First description of myxozoans from Syria: novel records of hexactinomyxon, triactinomyxon and endocapsa actinospore types

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ABSTRACT: Oligochaete worms collected in late March and early April 2005 from 3 freshwater biotopes in Syria were surveyed over an 11 wk period for myxosporean parasites (Myxozoa). Three types of novel actinospore stages were identified from 1 host species, *Psammoryctides albicola*. A hexactinomyxon was found in 6 *P. albicola* (7.5%) collected from a branch of the River Orontes, north of the city of Hama. A triactinomyxon and an endocapsa were found in single *P. albicola* specimens from the Al-Thaurah region of the Euphrates River (Lake Assad). No oligochaetes collected from Al-Ghab fish farm (Orontes region) released actinospores during the observation period. The present study is the first description of myxosporeans, including actinospore stages, from Syria. The 3 types described herein differ morphologically and molecularly (18S rDNA) from published records.

KEY WORDS: Myxozoa · Hexactinomyxon · Triactinomyxon · Endocapsa · *Psammoryctides albicola* · Orontes River · Euphrates River · Syria

INTRODUCTION

The first report on the occurrence of actinosporean parasites (Myxozoa) in invertebrates appeared over 100 years ago (Štolc 1899). Following the realisation that these spores were life cycle counterparts of myxosporean parasites in vertebrates, and are the infective stage for fish, the number of spore records from across the globe has increased rapidly over the last 20 years (Kent et al. 2001). In Syria fish parasites have not been the focus of many studies (Al-Samman 1992, 1996, Al-Samman et al. 2006) and research on myxosporeans started only very recently as a component of a bilateral research agreement between Hungary and Syria. As a result of this collaboration, we report here on the finding of new actinospore stages of Myxosporea in oligochaete hosts from Syrian freshwaters.

MATERIALS AND METHODS

Collection and screening of oligochaetes for actinospores. We conducted a survey of 3 biotopes in Syria (Fig. 1): (1) a dammed region of the Euphrates River (Lake Assad) near Al-Thaurah, (2) a branch of the Orontes River, 30 km south of Hama, and (3) the Al-Ghab region, Al-Sinn fish farm. Multiple sites were chosen to improve the chances of encountering infected oligochaetes. All samplings were conducted in late March or early April 2005. Mud samples and vegetation roots were collected and transported to the Fish Pathology Laboratory, Veterinary Faculty, Al-Baath University, Hama. Oligochaetes were washed from the mud on a dissection plate and picked up by pipette or gently separated from the roots of aquatic weed with forceps. After collection, the oligochaetes were placed
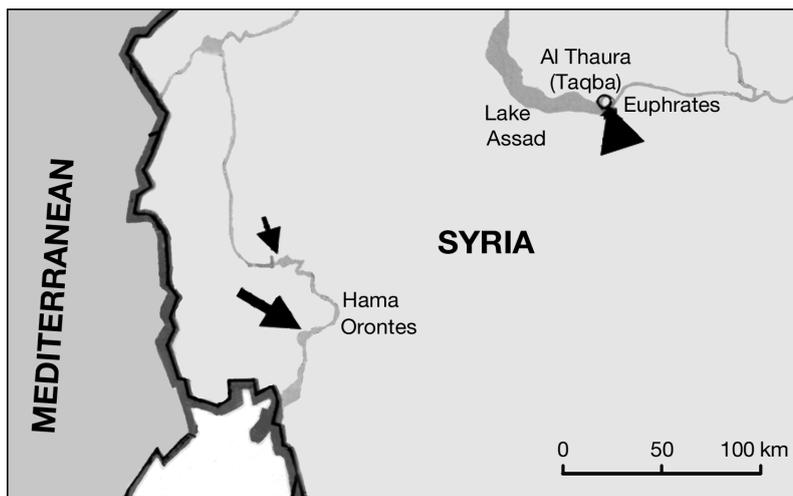
Fig. 1. Field locations in Syria: (1) Euphrates River (Lake Assad) (arrowhead); (2) branch of the Orontes River (large arrow); (3) Al-Sinn fish farm (Al-Ghab region) (small arrow)

individually into wells of 24 or 48 cell-well plates (as described by Yokoyama et al. 1991). The water layer above each oligochaete was then examined daily by stereo microscope for 3 d for the presence of released actinospores. After 7 d (Euphrates material) and 10 d (Orontes material) the live oligochaetes were relocated to our laboratory in Hungary in plastic tubes, and again placed into cell-well plates and monitored for actinospore release for a further 10 wk.

When examination indicated spore release, the water from a positive well was examined on a slide by light microscopy at high magnification and microphotographs were taken of spores using an Olympus BH-2 compound microscope equipped with a DP-10 digital camera. Subsequently, actinospores were drawn and measured, and their characters recorded according to the guidelines suggested by Lom et al. (1997). To determine the dimensions of hexactinomyxons, measurements of 20 mature spores from several hosts were averaged. Because of the limited number of triactinomyxon and endocapsa spores, only 3 mature spores for each were averaged. A sample of the released actinospores was fixed in 80% ethanol and sent to Oregon State University (OSU), USA, for molecular analyses.

Host identification. Representative oligochaetes from the 2 biotopes where infected worms were found were fixed in 80% ethanol and identified in Estonia by Tarmo Timm (see Timm 1997).

Histological analyses. Histological sections were made only from hexactinomyxon-infected oligochaetes. Heavily infected live oligochaetes were selected under a light microscope, fixed in 10% neutral buffered formalin and embedded in paraplast-wax. Five µm thick sections were stained with haematoxylin and eosin and photomicrographed.

Molecular analyses. The ethanol-fixed samples were spun for 3 min at 14 000 rpm (20 000 × g) to pellet the actinospores, the ethanol was removed, the sample rinsed in 500 µl molecular grade water (MGW), then respun and the water removed. The DNA was extracted using a QIAGEN DNeasy™ tissue kit (animal tissue protocol; Qiagen) and eluted in 2 steps of 30 and 20 µl buffer AE.

The 18S rRNA gene was amplified using the primers 18e (Hillis & Dixon 1991) and 18R (Whipps et al. 2003) in a 20 µl reaction which comprised: 0.5 µl extracted genomic DNA, 0.4 µl deoxyribonucleotide triphosphates (dNTPs) (10 mM each), 0.5 µl each primer (10 µM), 2 µl 10X *Taq* buffer, 1.2 µl 25 mM $MgCl_2$, 1 µl Rediload loading dye (Invitrogen), 0.25 µl *Taq* polymerase (1.25 U) (Promega) and 13.65 µl MGW. The PCR cycle profile was performed in a PTC-200 thermocycler (MJ Research) and consisted of an initial denaturation step of 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 45 s, 72°C for 120 s and finished with terminal extension at 72°C for 10 min, then rested at 4°C.

Three overlapping templates were generated from the 18e/18R fragment for sequencing: 18e and ACT1r (Hallett & Diamant 2001), ACT3f (Hallett & Diamant 2001) and MX3 (Andree et al. 1998), and either Kud3f (Whipps et al. 2003) (for hexactinomyxon) or ACT2f (Hallett & Diamant 2001) (for triactinomyxon) and 18R. Reagent amounts were scaled up to 50 µl reactions, included 1.25 µl of the 18e/18R template, and the above cycling profile used with the extension step shortened to 60 s. Aliquots of the resultant PCR products were electrophoresed through a 1% agarose 1X tris-acetate-EDTA buffer (TAE) gel stained with either 1% ethidium bromide or SYBR Safe (Invitrogen) alongside a 1 kb+ DNA ladder (Invitrogen) to confirm only a single amplicon of expected size was present.

Because no products were visible for the endocapsa sample (low genomic DNA concentration), the 18S rRNA gene was re-amplified as above but using the primers ERIB1 and ERIB10 (Barta et al. 1997) and the maximum amount (µl) of genomic template possible in a reaction. In the second round, a combination of primers was trialled on 0.5 µl template including MYX1f (Hallett & Diamant 2001) and ERIB10 from which 2 overlapping templates were generated for sequencing: MYX1f and ACT1r, and MyxGen4f and ERIB10. To amplify products for sequencing, 50 µl reaction volumes were used with 1.0 µl of template and the following cycle profile: 95°C for 2 min, followed by 35 cycles of 94°C for 20 s, 55°C for 30 s, 72°C for 45 s;

amplification was finished with terminal extension at 72°C for 10 min, then rested at 4°C.

Products were purified using a QIAquick PCR purification kit (Qiagen). DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies) and fragments were sequenced in both directions using the amplification primers (the triactinomyxon 18e/MYX1f template and endocapsa MyxGen4f/ERIB10 template was also sequenced with MYX1f and ACT2f, respectively) and ABI Big Dye Terminator chemistry on an Applied Biosystems (ABI) Capillary 3100 Genetic Analyzer at the OSU sequencing facility (Center for Gene Research and Biotechnology, Central Service Laboratory). The various forward and reverse sequence segments were aligned in BioEdit (Hall 1999) and ambiguous bases clarified using corresponding ABI chromatograms. Consensus sequences were submitted to GenBank. A standard nucleotide-nucleotide BLAST (blastn) search was conducted (Altschul et al. 1997).

RESULTS

Collection and screening of oligochaetes for actinospores

Oligochaetes collected from 2 (Orontes and Euphrates) of the 3 Syrian locations released 3 types of actinospores in the laboratory. The infected oligochaetes were identified as *Psammoryctides albicola* (mature and juvenile forms) (Fig. 2). Six (7.5%) of 80 oligochaetes examined from the branch of the Orontes River were infected with a novel hexactinomyxon type (Figs. 3 to 7). One host started to release spores the day after collection and 5 others began during the first week after relocation to Hungary; further actinospore release was observed for 6 wk.

A novel triactinomyxon (Fig. 8) and endocapsa (Figs. 9 to 11), both with low infection intensities, were found in *Psammoryctydes albicola* from the Al-Thaurah region of the Euphrates River (Lake Assad). These actinospore types were each released from only 1 host oligochaete.

None of the oligochaetes collected from Al-Sinn fish farm (Al-Ghab region) released actinospores during the 10 wk observation period.

Histological analyses

Histological examination of the hexactinomyxon-infected oligochaetes showed that pansporocysts were located in the intestinal epithelium (Figs. 12 & 13). In infected regions, almost all epithelial cells contained
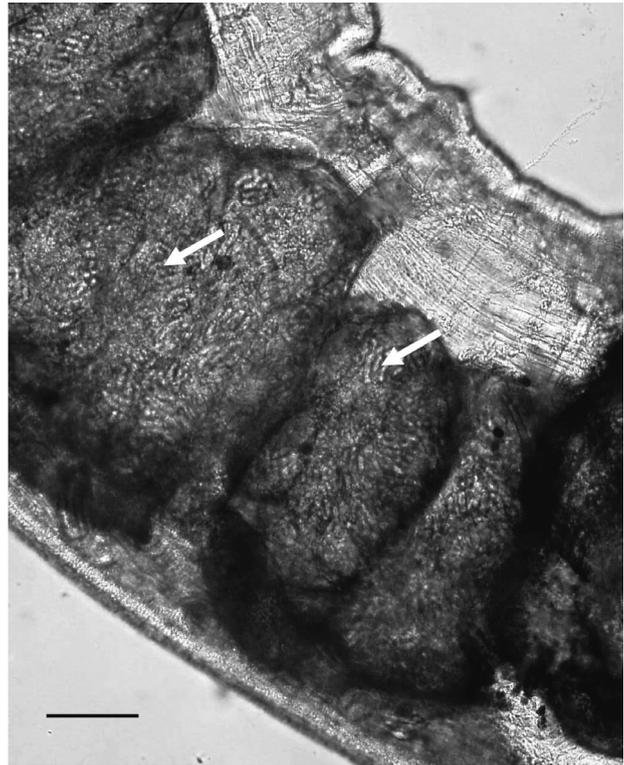

Fig. 2. Live *Psammoryctides albicola* from the Orontes River, infected with the novel hexactinomyxon; pansporocysts (arrows) are visible in the intestinal epithelium of the worm. Scale bar = 100 μm
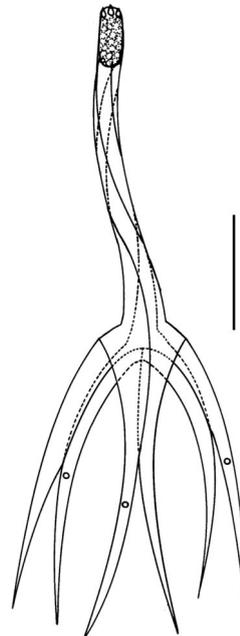

Fig. 3. Schematic drawing of the hexactinomyxon. Scale bar = 50 μm

pansporocysts with 8 developing spores clearly visible, and we were able to count the number of secondary cells (Fig. 13).
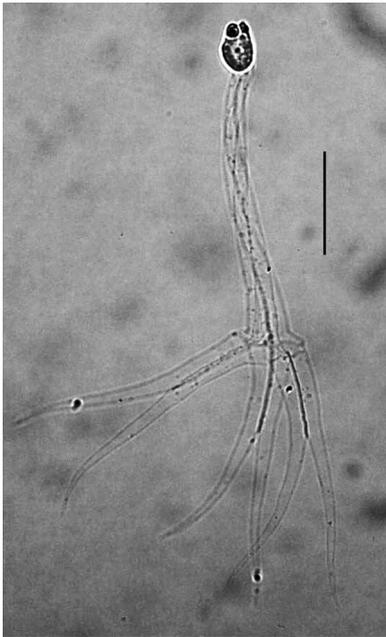
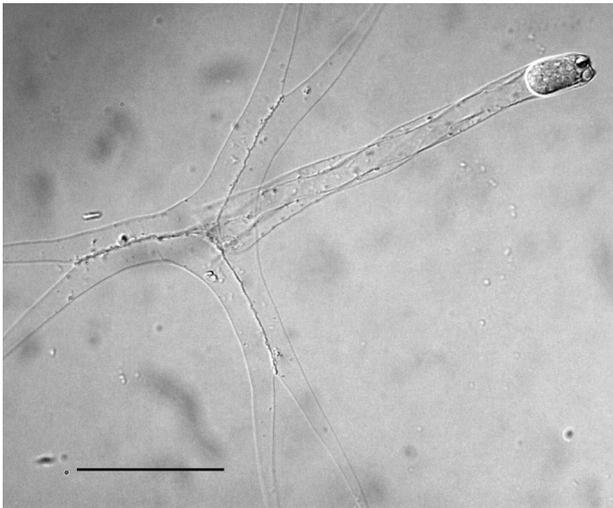Fig. 4. Hexactino-myxon spore: fresh, unstained spore undercoverslip. Scale bar = 50 μm

Fig. 5. Distinct valve sutures of the spore axis and processes of the hexactinomyxon. Scale bar = 50 μm
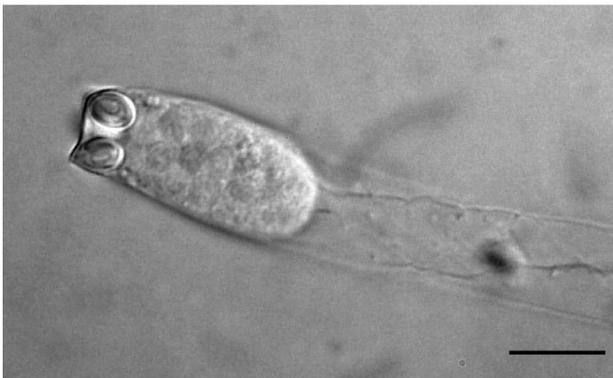

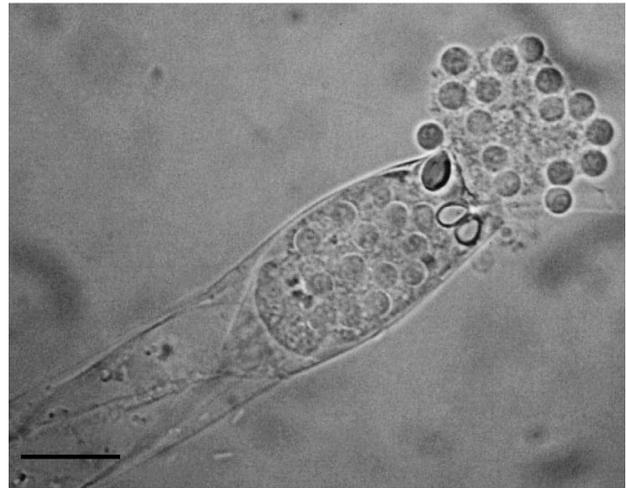Fig. 6. Spore body of the hexactinomyxon. Scale bar = 10 μm

Fig. 7. Spore body of the hexactinomyxon showing the germ cells. Scale bar = 10 μm

Fig. 8. Schematic drawing of the triactinomyxon, released by a *Psammoryctides albicola* from the Euphrates River (Lake Assad). Scale bar = 50 μm

Molecular analyses

The 18S rRNA gene was amplified from all 3 Syrian actinospores: 1950 bp for the hexactinomyxon (accession no. DQ473517), 1901 bp for the triactinomyxon (DQ473515) and 1915 bp for the endocapsa (DQ473516). None corresponded with any existing sequence in GenBank.
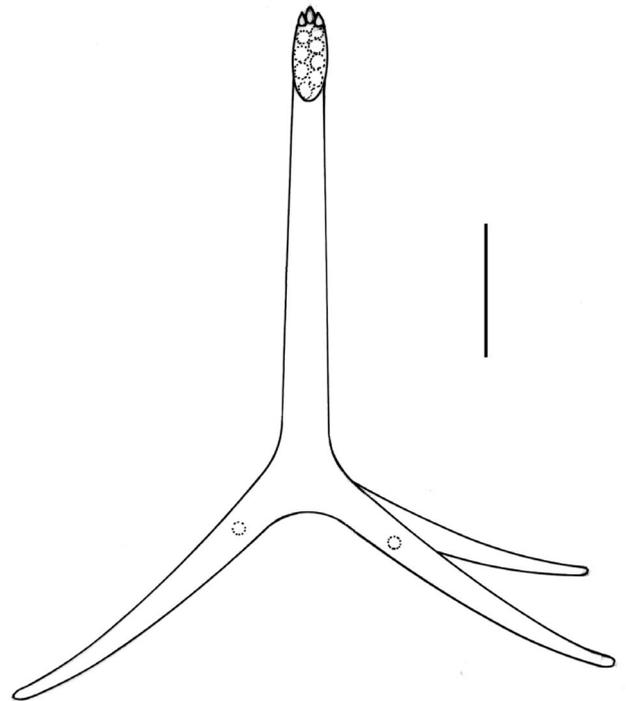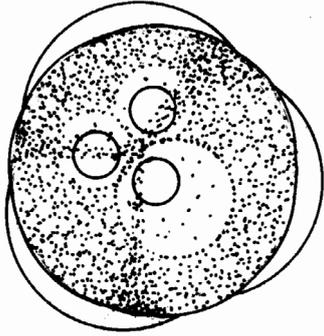
Fig. 9. Schematic drawing of the endocapsa released by a *Psammoryctides albicola* from the Euphrates River (Lake Assad). Scale bar = 10 µm

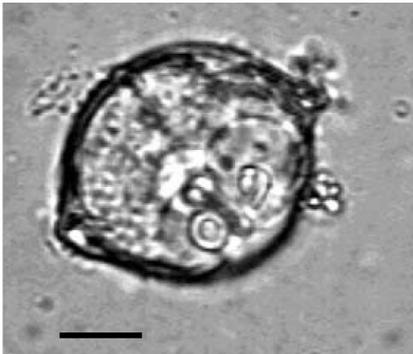

Fig. 10. Endocapsa spore. Fresh, unstained spore in semi-side view under coverslip. Scale bar = 10 µm
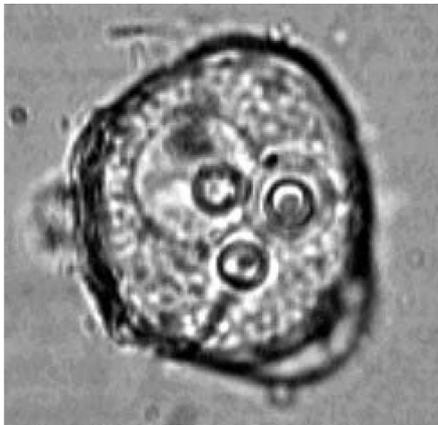
Fig. 11. Endocapsa spore. Fresh, unstained spore in apical view under coverslip. Scale bar = 10 µm

Fig. 12. Histological section (haematoxylin and eosin stained) of a *Psammoryctides albicola* oligochaete. Each segment contains abundant intestinal epithelial tissue infected with hexactinomyxon developmental stages. Scale bar = 50 µm
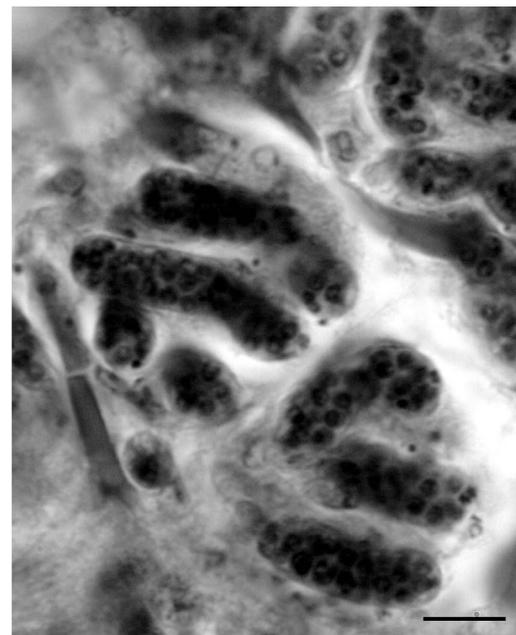
Fig. 13. Enlarged section of Fig. 12 (haematoxylin and eosin stained) showing pansporocysts containing spores, with secondary cells visible in their sporoplasms. Scale bar = 10 µm

DESCRIPTION OF THE NEW ACTINOSPORE TYPES

Hexactinomyxon type nov. (Figs. 3 to 7, Table 1)

Description: Mature spores are composed of a spore body, twisted style and 6 caudal processes (Figs. 3 & 4). The spore body is compact, oval, average length 28.5 µm (range 23.3–34.1 µm) and width 11.8 µm (9.3–13.9), contains 32 germ cells of 2.3 µm diameter and 3 polar capsules, measuring 4.7 × 3.0 µm (Figs. 6 & 7). The elongate style is 114.7 µm (86.1–129.4) long

Table 1. Morphometrics (in µm) of 8 previously described hexactinomyxons (Hex.). –: no data
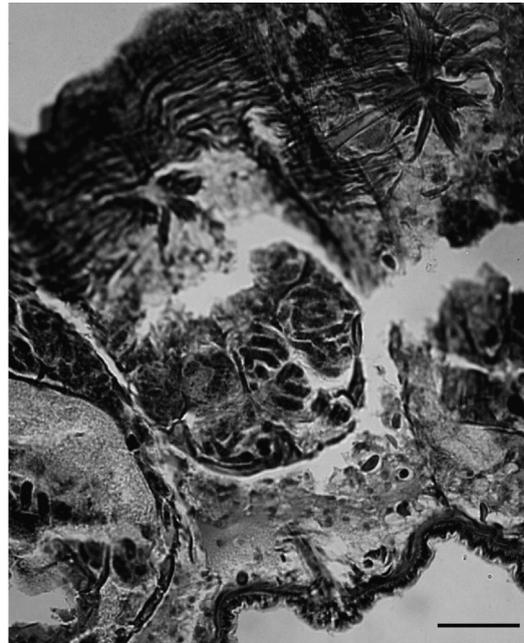
	Hexactinomyxon psammoryctis Štolc (1899)	Hexactinomyxon hedvigi Janiszweska (1955)	Myxobolus pavlovskii Ruidisch et al. (1991)	Hex. type 1 El-Mansy (2001)	Hex. type 2 El-Mansy (2001)	Hex. type 3 El-Mansy (2001)	Hex. type 1 Hallett et al. (2003)	Hex. type 2a Hallett et al. (2003)	Hex. type 2b Hallett et al. (2003)	Hex. Syrian type (present study)
Spore body length	20[a]	–	60 (55–66)[a]	30[a]	22[a]	42[a]	23 (19.4–27.2)	28.6 (25.9–31.1)	23.8 (22–28.5)	28.5 (23.2–34.1)
Spore body width	10	–	–	12	12	16	16.4 (14.3–18.1)	17.6 (15.5–18.1)	18.3 (15.5–20.7)	11.8 (9.3–13.9)
Style length	80	–	96.4 (90–102)	110	158	130	53.8 (42.7–62.2)	33.0 (29.8–42.7)	41.1 (33.7–47.9)	114.7 (86.1–129.4)
Style width at base	–	–	–	20	26	30	17.2 (14.3–23.3)	16.2 (12.9–19.4)	18.7 (15.5–20.7)	18.2 (15.3–23.3)
Spore axis	100	136–140[a]	170.1 (165–179)	140	180	172	76.6 (70–83)	61.9 (58–68)	64.9 (60.9–71.2)	142.5 (116.3–161.1)
Process total length	60	152–180	73.7 (68–83)	112	107.2	79.2	76.2 (59.6–93.2)	71.7 (67–75)	85.9 (77.7–97.1)	123.5 (104.7–147)
Process length to bifurcation	20	–	–	–	–	–	19.3 (12.9–25.9)	14.1 (13–15.5)	16.2 (12.9–25.9)	49.9 (37.2–53.5)
Process width before bifurcation	–	–	–	12	14.4	16	15.9 (14.3–18.1)	16.2 (14.3–19.4)	15.4 (12.9–18.1)	17.7 (15.3–22.4)
Process width after bifurcation	–	–	–	–	–	–	9.2 (7.8–12.3)	9.1 (7.5–10.4)	8.3 (7.8–9.1)	7.5 (7–9.3)
Polar capsules L × W	–	–	7.6 × 4.8 (6.8–8.2) × (4–5.5)	6 × 4	6 × 4	8 × 4	4.3 × 4.3	6.0 × 4.1	6.4 × 4.2	4.7 × 3.0
Number germ cells	>30	128	30	28–38	100–150	32–40	32	32	32	32
Germ cell diameter	–	–	–	2	1.5	3	–	–	3.6	2.3
Host	Psammoryctides barbatus	Tubifex tubifex	Tubifex tubifex	–	–	Limnodrilus hoffmeisteri	Limnodrilus hoffmeisteri	Limnodrilus udekemianus	Limnodrilus udekemianus	Psammoryctides albicola
Site of infection	Intestinal epithelium	Intestinal epithelium	Intestinal epithelium	–	–	–	Intestinal epithelium	Intestinal epithelium	Intestinal epithelium	Intestinal epithelium

[a] Not including polar capsules

and 18.2 µm (15.3–23.3) wide at base with prominent, curved, valve cell sutures (Fig. 5). The spore axis is 142.5 µm (116.3–161.1) long and widens to base where each valve cell divides into 2 equal projections (Figs. 4 & 5). One of each pair is fused to the nearest neighbouring process for ~40 % (49.9 µm, 37.2–53.5) of their total length (123.5 µm, 104.7–147.0). Width across 2 fused processes is 17.7 µm (15.3–22.4) and width of a single process after suture is 7.5 µm (7.0–9.3). Valve cell nuclei in processes are irregularly positioned.

Host: *Psammoryctides albicola* (Michaelsen) Hrabe.
Site in host: Intestinal epithelium.
Locality: Branch of Orontes River, 30 km south of Hama, Syria.
Prevalence of infection: 6 of 80 worms (7.5 %).
Phototypes: In the collection of Cs. Sz., Veterinary Medical Research Institute, Hungarian Academy of Sciences.
Specimens: Stored in 70 % ethanol deposited in the Parasitological Collection of the Hungarian Natural History Museum, accession numbers: HNHM 70043, 70044, 70045.

Remarks: Spore morphometrics do not correspond with any of the 8 hitherto described hexactinomyxon species/types (Table 1). The principle distinguishing character is the twisted spore axis, which was only reported by Štolc (1899) for *Hexactinomyxon psammoryctis*; however, all the dimensions of *H. psammoryctis* are smaller than those of our spore (particularly the processes, which are on average half the size of the Syrian hexactinomyxon). A BLAST search indicated that the Syrian hexactinomyxon 18S rDNA sequence is most similar to freshwater species of *Myxobolus* (Table 2). The BLAST alignment highlighted that the Syrian hexactinomyxon has 2 small insertion sites — one at ~650 bp and the second at ~1450 bp — that distinguish it from at least the 50 most similar myxozoans. Comparison with the only 2 members of the collective group for which there is sequence data, Hexactinomyxon types 1 and 2 of Hallett et al. 2003 (AY162271 and AY162272), showed 92 % (over 1430 bp) and 93 % (1262 bp) similarity, respectively.

Triactinomyxon type nov. (Fig. 8, Table 3)

Description: Mature spores are composed of a spore body, style and 3 caudal processes. The spore body is compact, oval, average length 30 µm and width 13 µm, contains 8 germ cells and 3 polar capsules measuring 3×2 µm. The elongate style is 130 µm long, and 17 µm wide at its base. The spore axis is 160 µm long. The average length of the approximately equal processes is 120 µm. Valve cell nuclei in processes are irregularly positioned.
Host: *Psammoryctides albicola* (Michaelsen) Hrabe.

Site in host: Not determined.
Locality: Euphrates River, Lake Assad, Al-Thaurah Region, Syria.
Prevalence of infection: 1 of 150 worms (0.77 %).
Phototypes: In the collection of Cs. Sz., Veterinary Medical Research Institute, Hungarian Academy of Sciences.
Specimens: All the spores were used for DNA extraction.

Remarks: More than 60 triactinomyxon types are described in the literature, of which 14 have 8 germ cells in their sporoplasm (Table 3). Of these, 3 have processes shorter than their spore axis or style. There are no dimensions for *Triactinomyxon petri*; however, it clearly differs morphologically to our triactinomyxon (the sporoplasm of *T. petri* fills its style and hence should perhaps be considered a Raabeia). Our type is most similar to Triactinomyxon type 3 of El-Mansy et al. (1998) but it is smaller in all dimensions (5.9 to 36 %) and is described from a different host oligochaete. Thus, the Syrian triactinomyxon does not correspond with any other triactinomyxon described to date. A BLAST search indicated affinity of the Syrian triactinomyxon with marine myxozoans (Table 2). The highest ranking known triactinomyxon in the search, *Triactinomyxon* sp. (AF306792), was 79 % similar to the Syrian triactinomyxon in a BioEdit pairwise alignment; (the actinospore stages of higher ranking myxosporeans are unknown and may include triactinomyxons).

Table 2. Percent similarity (determined by a pairwise alignment in BioEdit) of the Syrian actinospore 18S rDNA sequences with the top ranking sequences identified by a BLAST search

Syrian actinospore	BLAST sequence	Similarity (%)	No. bases compared
Hexactinomyxon	*Myxobolus algonquinensis* (AF378335)	89	1951
	M. macrocapsularis (AF507969)	87	1577
Triactinomyxon	*M. exiguus* (AY129317)	94	1571
	M. muelleri (AY129314)	93	1572
	Endocapsa rosulata (AF306791)	87	1782
	Sphaeractinomyxon ersei (AF306790)	83	1781
Endocapsa	*M. exiguus*	93	1581
	M. muelleri	92	1582
	E. rosulata	86	1791
	S. ersei	84	1793

Table 3. Comparison of the main features of documented triactinomyxon actinospores with 8 germ cells (GC). All measurements are in µm. –: no data

Species/type	Host	Process length	Spore axis *Style length	Spore body length	Source
Triactinomyxon Syrian type	*Psammoryctides albicola*	120	160 *130	30	Present study
Triactinomyxon ignotum	*Tubifex tubifex*	175–220	*140–170	30–50	Štolc (1899), Marques (1984)
T. ohridensis	*T. ohridensis* (*Criodrilus ohridensis*?)	30–40	*90–100	20–30	Georgevitch (1940), Marques (1984)
T. petri	*Lumbriculus* sp.	–	–	–	Georgevitch (1940), Marques (1984)
Triactinomyxon type 3 of El-Mansy et al. (1998)	*Nais elinguis*, *T. tubifex*	127.5	*150	47.1	El-Mansy et al. (1998)
Triactinomyxon type 4 of El-Mansy et al. (1998)	*Limnodrilus hoffmeisteri*	173.4	*137.7	41.2	El-Mansy et al. (1998)
Triactinomyxon type C of Xiao & Desser (1998)	*L. hoffmeisteri*	280–300	185–205	28–31 (sporoplasmic mass)	Xiao & Desser (1998)
Triactinomyxon of Hallett et al. (2004)	*T. tubifex*	2 long: 147.5–272.0 1 short: 70–176	121.5–178.5 *97–128	19.5–32.5	Hallett et al. (2004)
Triactinomyxon type 2 of Lowers & Bartholomew (2003) (6–8 GC)	Tubificid	126–250	*138–187	24–34	Lowers & Bartholomew (2003)
Triactinomyxon type 3 of Lowers & Bartholomew (2003)	Tubificid	238–289	*190–194	32–39	Lowers & Bartholomew (2003)
Triactinomyxon type 4 of Lowers & Bartholomew (2003) (8–10GC)	Tubificid	204–238	*88–117	49–68	Lowers & Bartholomew (2003)
Triactinomyxon type 5 of Lowers & Bartholomew (2003) (8–14GC)	Tubificid	90–155	*84–105	18–20	Lowers & Bartholomew (2003)
Triactinomyxon type 1 of Oumouna et al. (2003)	*Tubifex* sp.	155–165	*160–180	–	Oumouna et al. (2003)
Triactinomyxon stage of *Myxobolus pseudodispar*	*T. tubifex*	2 long: 190–204 1 short: 104–144	*145–173	47–53	Székely et al. (1999, 2001)
Triactinomyxon type 1 of Rácz & Timm (2002)	*T. tubifex*	168–254	*114–147	29–50	Rácz & Timm (2002)

Endocapsa type nov. (Figs. 9 to 11, Table 4)

Description: Mature spores are composed of a compact, round spore body, with 3 reduced lateral valve cell swellings which give the spore a triangular appearance in apical view. Spore diameter 27.6 µm (26 to 29.6 µm). Swellings are biconcave, not obviously conjoined, length 3.1 µm and width 13.2 µm. Polar capsules within prominent capsulogenic cells are 4 × 3.2 µm, non-protruding, and positioned under the valve cell junctions and perpendicular to the swellings. The number of germ cells could not be determined; however the sporoplasm is granular, which suggests that it is multinucleate. Suture line is distinct.

Table 4. Comparison of the main features of Endocapsa actinospores (all measurements in µm)

Species/type	Host	Process length	Spore body diameter	Source
Syrian endocapsa	*Psammoryctides albicola*	3.1	26–29.6	Present study
Endocapsa rosulata	*Heterodrilus* cf. *keenani* *Thalassodrilus* cf. *gurwitschi* *Heronidrilus* sp.	4–6	25–27	Hallett et al. (1999)
Endocapsa stepheni	*Heterodrilus* cf. *keenani* *Heterodrilus queenslandicus*	~4	25–28	Hallett et al. (1999)
Endocapsa type 1 of Hallett et al. (2001)	Tubificidae spp.	3–4	17–30	Hallett et al. (2001)

Host: *Psammoryctides albicola* (Michaelsen) Hrabe.
Site in host: Not determined.
Locality: Euphrates River, Lake Assad, Al-Thaurah region, Syria.
Prevalence of infection: 1 of 150 worms (0.77%).
Phototypes: In the collection of Cs. Sz., Veterinary Medical Research Institute, Hungarian Academy of Science.
Specimens: All the spores were used for DNA extraction.
Remarks: The reduced valve cell swellings (ratio of valve cell length:spore diameter = 0.1:1) and the submerged (non-protruding) polar capsules are consistent with the collective group Endocapsa (Hallett et al. 1999). It is unknown whether the swellings of the Syrian endocapsa arise after contact with water or are already present within the host. The Syrian type is most similar morphologically and morphometrically to *Endocapsa rosulata*, but its swellings are even more reduced and it was described from a freshwater oligochaete rather than a marine host. A BLAST search indicated affinity of the Syrian endocapsa with the same marine clade of myxozoans as for the Syrian triactinomyxon (Table 2). Comparison of these 2 Syrian actinospores in a pairwise alignment in BioEdit showed 94% similarity (over 1902 bp).

DISCUSSION

This is the first report on actinospore stages of myxosporeans described from Syria or indeed on any myxozoans from this country. A species of *Myxobolus* was isolated from the muscle of *Phoxinellus* sp. collected from the Orontes River, where the hexactinomyxon occurred (K. Molnár & Cs. Székely unpubl. data). Comparsion of their 18S rDNA sequences indicated the 2 stages were not the same species. There are reports of myxozoans from the neighbouring countries of Israel, Turkey and Iraq, but these organisms inhabit different river basins.

After the pioneering work of Štolc (1899), who described not only the first hexactinomyxon but the first actinospores, only a few authors have reported a total of 8 hexactinomyxon types or species from natural waters (Janiszewska 1955, Ruidisch et al. 1991, El-Mansy 2001, Hallett et al. 2003) (Table 1). The hexactinomyxon type from this study with its twisted style most closely resembled *Hexactinomyxon psammoryctis* (Štolc 1899), but differed substantially in most of its dimensions. In addition to the morphological examinations, the molecular comparison also indicated that the Syrian hexactinomyxon type (or its assumed myxospore stage) had not been studied before.

Among the hitherto clarified myxosporean life cycles reported in the literature, there is only one in which the intraoligochaete stage is a hexactinomyxon, that of *Myxobolus pavlovskii* (Ruidisch et al. 1991). It is likely that the type found in the present study is an actinospore stage of a fish or amphibian myxosporean parasite in line with other members of the class, and both the limited life cycle and molecular data for hexactinomyxons suggest affinity with the genus *Myxobolus.* DNA sequence data may prove to be the better indicator of alternate myxosporean stages since there is no clear morphological link between certain myxospores and actinospores: e.g. aurantiactinomyxon stages alternate with myxospores from at least 3 different myxosporean genera, *Henneguya, Hoferellus* and *Thelohanellus* (see Kent et al. 2001) and *Hennuguya* myxospores can also alternate with triactinomyxon actinospores (Kallert et al. 2005).

The most prevalent actinospores are triactinomyxons, of which more than 60 species or types have been described in the literature (Kent et al. 2001, Hallett et al. 2004, Rácz 2004). They are also the most common type of actinospore counterpart for the most speciose myxosporean genus, *Myxobolus.* The Syrian triactinomyxon was morphologically distinct from all of these and its 18S rDNA sequence did not match any myxozoan in GenBank. Morphologically, it most closely resembled Triactinomyxon type 3 of El-Mansy et al. 1998 from Hungary, but was smaller by 6 (process length) to 36% (spore body length). While considerable intraspecific morphometric variation is not uncommon among triactinomyxons (see Hallett et al.

2004), this plasticity may not apply to all members of the group. The Hungarian and Syrian triactinomyxons infect different oligochaete hosts (Table 3) and although host species is not a recognised criterion for distinguishing myxospore stages in fish (Lom & Arthur 1989), its use may be justified for freshwater actinospores, as 90% infect only a single oligochaete species (see Hallett et al. 2001). Thus, because the 2 records are not obviously the same species, we prefer to keep them separate until further data contradicts this decision. Thus, without additional unambiguous data such as DNA sequences it can be difficult to be definitive in a diagnosis.

Three endocapsa species or types have been described (Table 4; Hallett et al. 1999, 2001), all from marine oligochaetes. The Syrian endocapsa is the first freshwater member of this collective group and, interestingly, genetically it affiliates with marine myxozoans including *Myxobolus* spp. and the actinospores *Endocapsa rosulata* and *Sphaeractinomyxon ersei* (Table 2). The Syrian triactinomyxon is also most similar to this marine clade and both it and the Syrian endocapsa were collected from the same location (different to the Syrian hexactinomyxon). In phylogenetic analyses, this marine clade clusters separately from freshwater species (see Kent et al. 2001, Bahri et al. 2003, Cone et al. 2005). Note that there are 2 entries in GenBank for *Myxobolus muelleri*: a marine isolate AY129314 was obtained from mullet from Ichkeul Lake, Tunisia (Bahri et al. 2003), whereas a freshwater isolate AY325284 was collected from chub from the River Danube, Hungary (Eszterbauer 2004).

The marine affinities of both the endocapsa and the triactinomyxon, as indicated by their 18S rDNA, suggest that the ancestors of these myxosporean species may have made the transition from marine to freshwater relatively recently.

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