl, which contained approx. 10 to

Table 1. Primers used for PCR and/or sequencing.

Name	Sequence	Reference
18e	5′-CTG GTT GAT TCT GCC AGT-3′	Hillis & Dixon, 1991
18g'	5′-CGG TAC TAG CGA CGG GCG GTG TG-3′	Hillis & Dixon, 1991
MX5	5′- CTG CGG ACG GCT CAG TAA ATC AGT-3′	Andree et al., 1999
MX3	5′- CCA GGA CAT CTT AGG GCA TCA CAG A-3′	Andree et al., 1999
MB5r	5′-ACC GCT CCT GTT AAT CAT CAC C-3′	Eszterbauer, 2004

150 ng DNA, 1 × Taq PCR reaction buffer (MBI Fermentas, Lithuania), 1.25 mM $MgCl_2$, 0.2 mM dNTP mix (Sigma, USA), 50 pmol of each primer and 2 units of *Taq* DNA Polymerase (MBI Fermentas, Lithuania). MJ Research PTC-200 and Biometra T1 thermocycles were used for amplification. Amplification conditions in the first round were: 95°C for 50 sec, 56°C for 50 sec and 72°C for 80 sec for 35 cycles, with a terminal extension at 72°C for 7 min. This was followed in the second round with 95°C for 30 sec, 50°C for 30 sec and 72°C for 60 sec for 35 cycles, and was terminated with an extension period at 72°C for 7 min. The PCR products were electrophoresed in 1.0% agarose gels (Sigma, USA) in TBE buffer and then purified with Geneclean III Kit (Bio 101, USA). For samples with insufficient amount of amplified PCR product for direct DNA sequencing, purified DNA was cloned into a pGEM-T Vector System I (Promega) following the manufacturer's manual.

A fragment (*c*.850 bp) of the PCR products was sequenced in both directions with primers MX5 and MB5r (Table 1) using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyzer automated DNA sequencer (Applied Biosystems, USA). For sequence assembling, the STADEN Sequence Analysis Package version 2001.0 (Staden, 1996) was used. DNA sequence similarities were calculated with the Sequence Identity Matrix of the computer program BioEdit.

Phylogenetic analyses

Nucleotide sequences were aligned with the software MultAlin (Corpet, 1988) available online. The alignment was corrected manually using the GeneDoc sequence alignment editor program. The dataset for the alignment was chosen on the basis of the results of BLAST searches and a preliminary alignment, which contained almost all myxosporean sequences available in GenBank (data not shown). Gaps longer than 5 nucleotides were excluded. Phylogenetic calculations were performed with PHYLIP v3.5c and v3.6a (Felsenstein, 1997). The data were analysed with maximum likelihood (DNAML with transversion/transition ratio 1:1, 1:2 or 1:3, empirical base frequencies, one rate class for nucleotide substitution and global rearrangements) and distance matrix analysis (DNADIST using Kimura-2 parameter followed by FITCH with global rearrangements). Clade support was assessed with bootstrapping (100 replicates for maximum likelihood and 1,000 replicates for distance matrix method). *Ceratomyxa shasta* Noble, 1950 was chosen as the outgroup.

Descriptions of the actinosporean types examined

During the survey, 14 different actinosporean 'morphotypes' were found, which could be assigned to the collective groups triactinomyxon Štolc, 1899 (5), neoactinomyxum Granata, 1922 (4), aurantiactinomyxon Janiszewska, 1957 (3) guyenotia Naville, 1930 (1) and raabeia Janiszewska, 1955 (1). Measurements of the 'morphotypes' are listed in Table 2. The molecular characterisations are summarised in Table 3.

Guyenotia type (Figure 1a,b)

Description. Spores possess 3 finger-like caudal processes with rounded ends which are curved slightly downwards. In side view, bases of processes covered only posterior half of almost globular spore body. In apical view, spore body is spherical. Three drop-like polar capsules are positioned apically on spore body close to each other. Valve cell nuclei occur only distally, close to tip of caudal processes.

Host: *Branchiura sowerbyi* Beddard.
Localities: Fish farm, River Tisza.
Date of collection: May, June, July and September, 2003 from fish farm and September, 2003 from River Tisza.
Prevalence of infection: 1/20 (5%) in May, 3/31 (9.7%) in June, 2/27 (7.4%) inAugust, 2/11 (18.2%) in September and 8/109 (7.3%) during the whole period from fish farm and 1/41 (2.4%) in September and 1/338 (0.3%) during the whole period from the River Tisza.

Remarks. The dimensions of the guyenotia type examined greatly resembled the guyenotia type described previously from *Lumbriculus variegatus* (Müller) by Xiao & Desser (1998b) in Lake Sasajewun, Canada, apart from the length of polar capsules (1.6 μm, versus 3 μm in the latter case). The type described by these authors had 21 μm long and

Table 2. Mean measurements (μm) of actinosporean morphotypes examined. The minimum and maximum values are in parentheses.

Morphotype	Caudal processes		Spore body		Sporoplasm	Style		Total length	Polar cfsapsule	No. of secondary cells
	L	W	L	W	L	L	W			
Guyenotia (fish farm)	16 (15–19.5)	4.5 (4–5.5)	D: 10 (9.5–11)		n.d.	absent		–	L: 1.6 (1.4–2)	n.d.
(River Tisza)	17 (15–21)	4.5 (3.5–5.5)	D: 11 (10–12)		n.d.	absent		–	L: 1.6 (1.3–2)	n.d.
Aurantiactionmyxon 'A'	47 (37–58)	10 (8–12)	D: 20 (18–22)		n.d.	absent		–	L: 3 (2–3.3)	32
Aurantiactinomyxon 'B1'	24 (20–30)	9.8 (9–10)	D: 18 (17–20)		n.d.	absent		–	L: 2.5 (2–3)	n.d.
Aurantiactinomyxon 'B2'	16 (14–20)	8.4 (7–10.6)	D: 19 (18–21)		n.d.	absent		–	L: 2.6 (2–4.1)	n.d.
Triactinomyxon 'A'	150 (120–182)	13 (12–14)	37 (27–48)	12 (9–17)	30 (25–42)	241 (222–258)	11 (9–14)[a] 17 (14–21)[b]	278 (249–306)	L:5.5 × W:2.7	8
Triactinomyxon 'B'	140 (118–159)	14 (12–16)	31 (28–35)	9 (8–9)	26 (23–28)	205 (199–210)	8 (7–9)[a] 15 (13–16)[b]	236 (227–245)	L:5 × W:2.5	8
Triactinomyxon 'C1'	169 (132–185)	10 (9–13)	44 (38–52)	9 (8–11)	38 (33–45)	113 (107–117)	9 (8–13)[a] 19 (16–24)[b]	157 (145–169)	L:4 × W:3	8
Triactinomyxon 'C2'	173 (135.3–195.8)	13.7 (13.3–14.1)	n.d.	n.d.	n.d.	n.d.	7.7 (7.7–7.7)[a] 15.5 (14.2–17.8)[b]	221.6 (217.4–223.6)	n.d.	8
Triactinomyxon 'D'	287 (252.1–319.8)	12.81 (11–14.4)	41.465 (36.1–46.6)	14.256 (12.5–18.1)	33.98 (29.2–38.9)	160.75 (142.4–178.2)	16.37 (14.6–19.1)[a] 29.9 (26.5–37.8)[b]	202.2 (178.5–224.8)	L: 7.26 (6.8–8) W: 4.3 (3.9–5.3)	32

Neoactinomyxum 'A1'	8 (7–9)	19 (16–22)	D: 21 (19–24)	n.d.	absent	–	L: 2.6	16
Neoactinomyxum 'A2'	7 (5–8)	15 (14–18)	D: 21 (19–22)	n.d.	absent	–	L: 2.5 (2–3.2)	16
Neoactinomyxum 'B1'	8 (6–10)	15 (13–18)	D: 22 (20–24)	n.d.	absent	–	L: 2.6 (2–4)	*c.*16
Neoactinomyxum 'B2'	7 (6–10)	17 (16–19)	D: 21 (19–22)	n.d.	absent	–	L: 2.7 (2–3.5)	*c.*16
Raabeia	191 (170–213)	7 (6–9)	23 (21–25)	10 (9–11)	n.d.	absent	–	L: 4 (3–5) W: 2.5 (1.8–2.9)	*c.*16

L, length; W, width; D, diameter.
-, undefined measurement; n.d.: no data.
[a]: at the tip; [b] , at the base.

4.5–6.4 μm wide caudal processes, the spore body was 9.5μm long and 8.8μm wide. The guyenotia type detected also resembled the aurantiactinomyxon 'type 5' originating from the same fish farm previously by El-Mansy et al. (1998a) and the aurantiactinomyxon 'type 3' collected from Lake Balaton by El-Mansy et al. (1998b). The spore shape of the guyenotia type examined was similar to aurantiactinomyxon sp. 1 collected from *Branchiura sowerbyi* at a goldfish farm with a kidney enlargement disease enzootic (Yokoyama et al., 1993a). The authors unfortunately gave no measurement data for the actinosporeans; therefore, the comparison was based only on the photomicrographs. Furthermore, the measurements of the spore body were similar to *Aurantiactinomyxon pavinsis* Marques, 1984 ('petite form'), but the length of processes were longer in the guyenotia examined. The 18S rDNA sequence of *A. pavinsis* (AJ582006) studied by Holzer et al. (2004) was only less than 90% similar to the DNA sequence of the guyenotia type at hand. On the basis of the partial 18S rDNA sequences, the guyenotia type examined was 100% identical with *Sphaerospora* sp. ex *Carassius auratus auratus* L. (AY735411) originating from the same habitat, which was studied at DNA level by Eszterbauer & Székely (2004). A confusing new finding is that the DNA sequence of *Zschokkella* sp. (DQ118776) from the afferent bile-duct of *Carassius auratus auratus* submitted to GenBank recently, was found to be 99.9% similar to the present guyenotia sequence.

Aurantiactinomyxon types

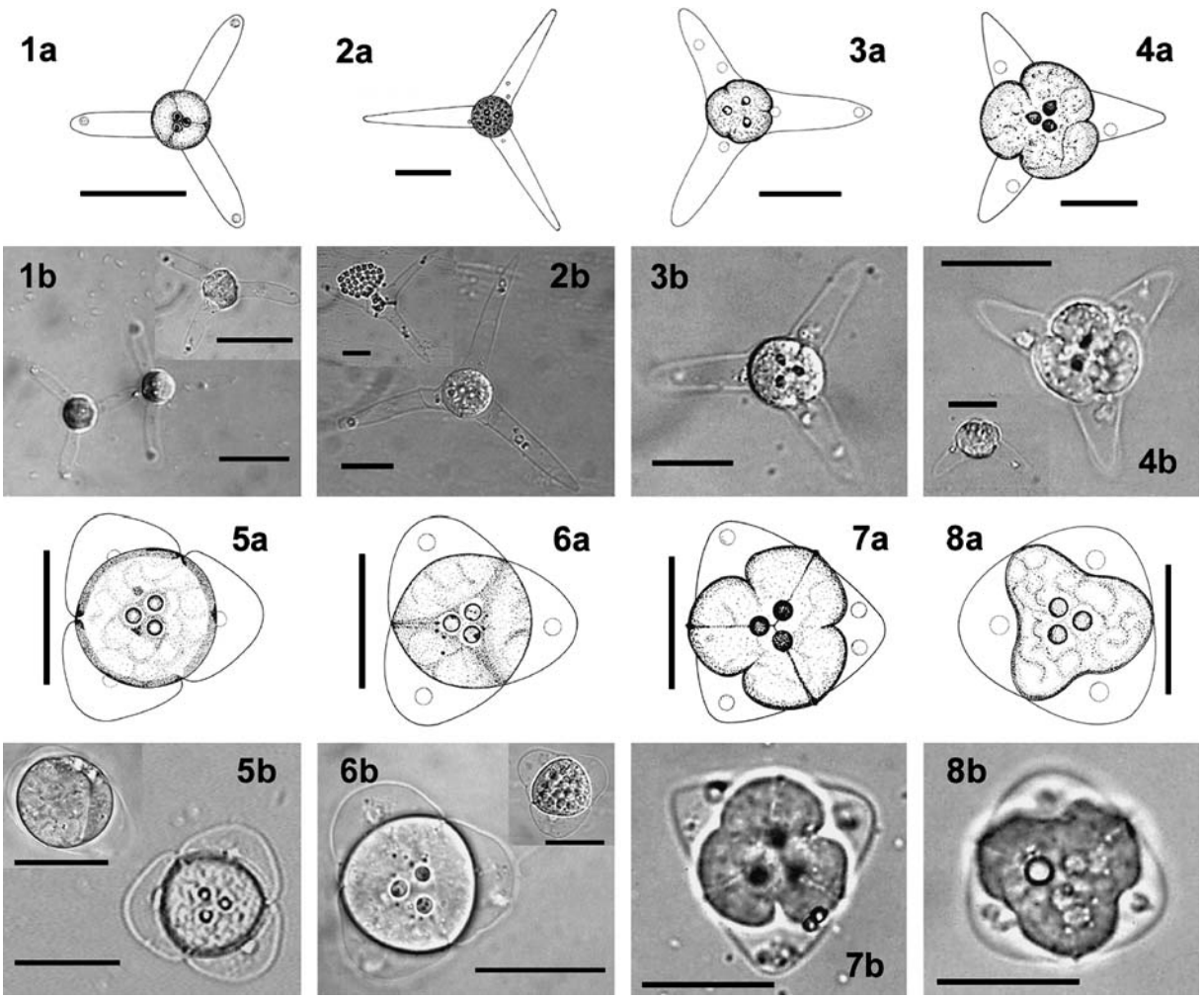
The Aurantiactinomyxon spores examined comprise a style-less spore body with 3 equal-sized, elongate, triangular or subtriangular processes. The bases of the processes embrace the spore body almost entirely.

Aurantiactinomyxon 'A' (Figure 2a,b)
Description. Spores possess 3 equal-sized, elongate caudal processes. Caudal processes straight, slightly rounded with almost pointed tips. Spore body spherical both in apical and side views. Three drop-like polar capsules present on top of spore body close to each other. Number of secondary cells in sporoplasm 32. Valve cell nuclei occur either distally or proximally in caudal processes.

Table 3. The summarised result of the molecular characterisation of actinosporean types examined.

Genotype	Morphotype	No. of samples sequenced	Sequence identities (%)	Genetic similarities*	GenBank acc. no.
Guyenotia	Guyenotia	10	99.9–100	**99.9–100**%: *Sphaerospora* sp. ex *Carassius auratus* (AY735411)	AY779063
AUR A	Aurantiactinomyxon 'A'	5	99.8–99.9	**99.8–99.9**%: *Thelohanellus hovorkai* (DQ231155) 89.9%: *Thelohanellus hovorkai* (AJ133419)[#]	'A'-1: DQ231153 'A'-2: DQ231154
AUR B	Aurantiactinomyxon 'B1' Aurantiactinomyxon 'B2'	3 2	99.9–100	87.4%: *Thelohanellus nikolskii* (DQ231156) 87.8%: *Thelohanellus hovorkai* (AJ133419)[§]	DQ231148
TAM A	Triactinomyxon 'A'	2	100	98.0%: *Myxobolus pseudodispar* ex *Rutilus rutilus* (AF380143)	DQ231145
TAM B	Triactinomyxon 'B'	1	–	97.8%: *Myxobolus pseudodispar* ex *Abramis brama* (AF380144)	DQ231143
TAM C	Triactinomyxon 'C1' Triactinomyxon 'C2'	1 1	100	96.4%: *Myxobolus pseudodispar* ex *Rutilus rutilus* (AF380145)	DQ231144
TAM D	Triactinomyxon 'D'	2	98.1	92.4–93.0%: TAM 'type 3' (AY495706) 98.2–**99.9**%: *Myxobolus* sp. ex fins of *Rutilus rutilus* (DQ231157)	'D'-1: DQ231146 'D'-2: DQ231147
NEO A	Neoactinomyxum 'A1' Neoactinomyxum 'A2'	2 9	99.9–100	88.4%: *Myxobolus pavlovskii* (AF507973) 89.0%: *Thelohanellus nikolskii* (DQ231156) 70.0%: *Endocapsa rosulata* (AF306791)	DQ231149
NEO B	Neoactinomyxum 'B1' Neoactinomyxum 'B2'	1	99.6–99.7	80.7%: *Sphaerospora renicola* (AY735410) 87.2–87.5%: *Myxobolus pavlovskii* (AF507973)	'B1': DQ231150 'B2'-1: DQ231151 'B2'-2: DQ231152
Raabeia	Raabeia	2	99.9	**99.4**%: *Myxobolus cultus* (AB121146)	DQ231142

* The most similar myxosporeans and other relevant species.
[#] Only 298 bp overlap in 18S rDNA sequences.
[§] Only 314 bp overlap in 18S rDNA sequences.



Figures 1–8. Actinosporean types examined. 1a,b. Guyenotia type actinospore infecting *Branchiura sowerbyi* originating from the fish farm and from the River Tisza. 2a,b. Aurantiactinomyxon 'A' from *B. sowerbyi* collected at the fish farm. Inset: released sporoplasm with secondary cells. 3a,b. Aurantiactinomyxon 'B1' from *B. sowerbyi* collected at the fish farm. 4a,b. Aurantiactinomyxon 'B2' from *B. sowerbyi* collected in the River Tisza. Inset: side view of the spore. 5a,b. Neoactinomyxum 'A1' released by *B. sowerbyi* collected at the fish farm. Inset: side view of the spore. 6a,b. Neoactinomyxum 'A2' from *B. sowerbyi* at the fish farm. Inset: Spore with noticeable secondary cells. 7a,b. Neoactinomyxum 'B1' from *B. sowerbyi* at the fish farm. 8a,b. Neoactinomyxum 'B2' from *B. sowerbyi* at the fish farm. *Scale-bars*: 20 μm.

Host: *Branchiura sowerbyi*.
Locality: Fish farm.
Date of collection: June and August, 2003.
Prevalence of infection: 3/31 (9.7%) in June, 1/27 (3.7%) in August and 4/109 (3.7%) during the whole period.

Remarks. The shape, measurements and the number of secondary cells of this type most closely resembled *Aurantiactinomyxon trifolium* Marques, 1984 described from *Tubifex* sp., as the latter aurantiactinomyxon had 40–45 μm long caudal processes and a 20–25 μm long spore body. Aurantiactinomyxon 'A' (AUR A) had a similar morphology to the aurantiactinomyxon 'type 4' recorded from this farm previously by El-Mansy et al. (1998a) and aurantiactinomyxon 'type 1' detected from Lake Balaton by El-Mansy et al. (1998b). Based on the 18S rDNA sequences, AUR A was found to be identical with *Thelohanellus hovorkai* Achmerov, 1964 (DQ231155) collected from the abdomen of *Cyprinus carpio* in Hungary

and with its aurantiactinomyxon counterpart obtained from an experimental infection performed in our laboratory previously (Rácz et al., unpublished). AUR A and the aurantiactinomyxon from the experiment were morphologically also very similar (similar spore shape and the same number of secondary cells), although slight differences were detectable in the length of caudal processes. The genetic similarity between AUR A and *T. hovorkai* from Japan (AJ133419) was only 89.9%, although the overlapping 18S rDNA fragment was rather short, 298 bp. The DNA sequence of *T. hovorkai* from Hungary (DQ231155) and the one collected in Japan (AJ133419) showed 94.3% similarity over a 714 bp long fragment.

Aurantiactinomyxon 'B1' (Figure 3a,b)
Description. Spores with 3 triangular, rounded or moderately pointed caudal processes, which curve slightly downwards. Caudal processes equal in length. In apical view, spore body has round-shape with 3 small indentations at base of each process. Three drop-like polar capsules occur on top of epispore. Number of secondary cells in sporoplasm not determined. Valve cell nuclei present either distally or proximally in caudal processes.

Host: *Branchiura sowerbyi*.
Locality: Fish farm.
Date of collection: June and August, 2003.
Prevalence of infection: 3/31 (9.7%) in June, 2/27 (7.4%) in August and 5/109 (4.6%) during the whole period.

Remarks. The shape and measurements of this type best resembled the actinospore of *Thelohanellus hovorkai* described by Yokoyama (1997) and studied later by Székely et al. (1998), but the slightly longer polar capsules (3.42 *vs* 2.5μm) and caudal processes (29 *vs* 24μm) differed from data described by Székely et al. (1998). However, the molecular results did not confirm the morphological findings. The DNA sequence similarity was only 87.8% between *T. hovorkai* originating from Japan (AJ133419) and the aurantiactinomyxon 'B1' examined. The present type was found to be morphologically very similar to the aurantiactinomyxon 'type 7' found previously by El-Mansy et al. (1998a) in the same farm.

Aurantiactinomyxon 'B2' (Figure 4a,b)
Description. Spores with 3 broad, triangular caudal processes, rounded at their ends and equal in length. These process extend downward at inclination of *c*.40° in side view. In apical view, spore body tends to have trefoil-like shape but is otherwise globular to moderately rounded. Three drop-like polar capsules occur at apex of spore body. Valve cell nuclei present proximally in caudal processes.

Host: *Branchiura sowerbyi*.
Locality: River Tisza.
Date of collection: June, 2003.
Prevalence of infection: 1/163 (0.61%) in June and 1/338 (0.3%) during the whole period.

Remarks. The shape and measurements of this type resembled the actinospore of *Thelohanellus nikolskii* Achmerov, 1955 (see Székely et al., 1998), a fin and scale parasite of the common carp *Cyprinus carpio*, but the genetic similarity was only 87.4%. Although, AUR B1 and B2 were morphologically rather different, since a 99.9–100% similarity was found in their 18S rDNA sequences, AUR B1 and B2 appear to be the same type (AUR B).

Neoactinomyxum types

Neoactinomyxum types studied have a style-less spore body with 3 small processes equal in size. The spores are triangular in apical view. The four neoactinomyxum 'morphotypes' examined also resembled members of collective group Endocapsa Hallett, Erséus & Lester 1999. However, as the Endocapsa collective group was described from the marine environment, the actinosporean types examined were classified as members of the collective group Neoactinomyxum Granata, 1922, which was described from freshwater oligochaetes.

Neoactinomyxum 'A1' (Figure 5a,b)
Description. Spore (spore body with caudal processes) elliptical in side view and triangular in apical view; spore body is globular in form. Three, small drop-like polar capsules. Number of secondary cells in sporoplasm 16. Valve cell nuclei present proximally in caudal processes.
Host: *Branchiura sowerbyi*.

Locality: Fish farm.
Date of collection: April and June, 2003.
Prevalence of infection: 1/12 (8.3%) in April, 2/31 (6.5%) in June and 3/109 (2.8%) during the whole period.

Remarks. This type did not resemble any known endocapsa or neoactinomyxum type described in the literature, either morphologically or at the DNA level. The most similar DNA sequence belongs to *Thelohanellus nikolskii*, which shares 89.0% identical nucleotides over a *c*.850 bp long 18S rDNA fragment.

Neoactinomyxum 'A2' (Figure 6a,b)
Description. Spore elliptical in side view and triangular in apical view. Spore body mainly rounded. Three small, spherical polar capsules. Number of secondary cells in sporoplasm 16. Valve cell nuclei located either distally or proximally in caudal processes.

Host: *Branchiura sowerbyi*.
Locality: Fish farm.
Date of collection: May, June, July, August and September, 2003.
Prevalence of infection: 2/20 (10%) in May, 3/31 (9.7%) in June, 2/8 (25%) in July, 2/27 (7.4%) in August and 3/11 (27.3%) in September, 12/109 (11%) during the whole period.

Remarks. NEO 'A2' had a similar morphology to *Endocapsa rosulata* Hallett, Erséus & Lester 1999, although the diameter of the spore body of NEO 'A2' (19–22 *vs* 25–27μm) and the length of polar capsules (2–3.2 *vs* 4–5μm) are smaller, and the shape of spore body is rather rounded in NEO 'A2'. Comparing the 18S rDNA sequences, only a 70.0% similarity was calculated over a 899 bp long alignment. The type NEO 'A2' did not resemble any known neoactinomyxum type described in the literature, either morphologically or genetically. The DNA sequences of NEO 'A2' samples are 99.9–100% identical with those of the NEO 'A1'. Despite the morphological difference in size and shape of the processes (and their junction with the spore body), they appear to be the same type (NEO A).

Neoactinomyxum 'B1' (Figure 7a,b)
Description. In apical view, spore body exhibits typical trefoil-shape within triangular actinospore formed by spore body and caudal processes. Three drop-like polar capsules. Number of secondary cells in sporoplasm not determined exactly, but most likely 16. Valve cell nuclei located either distally or proximally in caudal processes.

Host: *Branchiura sowerbyi*.
Locality: Fish farm.
Date of collection: June and July, 2003.
Prevalence of infection: 1/31 (3.2%) in June, 1/8 (12.5%) in July and 2/109 (1.8%) during the whole period.

Remarks. The shape and measurements of the type NEO 'B1' resembled the actinospore of *Sphaerospora renicola* Dyková & Lom, 1982, a myxosporean parasite of the kidneys of the common carp *Cyprinus carpio* (see Molnár et al., 1999). However, the molecular data did not confirm this association, as their genetic similarity was only 80.7% over a *c*.850 bp fragment of 18S rDNA. The morphology of this actinosporean type was also similar to the neoactinomyxum 'type 7' previously described by El-Mansy et al. (1998a) from this same fish farm. There was no endocapsa type found in literature similar to NEO 'B1'.

Neoactinomyxum 'B2' (Figure 8a,b)
Description. In apical view, spore body is trefoil-like in shape within triangular actinospore formed by spore body and strongly rounded caudal processes. Three drop-like polar capsules. Number of secondary cells in sporoplasm was not determined exactly, but is most likely 16. Valve cell nuclei located proximally in caudal processes.

Host: *Branchiura sowerbyi*.
Locality: Fish farm.
Date of collection: August, 2003.
Prevalence of infection: 1/27 (3.7%) in August, 1/109 (0.9%) during the whole period.

Remarks. There is no endocapsa type similar morphologically or genetically to NEO 'B2'. The dimensions of this type were approximately the same as measured previously by El-Mansy et al. (1998a) for neoactinomyxum 'type 1' (spore body width 21.2 μm; caudal process dimensions 8.5×16.4 μm) and 'type 4' (spore body width 22.3 μm; caudal process dimensions 7×16 μm) originating from the same fish farm as studied. Although, the shape of processes were slightly

different for NEO 'B1' and 'B2' (triangular *vs* rounded form), the DNA sequences of NEO 'B2' samples were 99.6–99.7% similar to those of the type NEO 'B1'; therefore, they appear to be the same type (NEO B).

Triactinomyxon types

The triactinomyxon (TAM) spores examined are characterised by 3 polar capsules, a sporoplasm, a style and 3 caudal processes resulting in an anchor-shaped assemblage.

Triactinomyxon 'A' (Figure 9a,b)
Description. Spore body cylindrical. Cylinder-shaped sporoplasm contains 8 secondary cells. Drop-like polar capsules present apically. Style comparatively long, gradually distends towards processes. Caudal processes slightly curved upward, tapering gradually towards distal end.

Host: *Tubifex newaensis* (Michaelsen).
Locality: River Tisza.
Date of collection: May, 2003.
Prevalence of infection (unseparated *Tubifex* + *Limnodrilus* stock): 1/40 (2.5%) in May and 1/117 (0.85%) during the entire period.

Remarks. Although the shape of the spore resembled the triactinomyxon stage of *Myxobolus pseudodispar* Gorbunova, 1936, due to its extremely long style and relatively short caudal processes, this triactinomyxon type differed from all known actinosporean types. The highest genetic similarity (98.0%) was obtained with *M. pseudodispar* ex *Rutilus rutilus* (AF380143).

Triactinomyxon 'B' (Figure 10a,b)
Description (based on 10 actinospores). Spore body cylindrical. Cylindrical sporoplasm contains 8 secondary cells. Polar capsules pyriform. Style very long and slightly widened toward tails. Caudal processes slightly curved upward, tapering gradually towards distal end.

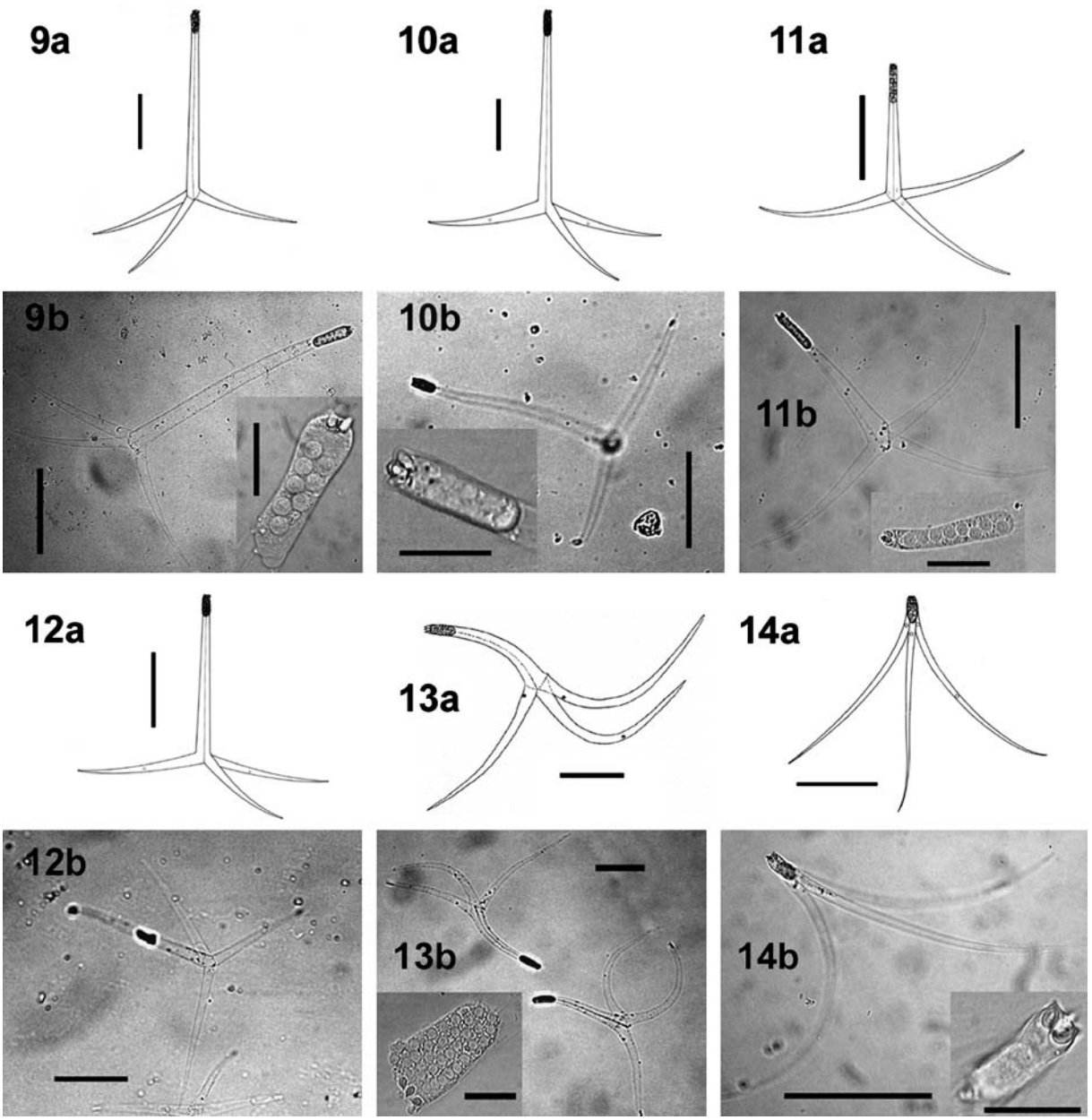
Host: *Tubifex tubifex* (Müller).
Locality: Fish farm.
Date of collection: September, 2003.
Prevalence of infection (unseparated *Tubifex* + *Limnodrilus* stock): 2/98 (2.04%) in September and 2/2924 (0.07%) during the entire period.

Remarks. Despite the *Myxobolus pseudodispar*-like spore shape, this TAM type differed from all known forms due to its extremely long style. Although the spore-shape of the TAM 'B' was similar to TAM 'A', the former bore a remarkably longer style and all of its measurements were greater than those of the latter, and only a 97.2% similarity was detected between their 18S rDNA fragments. It resembled the triactinomyxon type described by El-Mansy (2001) in Egypt, but the style of the Egyptian type was shorter and much wider (20.8–40 μm) while its caudal processes were longer (247.5 μm) and wider (42 μm). The greatest genetic similarity (97.8%) was calculated with *M. pseudodispar* ex *Abramis brama* (AF380144).

Triactinomyxon 'C1' (Figure 11a,b)
Description. Spore body cylindrical. Sporoplasm contains 8 secondary cells. Polar capsules pyriform. Style widened toward its base. Caudal processes slightly curved upward, tapering gradually towards distal ends, which are blunt.

Host: *Tubifex tubifex*.
Locality: Fish farm.
Date of collection: May, 2003.
Prevalence of infection (unseparated *Tubifex* + *Limnodrilus* stock): 1/1214 (0.08%) in May and 1/2924 (0.03%) during the entire period.

Remarks. The TAM 'C1' type examined resembled the triactinomyxon 'type 1' described by Oumouna et al. (2003) from a fish farm in Bavaria. The latter triactinomyxon had 160μm long caudal processes, a 170μm long spore axis (style and spore body) and polar capsules of 4 × 3μm. The dimensions of this TAM, the tapering style and the number of secondary cells revealed a great similarity with the TAM 'C1', but the caudal processes in this actinosporean type had sharply pointed ends. The triactinomyxon nov. (TAM SLH-2002, AY162270) described by Hallett et al. (2004) was also morphologically similar to TAM 'C1'. Although the length and width of the spore axis and caudal processes of TAM 'C1' were within the range measured by Hallett et al. (2004), the caudal processes were equal in length, unlike those of Hallett et al. (2004); therefore, the terms 'short' and 'long processes' were not applied. At the DNA level, a 96.1% similarity was detected between



Figures 9–14. Actinosporean types examined (continued). 9a,b. Triactinomyxon 'A' released by *Tubifex newaensis* originating from the River Tisza. Inset: spore body with 8 secondary cells. 10a,b. Triactinomyxon 'B' released by *Tubifex tubifex* from the fish farm. Inset: spore body with polar capsules and 8 secondary cells. 11a,b. Triactinomyxon 'C1' from *Tubifex tubifex* at the fish farm. Inset: spore body with 8 secondary cells. 12a,b. Triactinomyxon 'C2' from *Tubifex tubifex* at the fish farm. 13a,b. Triactinomyxon 'D' from *Tubifex tubifex* at the fish farm. Inset: spore body with polar capsules and *c*.32 secondary cells. 14a,b. Raabeia type actinospore released by *B. sowerbyi* from the fish farm. Inset: spore body with pyriform polar capsules. *Scale-bars*: 100 μm; insets 20 μm.

TAM 'C1' and TAM SLH-2002. The most similar DNA sequence belonged to *Myxobolus pseudodispar* ex *Rutilus rutilus* (AF380145) (96.4%).

Triactinomyxon 'C2' (Figure 12a,b)

Description (based on 9 actinospores). Spore body cylindrical. Sporoplasm contains 8 secondary cells.

[As spores are partly damaged, thus some features not measurable.]

Host: *Tubifex tubifex*.
Locality: Fish farm.
Date of collection: September, 2003.
Prevalence of infection (unseparated *Tubifex* + *Limnodrilus* stock): 1/98 (1.02%) in September and 1/2924 (0.03%) during the entire period.

Remarks. TAM 'C2' has a more distinct shape than TAM 'C1', as it possesses a distinctly longer style, even compared to the length of the caudal processes. However, their DNA sequences are 100% identical; therefore, they appear to be the same type (TAM C).

Triactinomyxon 'D' (Figure 13a,b)
Description (based on 10 actinospores). Spore body cylindrical. Barrel-like sporoplasm contains 32 secondary cells. Polar capsules pyriform. Style width extends notably towards caudal processes. Caudal processes curve upward and have sharply pointed ends.

Host: *Tubifex tubifex*.
Locality: Fish farm.
Date of collection: April and May, 2002.
Prevalence of infection: No data.

Remarks. The long caudal processes, the relatively short style and the number of secondary cells (32) of TAM 'D' resemble the TAM of *Myxobolus bramae* Reuss, 1906 as described by Eszterbauer et al. (2000), but almost all the measurements were smaller in the latter case. At the DNA level, the most similar actinosporean is the triactinomyxon 'type 3' (AY495706) with a 92.4–93.0% similarity. On the basis of the DNA sequence analysis, it is assumed that TAM 'D'-1 represents the actinospore of a histozoic *Myxobolus* species (DQ231157) developing cysts in the fins of *Rutilus rutilus*, as only one nucleotide difference was determined over a 895 bp DNA fragment.

Raabeia type (Figure 14a,b)

Description. Raabeia spores have 3 relatively long caudal processes and no style. Processes slightly curved upward, tapering gradually to sharply pointed end. Spore body flask- or barrel-shaped. Polar capsules pyriform. Number of secondary cells not determined accurately, but most likely 16.

Host: *B. sowerbyi*.
Locality: Fish farm.
Date of collection: May, 2003.
Prevalence of infection: 1/20 (5%) in May and 1/109 (0.9%) over the entire period.

Remarks. The dimensions of raabeia type examined are approximately the same as those measured by Xiao & Desser (1998a) for raabeia type 'E' detected in *T. tubifex* from Lake Sasajewun, Canada. The dimensions and the shape of the sporoplasm of the raabeia type detected by us greatly resemble those described from a Scottish salmon hatchery by McGeorge et al. (1997), although there are differences in the measurements of, for example, the polar capsules (7×5 *vs* 4 × 2.5μm). The raabeia type examined also resembles the raabeia 'type 4' described previously from this farm by El-Mansy et al. (1998a) and raabeia 'type 2' recorded in Lake Balaton by El-Mansy et al. (1998b), with the polar capsules being larger (5.7×4 *vs* 4 × 2.5μm) in the latter two types. Its morphotype also resembled the raabeia type detected in *B. sowerbyi* by Yokoyama et al. (1995), although the spore body in the latter type is more cylindrical than of flask-shaped. At the DNA level, the raabeia type examined was 99.4% similar to the DNA sequence of *Myxobolus cultus* Yokoyama, Ogawa & Wakabayashi, 1995.

General remarks on the occurrence of actinosporean stages

At the fish farm, 38 of 109 (35%) of the *Branchiura sowerbyi* specimens and six of 2302 (0.26%) *Limnodrilus* + *Tubifex* specimens were found to produce actinospores over the entire sampling period. At the River Tisza, only three of 332 *Branchiura* specimens (0.9%) and 2 of 117 *Limnodrilus* + *Tubifex* specimens (1.7%) released actinospores.

PCR and sequencing

The universal 18e-18g' primers and the primer pair MX5-MX3 specific for the family Myxobolidae

successfully amplified fragments of *c*.1,900 and *c*.1,600 bp of the 18S rDNA from every sample examined. In some cases, the PCR also produced non-specific fragments. In these cases, the DNA fragment of the expected size was isolated from the agarose gel and purified with a Geneclean III Kit (BIO 101) before the next step.

Approximately 850 bp long 18S rDNA fragments of 43 samples were sequenced. Samples examined belonged to 10 'genotypes' (with a $<2\%$ difference in the 18S rDNA sequences), from which six were determined as novel actinosporean types. The 18S rDNA sequences obtained were deposited in GenBank under the accession numbers listed in Table 3.

Phylogenetic analyses

Phylogenetic analyses were based on the final edited alignment which was 894 bp in length and contained 38 myxosporean taxa. The distance matrix analysis confirmed the clustering pattern of the maximum likelihood analysis, in several cases with different bootstrap values (Figure 15). In the phylogenetic tree, the guyenotia type actinospore, whose DNA sequence was found to be identical with that of *Sphaerospora* sp. ex *Carassius auratus auratus* (AY735411), clustered with *Myxidium truttae* Léger, 1930 and its counterpart Raabeia 'type 3' (AJ582061). The two aurantiactinomyxon and neoactinomyxum genotypes (AUR A, B and NEO A, B) composed a new cluster within the myxosporean phylogenetic tree and located distantly from the other myxosporeans available in GenBank. The 18S rDNA sequences of AUR A samples were 99.8–100% identical with the *Thelohanellus hovorkai* (DQ231155) sequence obtained from an experimental infection study previously performed in our laboratory (Rácz et al., unpublished). This branch included *T. nikolskii* (DQ231156), another common parasite of cultured carp. Three of the triactinomyxon genotypes (TAM A, B and C) comprised a group with several *Myxobolus pseudodispar* isolates and a TAM type (AY162270) described by Hallett et al. (2004), in which 100% identities were found in none of the cases. TAM D, the fourth triactinomyxon 'genotype', located within the branch of gill-parasitic *Myxobolus* species, while the raabeia type examined was found to be identical with *M. cultus* (AB121146) and clustered with *M. lentisuturalis* Dykova, Fiala & Nie, 2002 (AY119688) and the actinospore type hungactinomyxon Rácz, Eszterbauer, Molnár, 2005 (AY779062) described previously by Rácz et al. (2005).

Discussion

Apart from the early studies on actinosporeans (Štolc, 1899; Janiszewska, 1955, 1957; Marques, 1982, 1984), surveys on actinosporeans in a certain habitat or geographical region were intended to investigate the morphological diversity of the actinosporean fauna and prevalence of infection in different oligochaete species. These surveys have shown that the prevalence of actinospore production tended to be rather low in oligochaetes from natural waters (Yokoyama et al., 1991; Xiao & Desser, 1998a, b, c; El-Mansy et al., 1998b; Oumouna et al., 2003; Negredo & Mulcahy, 2001; Özer et al., 2002). Most studies were based on a short-term examination of collected oligochaetes (Yokoyama et al., 1993b), although, in some cases, authors observed the oligochaete samples for a longer period, occasionally for a year (El-Mansy et al., 1998a, b). As in the case of our investigation, Xiao & Desser (1998a, b, c) studied the oligochaete worms collected from Lake Sasajewun in Canada for four weeks, and determined 0.03, 0.02 and 0.14% prevalence values in the case of neoactinomyxum, aurantiactinomyxon and antonactinomyxon Janiszewska, 1957 types, respectively. Özer et al. (2002) studied the oligochaete fauna of a Scottish salmon hatchery and detected a total 2.9% prevalence, although this was equivalent only to 0.001–0.9% per actinosporean type. Holzer et al. (2004) reported similarly low prevalence values for the actinosporean fauna of a Scottish highland stream, although, in the case of *Aurantiactinomyxon pavinsis* Marques, 1984, the prevalence detected reached the value of 33.3%.

In the case of the actinospore prevalence detected in *Branchiura sowerbyi* worms, remarkable differences were determined between the prevalence values obtained at the fish farm and in the River Tisza. In contrast to the 50% prevalence occasionally detected at the fish farm, the prevalence of infection was only 0.9% in the River Tisza. Interestingly, in the case of

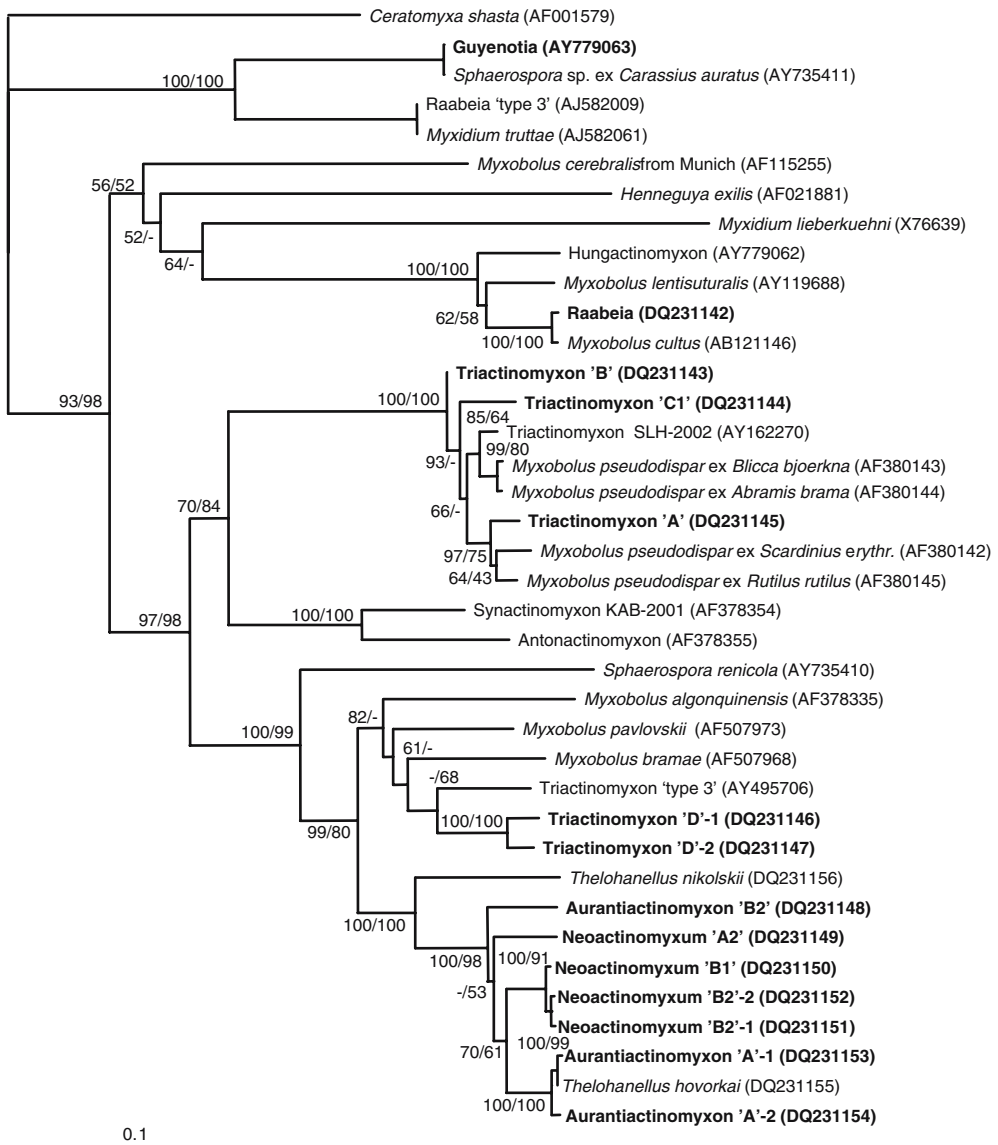


Figure 15. Phylogenetic tree generated by distance matrix and maximum likelihood analyses of the 18S rDNA sequences of myxosporeans and rooted by *Ceratomyxa shasta*. The numbers at the nodes indicate bootstrap confidence levels in percent (distance matrix method: 1,000 repetitions; maximum likelihood: 100 repetitions, Tv/Ts 1:2;). GenBank accession numbers are in parentheses. The distance scale is shown beside the tree. Actinosporeans examined in the present study are in bold.

the *Tubifex* + *Limnodrilu*s sp., an opposite tendency became apparent, when only 0.26% prevalence was found at the fish farm, while 1.7% was observed in the River Tisza. It seems that the likelihood of infection by Myxosporea is not really dependent on the habitat, as one might expect, and the 'physical' effect of flowing water does not necessarily decrease the prevalence of actinospores in oligochaete hosts. In the case of the parasitologically well-studied fish farm, the detailed comparison of the actinosporean types examined and actinosporeans formerly found by El-Mansy et al. (1998a) was intended. As these authors studied the actinosporean fauna only phenotypically, a comparison of the DNA sequence data was not possible. Due to the high morphological variability often detected within a given actinosporean type (Hallett et al., 2002,

2004; personal observations), we were not able to identify with certainty the actinosporean types examined on the basis of the previous actinosporean descriptions by El-Mansy et al. (1998a, b), which lacked detailed spore descriptions and differential diagnoses in most cases. Therefore, no conclusions were drawn in relation to the possible changes that might have appeared in the actinosporean fauna of the fish farm studied.

The 10 actinosporean 'genotypes' determined in this study represented 14 different 'morphotypes'. The phenotypic differences varied within the 'genotypes', but in most cases the spore shape was similar and any differences were morphometric. However, in some other cases two remarkably different spore 'morphotypes' belonged to the same 'genotype' (e.g. AUR 'B1' and 'B2'). Hallett et al. (2002) also reported similar results, when they found two aurantiactinomyxon types that differed considerably in the length of their processes, while their 18S rDNA sequences were 100% identical.

Analysis of DNA sequence data assigned the actinospores to four myxosporean species. The raabeia type examined shared 99.4% base pairs with *Myxobolus cultus* that develops cysts in the gill-arches of goldfish *Carassius auratus auratus*. This was the first detection of this parasite in Hungary. The myxospore of *M. cultus* has not so far been identified in fish from the examined habitats in Hungary, probably because of the small size and unusual localisation of the cysts in the fish host.

The DNA sequence of the aurantiactinomyxon 'A' (AUR 'A') type was 99.8–100% identical with the actinospore and myxospore of *Thelohanellus hovorkai* obtained from an experimental infection study performed in our laboratory previously (Rácz et al., unpublished). In GenBank, there is a short DNA sequence (710 bp) of *T. hovorkai* (AJ133419) collected from the common carp *Cyprinus carpio* in Japan (Anderson et al., 2000). Although the myxospores used for experimental infections were identified in both cases as *T. hovorkai*, neither the actinospores examined in the present study nor those obtained from the experiment performed in our laboratory were identical either morphologically or at DNA level with the aurantiactinomyxon described by Yokoyama (1997), which was studied experimentally later by Székely et al. (1998) and by Anderson et al. (2000) at the DNA level.

At the DNA level, an isolate of the triactinomyxon type 'D' (TAM 'D') was 99.9% identical (1 nt difference over a *c*.850 bp DNA fragment) with a *Myxobolus* sp. developing in the subcutaneous connective tissue of the fins of roach *Rutilus rutilus*, a species that has already been studied histologically by Molnár (2002). TAM 'D' clustered in the phylogenetic tree within the clade of *Myxobolus* species which develop in different connective tissues within the gills of fish. Its phylogenetic position confirms the theory of the correlation between the tissue specificity and genetic relationships of *Myxobolus* species previously proposed by Eszterbauer (2004).

The genetic classification of the myxospore counterpart of the guyenotia type examined became complicated by the recent GenBank submission of the DNA sequence of a *Zschokkella* sp. (originating from the bile-duct of goldfish from the USA), which was 99.9% similar to the present guyenotia species and its assumed myxosporean life stage, *Sphaerospora* sp., from the kidney tubules of goldfish in Hungary. To resolve this conflict, further investigations of the myxosporean parasites of goldfish are required urgently. During the detailed parasitological surveys made on the goldfish fauna at the examined fish farm during recent decades, no *Zschokkella* or *Myxidium* species which possesses a similar morphology to *Zschokkella* spp. has been detected either in the kidney tubules or in the bile-duct of goldfish (Molnár, personal communication). Therefore, the likelihood of contamination of the *Sphaerospora*-containing kidney samples by a species of *Zschokkella* and/or *Myxidium* is rather low, although it certainly cannot be excluded.

Fewer myxospore-actinospore pairs were confirmed than expected via the DNA sequence analysis, although the examined fish farm is frequently studied parasitologically. Myxosporeans have a wide range of vertebrate hosts, but only the fish hosts so far have been studied at this region. Reptiles and amphibians may also be infected by Myxosporea, and some of the actinosporean types found in our study may belong to these parasite species. Another reason for the low number of matching pairs found might be the periodic (discontinuous) sampling of actinosporeans. As the actinospore release by oligochaetes is seasonal and intensive actinospore production usually lasts only for two to four weeks (El-Mansy

et al, 1998a), it is possible that the release of some actinosporeans occurrred between the dates of two sample collections.

In most actinospore types examined, morphological variability was detected, and the 10 'genotypes' distinguished consisted of 14 different 'morphotypes'. Generally, our findings suggest that morphometric variation is rather common within a given actinosporean type and that the only reliable morphological features are the number of secondary cells and the shape of spore body. The *Myxobolus pseudodispar*-like triactinomyxons (TAM A, B, C) represent, to a certain extent, an exception to this idea. Although variability in the measurements was also detected, the general spore shape was similar and the number of secondary cells was identical at all three TAM types. *M. pseudodispar* is a common muscle-infecting parasite of cyprinids, whose development was studied in detail by Székely et al. (1999, 2001). Due to the phylogenetic analysis conducted on several *M. pseudodispar* isolates originating from four different cyprinid hosts, authors have already questioned the uniformity of this species as the difference in 18S rDNA sequences reached 3% in some cases (Molnár et al., 2002). Doubts have arisen over the unity of *M. pseudodispar* when none of the three TAM types examined (TAM A, B, C) was identical with any of the *M. pseudodispar* myxospore isolates, exhibiting only 96.4–99.0% similarities in their 18S rDNA. It is difficult to interpret the apparent high genetic difference within the species *M. pseudodispar*, especially because such a difference has not been detected in any other myxosporean species studied so far, as the 'usual' or 'accepted' intraspecific difference seemed to be $< 1\%$ in the 18S rDNA (e.g. Holzer et al., 2004). Nonetheless, the results suggest that this taxon might have reached a stage of species-segregation in its evolution, which may explain the relatively wide range of host species (both vertebrates and oligochaetes).

Variation might also be generated by the high genetic variability among oligochaete individuals of the cryptic species *Tubifex tubifex*. Although the genetic variability of the oligochaete hosts was not investigated in their study, Hallett et al. (2004) reported that variability can be expected within an actinosporean type originating from more than one oligochaete hosts. There are several other factors which may influence the occurence of morphological variations in actinospores, such as seasonality, environmental conditions (e.g. type of water), age, gender or size of the oligochaete host, etc., which should be taken into account in species or type descriptions.

Determining specific criteria may also cause difficulties. The 'traditional' species concepts (such as the biological and morphological concepts) have been successfully employed in the cases of self-fertilising taxa; however, these might be of limited utility in the case of myxozoans, since mating is most likely reduced to postmeiotic autogamy of single clonal lineages, as suggested for *Myxobolus cerebralis* Hofer, 1903 (El-Matbouli et al., 1995; El-Matbouli & Hoffmann, 1998). The probable absence of intraspecific sexual reproduction could result in several individual lineages, which may differ in one or more genotypic/phenotypic characters. It is likely that these distinct lineages do not produce new spore-shapes but variations in size or even in host or tissue tropism, which cannot be measured directly by the difference in 18S rDNA. Kallert et al. (2005a) suggested that many difficulties caused by a morphological approach would be solved by the application of the phylogenetic species recognition concept (PSR) (Taylor et al., 2000; Agapow et al., 2004) which would help in clarifying genetic distances between myxosporean species. A single gene genealogy (e.g. 18S rDNA) may raise taxonomic uncertainty, as decisions on the limits of a species are rather subjective. As the PSR relies on the concordance of more than one gene genealogy, it is able to avoid subjectivity and underlie a congruence analysis.

The present molecular biological investigations revealed that, for actinosporeans, different 'morphotypes' might belong to the same 'genotype'. Furthermore, results confirmed that the identification of actinosporean types based solely on the traditional morphological features may be false, thus sequence analysis of 18S rDNA and/or other genes is recommended in species descriptions.

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