



Redescription and phylogenetic position of *Myxobolus aeglefini* and *Myxobolus platessae* n. comb. (Myxosporea), parasites in the cartilage of some North Atlantic marine fishes, with notes on the phylogeny and classification of the Platysporina

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ABSTRACT

Myxobolus aeglefini Auerbach, 1906 was originally described from cranial cartilage of North sea haddock (*Melanogrammus aeglefinus*), but has subsequently been recorded from cartilaginous tissues of a range of other gadoid hosts, from pleuronectids and from lumpsucker (*Cyclopterus lumpus*) in the North Atlantic and from a zoarcid fish in the Japan Sea (Pacific).

We obtained partial small-subunit rDNA sequences of *Myxobolus aeglefini* from gadoids and pleuronectids from Norway and Iceland. The sequences from gadoids and pleuronectids represented two different genotypes, showing 98.2% identity. Morphometric studies on the spores from selected gadoids and pleuronectids revealed slight but statistically significant differences in spore dimensions associated with the genotypes, the spores from pleuronectids were thicker and with larger polar capsules. We identify the morpho- and genotype from gadoids with *Myxobolus aeglefini* sensu Auerbach, and the one from pleuronectids with *Sphaerospora platessae* Woodcock, 1904 as *Myxobolus platessae* n. comb. The latter species was originally described from Irish Sea plaice (*Pleuronectes platessa*). *Myxobolus albi* Picon et al., 2009 described from the common goby *Pomatoschistus microps* in Scotland is a synonym of *M. aeglefini*. The Pacific *Myxobolus aeglefini* represents a separate species, showing only 97.4–97.6% identity to the Atlantic species. In phylogenetic analyses based on SSU rDNA sequences, these and some related marine chondrotropic *Myxobolus* spp. form a distinct well supported group. This clusters with freshwater and marine myxobolids and *Triangula* and *Cardimyxobolus* species, in a basal clade in the phylogeny of the Platysporina. Members of family Myxobolidae, *Ortholinea* spp. (currently Ortholineidae) and sequences of some other urinary system infecting myxosporeans form a well supported clade among members of the suborder Platysporina. Based on phylogenetic analyses, we propose the following changes to the classification of Myxosporea: i) Ortholineidae is dismantled and *Ortholinea* spp. transferred to Myxobolidae, and ii) Myxobolidae is transferred from suborder Variisporina to Platysporina.

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1. Introduction

Myxobolus aeglefini Auerbach, 1906 was originally described from cavities in cranial bones and cartilage of haddock (*Melanogrammus aeglefinus* (L.)) [1]. The infected haddock were caught in the North Sea according to Auerbach [2]. A similar parasite was found by Johnstone

& Woodcock [3,4] in Norway pout (*Trisopterus esmarkii* (Nilsson)) from Morecambe Bay, Irish Sea, and described as *Myxobolus esmarkii* Woodcock, 1906. These were subsequently considered synonymous [5,6,7]. Several additional gadoids have later been found to host *M. aeglefini* [8]. However, the host range of *M. aeglefini* has also been expanded to nongadoids, mostly pleuronectid flatfish [7,8,9]. However, *Sphaerospora platessae* Woodcock, 1904 was described from the cartilage in the otic capsules of Irish Sea plaice (*Pleuronectes platessa* L.), on the basis of a spore smear [10,11]. Being otherwise *Myxobolus*-like, Woodcock [10,11] interpreted the spores in the smears as spherical, therefore inclining towards placement in the genus *Sphaerospora* Thélohan, 1892. Nielsen et al. [12] did not find evidence for genetic

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differences between *M. 'aeglefini'* from gadoid and pleuronectid hosts in the Kattegat off Denmark, examining ribosomal small subunit gene (SSU rDNA) sequences with RFLP (restriction fragment length polymorphism). Hence, *S. platessae* could represent a senior synonym of *M. 'aeglefini'*.

Recently *Myxobolus albi* Picon-Camacho, Holzer, Freeman, Morris et Shinn, 2009 was described from the gill cartilage of the common goby *Pomatoschistus microps* (Krøyer) in Scotland [13]. A partial SSU rDNA sequence was provided for *M. albi*. Sequence identity led Cavin et al. [14] to consider a *M. 'aeglefini'* like myxosporean in the cartilage of Maine lumpfishes (*Cyclopterus lumpus* L.) as *M. albi*. This fish species has previously also been recorded as a host of *M. 'aeglefini'* [8,15].

All these marine cartilage infecting, nominal myxosporean species are very similar, differing slightly in their spore dimensions. However, spore measurements were obtained from material treated in different ways, such as fresh, fixed and air-dried stained smears. The methods employed could be responsible for the differences observed in spore measurements.

We therefore collected *Myxobolus* sp. spores from the cartilage from a range of gadoid and non-gadoid hosts, including the type hosts for *Myxobolus 'aeglefini'*, *Myxobolus esmarkii* and *Sphaerospora platessae*. We aimed at comparing the spore morphology and SSU rDNA sequences of the *M. 'aeglefini'*-like myxosporeans from gadoid and pleuronectid hosts, and reveal their phylogenetic position within Myxosporidia.

2. Material & methods

2.1. Samples

Fish with cranial and scleral *Myxobolus* spp. infections were collected both in Norway and Iceland (Table 1). The Norwegian material consists of samples of infected tissue from 2 haddock, 4 cod (*Gadus morhua* L.), 2 Norway pout, 1 silvery pout (*Gadiculus thori* Schmidt), 1 blue whiting (*Micromesistius poutassou* (Risso)), 2 ling (*Molva molva* (L.)), 1 flounder (*Platichthys flesus* (L.)) and 2 lemon sole (*Microstomus kitt* (Walbaum)). The Icelandic material represents 3 haddock, 2 cod, 2 plaice, 1 dab (*Limanda limanda* (L.)) and 2 flounder. *Myxobolus* spp. infections were verified by microscopy, and image series of fresh spores (1000× magnification) kept from some infected hosts for measurements. Corresponding samples for DNA were stored in 96% ethanol or transferred directly into DNA lysis buffer for extraction.

Table 1

Overview of the origin of the samples of *Myxobolus* spp. studied. Those used in the morphological study and providing spore measurements indicated under 'Morph.' Samples from which partial SSU rDNA sequences were obtained are indicated by their GenBank accession numbers. n = number, W = western, N = northern, SW = southwestern.

Host	Area	Position	Morph.	SSU rDNA sequence
Haddock	W Norway	60°56.4'N 4°57.0'E	X	KX886718
Haddock	W Norway	60°09.4'N 5°09.4'E	–	KX886719
Haddock (n = 2)	Iceland	? (from fish receiver)	X	KX886730
Cod (n = 2)	W Norway	60°52.9'N 4°52.0'E	X	KX886720
Cod	N Norway	70°45.4'N 25°58.1'E	X	KX886721
Cod	mid Norway	63°48.5'N 11°23.5'E	X	–
Cod (n = 2)	SW Iceland	64°09.1'N 21°55.7'W	X	KX886731 ^a
Norway pout	W Norway	60°15.2'N 5°18.5'E	–	KX886722
Norway pout	W Norway	60°16.3'N 5°10.7'E	–	KX886723
Silvery pout	W Norway	60°16.3'N 5°10.7'E	–	KX886724
Blue whiting	W Norway	60°16.3'N 5°10.7'E	–	KX886725
Ling	W Norway	60°09.4'N 5°09.4'E	–	KX886726
Ling	W Norway	60°16.4'N 5°13.3'E	–	KX886727
Plaice (n = 2)	SW Iceland	Faxaflói, exact position unknown ^b	X	KX886732 ^c
Dab	SW Iceland	64° 09.1'N 21°55.7'W	X	KX886733
Flounder	W Norway	60°16.4'N 5°13.3'E	–	KX886728
Flounder (n = 2)	SW Iceland	63°51.5'N 21°43.2'W	–	KX886734
Lemon sole (n = 2)	W Norway	60°35.2'N 4°49.0'E	–	KX886729

^a Partial LSU sequence KX886736.

^b From fish dealer.

^c Partial LSU sequence KX886737.

Table 2

Measurements of *Myxobolus 'aeglefini'* from haddock (type host) and cod. L = length, W = width, PC = polar capsule, SD = standard deviation, N = number of measurements.

	Haddock (2 fish)				Cod (5 fish)			
Measurements	Mean	SD	Range	N	Mean	SD	Range	N
Length (μm)	10.2	0.4	9.2–10.8	75	10.2	0.4	9.0–11.3	129
Width (μm)	9.9	0.4	9.0–10.6	76	9.8	0.3	9.0–10.5	131
L/W ratio	1.03	0.03	0.95–1.12	66	1.04	0.03	0.96–1.14	125
PC region length (μm)	5.5	0.4	4.8–6.4	42	5.6	0.4	4.5–6.7	106
PC region/L (%)	54	2	48–61	40	54	3	44–65	104
PC length (μm)	4.8	0.3	4.0–5.5	90	4.8	0.4	4.0–5.6	178
PC diameter (μm)	3.1	0.2	2.5–3.7	96	3.1	0.2	2.6–3.6	191
Thickness (μm)	7.1	0.3	6.6–7.7	13	6.9	0.4	5.8–7.7	73

The myxosporean *Triangula percae* Langdon, 1987, was sampled from redfin perch (*Perca fluviatilis* L.) from Lake Nagambie, Victoria, Australia. Myxospores were identified using microscopy and samples taken for DNA analysis.

2.2. Measurements

Spore measurements were taken from images using the software ImageJ (1.45 s) according to the recommendations of Lom & Arthur [16]. In addition, we measured the distance from anterior end to the midpoint of a line between the posterior end of each polar capsule (PC) ('PC region length'), which was used to calculate a PC region/length index describing the posterior extent of the polar capsules in the spore (% of length). When clearly seen, the number of coils of the polar filament was noted, and the diameter of the coils measured. The angles between the polar filament coils and the PC axis, and between the PC axes were also measured using ImageJ, from spores in perfect valvular view. Statistical analyses on spore measurements were done with Student's *t*-tests.

2.3. DNA analyses

DNA was extracted from the samples using the DNeasy® Tissue Kit protocol for animal tissues (Qiagen, Hilden, Germany). Different PCR's were performed on the Norwegian and Icelandic samples. The PCR primer combinations used to amplify SSU rDNA from the Norwegian samples were Mybo-F/18 g (see [17]) and Myxospec-F [18]/Mbol-R1,

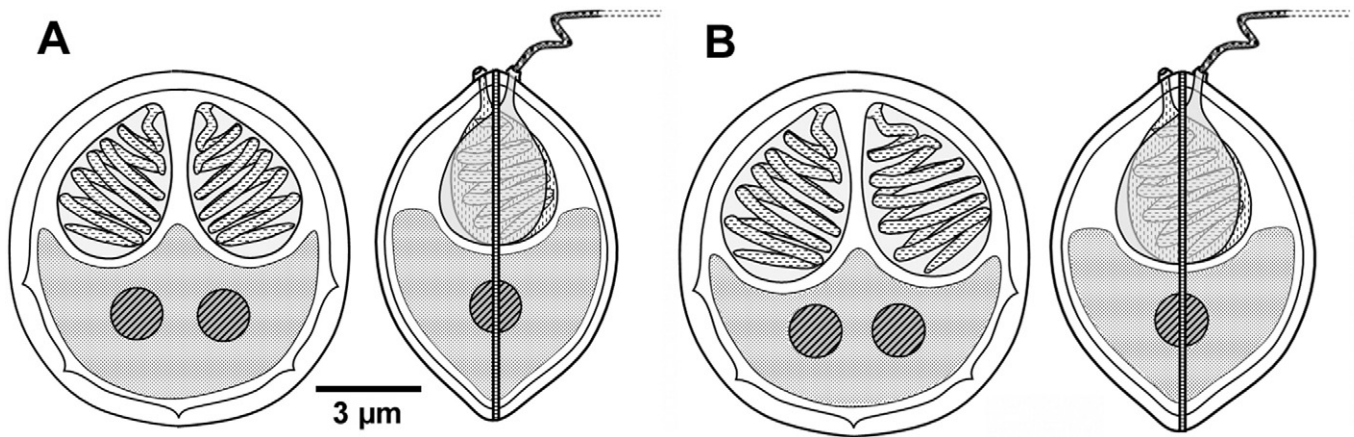


Fig. 1. A. Line drawing of *Myxobolus* 'aeglefini' from haddock in valvular and lateral sutural view. B. *Myxobolus platessae* n. comb. from plaice.

both PCR's with annealing temperature 57 °C. The sequences of the novel primers are 5'-tggtgatagcatggaacgaacaattg-3' (Mybo-F) and 5'-catgcaccaccatccaacg-3' (Mbol-R1). The 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 reverse and forward primer, 2 U of thermostable DNA polymerase (GoTaq) and 26 µl dH₂O. The PCR conditions were as previously described [19]. The PCR products were cleaned with ExoSAP-IT® (Affymetrix Inc.) and then sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit. The PCR amplifications for SSU rDNA from the Icelandic samples employed the primer

combinations M-alb-430fwd/M-alb-1470rev, and 1430fwd/18gM [20]. The sequences of the novel primers are 5'-aagacagcaggcgcaac-3' (M-alb-430fwd), 5'-tctcgctcgtttaaggaatc-3' (M-alb-1470rev). The PCR conditions were as previously [20], but extension was 45 s. Partial LSU sequences were obtained from two Icelandic samples using the primers NLF-184/NLR 1270 + NLR-1694, as described in Bartošová et al. [21]. The PCR amplifications for the Australian samples were done using the method described by Freeman et al. [20]. The sequencing was performed using the amplification primers, in both forward and reverse directions for all PCR products. The sequence data were assembled by eye or with the Vector NTI 11 software (Invitrogen).

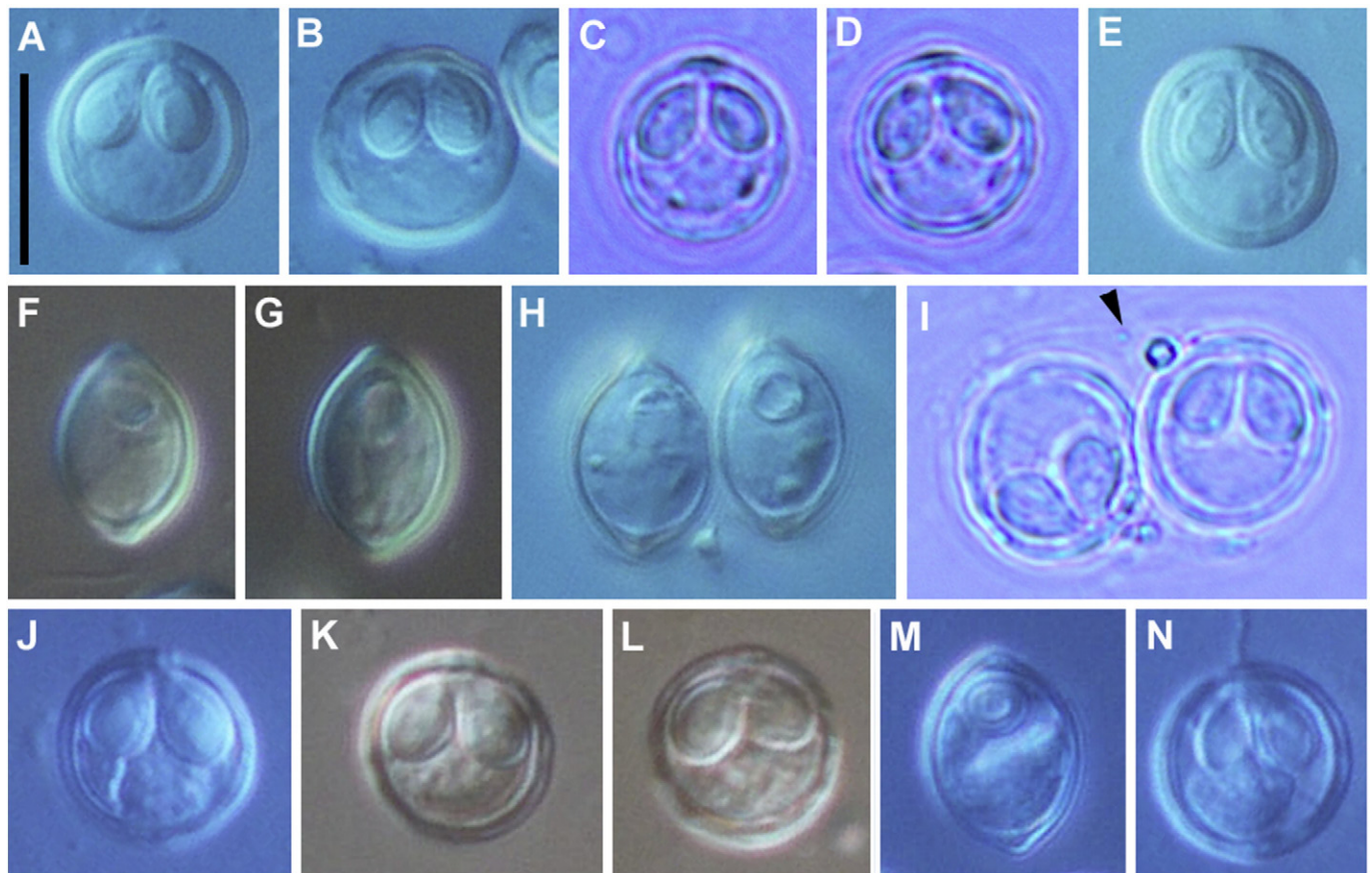


Fig. 2. *Myxobolus* spp. spores from the cartilage of marine fishes. A–I *Myxobolus* 'aeglefini', J–N *Myxobolus platessae* n. comb. A–E, I, J–L in valvular view, F–H, M in sutural view. I two spores in pansporoblast membrane (arrowhead). N with one polar capsule extruded. A–B, F–G, I from haddock, C–E, H from cod, J–N from plaice. All to some scale, scale in A 10 µm.

Table 3

Measurements of *Myxobolus platessae* n. comb. from plaice (type host) and dab. L = length, W = width, PC = polar capsule, SD = standard deviation, N = number of measurements.

	Plaice (1 fish)				Dab (1 fish)			
Measurements	Mean	SD	Range	N	Mean	SD	Range	N
Length (µm)	10.3	0.3	9.8–10.9	53	10.5	0.3	10.0–11.2	20
Width (µm)	10.1	0.2	9.7–10.7	40	10.3	0.3	9.6–10.7	18
L/W ratio	1.01	0.02	0.96–1.08	34	1.03	0.03	0.99–1.08	17
PC region length (µm)	5.8	0.3	5.1–6.3	43	6.1	0.1	6.0–6.3	4
PC region/L (%)	57	3	50–61	43	59	1	57–60	4
PC length (µm)	5.3	0.2	4.8–5.8	53	5.5	0.3	5.1–5.9	10
PC diameter (µm)	3.5	0.2	3.0–3.9	65	3.6	0.2	3.3–3.9	11
Thickness (µm)	7.6	0.3	6.8–8.0	15				0

2.4. Phylogenetic analyses

CLUSTAL X [22] was used for the initial SSU rDNA sequence alignments of taxa chosen to cover the complete phylogenetic range of the Platysporina including the urinary-infecting groups Myxobolidae Shul'man, 1953 and Ortholineidae Lom et Noble, 1984, currently assigned to the Variisporina. Our preliminary analyses revealed that *Triangula percae* was a basal member of the Platysporina, and this sequence (KX886735) was therefore included to improve resolution. The final alignment was manually edited using the BioEdit sequence alignment editor [23] and contained 2524 characters and 127 taxa including the novel sequences. Phylogenetic analyses were performed using the maximum likelihood methodology in PhyML [24] with the general time-reversible substitution model GTR + G6 + I selected as the most suitable, with 1000 bootstrap repeats. Bayesian inference (BI) analysis was performed using MrBayes v. 3.2.1 [25]. For the BI analysis, models of nucleotide substitution were first evaluated for the alignment using MrModeltest v. 2.2 [26]. The most parameter-rich evolutionary model based on the AIC was the general time-reversible, GTR + I + G model of evolution. Therefore, the settings used for the analysis were nst = 6, with the gamma-distributed rate variation across sites and a proportion of invariable sites (rates = invgamma). The priors on state frequency were left at the default setting (Prset statefreqpr = dirichlet (1,1,1,1)). Posterior probability distributions were generated using the Markov Chain Monte Carlo (MCMC) method with four chains being run simultaneously for 2,000,000 generations. Burn in was set at 2500 and trees were sampled every 100 generations making a total of 7500 trees used to compile the majority rule consensus trees.

Percentage divergence matrices were constructed from selected aligned taxa in CLUSTAL X using the neighbour-joining method based on the Kimura 2-parameter model [27].

3. Results

3.1. Sites

In gadids, foci of infection occurred in both the sclera of the eye and in the cranial cartilage, particularly around the cranial cavity. In heavily

infected eyes, confluent white irregular masses occurred. In lighter infections with separate foci, these could appear entire (0.4–1 mm in diameter), but often showed a lobed star-like growth, reaching 7 mm in diameter.

In pleuronectids, small foci (typically 0.4 mm) occurred in the cranium, most commonly in the inner part towards the cranial cavity and surrounding the stato-acoustic organs. Foci also occurred in the sclera of the eyes. A yellowish mass was seen in the otic capsules of some heavily infected plaice and lemon sole from Norway, containing large numbers of free spores.

Microscopy on preparations from foci in both gadids and pleuronectids revealed myxoboloid spores that occurred free or in pairs within pansporoblasts released from destroyed plasmodia.

3.2. Description of spores

3.2.1. *Myxobolus 'aeglefini'* from gadids (Table 2; Fig. 1A, Fig. 2A–I)

Spores rounded in valvular view, biconvex in sutural view. Slight elevation often apparent in association with PC openings. Valves smooth, thick (0.4–0.5 µm). Suture straight, in ridge produced by the valves, protruding 0.5–0.7 µm. Notches at sutural edge occasionally evident, most commonly 4–7 in posterior part but up to 9 seen. Polar capsules pyriform, equal, with 5–6 coils of polar filament (N = 50 observations). Coils perpendicular or oblique to PC axis in valvular view, angles 43–90°. Coil diameter 2.1 µm (1.9–2.3 µm, N = 36), representing 64 ± 4% (56–75%) of PC diameter. Apparently completely extruded polar filaments 29 (25–33) µm long (N = 18). Angle between PC axes in valvular view 49–81° (66 ± 7°) (N = 131).

3.2.2. *Myxobolus* sp. from flatfish (Table 3, Fig. 1B; Fig. 2J–N)

Spores rounded in valvular view, biconvex in sutural view. Slight elevation occasionally apparent in association with PC openings. Valves smooth, thick (0.5 µm). Suture straight, in ridge produced by the valves, protruding 0.5–0.7 µm. Notches at sutural edge occasionally evident, most commonly 4–6 in posterior part but up to 9 seen. Polar capsules pyriform, equal, with 5–6 coils of polar filament (N = 20 observations). Coils perpendicular or oblique to PC axis in valvular view, angles 58–90°. Coil diameter 2.1 µm (1.9–2.5 µm, N = 26), representing 63 ± 4% (57–70%) of PC diameter. Apparently completely extruded polar filaments 31–42 µm long (N = 16). Angle between PC axes in valvular view 55–81° (68 ± 5°) (N = 65).

3.3. Comparison of spores from gadids and pleuronectids

The spores of *M. 'aeglefini'* and *Myxobolus* sp. from pleuronectids are very similar in dimensions and polar capsule arrangement. However, the polar capsules of *Myxobolus* sp. are significantly longer ($T = 10.2$, $P < 0.001$), and with a larger diameter ($T = 14.9$, $P < 0.001$) than those of *M. 'aeglefini'*. This is reflected in a significantly longer polar capsule region in the spores of *Myxobolus* sp. ($T = 5.0$, $P < 0.001$). The spores of *Myxobolus* sp. also tend to be wider ($T = 5.1$, $P < 0.001$) and thicker ($T = 5.9$, $P < 0.001$).

Table 4

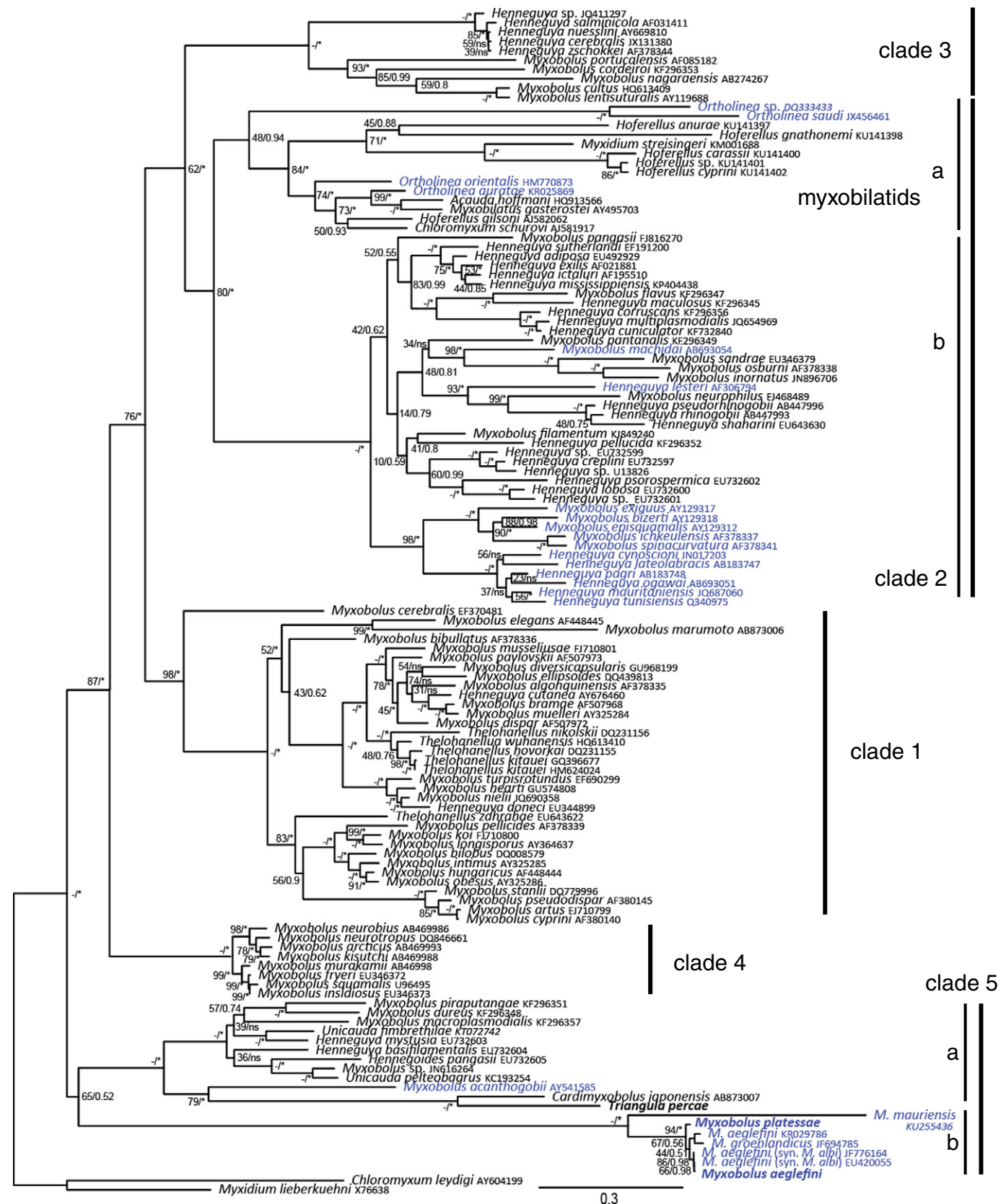
Percentage identities of SSU rDNA sequences, above diagonal, and number of bases compared, below diagonal, for chondrotropic *Myxobolus* spp. in Clade-5b of the Platysporina.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
(1) <i>Myxobolus 'aeglefini'</i> (gadoids, this study)	–	98.24	100	99.93	97.60	97.22	82.49
(2) <i>Myxobolus platessae</i> (pleuronectid flatfish, this study)	1479	–	98.24	98.30	97.36	97.22	82.43
(3) <i>M. 'aeglefini'</i> (syn <i>M. albi</i>) (common goby: EU420055)	1479	1479	–	99.87	97.76	96.46	82.40
(4) <i>M. 'aeglefini'</i> (syn <i>M. albi</i>) L (Atlantic lumpfish: JF776164)	1469	1469	1496	–	97.86	97.32	82.43
(5) <i>M. 'aeglefini'</i> (porous-head eelpout: KR029786)	1476	1476	1521	1493	–	97.96	82.93
(6) <i>M. groenlandicus</i> (Greenland halibut: JF694785)	1477	1477	1555	1494	1520	–	83.52
(7) <i>M. mauriensis</i> (river herring; <i>Alosa</i> spp. KU255436)	1456	1457	1489	1446	1470	1572	

3.4. Comparison of SSU rDNA sequences

The partial SSU rDNA sequences obtained from 15 samples representing 7 gadoid species were identical and there were no ambiguous positions. The partial sequences from 8 samples from 4

pleuronectid species were also identical, but two ambiguous positions occurred. The sequences from gadoids and pleuronectids differed by 25 substitutions and an indel, disregarding two ambiguous positions. Table 4 shows the percentage identities for *M. aeglefini* to related species, with a 98.24% identity to its closest relative, *M. platessae*. The



most basal member of the clade *Myxobolus mauriensis* Lovy et Hutcheson, 2016 is more distantly related with a percentage identity of only 82.49%.

3.5. Phylogenetic position

The *Myxobolus* sequences obtained in the current study, from both gadoids and pleuronectids, formed a well-supported clade with the related marine species *Myxobolus groenlandicus* Buchmann, Skovgaard et Kania, 2012 and *M. mauriensis* and the synonymous species *M. albi* (Fig. 3). This clade formed as a sister to another robustly supported group that contained numerous freshwater myxobolids (members of genera *Myxobolus* Bütschli, 1882, *Henneguya* Thélohan, 1892, *Hennegoides* Lom, Tonguthai et Dyková, 1991 and *Unicauda* Davis, 1944) and the sequences for *Cardimyxobolus japonensis* Li et Sato, 2014 and *Triangula percae* (sequence from this study). The latter two are both freshwater species, in genera currently assigned to the Ortholineidae (Variisporina), and formed a well-supported sub-clade with the marine myxobolid *Myxobolus acanthogobii* Hoshina, 1952. This whole grouping (Clades 5a/b) was only moderately supported but was very robustly placed as the most basal clade in the phylogeny of the Platysporina. Four other major clades were highly supported in both analyses. Clade 1 was dominated by *Myxobolus* spp. and *Thelohanellus* spp., Clade 3 with *Henneguya* and *Myxobolus* spp. and Clade 4 with salmonid *Myxobolus* spp. These clades contain only freshwater species. Clade 2 contained two subclades, both with freshwater and marine members. The largest subclade (Clade 2b) contained members of the platysporine genera *Henneguya* and *Myxobolus*, while the other subclade (Clade 2a) harboured currently non-platysporine members. These were mainly myxobolids (genera *Myxobolus* Davis, 1944; *Acauda* Whipps, 2011 and *Hoferellus* Berg, 1898) and *Ortholinea* spp. (Ortholineidae), but also included *Myxidium streisingeri* Whipps, Murray et Kent, 2015 from zebrafish, *Danio rerio* (Hamilton) and *Chloromyxum schurovi* Shul'man et Ieshko, 2003 from Atlantic salmon, *Salmo salar* L. (Fig. 3).

4. Discussion

4.1. Identification of *Myxobolus* 'aeglefini'

Myxobolus 'aeglefini' was originally described from haddock caught in the North Sea off Germany [1,2,28]. The spore measurements reported by Auerbach [1] are large compared to those obtained from haddock in the present study. He found them to be 10.8–11.7 long, 9.9–10.4 µm wide, and 7.2–9 µm thick. Polar capsule length was reported to be 4.5–5 µm. Hence particularly Auerbach's [1] spore lengths exceed the measurements obtained in the present study. However, Karlsbakk [29] found that the spore dimensions of 8 myxosporeans described by Auerbach [5,30–32] from Norway generally were smaller than in the original descriptions, with an apparent systematical difference. The spore dimensions tended to be some 89% of those originally reported. Such a correction of Auerbach's [1] measurements place them close to those obtained in the present study. Also, more recent studies of *M. 'aeglefini'* from gadids [33,34] have found spores significantly smaller than the

ranges given by Auerbach [1]. The use of an ocular scale when measuring spores is much less accurate than the now widespread use of images. Therefore, spore measurements from images tend to show less variation than reported in older studies.

Our partial SSU rDNA sequences of *Myxobolus* sp. from cartilage in a range of gadoids over a large geographic area are identical. These therefore represent a single species, which despite some smaller dimensions compared with the original description must be identified with *M. 'aeglefini'*.

4.2. Establishment of *Myxobolus platessae* n. comb.

Sphaerospora platessae Woodcock, 1904 was briefly described on the basis of spores in dried smears from cysts in the otic capsule cartilage of plaice, caught in the northeastern Irish Sea. Spores were round in the smears, and Woodcock [10,11] interpreted them as likely to have been spherical. They measured 8–9 µm in diameter, had smooth valves and two prominent polar capsules. Extruded polar filaments were reported to reach 70 µm. This species have subsequently never been recorded again. However, dab, plaice and other flatfish species have been recorded as a host of *Myxobolus* 'aeglefini' in Irish waters [9,35], North Sea [7, 36] and Kattegat [37]. The parasite occurs particularly in cranial cartilage, including the otic capsules [7], hence sharing both host, site and tissue preferences with *S. platessae*. Kabata [7] provided the following average measurements of *Myxobolus* 'aeglefini' spores from plaice; length 11.2 µm, width 10.6 µm, thickness 7.0 µm and polar capsule length 5.9 µm. While his spores were measured after lugol staining, their dimensions are most similar to the present ones from plaice and dab, particularly polar capsule size. His images show large polar capsules extending clearly post-equatorially in the spores, the only useful morphological characters found in the present study that may help separate *M. 'aeglefini'* from gadids and flatfish. The morphological differences between *Myxobolus* 'aeglefini' from plaice and blue whiting led Gaevskaya & Kovaleva [34] to suspect that the plaice parasite could be a separate species. Based on differences in the morphology of the spores, different hosts, and distinct SSU rDNA sequences, we consider our material from gadoids and pleuronectids to represent two separate species, which we identify with *Myxobolus* 'aeglefini' Auerbach, 1906 and *Sphaerospora platessae* Woodcock, 1904 respectively, the latter transferred to genus *Myxobolus* as *Myxobolus platessae* (Woodcock, 1904) n. comb.

4.3. Synonymy and host range of *M. 'aeglefini'*

Our sequences of *Myxobolus* 'aeglefini' from gadoids show very high identity (99.5%) with a sequence (EU420055) of *M. albi*, described from the gill cartilage of common goby in Scotland [13]. This *M. albi* sequence was submitted with some errors which have now been corrected, and proves to be 100% identical to our *M. 'aeglefini'* sequences. The morphology of the *M. albi* spores is similar to *M. 'aeglefini'*, but the dimensions reported are slightly smaller. *Myxobolus albi* is here considered a synonym of *M. 'aeglefini'*. *Myxobolus albi* infections were also detected in the cartilage of captive lumpsucker originating in Maine [14]. This identification was based on sequence similarity; lumpsucker has previously

Fig. 3. Maximum likelihood (ML) topology of 127 myxosporean taxa from the Platysporina (outgroup Variisporina), inferred using the GTR + G6 + I model of nucleotide substitutions, a gamma-distribution and invariable sites on an alignment of 2524 characters of 18S rDNA sequences. Numbers at the nodes represent ML bootstrap percentages/and Bayesian posterior probabilities; (—/—) represents full support for both methodologies, (ns) denotes a different branching for the Bayesian tree. Taxa in blue are found in marine fish, with sequences from the present study in bold. There are five major well-supported clades identified (labelled 1–5). All *Myxobolus* sequences obtained in the current study formed a well-supported clade with the related species *Myxobolus groenlandicus* and *Myxobolus mauriensis* and the synonymous species *Myxobolus albi*. This clade formed as a sister to another robustly supported group that contained numerous members from the Myxobolidae and the sequences for *Cardimyxobolus japonensis* and *Triangula percae* (bold, this study), which formed a well-supported sub-clade with *Myxobolus acanthogobii*. This whole grouping (Clades 5a/b) was only moderately supported but was very robustly placed as the most basal clade in the phylogeny of the Platysporina. Clade 1 is dominated by *Myxobolus* and *Thelohanellus* spp., Clade 3 with *Henneguya* and *Myxobolus* spp. and Clade 4 with salmonid-infecting *Myxobolus* spp. Clade 2 contained a major subclade (Clade 2b) with members of the platysporine genera *Henneguya* and *Myxobolus*, and a subclade with currently non-platysporine members from the Myxobolidae and Ortholineidae (Clade 2a).

been reported to be infected with *M. 'aeglefini'* [8,15]. However, the *M. albi* sequence from lumpsucker (JF776164) show 99.9% identity (see Table 4) with our *M. 'aeglefini'* sequences. Hence both common goby and lumpsucker are hosts to *Myxobolus 'aeglefini'*, evidence suggesting that this myxosporean is not specific to gadoids, and demonstrates low host specificity.

Therefore, this could mean that flatfish could become infected also with *M. 'aeglefini'*. However, the present observations based on 8 flatfish individuals from 4 species, both from Iceland and Norway, suggest they only host *M. platessae* n. comb. infections. The sequence assigned to *M. 'aeglefini'* from the porous-head eelpout *Bothrocara hollandi* (Jordan & Hubbs) from Korea (KR029786) [38], only has an identity of 97.6% to our sequences for *M. 'aeglefini'* in this study, which suggests that it is a novel species. *Myxobolus lairdi* Moser et Noble, 1977 from roundnose grenadiers *Coryphaenoides rupestris* Gunnerus (Macrouridae) in western Norway [39] is also very similar to *M. 'aeglefini'*, but this possible synonymy needs to be confirmed by rDNA sequencing as valid species similar to *M. 'aeglefini'* do exist.

4.4. Phylogenetic relationships

Myxobolus 'aeglefini' and *M. platessae* n. comb. groups closely with *M. groenlandicus* and a *M. 'aeglefini'* sequence from Korea, and the recently described *M. mauriensis*. These are all from marine fishes, and the Atlantic species *M. 'aeglefini'*, *M. platessae* n. comb., *M. groenlandicus* and *M. mauriensis* are tissue specific, developing in cartilage. *Myxobolus groenlandicus* cause cartilage hypertrophy producing cylindrical structures at the position of the proximal pterygiophores of the unpaired fins in Greenland halibut (*Reinhardtius hippoglossoides* (Walbaum)), affecting adjoining musculature [40]. *Myxobolus mauriensis* produce pseudocysts in the pleural ribs of river herrings (*Alosa* spp.), also extending into the musculature [41]. However, *M. 'aeglefini'* from the Korean zoarcid *B. hollandi* was reported to produce pseudocysts in the musculature [38]. Their distribution in the musculature seems compatible with a possible origin from ribs. Indeed, such pseudocysts in the same host from Japanese waters were found to be encased in cartilage [42]. Hence Clade 5b appears to represent marine chondrotropic *Myxobolus* spp. These species also share remarkably similar myxospore morphology.

The basal position of the Clade 5 suggests that the ancestral form of the Platysporina could have been *Myxobolus*-like. The distribution of *Henneguya* spp. and *Thelohanellus* spp. in different clades show that the evolution of valvular appendages and polar capsule losses in the myxospores are convergent [43,44]. Clade 5 also includes the species *Cardimyxobolus japonensis* and *Triangula percae* (this study), the only members of these genera from which SSU rDNA sequences are currently available. But neither of these are generic type species. The genera *Cardimyxobolus* Ma, Dong et Wang, 1982 and *Triangula* Chen et Hsieh, 1984 are currently classified within the family Ortholineidae [45]. However, the present findings suggest they may be basal Platysporina. Such a position is also supported by their being histozoic and possessing smooth valves, as opposed to ridged spores and coelozoic development in the urinary system that is typical for *Ortholinea* spp. and myxobolids (see below).

Our analysis of the Platysporina based on the SSU rDNA sequences provides support for four major clades in addition to the basal Clade 5. These major clades could represent families or even superfamilies in a future revision of the group, now difficult due to the lack of suitable defining characters (synapomorphies). However, our analysis provides robust support for an inclusion of Myxobolidae and *Ortholinea* spp. (Ortholineidae) in the Platysporina, these families are currently classified in the Variisporina [45,46]. Family Ortholineidae is not supported by phylogenetic analyses, some *Ortholinea* spp. are close to *Myxobolus gasterostei* Parisi, 1912 (type species of *Myxobolus*) ([47,48], present study). *Myxidium streisingeri* also groups in this clade, a species showing several traits in common with genus *Neomyxobolus* Chen et Hsieh, 1960,

currently placed in the Ortholineidae. This includes coelozoic development in the urinary system and 3 prominent sutural ridges. At variance with *Neomyxobolus* spp. the polar capsules in *M. streisingeri* are placed at the spore ends [49], which could represent a derivation from the typical *Neomyxobolus* spore organisation. However, the phylogenetic placement of *Neomyxobolus ophiocephalus* Chen et Hsieh, 1960, the type species of *Neomyxobolus*, is currently unknown, hampering this transfer now. The occurrence of the sequence of *Chloromyxum schurovi* in the Myxobolidae-clade is problematic. Firstly, its congeners from freshwater teleosts group in another major clade, the 'freshwater Gb clade' [18]. Secondly, it is very similar to sequences of *Myxidium giardi* Cépède, 1906 (AJ582213; 99.3% identity) and *Zschokkella* sp. (AJ581918; 98.1% identity) from eel (*Anguilla anguilla* L.), representing different myxosporean genera. The sequences of these myxosporeans therefore need confirmation.

4.5. Revision of Platysporina

We propose to transfer Family Myxobolidae from Variisporina to Platysporina. This is based on the present and some previous phylogenetic analyses [18,20,50] on SSU rDNA sequences. Family Ortholineidae is dismantled and *Ortholinea* spp. transferred to Myxobolidae. *Cardimyxobolus*, *Neomyxobolus* and *Triangula* are transferred to Platysporina but must be considered *incertae sedis*, pending the sequencing of the generic type species and a revision (split) of family Myxobolidae. Genus *Kentmoseria* Lom et Dyková, 1995 is retained in the Variisporina, and is provisionally placed in family Sinuolineidae Shul'man, 1959.

Suborder Platysporina Kudo, 1920 emend.

Emendation based on diagnosis in Lom & Dyková [45].

Spores as a rule flattened parallel to the sutural plane, bilaterally symmetrical. Two polar capsules, one occasionally rudimentary or absent. Polar capsules generally positioned at or near spore apex, usually positioned in the sutural plane; but occasionally in plane perpendicular to this. Typically histozoic in various tissues, occasionally coelozoic in the urinary system. Plasmodia polysporic; sporogony in pansporoblasts. Plasmodia up to several mm in size, when histozoic usually enveloped by the connective tissue of the host and appear like small cysts. Parasites of freshwater and marine teleosts, occasionally in amphibians; invertebrate hosts Oligochaeta.

Family Myxobolidae Shul'man, 1953 emend.

Emendation based on diagnosis in Whipps [51].

Spores elongated, spherical or compressed, with 2 striated valves, and 2 polar capsules at 1 end of the spore. Suture straight, perpendicular to polar capsule plane. Spores with or without caudal projections or filaments. Polysporic plasmodia; sporogony in pansporoblasts. Parasites of urinary system of freshwater and marine fishes.

Three life cycles known, with triactinomyxon type actinospores developing in the intestinal epithelium of oligochaeta [48,52,53]. The family includes 4 genera: *Myxobolus*; *Acauda*; *Hoferellus* and *Ortholinea* Shul'man, 1962.

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References

- [1] M. Auerbach, Ein *Myxobolus* im Kopfe von *Gadus aeglefinus* L., Zool. Anz. 30 (1906) 568–570.
- [2] M. Auerbach, Unsere heutigen Kenntnisse über die geographische Verbreitung der Myxosporidien, Zool. Jahrb. Abt. Syst. 30 (1911) 471–494.
- [3] J. Johnstone, H.M. Woodcock, On a myxosporidian infection of *Gadus esmarkii*. With a note on the identification of the parasite, Rep. Lancashire Sea-Fish. Lab. 15 (1906) 204–208.

- [4] J. Johnstone, H.M. Woodcock, On a myxosporidian infection of *Gadus esmarkii*, with a note on the identification of the parasite, *Trans. Liverpool Biol. Soc.* 21 (1907) 304–308.
- [5] M. Auerbach, Bemerkungen über Myxosporidien, *Zool. Anz.* 34 (1909) 65–82.
- [6] R.R. Kudo, Studies on Myxosporidia. Synopsis of genera and species of Myxosporidia, Illinois, Biol. Monogr. 5 (1920) 241–503 [= 1–265].
- [7] Z. Kabata, Note on a new host of *Myxobolus 'aeglefini'*, *Parasitology* 47 (1957) 165–168.
- [8] P. van Banning, in: C.J. Sindermann (Ed.), Cranial Myxosporidiosis of Fishes, ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish, 1984 (4 pp).
- [9] M.F. Mulcahy, Diseases of flatfish, *Bull. Eur. Assoc. Fish Pathol.* 22 (2002) 86–94.
- [10] H.M. Woodcock, On myxosporidia in flat-fish, *Rep. Lancashire Sea Fish. Lab.* 12 (13) (1904) 46–62.
- [11] H.M. Woodcock, On myxosporidia in flat-fish, *Proc. Trans. Liverpool Biol. Soc.* 18 (1904) 126–142.
- [12] C.V. Nielsen, M. Køie, C. Szekely, K. Buchmann, Comparative analysis of 18S rRNA genes from *Myxobolus 'aeglefini'* Auerbach, 1906 isolated from cod (*Gadus morhua*), plaice (*Pleuronectes platessa*) and dab (*Limanda limanda*), using PCR-RFLP, *Bull. Eur. Assoc. Fish Pathol.* 22 (2002) 201–205.
- [13] S.M. Picon-Camacho, A.S. Holzer, M.A. Freeman, D.J. Morris, A.P. Shinn, *Myxobolus albi* n. sp. (Myxozoa) from the gills of the common goby *Pomatoschistus microps* Krøyer (Teleostei: Gobiidae), *J. Eukaryot. Microbiol.* 56 (2009) 421–427.
- [14] J.M. Cavin, S.L. Donahoe, S. Frasca Jr., C.J. Innis, M.J. Kinsel, T. Kurobe, L.M. Naples, A. Nyaok, C.P. Poll, E.P.S. Weber III, *Myxobolus albi* infection in cartilage of captive lumpfish (*Cyclopterus lumpus*), *J. Vet. Diagn. Investig.* 24 (2012) 516–524.
- [15] J. Lom, Protozoa causing diseases in marine fishes, *Am. Fish. Soc. Spec. Publ.* 5 (1970) 101–123.
- [16] J. Lom, J.R. Arthur, A guideline for the preparation of species descriptions in Myxosporea, *J. Fish Dis.* 12 (1989) 151–156.
- [17] E. Karlsbakk, M. Køie, 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 (2011) 139–145.
- [18] I. Fiala, The phylogeny of Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis, *Int. J. Parasitol.* 36 (2006) 1521–1534.
- [19] M. Køie, E. Karlsbakk, A. Nylund, The marine herring myxozoan *Ceratomyxa auerbachii* (Myxozoa: Ceratomyxidae) uses *Chone infundibuliformis* (Annelida: Polychaeta: Sabellidae) as invertebrate host, *Folia Parasitol.* 55 (2008) 100–104.
- [20] M.A. Freeman, H. Yokoyama, K. Ogawa, Description and phylogeny of *Ceratomyxa anko* sp. n. and *Zschokkella lophii* sp. n. from the Japanese anglerfish, *Lophius litulon* (Jordan), *J. Fish Dis.* 31 (2008) 921–930.
- [21] P. Bartošová, I. Fiala, V. Hypša, 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. Phylogenet. Evol.* 53 (2009) 81–93.
- [22] J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acids Res.* 25 (1997) 4876–4882.
- [23] T.A. Hall, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucleic Acids Symp. Ser.* 41 (1999) 95–98.
- [24] S. Guindon, J.F. Dufayard, V. Lefort, M. Anisimov, W. Hordijk, O. Gascuel, New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0, *Syst. Biol.* 59 (2010) 307–321.
- [25] F. Ronquist, J.P. Huelsenbeck, MrBayes 3: Bayesian phylogenetic inference under mixed models, *Bioinformatics* 19 (2003) 1572–1574.
- [26] J.A.A. Nylander, F. Ronquist, J.P. Huelsenbeck, J.L. Nieves-Aldrey, Bayesian phylogenetic analysis of combined data, *Syst. Biol.* 53 (2004) 47–67.
- [27] N. Saitou, M. Nei, The neighbour-joining method: a new method for reconstructing phylogenetic trees, *Mol. Biol. Evol.* 4 (1987) 406–425.
- [28] M. Auerbach, Weitere Mitteilungen über *Myxobolus 'aeglefini'* Auerb., *Zool. Anz.* 31 (1907) 115–119.
- [29] E. Karlsbakk, The dimensions of the spores of some marine Myxosporea described from Norwegian marine fishes by M. Auerbach 1909–1910, Book of Abstracts 16th International Conference on Diseases of Fish and Shellfish Tampere September 2–6, 2013, p. 208.
- [30] M. Auerbach, Bericht über eine Studienreise nach Bergen, *Verhandl. Naturwiss. Vereins Karlsruhe* 21 (1909) 37–73.
- [31] M. Auerbach, Biologische und morphologische Bemerkungen über Myxosporidien, *Zool. Anz.* 35 (1910) 57–63.
- [32] M. Auerbach, Cnidosporidienstudien, *Zool. Anz.* 35 (1910) 767–777.
- [33] R.A. Shotter, Notes on myxosporidian parasites of whiting *Odontogadus merlangus* (L.) from the northern Irish Sea, *Rep. Mar. Biol. Statn. Port Erin* 83 (1971) 51–54.
- [34] A.V. Gaevskaya, A.A. Kovaleva, On a disease of blue whiting from the northeast Atlantic caused by the myxosporidian *Myxobolus 'aeglefini'*, Biological Fisheries Research in the Atlantic Ocean, Atlanticheskij Nauchno-Issledovatel'skij Institut Rybnogo Khozajstva i Okeanografii (AtlantNIRO), Kaliningrad 1976, pp. 164–167 (in Russian).
- [35] R.E. Richards, M.F. Mulcahy, The myxosporidian parasite *Myxobolus 'aeglefini'* (Auerbach 1906) in pleuronectids in Irish coastal waters, with particular reference to Cork Harbour, Ireland, IVth International Symposium of Fish Parasitology, Munich, Germany, October 3–7, 1995, Program and book of abstracts, *Inst. Zool. Fish Biol. Fish Dis. Univ. Munich, Germany* 1995, pp. 0–42.
- [36] P. van Banning, Long-term recording of some fish diseases using general fishery research surveys in the south-east part of the North Sea, *Dis. Aquat. Org.* 3 (1987) 1–11.
- [37] J. Thulin, J. Höglund, E. Lindesjö, Fish Diseases in Coastal Waters of Sweden, Naturvårdsverket, Solna, 1989 (in Swedish).
- [38] C.-H. Jeon, J.-H. Kim, *Myxobolus 'aeglefini'* (Myxozoa: Myxobolidae) infection in muscles of porous-head eelpout (*Bothrocara hollandi*), *J. Fish Pathol.* 28 (2015) 79–85.
- [39] M. Moser, E.R. Noble, Three genera of myxosporidia (Protozoa) in macrourid fishes, *Int. J. Parasitol.* 7 (1977) 93–96.
- [40] K. Buchmann, A. Skovgaard, P.W. Kania, *Myxobolus groenlandicus* n. sp. (Myxozoa) distorting skeletal structures and musculature of Greenland halibut *Reinhardtius hippoglossoides* (Teleostei: Pleuronectidae), *Dis. Aquat. Organ.* 98 (2012) 133–141.
- [41] J. Lom, J.M. Hutcheson, *Myxobolus mauriensis* n. sp. infecting rib cartilage of young-of-the-year river herring in New Jersey: Notes on pathology, prevalence, and genetics, *J. Parasitol.* 102 (2016) (10.1645/15-939).
- [42] H. Yokoyama, S. Wakabayashi, *Myxobolus 'aeglefini'* found in the skeletal muscle of porous-head eelpout *Allolepis hollandi* from the Sea of Japan, *Fish. Sci.* 66 (2000) 963–966.
- [43] I. Fiala, P. Bartošová, History of myxozoan character evolution on the basis of rDNA and EF-2 data, *BMC Evol. Biol.* 10 (2010) 228.
- [44] I. Fiala, P. Bartošová-Sojková, B. Okamura, H. Hartikainen, Adaptive radiation and evolution within the Myxozoa, in: B. Okamura, B. Gruhl, J.L. Bartholomew (Eds.), *Myxozoan Evolution, Ecology and Development*, Springer Int. Publ, Cham 2015, pp. 69–84.
- [45] J. Lom, I. Dykova, Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species, *Folia Parasitol.* 53 (2006) 1–36.
- [46] I. Fiala, P. Bartošová-Sojková, C.M. Whipps, Classification and phylogenetics of Myxozoa, in: B. Okamura, B. Gruhl, J.L. Bartholomew (Eds.), *Myxozoan Evolution, Ecology and Development*, Springer Int. Publ, Cham 2015, pp. 85–110.
- [47] L.F. Rangel, S. Rocha, M.H. Borkhanuddin, G. Cech, R. Castro, G. Casal, C. Azevedo, R. Severino, C. Szekely, M.J. Santos, *Ortholinea auratae* n. sp. (Myxozoa, Ortholineidae) infecting the urinary bladder of the gilthead seabream *Sparus aurata* (Teleostei, Sparidae), in a Portuguese fish farm, *Parasitol. Res.* 113 (2014) 3427–3437.
- [48] L.F. Rangel, S. Rocha, G. Casal, R. Castro, R. Severino, C. Azevedo, F. Cavaleiro, M.J. Santos, Life cycle inference and phylogeny of *Ortholinea labracis* n. sp. (Myxosporea: Ortholineidae), a parasite of the European seabass *Dicentrarchus labrax* (Teleostei: Moronidae), in a Portuguese fish farm, *J. Fish Dis.* 39 (2016), <http://dx.doi.org/10.1111/jfd.12508>.
- [49] C.M. Whipps, K.N. Murray, M.L. Kent, Occurrence of a myxozoan parasite *Myxidium streisingeri* n. sp. in laboratory zebrafish *Danio rerio*, *J. Parasitol.* 101 (2015) 86–90.
- [50] E. Karlsbakk, M. Køie, *Bipteria formosa* (Kovaleva et Gaevskaya, 1979) comb. n. (Myxozoa: Myxosporea) in whiting *Merlangius merlangus* (Teleostei: Gadidae) from Denmark, *Folia Parasitol.* 56, 2009 86–90.
- [51] C.M. Whipps, Interrenal disease in bluegills (*Lepomis macrochirus*) caused by a new genus and species of myxozoan, *J. Parasitol.* 97 (2011) 1159–1165.
- [52] S.D. Atkinson, J.L. Bartholomew, Alternate spore stages of *Myxobolus gasterostei*, a myxosporean parasite of three-spined sticklebacks (*Gasterosteus aculeatus*) and oligochaetes (*Nais communis*), *Parasitol. Res.* 104 (2009) 1173–1181.
- [53] L.F. Rangel, S. Rocha, R. Castro, R. Severino, G. Casal, C. Azevedo, M.J. Santos, The life cycle of *Ortholinea auratae* (Myxozoa: Ortholineidae) involves an actinospore of the triactinomyxon morphotype infecting a marine oligochaete, *Parasitol. Res.* 114 (2015) 2671–2678.