Sphaeromyxa artedielli sp. n. (Myxozoa: Sphaeromyxidae), a parasite of sculpins (Cottidae) in northern Norway

Egil Karlsbakk¹, Ann-Cathrine Bårdsgjære Einen¹ and Pavla Bartošová²

¹Institute of Marine Research, Bergen, Norway;

²Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

Abstract: *Sphaeromyxa artedielli* sp. n. is described from the gall bladder of the Atlantic hookear sculpin *Artediellus atlanticus* Jordan et Evermann (Cottidae; type host) from northern Norway. The parasite was also found to infect *Triglops murrayi* Günther (Cottidae). Spores are produced in disporic pansporoblasts in large flat plasmodia. Spores are straight and fusiform with truncated ends, and measure 16.5–18.7 $\mu\text{m} \times 4.9\text{--}6.2\ \mu\text{m}$. Valves are thick, striated and suture line is straight. Two equal ovoid polar capsules measure 4.2–6.8 $\mu\text{m} \times 2.9\text{--}4.4\ \mu\text{m}$ and contain irregularly folded polar filaments. Distinctive features include spore shape and size, spore length/width relationship, striated valves, equal polar capsules and a short intercapsular distance. *Sphaeromyxa bonaerensis* Timi et Sardella, 1998, *Sphaeromyxa cannolii* Sears, Anderson et Greiner, 2011, and *Sphaeromyxa sevastopoli* Naidenova, 1970 produce straight spores with truncated ends that are of similar length as those of the new species. *Sphaeromyxa cannolii* differs in showing smooth spores with unequal polar capsules. The new species differs from *S. bonaerensis* and *S. sevastopoli* in significantly wider spores and polar capsules. *Sphaeromyxa balbianii* Thélohan, 1892, a species originally described with significantly smaller spores than *S. artedielli* sp. n., has previously been recorded from *T. murrayi*. We show that *S. artedielli* sp. n. differs from *S. balbianii* from the type host *Gaidropsarus vulgaris* (Cloquet) by its SSU rDNA sequence, and suggest that Atlantic records of *Sphaeromyxa* spp. from *T. murrayi* represent *S. artedielli* sp. n. The closest relative to *S. artedielli* sp. n. according to the SSU rDNA sequences, *S. longa* Dunkerly, 1921, differs clearly by spore size and shape. In the SSU rDNA-based phylogenetic analyses, *S. artedielli* sp. n. groups with other *Sphaeromyxa* spp. with straight spores and truncated ends in a clade that represents a sister-group to *Sphaeromyxa* spp. with arcuate spores and rounded ends. Our results indicate that an SSU rDNA pseudogene is present in *S. balbianii*.

Keywords: Myxosporeans, marine fish, new species, *Artediellus atlanticus*, *Triglops murrayi*, SSU rRNA gene sequences, pseudogene, northern Atlantic Ocean, *Sphaeromyxa balbianii*

The genus *Sphaeromyxa* Thélohan, 1892 currently contains 46 species, which are coelozoic parasites in the bile ducts and gall bladder of marine fishes. Most species produce a large number of spores in very large flat plasmodia (up to 22 mm in diameter). The spores contain two polar capsules, one at each spore end. These contain a polar filament, which is thick when extruded, broad at the base and tapering towards the ends. The polar filament in *Sphaeromyxa* spp. is folded upon itself several times inside the polar capsule, whereas it is thin and coiled in other myxosporeans (Lom and Dyková 2006).

Different *Sphaeromyxa* spp. produce spores of two main types: either straight fusiform spores with oval polar capsules and usually truncate ends, or arcuate spores with pyriform polar capsules that usually have rounded spore ends. Laird (1953) referred to these as members of the ‘balbianii’ and ‘incurvata’ groups, respectively. Separation of the species in these two groups is also supported by phylogenetic studies (Kristmundsson and Freeman 2013, Whipps and Font 2013).

During a survey on myxosporean diversity of Norwegian marine fishes, infections with a *Sphaeromyxa* species belonging to the ‘balbianii’ group were discovered in two sculpin species caught in relatively deep waters in northern Norway. This species was found to be new to science and is described below.

MATERIALS AND METHODS

The marine sculpins *Artediellus atlanticus* Jordan et Evermann and *Triglops murrayi* Günther were collected by bottom trawl during a research cruise along the coast of northern Norway with RV ‘Helmer Hanssen’ from 17 October to 12 November 2011 (Table 1). The sculpins were immediately examined post capture. Fresh bile samples were examined in a microscope (Olympus CX41) equipped with a digital camera (Olympus SC30) and digital image series of the spores and plasmodia were taken. Plasmodia and spore measurements were taken from the images using the Image J software. Air dried smears were kept from infected samples; these were stained with Hemacolor® (Merck Millipore, Billerica, MA). Ethanol-fixed (96%) samples

Table 1. Specimens of the sculpins *Artediellus atlanticus* (AA) and *Triglops murrayi* (TM) found infected with *Sphaeromyxa artedielli* sp. n. in the gall bladder.

Sequence isolate	Date	Position	Depth (m)	Host	Total length of host fish (mm)	Stages of <i>S. artedielli</i> observed in bile	GenBank Accession No.
HH-3	30 October 2011	70°14.4'N, 18°28.2'E	181–195	AA	90	spores, plasmodia	KF135220
HH-72	30 October 2011	70°14.4'N, 18°28.2'E	181–195	AA	93	plasmodia	KF135221
HH-73	30 October 2011	70°14.4'N, 18°28.2'E	181–195	AA	95	plasmodia	KF135222
HH-38	5 November 2011	67°13.2'N, 11°14.5'E	177–184	TM	95	spores, plasmodia	KF135223
HH-39	5 November 2011	67°39.9'N, 11°29.0'E	145–151	TM	102	spores, plasmodia	KF135224
-	5 November 2011	67°39.9'N, 11°29.0'E	145–151	TM	98	spores, plasmodia	not sequenced

of the new species spores and plasmodia were kept for subsequent DNA isolation. Ethanol-fixed samples of *Sphaeromyxa balbianii* spores (isolate M0167) from the type host, *Gaidropsarus vulgaris* (Cloquet), were available at the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR and originated from the same sampling lot in Scotland as *S. balbianii* sample (isolate M0312), for which SSU and LSU rDNA sequences have been published previously (Bartošová et al. 2009).

DNA was extracted from dried (60 °C, ethanol-free) samples of myxosporean spores and plasmodia using the DNeasy® Tissue Kit protocol for animal tissues (Qiagen, Hilden, Germany). The following forward/reverse primers were used for PCR to amplify the SSU rDNA of *S. artedielli*: Erib1/1430rev, MyxF2/Myxgen4R and Myxgen4F/RosR2 (see Barta et al. 1997, Kent et al. 2000, Freeman et al. 2008, Køie et al. 2008), with annealing temperatures of 52 °C, 58 °C and 52 °C, respectively. The novel *S. balbianii* sequences were obtained with the Erib1/Erib10 primers (Barta et al. 1997) with an annealing temperature of 50 °C.

PCR amplifications were performed in a total volume of 50 µl using 2 µl of template DNA and a reaction mixture consisting of 10 µl 5× PCR buffer, 3 µl 25 mM MgCl₂, 5 µl 10 mM dNTP, 2 µl (10 mM) of the forward and reverse primer, 2 U of thermostable DNA polymerase (GoTaq®, Promega, Madison, WI), and 26 µl dH₂O. PCR conditions were used as previously described (Køie et al. 2008). PCR products were cleaned with ExoSAP-IT® (Affymetrix Inc., Santa Clara, CA) and then sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit. The sequencing of both sense and anti-sense strands was done using the PCR amplification primers. The sequence data were assembled with the Vector NTI 11 software (Invitrogen, Carlsbad, CA) and GenBank searches were done with Blast (2.0).

The phylogenetic relationships between *S. artedielli* and other *Sphaeromyxa* spp. were inferred by maximum likelihood (ML) and maximum parsimony (MP) analyses using MEGA 5.1 (Tamura et al. 2011). SSU rDNA sequences were aligned with Clustal W (AlignX, Vector NTI) with default settings. A GTR + Γ + I model of nucleotide substitution was selected (AICc) for the ML analysis. Nodal supports were calculated using 500 bootstrap replications for both analyses.

RESULTS

Sphaeromyxa artedielli sp. n.

Figs. 1, 2

Plasmodia large, flat, round to oval or elongate, sometimes bilobed. Small plasmodia rounded to bilobed, often with a brushy region at one side (filopodia/rhizoids) (Fig. 1B). Large polysporic plasmodia with distinct

Table 2. Measurements of spores of *Sphaeromyxa artedielli* sp. n. from *Artediellus atlanticus* (type host) and from *Triglops murrayi*.

Host	<i>Artediellus atlanticus</i>					<i>Triglops murrayi</i>				
Measurement	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N
Length (L)	17.8	0.5	16.5	18.7	31	17.4	0.7	14.9	18.9	48
Width (W)	5.4	0.3	4.9	6.2	19	5.7	0.2	5.3	6.1	27
Thickness	5.4	0.3	4.9	6.1	14	5.7	0.3	5.1	6.3	21
PC length	5.6	0.4	4.2	6.8	64	5.5	0.3	4.5	6.3	96
PC diameter	3.5	0.3	2.9	4.4	63	3.6	0.3	3.1	4.3	96
PC-PC	5.3	0.3	4.6	5.8	31	5.0	0.5	4.1	6.2	48
End-dish diameter	2.1	0.4	1.2	3.1	59	2.3	0.4	1.3	3.0	95
ID/PC index	0.9	0.1	0.8	1.2	31	0.9	0.1	0.7	1.2	48
L/W index	3.3	0.2	3.0	3.7	17	3.1	0.2	2.8	3.4	25

SD – standard deviation; N – number of measurements; PC – polar capsule; ID/PC index – ratio of intercapsular distance (PC–PC) and mean PC length of each spore.

transparent ectoplasm, measuring 3–8 µm in thickness at plasmodium margin, with distinct mucoid zone externally, 3.5–5.5 µm in thickness (Fig. 1A). Plasmodia without spores (N = 33) up to 107 µm in diameter; plasmodium with immature spores measuring 450 × 440 µm. Plasmodia with mature spores 0.5–2.2 mm in diameter (area 0.18–3.0 mm²). A plasmodium with area 1.65 mm² estimated to contain about 4700 spores. Pansporoblasts distinct, vacuole-like, round to oval, each containing two spores (Fig. 1A). Pansporoblasts with spores 17–25 µm in mean diameter (N = 12).

Spore measurements in Table 2. Spores fusiform with truncate ends. Valves with 6–7 faint striations, parallel to longitudinal axis of spore. Spore thickness equal to width. Valves thick, 0.4–0.5 µm (mean 0.44; N = 33). Suture line indistinct, straight (Fig. 2C), occasionally slightly sigmoid (Fig. 2D). Polar capsules at each spore end, equal, ovoid, often with truncated apical end. Polar filament irregularly arranged in 1.5–2.5 visible folds/coils, roughly parallel to longitudinal axis of polar capsule. Nuclei of capsulogenic cells distinct, positioned posterolaterally to polar capsules in valvular view. Intercapsular distance equal to or shorter than polar capsule length. Sporoplasm binucleate. Immature spores with more pointed *Myxidium*-like ends, longer (18–20 µm) than mature spores.

Fig. 1. Images of fresh *Sphaeromyxa artedielli* sp. n. plasmodia and spores from the bile of sculpins, *Triglops murrayi* (A, B, D, E) and *Arctiellus atlanticus* (C). **A** – margin of a large plasmodium showing granular endoplasm with disporic pansporoblasts and transparent ectoplasm; a mucoid zone is seen outside the ectoplasm (arrow); **B** – small plasmodium with a brushy zone (arrow – rhizoids); **C–E** – spores. Scale bar is same for A–E.

Fig. 2. Line drawings of *Sphaeromyxa artedielli* sp. n. spores from *Arctiellus atlanticus*. **A, B** – valvular view; **C, D** – sutural view (**D** – spore with slightly sigmoid suture line).

Table 3. *Sphaeromyxa* spp. having straight spores with truncate flattened ends. Data for *S. artedielli* sp. n. given from both hosts (*Artediellus atlanticus* and *Triglops murrayi*, N = 79).

Species	Type host (family)	Locality	Length	Width	PC length	PC width	ID/PC Index*	Valves	Reference
<i>Sphaeromyxa artedielli</i> sp. n.	<i>Artediellus atlanticus</i>, <i>Triglops murrayi</i> (Cottidae)	Northern Norway	14.9–18.9 (17.5)	4.9–6.2 (5.6)	4.2–6.8 (5.6)	2.9–4.4 (3.6)	0.7–1.2 (0.9)	Str	Present study
<i>Sphaeromyxa balbianii</i> Thélohan, 1892	<i>Gaidropsarus vulgaris</i> (Lotidae)	Roscoff, France	13–16; 15	5	N/A	N/A	> 1.9	Str	Thélohan 1892, 1895
<i>Sphaeromyxa bonaerensis</i> Timi et Sardella, 1998	<i>Anchoa marini</i> (Engraulidae)	Mar del Plata, Argentina	16.5–18.8 (17.3)	3.7–4.8 (4.5)	3.0–4.5 (4.1)	1.9–3.3 (2.8)	1.1–1.3	Str	Timi and Sardella 1998
<i>Sphaeromyxa cannolii</i> Sears, Anderson et Greiner, 2011	<i>Hippocampus erectus</i> (Syngnathidae)	Gulf of Mexico	17–18 (17.6)	5–6 (5.5)	4.2–5.4 (4.8) + 4.4–5.9 (5.2)	2.4–3.3 (3.0) + 2.4–3.5 (2.8)	1.1–1.3	Sm	Sears et al. 2011
<i>Sphaeromyxa japonica</i> Aseeva, 2002	<i>Triglops murrayi</i> (Cottidae)	Japan Sea	18.5–23.7	4.3–4.5	5.0–6.3	3.8	1.6–2.1	Str	Aseeva 2002
<i>Sphaeromyxa magna</i> Zhukov, 1964	<i>Liparis gibbosus</i> (Liparidae)	Bering Sea	23	6.4	8.5	4	1.0–1.1	Str	Zhukov 1964
<i>Sphaeromyxa minuta</i> Polyansky, 1955	<i>Hippoglossus hippoglossus</i> (Pleuronectidae)	Barents Sea	13.5–16.2	2.7–3.6	2.7–4.5	N/A	1.4–1.6	Sm	Polyansky 1955
<i>Sphaeromyxa opisthopterae</i> Sarkar, 1999	<i>Opisthopterus tardoore</i> (Clupeidae)	Bay of Bengal	9–12.7 (11.5)	3.3–4.5 (3.9)	2.6–3.8 (3.6)	1.8–3.0 (2.8)	1.1–1.8	Str	Sarkar 1999
<i>Sphaeromyxa schulmani</i> Kovaleva et Gaevskaya, 1982	<i>Salilota australis</i> (Moridae)	SW Atlantic	18.6–20.0	4–6.0	4.6–6.0	2.6–3.3	1.3–1.8	Sm	Kovaleva and Gaevskaya 1982
<i>Sphaeromyxa sevastopoli</i> Naidenova, 1970	<i>Neogobius fluviatilis</i> (Gobiidae)	Black Sea	18.2–18.3	4.2–4.6	5.6–5.8	3.1–3.3	0.9–1.3	Str	Naidenova 1970
<i>Sphaeromyxa zaharoni</i> Diamant, Whipps et Kent, 2004	<i>Pterois miles</i> (Scorpaenidae)	Red Sea	13.7–15.1 (14.5)	4.2–5.5 (4.8)	3.3–5.6 (4.8)	2.5–4.0 (3.4)	1.0–1.3	Str	Diamant et al. 2004

PC – Polar capsule; ID/PC index – the ratio of the intercapsular distance to the mean PC length; Str – striated valves; Sm – smooth valves; N/A – not given; *source: Thélohan 1895 – plate VIII Figs. 58, 59 (N = 2); Timi and Sardella 1998 – Figs. 1, 4 (N = 2); Sears et al. 2011 – Fig. 1b (N = 4); Aseeva 2002 – Fig. 1 (N = 6); Zhukov 1964 – Fig. 26 (N = 2); Polyansky 1955 – Fig. 9 (N = 4); Sarkar 1999 – Fig. 1 (N = 7); Kovaleva and Gaevskaya 1982 – Fig. 1 (N = 4); Naidenova 1970 – Fig. 2 (N = 3); Diamant et al. 2004 – Figs. 1, 5, 6 (N = 6).

Type host: *Artediellus atlanticus* Jordan et Evermann, (Scorpaeniformes: Cottidae).

Other host: *Triglops murrayi* Günther (Scorpaeniformes: Cottidae).

Type locality: Off Grøtøya, Troms county, Northern Norway (70°24'N, 18°47'E; depth of 181–195 m).

Site of infection: Gall bladder.

Prevalence: *Artediellus atlanticus* – 100% (n = 4); *Triglops murrayi* – 75% (n = 4).

Deposition of specimens: University Museum Bergen, Zoological Collections; syntype slide, stained smear of *S. artedielli* sp. n. from *A. atlanticus* (HH-3; ZMBN-91884), ethanol sample (HH-3; ZMBN-91885). Vouchers: stained smear from *T. murrayi* (HH-39; ZMBN-91886), ethanol sample (HH-39, ZMBN-91887).

E t y m o l o g y: Named after the generic name of the type host.

Remarks. Comparative measurements of *S. artedielli* and similar members of the ‘balbianii’ group are provided in Table 3. *Sphaeromyxa diacanthusa* Sarkar, 2004, *S. intermediata* Moser et Noble, 1977, *S. pultai* Tripathi, 1954, and *S. reinhardtii* Jameson, 1929 were also described as having truncate ends, but the spore ends in these species are more rounded (convex) than in the species listed in Table 3. *Sphaeromyxa artedielli* shows similar spore length as *S. bonaerensis* Timi et Sardella, 1998, *S. cannolii* Sears,

Anderson et Greiner, 2011, and *S. sevastopoli* Naidenova, 1970. From these, the new species differs in spore width (narrower in *S. bonaerensis* and *S. sevastopoli*), polar capsule size (smaller in *S. bonaerensis*), polar capsule diameter (smaller in *S. sevastopoli*) or in having surface striations and equal-sized polar capsules (smooth valves and unequal polar capsules in *S. cannolii*). The hosts and geographic locations for these species also differ.

Sphaeromyxa minuta Polyansky, 1955 was described from halibut, *Hippoglossus hippoglossus* (Linnaeus), in the same region (Barents Sea). This species shows shorter and markedly narrower spores than *S. artedielli* and smaller polar capsules. Polyansky (1955) obtained measurements from stained smears, a method that could cause spore shrinking. However, he did not observe striations on the spore valves and the relative proportions of the spores such as the ID/PC index differs clearly.

Sphaeromyxa spp. in the ‘balbianii’ group have previously been detected in Arcto-boreal cottids in both the North Atlantic (Shul’man 1966, Lom 1970, 1984, Khan et al. 1986, Lom and Dyková 1992) and the North Pacific (Zhukov 1964, Aseeva 2002). *Sphaeromyxa japonica* Aseeva, 2002 from sculpins in the Sea of Japan shows larger spores, but also clearly differs in the relative extent of the intercapsular distance (ID/PC index > 1.6 in *S. japonica*).

Lom (1970, 1984) presented images of *S. balbianii* Thélohan, 1892 from *Triglops murrayi*, according to Khan et al. (1986) caught in the Grand Banks area off Newfoundland (see Lom and Laird 1969). Khan et al. (1986) also found 100% of *T. murrayi* ($n = 7$) from southern Labrador and the Grand Banks area infected by *S. balbianii*. Based on the dimensions of *S. balbianii* given by Parisi, 1912, Khan et al. (1986) concurred with Lom's (1970) identification. However, the dimensions given by Parisi (1912) for *S. balbianii* from sardines, *Sardina pilchardus* (Walbaum), are larger than those of Thélohan (1892, 1895, see Table 3) from *Gaidropsarus vulgaris* (Cloquet) with spores measuring $15\text{--}20\text{ }\mu\text{m} \times 5\text{--}6\text{ }\mu\text{m}$ and polar capsules measuring $7\text{ }\mu\text{m} \times 4.7\text{ }\mu\text{m}$. These dimensions are more in agreement with those of *S. artedielli* and the images presented by Lom (1970, 1984) do suggest that they may represent the same species.

Lom and Dyková (1992) presented an image of *S. magna* Zhukov, 1964 from *T. murrayi*, presumably representing the same material previously identified as *S. balbianii* (see Lom 1970, 1984). *Sphaeromyxa magna* was originally described from a Pacific liparid fish and differs clearly from *S. artedielli* in spore dimensions but not in shape or relative proportions.

Other myxosporean species were not detected in the bile, and myxosporean spores were not observed in the urinary system of the studied sculpins.

SSU rDNA sequences

Sequences of *S. artedielli* sp. n. were obtained from all three sculpins infected with spores that were measured. Almost identical sequences were also obtained from isolates from two sculpins containing large flat plasmodia without spores. The three partial SSU rDNA sequences of *S. artedielli* from *A. atlanticus* were identical over 1809 positions compared, except for an ambiguous signal (double peak) at position 1139 (HH3, HH72). The two partial SSU rDNA sequences of *S. artedielli* from *T. murrayi* were also identical, except for ambiguous signals at positions 715 and 884 in HH38 (1883 positions compared). However, there were 19 unambiguous substitutions and one nt gap in the sequences from *T. murrayi* when compared to those from *A. atlanticus* (98.9% identity, 1809 positions compared).

Phylogenetic analyses

The phylogenetic affinities of *Sphaeromyxa artedielli* sp. n. with other myxosporeans were firstly examined by ML analysis (GTR I + G) with 23 ingroup sequences (801 positions). The sequences were selected on the basis of Blast searches (Blastn and MegaBlast). The same relationships among *Sphaeromyxa* spp. were also observed in an analysis particularly restricted to sphaeromyxids as ingroups (Fig. 3). Both ML and MP analyses placed *S. artedielli* in a well supported clade with members of

Fig. 3. The phylogenetic position of *Sphaeromyxa artedielli* sp. n. among other *Sphaeromyxa* spp. Maximum likelihood tree based on partial SSU rDNA sequences (869 positions), including 11 *Sphaeromyxa* SSU rDNA sequences as ingroups and *Cystodiscus melleni* and *Myxidium coryphaenoideum* as outgroups. Nodal supports are indicated for ML (upper) and MP (lower). PG – likely pseudogene sequences. New sequences obtained in the present study indicated in bold.

the 'balbianii' group, in a subclade containing *S. longa* Dunkerly, 1921. The members of the 'incurvata' group sequenced so far, *Sphaeromyxa hellandi* Auerbach, 1909, *S. kenti* Whipps et Font, 2013 and *S. lycodi* Kristmundsson et Freeman, 2013, constituted a second major clade of the genus.

SSU rDNA variation in *Sphaeromyxa balbianii*

Two sequence variants of *S. balbianii* SSU rDNA (GenBank Accession numbers KF135225–6) were identified by PCR with primers Erib1–Erib10. The primers amplified two fragments of a different length corresponding to 1977 bp and 1480 bp. Their length difference was due to the presence of two indels. A long insertion (491 nt) within the longer sequence started from the conservative region positioned just after the end of the variable region 5 (V5) and ranged into V8 of the *S. balbianii* SSU rRNA gene. A second (6 nt) insertion also occurred in the V8 region. Besides the presence of these indels, the two sequences differed at 12 positions spread within the rest of the SSU rDNA.

DISCUSSION

The large, flat, polysporic plasmodia as observed in *Sphaeromyxa artedielli* sp. n. are the common type in the genus *Sphaeromyxa* – see Lom and Dyková (2006).

The large plasmodia showed a clear ectoplasmic region, which was not apparent in small plasmodia. The latter showed regions with rhizoids, suggesting that they occur attached to the gall bladder epithelium. We observed a distinct mucoid layer external to the margin of the large plasmodia. Previous ultrastructural studies on *Sphaeromyxa* spp. suggest this may represent a zone with mucus penetrated with microvillousities of the plasmodium (Gracia et al. 1997, Lom 2004).

Most members of the ‘balbianii’ group show truncate spore ends. *Sphaeromyxa artedielli* spores observed from both the valvular and sutural views show this truncation, suggesting that the spore ends are disc-like. Since there are few good characters in the ‘balbianii’ group, the diameter of the disc is an additional feature that may be used in species discrimination. We also found the ratio of intercapsular distance to (mean) polar capsule length (ID/PC index) to be useful in distinguishing some species. This is a character that can be readily recognized in light microscopy or from images. The fine longitudinal striations seen on the spores represent clefts between ridges on the valves (Lom 2004).

To date rDNA sequences of six *Sphaeromyxa* species have been deposited in GenBank. The SSU rDNA sequences of *S. artedielli* show the highest percentage of identity (aligned positions, neglecting gaps) with *S. longa* (DQ377691; 95.7%; 903 positions compared), *S. zaharoni* (AY538662; 94.6%; 1867 positions compared) and *S. balbianii* (KF135225–6, FJ417045; 94.6%, 94.6%, 93.3%; 1806, 1309 and 1116 positions compared, respectively). Our results show that *S. artedielli* is distinguished from *S. balbianii*, with which it may have been misidentified in the past (see above), by both morphological and molecular differences. The closest relative to *S. artedielli* according to the SSU rDNA sequences, *S. longa*, differs clearly by having longer, slightly arcuate spores, with more rounded ends.

To provide a proper sequence comparison of *S. artedielli* with *S. balbianii* (see above), the type species of genus *Sphaeromyxa*, full-length SSU rDNA sequences of *S. balbianii* were obtained in this study. Two SSU rDNA sequence variants of a different length were found. These represent the first significant rDNA genomic variation to be reported in the phylum Myxozoa. Such sequence variation has been found frequently in some microsporidians (Lom et al. 2001, Tay et al. 2005) and in parasitic protists (e.g. Pecher et al. 2004). Such a large sequence variation has been attributed to the existence of pseudogenes in addition to functional rRNA genes (Lom et al. 2001, Pecher et al. 2004), but also to strand jumping during PCR amplification (Brown et al. 2004).

Another explanation for several rDNA variants in a single host specimen is multiple infections by different genotypes. Our data indicate that the shorter copy of the SSU rRNA gene most probably represents a pseudogene in the

S. balbianii genome. The basis for this interpretation is that a substantial part of the large deletion contains conservative regions of the SSU rDNA, and their lack certainly affects the function of the rRNA molecule. In addition to indels, the two copies of the SSU rDNA in *S. balbianii* also differ at a number of positions. Such polymorphism, normally seen as ambiguous signals, was also observed in *S. artedielli* and is common intragenomic variation in the Myxozoa (Bartošová et al. 2009, Køie et al. 2013).

The almost complete SSU rDNA sequences of *S. artedielli* obtained from the host species *A. atlanticus* and *T. murrayi* diverged by 1.1%, but were found to be consistent within host species. This may suggest that these sculpins host particular genotypes of the myxosporean. Similar patterns have been seen in other Myxosporidia (Atkinson et al. 2011, Bartošová and Fiala 2011, Køie et al. 2013). The clupeid fish species *Sprattus sprattus* (Linnaeus) and *Clupea harengus* Linnaeus are both infected by Parvicapsulidae gen. sp. and *Ortholinea orientalis* (Shul'man et Shul'man-Albova, 1953) in the northeast Atlantic, but apparently by particular genotypes of the parasites (Karlsbakk and Køie 2011, Køie et al. 2013). However, more intensive sampling is needed to substantiate these interesting patterns, which may relate to the existence of cryptic myxosporean species.

Phylogenetic analyses placed *S. artedielli* in a well supported clade containing members of the ‘balbianii’ group of the *Sphaeromyxa* clade, in a subclade together with *S. longa*. The inclusion of *S. artedielli* in the analyses supports a bipartition of species of the genus *Sphaeromyxa* into two clades (Kristmundsson and Freeman 2013, Whipps and Font 2013). These clades represent species morphologically belonging to the ‘balbianii’ and ‘incurvata’ groups of Laird (1953), respectively. The divergence in the SSU rDNA sequences between the members of the ‘balbianii’ group and those of the ‘incurvata’ group sequenced so far is large, 13–16%. This suggests that these clades and morphological types should be assigned to different genera within the family Sphaeromyxidae. The arcuate spore shape, twisted valves and rounded spore ends may represent synapomorphies of the ‘incurvata’ clade.

However, a better basis for the establishment of such a taxon is needed; indeed some species appear to be intermediate. Since there are few developmental and morphological characters readily available in myxosporeans, molecular characters must also be considered. These may represent substitutions or indels in the conserved (core) parts of the rDNA (e.g. Bass et al. 2009) or size, composition and morphology of the often expanded variable regions in the Myxosporidia (Holzer et al. 2007, Jirků et al. 2007). Therefore, a better coverage of the genus with respect to rDNA sequences is required.

A problem encountered in the present study, valid for many groups of parasites with few morphological charac-

ters, is an expansion of the morphological species conception due to supplementary descriptions of a species from different hosts and localities worldwide. This applies to *S. balbianii*, which has been recorded from numerous species of shallow water fishes in Europe, West Africa, Atlantic and Pacific coast of North America and from Brazil. It is likely that these records from Arctic to tropical waters refer to several morphologically similar species 'of the 'balbianii' group, and sequence information would be valuable in order to distinguish as well as to characterize them. Therefore, to solve many of the taxonomic

problems in the Myxosporea, redescrptions of many of the 'old' and poorly described species should include the material from their type hosts and the localities, accompanied with molecular data (preferably rDNA sequences).

Acknowledgements. The present study was supported by the Norwegian Biodiversity Information Centre Project No. 701 842 19 (E.K.), and by the Czech Science Foundation (P506/11/P724 to P.B.). We would like to thank Dr. Hana Pecková for technical assistance (Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR).

REFERENCES

- ASEEVA N.L. 2002: New species of myxosporeans (Myxozoa, Myxosporea) from sculpins of the northwestern Japan Sea. *Acta Parasitol.* 47: 179–189.
- ATKINSON S.D., JONES S.R.M., ADLARD R.D., BARTHOLOMEW J.L. 2011: Geographical and host distribution patterns of *Parvicapsula minibicornis* (Myxozoa) small subunit ribosomal RNA genetic types. *Parasitology* 138: 969–977.
- BARTA J.R., MARTIN D.S., LIBERATOR P.A., DASHKEVICZ M., ANDERSON J.W., FEIGNER S.D., ELBRECHT A., PERKINS-BARROW A., JENKINS M.C., DANFORTH H.D., RUFF M.D., PROFIOUS-JUCHELKA H. 1997: Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. *J. Parasitol.* 83: 262–271.
- BARTOŠOVÁ P., FIALA I. 2011: Molecular evidence for the existence of cryptic species assemblages of several myxosporeans (Myxozoa). *Parasitol. Res.* 108: 573–583.
- BARTOŠOVÁ P., FIALA I., HYPŠA V. 2009: Concatenated SSU and LSU rDNA data confirm the main evolutionary trends within myxosporeans (Myxozoa: Myxosporea) and provide an effective tool for their molecular phylogenetics. *Mol. Phyl. Evol.* 53: 81–93.
- BASS D., CHAO E.E.-Y., NIKOLAEV S., YABUKI A., ISHIDA K., BERNEYA C., PAKZAD U., WYLEZICH C., CAVALIER-SMITH T. 2009: Phylogeny of novel naked filose and reticulose Cercaria: Granofilosea cl. n. and Proteomyxidea revised. *Protist* 160: 75–109.
- BROWN G.D., HUDSON K.L., REECE K.S. 2004: Multiple polymorphic sites at the ITS and ATAN loci in cultured isolates of *Perkinsus marinus*. *J. Euk. Microbiol.* 51: 312–320.
- DIAMANT A., WHIPPS C.M., KENT M.L. 2004: A new species of *Sphaeromyxa* (Myxosporea: Sphaeromyxina: Sphaeromyxidae) in devil firefish, *Pterois miles* (Scorpaenidae), from the northern Red Sea: morphology, ultrastructure, and phylogeny. *J. Parasitol.* 90: 1434–1442.
- FREEMAN M.A., YOKOYAMA H., OGAWA K. 2008: Description and phylogeny of *Ceratomyxa anko* sp. n. and *Zschokkella lophii* sp. n. from the Japanese anglerfish, *Lophius litulon* (Jordan). *J. Fish Dis.* 31: 921–930.
- GRACIA M.P., MAILLO P.A., AMIGÓ J.M., SALVADÓ H. 1997: Ultrastructural study of *Sphaeromyxa balbianii* Thélohan 1892 (Myxozoa, Myxosporea: Bivalvulida), a parasite of *Cepola macrophthalma* Linnaeus, 1758. *Acta Protozool.* 36: 171–179.
- HOLZER A.S., WOOTTEN R., SOMMERVILLE C. 2007: The secondary structure of the unusually long 18S ribosomal RNA of the myxozoan *Sphaerospora truttae* and structural evolutionary trends in the Myxozoa. *Int. J. Parasitol.* 37: 1281–1295.
- JIRKŮ M., FIALA I., MODRÝ D. 2007: Tracing the genus *Sphaerospora*: rediscovery, redescription and phylogeny of the *Sphaerospora ranae* (Morelle, 1929) n. comb. (Myxosporea, Sphaerosporidae), with emendation of the genus *Sphaerospora*. *Parasitology* 134: 1727–1739.
- KARLSBAKK E., KØIE M. 2011: Morphology and SSU rDNA sequences of *Ortholinea orientalis* (Shul'man and Shul'man-Albova, 1953) (Myxozoa, Ortholineidae) from *Clupea harengus* and *Sprattus sprattus* (Clupeidae) from Denmark. *Parasitol. Res.* 109: 139–145.
- KENT M.L., KHATTRA J., HEDRICK R.P., DEVLIN R.H. 2000: *Tetracapsula renicola* n. sp. (Myxozoa: Saccosporidae); the PKX myxozoan – the cause of proliferative kidney disease of salmonid fishes. *J. Parasitol.* 86: 103–111.
- KHAN R.A., BOWERING W.R., BURGEON C., LEAR H., PIPPY J.H. 1986: Myxosporean parasites of marine fish from the continental shelf off Newfoundland and Labrador. *Can. J. Zool.* 64: 2218–2226.
- KØIE M., KARLSBAKK E., EINEN A.-C.B., NYLUND A. 2013: A parvicapsulid (Myxozoa) infecting *Sprattus sprattus* and *Clupea harengus* (Clupeidae) in the Northeast Atlantic uses *Hydroides norvegicus* (Serpulidae) as invertebrate host. *Folia Parasitol.* 60: 149–154.
- KØIE M., KARLSBAKK E., NYLUND A. 2008: The marine herring myxozoan *Ceratomyxa auerbachii* (Myxozoa, Ceratomyxidae) uses *Chone infundibuliformis* (Annelida: Polychaeta: Sabelliidae) as invertebrate host. *Folia Parasitol.* 55: 100–104.
- KOVALEVA A.A., GAEVSKAYA A.V. 1982: [New data on Myxosporidia from the south-western Atlantic fishes.] *Parazitologiya* 16: 353–359. (In Russian.)
- KRISTMUNDSSON Á., FREEMAN M.A. 2013: Sphaeromyxids form part of a diverse group of myxosporeans infecting the hepatic biliary systems of a wide range of host organisms. *Parasites & Vectors* 6: 51.
- LAIRD M. 1953: The Protozoa of New Zealand intertidal zone fishes. *Trans. Roy. Soc. New Zeal.* 81: 79–143.
- LOM J. 1970: Protozoa causing diseases in marine fishes. *Am. Fish. Soc., Spec. Publ.* 5: 101–123.
- LOM J. 1984: Diseases caused by protists. In: O. Kinne (Ed.), *Diseases of Marine Animals*. Vol. IV, Part 1. John Wiley & Sons, Chichester, pp. 114–168.
- LOM J. 2004: Morphology and ultrastructure of *Sphaeromyxa noblei* sp. n. (Myxozoa), parasite of *Heteroclinus whiteleggii* (Pisces) from Australian New South Wales coast. *Folia Parasitol.* 51: 19–26.
- LOM J., DYKOVÁ I. 1992: *Protozoan Parasites of Fishes*. Elsevier, Amsterdam, 315 pp.

- LOM J., DYKOVÁ I. 2006: Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitol.* 53: 1–36.
- LOM J., LAIRD M. 1969: Parasitic Protozoa from marine and euryhaline fish of Newfoundland and New Brunswick. I. Peritrichous ciliates. *Can. J. Zool.* 47: 1367–1380.
- LOM J., NILSEN F., DYKOVÁ I. 2001: *Thelohania contejeani* Henneguy, 1892: dimorphic life cycle and taxonomic affinities, as indicated by ultrastructural and molecular study. *Parasitol. Res.* 87: 860–872.
- NAIDENOVA N.N. 1970: [Parasite fauna of fishes in the family Gobiidae from the Azov Sea.] *Biol. Morya, Kiev* 20: 84–113. (In Russian.)
- PARISI B. 1912: Primo contributo alla distribuzione geografica dei Missosporidi in Italia. *Atti, Soc. Ital. Sci. Nat. Mus. Civ.* 50: 283–299.
- PECHER W.T., ROBLEDO J.A.F., VASTA G.R. 2004: Identification of a second rRNA gene unit in the *Perkinsus andrewsi* genome. *J. Euk. Microbiol.* 51: 234–245.
- POLYANSKY YU.I. 1955: Parasites of the Fish of the Barents Sea. *Trudy Zool. Inst. AN SSSR* 19, 158 pp. (In Russian; English translation by Israel program for scientific translations, Jerusalem 1966.)
- SARKAR N.K. 1999: *Ortholinea gadusiae* sp. n. and *Sphaeromyxa opisthopterae* sp. n. (Myxozoa: Myxosporidia) from the clupeid fish of the Bay of Bengal, West Bengal, India. *Acta Protozool.* 38: 145–153.
- SEARS B.F., ANDERSON P., GREINER E.C. 2011: A new species of myxosporidian (Sphaeromyxidae), a parasite of lined seahorses, *Hippocampus erectus*, from the Gulf of Mexico. *J. Parasitol.* 97: 713–716.
- SHUL'MAN S.S. 1966: Myxosporidian Fauna of the SSSR. Nauka, Moskva-Leningrad, 504 pp. (In Russian, English translation by Amerind Publishing Co., New Delhi, 1988, 631 pp.)
- TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M., KUMAR S. 2011: MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731–2739.
- TAY W.T., O'MAHONY E.M., PAXTON R.J. 2005: Complete rRNA gene sequences reveal that the microsporidium *Nosema bombi* infects diverse bumblebee (*Bombus* spp.) hosts and contains multiple polymorphic sites. *J. Euk. Microbiol.* 52: 505–513.
- THÉLOHAN P. 1892: Myxosporidies de la vésicule biliaire des poissons. Espèces nouvelles. *Compt. Rend. Hebd. Séanc. Acad. Sci., Paris* 115: 1091–1094.
- THÉLOHAN P. 1895: Recherches sur les Myxosporidies. *Bull. Sci. France Belg.* 26: 100–394.
- TIMI J.T., SARDELLA N.H. 1998: Myxosporidia and coccidians parasitic on engraulid fishes from the coasts of Argentina and Uruguay. *Parasite* 5: 331–339.
- WHIPPS C.M., FONT W.F. 2013: Interaction of two myxozoan parasites from naked goby *Gobiosoma bosc*, in Lake Pontchartrain, Louisiana. *J. Parasitol.* 99: 441–447.
- ZHUKOV E.V. 1964: [Parasitofauna of fishes of Chukotka. III. Protozoa of marine and freshwater fishes. General conclusions.] *Parazitol. Sbornik* 22: 224–262. (In Russian.)

Received 27 May 2013

Accepted 7 July 2013