Myxozoans in high Arctic: Survey on the central part of Svalbard archipelago

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A B S T R A C T

Myxosporeans (Myxozoa), microscopic metazoan parasitic organisms, are poorly studied in the Arctic region. Our survey of benthic and pelagic fish (n = 234) collected in Isfjorden (Svalbard, Norway) together with detailed morphological and molecular examination revealed the presence of nine myxosporean species. We compared observed myxosporean diversity with diversity documented in regions close to the Arctic and revealed that water depth rather than geographic distribution is an important factor influencing myxosporean fauna.

We describe three new myxosporean species: Zschokkella siegfriedi n. sp. from kidney of Boreogadus saida, Parvicapsula petuniae n. sp. from the urinary bladder of Gymnocephalus tricuspid, and Sinulina arctica n. sp. from the urinary bladder of Myxocephalus scorpius. We characterise Latyspora-like organism from kidney of Cuplea harense. We provide new data for Ceratomyxa porrecta, Myxidium gadi, Myxidium finnarchicum, Schulmania aenigmatoa, and Parvicapsula irregularis comb. nov. The phylogenetic analyses including the newly obtained SSU and LSU rDNA data revealed that most of the species studied cluster in the marine urinary clade within the marine myxosporean lineage. Newly obtained sequences including the first molecular data for the member of the genus Schulmania, substantially enriched the Zschokkella subclade. C. porrecta and the two Myxidium species cluster within the Ceratomyxa and marine Myxidium clade, respectively.

Newly described species, Z. siegfriedi n. sp., was revealed to be morphologically indistinguishable but genetically diverse from Zschokkella hilden known from numerous gadid fish. Therefore, we consider Z. siegfriedi to be a cryptic myxosporean species that might be misidentified with Z. hilden. A Latyspora-like organism was found to be taxonomically problematic due to its suture line and its distant phylogenetic position from the type species Latyspora scamboromori did not allow us to assign it to the genus Latyspora. Based on an increased taxon sampling and SSU + LSU rDNA-based phylogeny, evolutionary trends within the marine urinary clade are investigated.

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1. Introduction

Arctic ecosystems draw our attention due to their rapid responses to climate change (Post et al., 2009). The Arctic region can be defined as north of the Arctic Circle, and consists of the Arctic Ocean, northern part of Eurasia and North America, Greenland, Iceland, Svalbard archipelago etc. The Arctic can be divided into the Low Arctic and High Arctic, according to various environmental and biological characteristics. The Svalbard archipelago is located in the High Arctic. The Arctic Ocean is the most extreme ocean in regard to the seasonality of light and its seasonally fluctuating ice cover. In general, species richness is lower in the Arctic than at lower latitudes and is to some degree constrained by biotic and abiotic mechanisms that define species occurrences and associations (Hoberg and Kutz, 2013). Furthermore, species richness tends to decline from low to high Arctic (Payer et al., 2013). Low numbers of host species is usually correlated to low numbers of parasites. Moreover, water temperature may influence transmission dynamics and parasite development (e.g. Kerans et al., 2005). Arctic fjords in the west coast of the Svalbard archipelago region are exceptional in terms of significantly higher temperatures caused by the Gulf Stream. Variations in the number of parasites were found in morphotypes of three spine sticklebacks living in different temperatures; higher numbers of parasites were found in the morphotype from the deep-cold water habitat compared to...
two warmer water dwelling morphotypes in the same Iceland lake (Karvonen et al., 2013). The enriching effect of warmer temperatures on higher abundance and species richness of ectoparasites was demonstrated in more than 100 fish hosts. This effect is not an artefact, but rather an indication of the importance of temperature in the diversification of fish parasites in the tropics (Poulin and Rohde, 1997).

Myxosporean fauna has been poorly studied in the Arctic region. One of the most parasitologically and ecologically studied marine fish with high economical importance occurring in sub-Arctic and Arctic waters is the Atlantic cod Gadus morhua (Hemmingsen and MacKenzie, 2001; Perdiguer-Olano et al., 2008). Apart from a number of protozoan and metazoan parasites (mostly helminths), 11 myxosporean species have been found in Atlantic cod (Hemmingsen and MacKenzie, 2001; Koe et al., 2007a; Holzer et al., 2010). A survey of parasite fauna of Atlantic cod revealed relatively rich and abundant regional macroparasite fauna dominated mostly by generalist parasites with Arctic-Boreal distribution in six localities in the North East Atlantic (Perdiguer-Olano et al., 2008). These high-level fauna comparisons suggest that differences in the feeding behaviour of cod amongst localities which could affect the prevalence and abundance of parasite species. Kerans et al. (2005) found that water temperature influenced parasite development rates and was a primary determinant for the release of actinospores of the myxozoan Myoxybus cereblais in strains of its definitive host Tubifex tubifex. In addition to latitudinal temperature gradients, sea depth is an important factor for parasite ecology. Low parasite richness was reported in different mesop- and bathypelagic fishes in comparison to bentopelagic species in the Arctic Ocean (Klimpel et al., 2006).

This study is focused on the Myxozoa, microscopic metazoan parasites characterised by simplified bodies. Evolutionary history of the Myxozoa has been questioned until recent molecular evidence proved the cnidarian origin (Jiménez-Guri et al., 2007; Holland et al., 2011). Myxozoa infect various organs in the vertebrate, mainly fish, hosts: cestozoic species multiply in the cavities of body organs (gall bladder, urinary tract, renal corpuscles etc.) whereas histozoic species are intercellular in various tissues (liver, skin, kidney, testes etc.). The phylum Myxozoa is divided into two classes: Malacosporea with only three described species and Myxosporea with the overwhelming majority of the myxozoan species. Until now, approximately 2010 myxosporean species assigned to 60 genera have been described (Morris, 2010). Myxospore genera are characterised by the morphology of the spore: spore shape, number of spore valves and polar capsules (PCs), and position of suture lines towards the PCs are considered the main taxonomic features. However, many myxospore morphological features are not synapomorphic since great discrepancies were found between the classic taxonomic approach and the phylogenetic relationships (Holzer et al., 2004; Fiala and Bartošová, 2010).

Myxosporeans form two main phylogenetic lineages according to host habitat, i.e. marine and freshwater (Fiala, 2006), plus a recently revised third basal sphaerosporid lineage (Bartošová et al., 2013). The marine lineage exclusively consists of marine species with the exception of Ceratomyxa shasta. There are five clades within the marine lineage: the marine Myxidium clade, the Ceratomyxa clade, the Enteromyxum clade, the Kudoa clade and the marine urinary clade divided into the Parvicapsula and Zschokkella subclade (Fiala, 2006; Bartošová et al., 2011). With the exception of the Enteromyxum clade, the remaining clades include non-monophyletic genera. The clustering of species in particular clades follows tissue tropism criterion rather than myxospore morphology (Holzer et al., 2004; Fiala, 2006). The marine urinary clade is typical in this respect: phylogenetically closely related myxosporeans of the genera Parvicapsula, Gadimyxa, Sphaerospora, Sinuolinea, Latyspora, and Zschokkella differ in spore morphology but predominantly infect the excretory tract (Bartošová et al., 2011). However, some species of the Parvicapsula subclade also infect other sites such as the epithelium of the gall bladder, the intestine, the pseudobranch and testicles. The monophyly of the genus Parvicapsula was disrupted by clustering of Gadimyxa spp. with parvicipaludis as well as by the sister relationship of P. miniboricornis and Sphaerospora testicularis (Kaie et al., 2007a; Bartošová et al., 2011). The Zschokkella subclade contains species of the polyphyletic genus Zschokkella including its type species Z. hildae as well as type species of the genera Latyspora and Sinuolinea (Bartošová et al., 2011; Dyková et al., 2013). The Zschokkella subclade is characterised by species with high variability in myxospore shape with the position of PCs ranging from set at opposite ends of the spore, to directly next to each other.

This paper attempts to characterise myxosporean fauna on the Svalbard archipelago: (i) detailed morphological and molecular characterisation of myxosporean species; (ii) phylogeny and evolutionary trends; (iii) comparison of parasite diversity from the Arctic with other regions.

2. Material and methods

2.1. Fish hosts

Eight species of teleost fish were collected in part of the Billefjorden, Isfjorden, Petunia Bay (78° 69’ N, 16° 53’ E) in the central part of Svalbard archipelago during the summer season (July and August 2011). A total of 234 individuals of 8 fish species from 7 families were dissected. Families, namely Cottidae: Myxoceophilus scorpius (Linnaeus, 1758) (n = 98), Gymnocephalus tricuspid (Reinhardt, 1830) (n = 22); Clupeidae: Clupea harengus Linnaeus, 1758 (n = 66); Osmeridae: Mallotus villosus (Müller, 1776) (n = 16); Gadidae: Boreogadus saida (Lepechin, 1774) (n = 14); Pleuronectidae: Hippoglossoides platessoides (Fabricius, 1780) (n = 9); Myctophidae: Lumipena lumtapetaefomis (Walbaum, 1792) (n = 8); and Salmonidae: Salmo salar Linnaeus, 1758 (n = 1), Fish were caught using gill-nets in litoral habitat (maximum depth of gillnets was 40 m). After euthanasia all organs were checked for the presence of the Myxozoa in squash preparations by light microscopy (Olympus BX 53). Contents of gall and urinary bladders were examined fresh, under cover slips, on slides covered with a thin layer of 1% agar. In some cases we failed to obtain samples of gall and urinary bladders and the missing data are considered in prevalence records within species descriptions. A DNA sample of Parvicapsula miniboricorns was obtained from the kidney of Oncorhynchus nerka (Walbaum, 1792) in Cultus Lake (British Columbia, Canada).

2.2. Myxosporean collection and documentation

Pictures of fresh spores were made using an Olympus BX 53 microscope with Nomarski differential interference contrast equipped with an Olympus DP72 digital camera. Measurements of spores were analysed in ImageJ v.1.44p (Wayne Rasband, http://imagej.nih.gov/ij). Measurements are presented in micrometres. Means, standard deviation (SD) and range in the parentheses were calculated for each spore dimension. Range of plasmodia size is followed by mean and median in parentheses. For examination of fine structure of myxosporean spores and plasmodia by transmission electron microscopy (TEM), whole urinary bladders as well as samples of their contents and kidney tissue were fixed in cacodylate buffered 3% glutaraldehyde at 4 °C, rinsed in 0.1 M cacodylate buffer and postfixed in 1% osmium tetroxide. After graded acetone dehydration, the samples were embedded in Spurr’s resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a JEOL JEM 1010 electron
microscope operating at 80 kV. Images were collected with MegaView II soft paving system using analYSIS software.

For histological examination, organs were fixed for 24 h in Davidson fixative, stored in 70% ethanol; samples were routinely dehydrated and embedded into paraffin. Sections were stained by haematoxylin and eosin (HE) and Giemsa. Positive samples were preserved in TNEs buffer (10 mM Tris–HCl pH 8, 125 mM NaCl, 10 mM EDTA, 0.5% SDS, 4 M urea) for DNA isolation and selected samples were molecularly characterised by sequencing of rRNA genes.

2.3. DNA isolation and PCR

Total DNA was extracted by standard phenol–chloroform method after digestion with protease K (100 μg ml⁻¹) overnight at 55°C. The extracted DNA was resuspended in 100 μl of sterile dd H₂O and kept at 4°C. SSU rDNA sequences were obtained by PCR using universal eukaryotic ERIB1-ERIB10 primers and/or the combination of MyxospecF-ERIB10 and ERIB1-MyxospecR primers (Barta et al., 1997; Fiala, 2006). If the primary PCR failed, the reaction with ERIB primers was followed by nested PCR with combinations of MyxospecF-ERIB10, ERIB1-MyxospecR, and/or MyxospecF-MyxospecR primers. PCRs of the LSU rDNA were carried out in a 25 μl reactions using 1× Taq buffer, 250 μM of each dNTPs, 10 pmol of each primer, 0.5 μl of DNA and sterile dd H₂O. Cycling parameters for the primary/nested PCR were as follows: denaturation at 95°C for 3 min, then 30 cycles of amplification at 95°C for 1 min, 48°C/50°C for 1 min, 72°C for 2/1 min and followed by a 10 min of extension at 72°C. If above mentioned PCR combinations failed to amplify the desired product TITANIUM Taq DNA polymerase (BD Biosciences Clontech) was instead used instead of Taq-Purple polymerase. PCRs were conducted in 10 μl reactions with 0.025 μl μl⁻¹ TITANIUM Taq DNA polymerase, 10 × buffer containing 5 mM MgCl₂, 0.2 mM of each dNTPs, 0.5 mM of each primer, and 0.5 μl DNA. Cycling parameters for the primary/nested PCR were as follows: denaturation at 95°C for 2 min, then 30 cycles of amplification at 95°C for 40 s, 52°C/56°C for 40 s, 68°C for 1 min 40 s/1 min and followed by 8 × min of extension at 68°C. The 3° end of the LSU rDNA was obtained using the NLF1050-NLR3284 primer set (Bartošová et al., 2009; Van der Auwera et al., 2004). When these PCRs failed to amplify the desired products, a nested PCR approach with NLF1280-NLR3113 (Bartošová et al., 2009; Van der Auwera et al., 2004) primers was used. The LSU rDNA of P. minibicornis failed to amplify with primers listed above but was amplified by nested PCR using primers 28Scer5F1-28Scer5R1 (first PCR) and 28Scer5F2-28Scer5R2 (second PCR) according to Fiala et al. (in prep.). PCRs of the LSU rDNA were carried out in a 25 μl reactions using 1× LA buffer, 0.5 μl DMSO, 250 μM of each dNTPs, 10 pmol of each primer, LA DNA polymerase (Top-Bio, Czech Republic), 1 μl of DNA, and sterile dd H₂O. Cycling parameters of LSU rDNA samples in the primary/nested PCR reactions were denaturation at 95°C for 5 min, then 30 cycles of amplification at 95°C for 1 min, 50°C/54°C for 1 min, 68°C for 2 min/1 min 40 s and followed by 8 min of extension at 68°C. All PCR products were purified using Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., USA). PCR products were sequenced directly or cloned into pDrive Cloning vector (Qiagen, Germany) and transformed into the competent Escherichia coli strains XL-1. PCR products or plasmid were sequenced on an ABI PRISM 3130XL automatic sequencer (Applied Biosystems, Czech Republic).

2.4. Phylogenetic analyses

The overlapping partial sequences of both SSU and LSU rDNA markers were assembled into the contigs in the SeqMan II program v5.05 (DNASTAR Inc., Madison, Wisconsin). The SSU and LSU rDNA alignments were created in program MAFFT v6.864 (Katoh et al., 2002) using L-INS-i strategy and default parameters. Alignments contain newly obtained sequences and sequences retrieved from GenBank. Highly variable parts of the alignments were determined and excluded in SeaView v4 (Gouy et al., 2010) by Gblocks (Castresana, 2000) using less stringent parameters and slightly adjusted by eye mainly at the beginning and at the end of the alignment.

Five alignments were assembled: SSU rDNA-muc alignment with all newly sequenced myxospores and all sequences of taxa within the marine urinary clade available in GenBank (1491 characters) plus the representatives of the other marine clades; LSU rDNA alignment (1987 characters) with all newly sequenced LSU rDNA and those ones available in GenBank; concatenated SSU rDNA-muc + LSU rDNA alignment (3487 characters); SSU rDNA-mar-myxid alignment focused on the marine Myxidium clade (1549 characters); and SSU rDNA-cer alignment focused on the Ceratomyxa clade (1389 characters). Three myxosporean species from the freshwater lineage were selected as outgroup in the analyses of SSU rDNA-muc alignment and SSU rDNA + LSU rDNA alignment. Outgroups for the SSU rDNA-mar-myxid alignment, SSU rDNA-cer alignment, and LSU rDNA alignment were selected as follows: two species from Zschokkella subclade, three ceratomyxids from elasmobranchs and two species from the freshwater lineage, respectively.

Phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI). ML was done in the RAxML v7.0.3. (Stamatakis, 2006) with GTR GAMMA model of evolution. MP was performed in the PAUP+ v4.0b10 (Swofford, 2003) with heuristic search with random taxa addition and the TBR swapping algorithm. All characters were treated as unordered, Ts:Tv ratio was set to 1:2 and gaps were treated as missing data. BI was computed in the MrBayes v3.0 (Ronquist and Huelsenbeck, 2003) with the GTR + Γ + I model of evolution. Posterior probabilities were calculated over 1,000,000 generations via two independent runs of four simultaneous Markov chains Monte Carlo chains with every 100th tree saved. Tracer v1.4.1 (Rambaut and Drummond, 2007) was used to set the length of burn-in period. For ML and MP, the bootstrap supports were calculated from 500 replicates. Genetic distances (converted to similarities in %) were computed in PAUP+ v4.0b10 with default P parameter from the SSU rDNA-muc and SSU rDNA-mar-myxid alignments.

3. Results

3.1. Findings of myxosporean infections

A total of five (i.e. M. scirpis, G. tricuspis, C. harengus, B. saida, H. platessoides) out of eight fish species were positive for the presence of Myxozoa (Table 1). 18% out of all dissected fishes were infected by Myxospora. Two fish were infected with more than one myxosporean: M. scirpis with four myxosporeans and H. platessoides with two myxosporeans. In this fish species, several concomitant infections occurred (stated in the species descriptions below). The highest prevalence of myxosporean infection was observed in H. platessoides and B. saida (Table 1).

We obtained 9 SSU rDNA sequences of Parvicapsula irregularis, P. petuniae, Zschokkella siegfriedi, Sinuolinea arctica, Schulmania aenigmatosa, Latyspora-like organism, Ceratomyxa porrecta, Myxidium gadi, and M. finnmarchicum. We obtained 6 LSU rDNA sequences of P. petuniae, P. minibicornis (from Gasterosteus aculeatus; Oregon, USA), Latyspora-like organism, P. irregularis, S. arctica, and S. aenigmatosa.
| Myxosporean species         | Host          | Site of infection | Prevalence | Spores | PCs | Plasmodia | References                  |
|----------------------------|---------------|------------------|------------|--------|-----|-----------|-----------------------------|
| **Ceratomyxa porrecta**     | *Myoxocephalus scorpius* | gb    | 4% (3/79) | L: 2.9 ± 0.4 (2.3–3.2) | W: 29.1 ± 4.8 (25.5–34.4) | T: – | L: 3.9 ± 0.5 × 10.2 ± 2.2 (3.5–13.8) | This study                  |
| **Myxidium gadi**           | gb            | 6% (3/79)        | L: 4–5     | W: 11.3 ± 0.1 (11.2–11.4) | T: 5.3 ± 2.0 (3.9–6.7) | – | 3 ± 3 | – | Dogiel (1948)                |
| **Myxidium finnmarchicum**  | gb            | 7% (6/79)        | L: 15.3 ± 1.6 (11.1–17.8) | W: 9.2 ± 1.3 (7.2–10.1) | T: 9 ± 0.5 (8.4–9.7) | – | 4.8–6.4 × 3.2–4.8 | This study                  |
| **Sinuolinea arctica**      | ub            | 10% (5/48)       | L: 17.6–22.4 | W: 6.4–6.9 | – | – | 24.0 ± 3.2 (22.2–27.7) × 27.5 ± 3.6 (25.0–31.6) | This study                  |
| **Parvicapsula petuniae**   | *Gymnocanthus tricusps* | ub, kidney | 9% (2/22) | L: 11.0 ± 0.7 (9.9–12.3) | W: 7.9 ± 0.6 (7.4–8.3) | T: 8.7 ± 2.1 (6.7–11.9) | – | 6.4–6.4 × 3.2–4.8 | This study                  |
| **Zschokkella siegfriedi**  | *Boreogadus saida* | kidney | 43% (6/14) | L: 17.4 ± 0.7 (16.7–18.2) | W: 10.5 ± 1.2 (9.2–11.6) | T: 9.8 ± 0.7 (8.5–11.0) | – | 20.8 ± 5.3 (14–25.1) | This study                  |
| **Parvicapsula irregularis**| *Hippoglossoides platessoides* | ub, kidney | 44% (4/9) | L: 11.0 ± 0.7 (11.0–15.1) | W: 7.9 ± 0.6 (6.1–10.4) | T: 8.7 ± 2.1 (7.4–9.0) | – | 16.6 ± 4.2 (11.5–24.0) | This study                  |
| **Schulmania aenigmatosa**  | ub            | 22% (2/9)        | L: 8.0–11.0 (mean 10.6) | W: 6.0–9.0 (mean 7.1) | T: 16.9 ± 1.6 (15.0–19.5) | – | 2.2 | – | Kabata (1962)                |
| **Latyspora-like organism** | *Clupea harengus* | kidney | 14% (9/66) | L: 19.9–23.1 | W: 11.9–13.3 | T: 11.9–16.0 | – | 26.6–42.0 × 31.9–55.9 | This study                  |
3.2. Myxosporean species

3.2.1. Additional data on described species

*Ceratomyxa porrecta* Dogiel, 1948 (Fig. 1A).

Type host: Gymnocanthus herzensteini Jordan and Starks, 1904.

Other hosts: *M. scorpius* (Linnaeus, 1758), shorthorn sculpin, average standard length 18.9 cm; *Bero elegans* (Steindachner, 1881); *Myxocephalus brandtii* (Steindachner, 1867).

Type locality: Peter the Great Bay, Japan Sea.

Other locality: Greenland Sea, part of the Billefjorden, Isfjorden, Petunia Bay in the central part of Svalbard archipelago (78° 69′ N, 16° 53′ E).

Description of sporogonic stages: disporic plasmodia with filopodia; for dimensions see Table 1.

Description of myxospores: crescent shape with markedly elongated shell valves; PCs with a straight central shaft of the filament, located close to the suture line in a plane perpendicular to it; posterior angle 220°; for dimensions see Table 1.

Fig. 1. Mature spores and plasmodia. (A-N) Myxospores and myxosporean plasmodial stages as seen in Nomarski differential interference contrast. Measurements are listed in Table 1. (A) Mature spore of *Ceratomyxa porrecta*. (B) Spores of *Schulmania aenigmatosa* with focus on polar capsules (left) and sinuous valve suture (right). (C) Plasmodial stages (left) and mature spore of *Parvicapsula irregularis* (right). (D) Mature spores of *Parvicapsula petuniae*. (E) Mature spore of *Myxidium gadi*. (F) Mature spore of *Myxidium finnarchicum*. (G, H) Spores of *Sinuolinea arctica* in frontal (G) and sutural (H) view. (I, J) Plasmodial stages of *Zschokkella siegfriedi*. (K) Mature spores of *Zschokkella siegfriedi*. (L) Plasmodial stage of *Latyspora*-like organism. (M, N) *Latyspora*-like organism spores with focus on polar capsules and part of valve suture, respectively.
Localization of sporogonic stages: coelozoic, gall bladder.

Prevalence: 4% (3 of 79 gall bladders; 1 sample co-infected with Myxidium finnmarchicum).  

Pathology: no material available for evaluation the species pathogenicity.

Materials deposited: DNA sample (nr. 1373) stored in –80 °C in the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR; SSU (GenBank accession No. KF874235) rDNA sequence.

Remarks: Ceratomyxa porrecta has identical spore shape with original description of C. porrecta (Dogiel, 1948). Measurements differences (see Table 1): PCs are remarkably larger (3 × 3 μm), and spore length is longer (4.5 μm) in Dogiel’s description of C. porrecta contrary to our measurements of C. porrecta from this study (PC 1.9 × 1.7 μm; spore length 2.9 μm). The type host of C. porrecta is G. herzensteini Jordan and Starks, 1904. We suggest that M. scorpis is another host for C. porrecta. Although our material does not originate from the type host of C. porrecta and there is no sequence data for C. porrecta. We assign our molecular and morphological findings to C. porrecta based on identical spore morphology and on the close genetic relationship of hosts M. scorpis and M. brandtii (Knope, 2013).

Myxidium gadi Georgévitch, 1916 (Fig. 1E).

Type host: Pollachius pollachius (Linnaeus, 1758), pollack.

Other hosts: M. scorpis (Linnaeus, 1758), shorthorn sculpin, average standard length 18.5 cm; G. morhua Linnaeus, 1758, Atlantic cod; Pollachius virens (Linnaeus, 1758), saithe; Merlangius merlangus (Linnaeus, 1758), whiting; Melanogrammus aeglefinus (Linnaeus, 1758), haddock; Pleuronectes flesus Linnaeus, 1758, European flounder; Solea solea (Linnaeus, 1758), common sole.

Type locality: Roscoff, off France coast.

Other localities: Barents Sea, White Sea, Atlantic Ocean: off Canada coast, Greenland Sea, part of the Billefjorden, Isfjorden, Petunia Bay in the central part of Svalbard archipelago (78° 69’ N, 16° 53’ E).

Description of sporogonic stages: plasmodia not observed in our material.

Description of myxospores: fusiform shape with pointed ends; pyriform PCs at each end of the spore, for dimensions see Table 1.

Localization of sporogonic stages: coelozoic, gall bladder.

Prevalence: 6% (5 of 79 gall bladders).

Pathology: no material available for evaluation the species pathogenicity.

Materials deposited: DNA sample (nr. 1320) stored in –80 °C in the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR; SSU (GenBank accession No. KF874236) rDNA sequence.

Remarks: Myxidium gadi has a wide host species spectrum and has been reported from five gadids and two flatfish (MacKenzie et al., 2010; Shulman, 1966). M. scorpis is a new host for M. gadi, broadening its host spectrum in the family Coditidae, as spore measurements of our material from shorthorn sculpin basically correspond to the original description of M. gadi from P. pollachius (Table 1). Moreover, M. gadi from shorthorn sculpin and M. gadi from haddock are genetically highly similar (98.8%; Supplementary Fig. 2A, Table 2). Moreover intraspecific variability based on partial SSU rDNA sequence of M. gadi is high, on the edge of the border resolving two species. This variability can be driven by wide host spectrum with intermixing infra-populations of M. gadi. Infrapopulation of M. gadi on the edge of distribution area can be for some period of time without any gene flow with the rest of infrapopulations. Four Myxidium species were found in cottids: M. scorpis Schulman-Albowa, 1950, described from atypical infection site in the urinary bladder of M. scorpius; M. arcticum Zhuikov, 1962 described from Myxoeozaphal us arcticaillaris; M. japonicum Dogiel, 1948 described from Myxoezoaphal us branditii; and M. myxoeozaphal Fantham, Porter and Richardson, 1940 described from Myxoeozaphal us octodercemspinosus. Myxidium myxoeozaphal appears to be identical with M. incurvatum based on their similar morphology, morphometrics and distribution area, although Fantam et al. (1940) noted that the parasite was larger than M. incurvatum (Khan et al., 1986) and both myxosporeans also differ in host species and molecularly. Generally, marine Myxidium species clustering within the marine Myxidium clade have fusiform or S-shape spores. The spore measurements and shape of M. gadi correspond to those of M. scorpius described from the same host but they differ in tissue tropism and PCs size. Myxidium scorpius described from an atypical infection site in the urinary bladder has slightly smaller PCs (1.8–2.0 μm) than M. gadi (3.4 × 2.8 μm) originating from gall bladder of M. scorpius (Table 1).

Myxidium finnmarchicum MacKenzie et al., 2010 (Fig. 1F).

Type host: Merlangius merlangus (Linnaeus, 1758), Whiting.

Other host: M. scorpis (Linnaeus, 1758), Shorthorn sculpin, average standard length 16.5 cm.

Type locality: off Sørøya, North Norway (70° 47’ N, 22° 58’ E).

Other localities: Greenland Sea, part of the Billefjorden, Isfjorden, Petunia Bay in the central part of Svalbard archipelago (78° 69’ N, 16° 53’ E).

Description of sporogonic stages: spherical disporic plasmodia; for dimensions see Table 1.

Description of myxospores: sigmoid shape with pointed ends; fine transverse ridges; pyriform PCs at each end of the spore, for dimensions see Table 1.

Localization of sporogonic stages: coelozoic, gall bladder.

Prevalence: 7% (6 of 79 gall bladders; 2 samples co-infected with Ceratomyxa porrecta).

Pathology: no material available for evaluation the species pathogenicity.

Materials deposited: DNA sample (nr. 1610) stored in –80 °C in the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR; SSU rDNA sequence (GenBank accession No. KF874237).

Remarks: M. scorpis is a new host for M. finnmarchicum, broadening its host species spectrum in the family Cottidae. Myxidium finnmarchicum was described with 4–6 fine longitudinal striations (MacKenzie et al., 2010) which were not observed in this study. On the other side, we observed fine transverse ridges on M. finnmarchicum spores under the light microscope. However, scanning electron micrographs are required for re-evaluation of the spore surface. SSU rDNA sequences of the myxosporean from our three samples were almost identical with SSU rDNA data of M. finnmarchicum. Myxidium finnmarchicum has a similar spore shape as M. gadi but differs in larger spore size and genetic similarity is 94.4% (Supplementary Table 2).

Schulmania enigmaticosa (Koval’eva et al., 1983) (Fig. 1B, Figs. 2 and 3).  

Type host: H. platessoides (Fabricius, 1780) American plaice, average standard length 10.5 cm.

Other hosts: Hippoglossoides robustus Gill and Townsend, 1897, Bering flounder; Hippoglossoides elassodon Jordan and Gilbert, 1881, Flathead sole.

Type locality: south off the Labrador.

Other localities: Sea of Okhotsk, Chukchi Sea, Bering Sea, Greenland Sea, part of the Billefjorden, Isfjorden, Petunia Bay in the central part of Svalbard archipelago (78° 69’ N, 16° 53’ E).

Description of sporogonic stages: spherical disporic plasmodia; ectoplasm separated from endoplasm; for dimensions see Table 1.

Description of myxospores: spores notably large, inversely pyramidal in sputal view with pointed posterior pole; suture line waved; lateral wings partially visible in the light microscope and clearly in TEM, lateral wings on spore present as pocket-like extensions separated from the spore body by membrane; PCs in posterior pole apposed closely to each other and discharging forward in the direction slightly toward the axis of the spore, 7 coils of polar filament; for dimensions see Table 1.
Schulmania aenigmatosa is the first sequenced member of the genus with considerable degree of irregularity, widest diameter of spore about wider in frontal view compared to the original description of Schulmania aenigmatosa. Ultrathin section of almost mature spore of Schulmania aenigmatosa with lateral wings (LW) typical for the genus. CC capsulogenic cell, CC with polar capsule.

Localization of sporogonic stages: coelozoic, renal tubules.

Prevalence: 22% (2 of 9 urinary bladders; 2 samples co-infected with Parvicapsula irregularis).

Pathology: high numbers of rodlet cells observed in epithelium of heavily infected segments of renal tubules (Fig. 3); even seen in early infections but not present in epithelium of collecting ducts and urinary bladder.

Materials deposited: DNA sample (nr. 1376) stored in –80°C in the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR; SSU (GenBank accession No. KF874229) and LSU (GenBank accession No. KF874226) DNA sequences.

Remarks: Kabata (1962) described Sphaerospora irregularis from American plaice in northern North Sea. This species was later assigned to other genera: Myxoproteus (Gaevskaya and Kovaleva, 1984), and Ortholinea (Arthur and Lom, 1985). After re-examination, Keie et al. (2007b) suggested S. irregularis may belong to Parvicapsula. Despite S. irregularis was reported from another host, Pleuronectes platessa (MacKenzie et al., 1976), this report most probably corresponds to Parvicapsula bicornis later described from this host (Keie et al., 2007b). Unfortunately, the report of “S. irregularis” by MacKenzie et al. (1976) lacked sufficient morphological documentation and comparison with similar species. Therefore, P. bicornis from P. platessa was regarded as syn. part. of S. irregularis (Keie et al., 2007b). Since this species is now re-examined and molecularly characterised we claim that P. bicornis and the re-described P. irregularis are two morphological and molecularly different species. S. testicularis as the closest relative of P. irregularis has a wider and thicker spore.

3.2.2. Description of new taxa

Zschokkella siegfriedi n. sp. (Fig. 11–K, Figs. 4–6).

Family Myxidiidae Thélohan, 1892.

Genus Zschokkella Auerbach, 1910.

Type host: B. saida (Lepechin, 1774), Polar cod (officially accepted common name; commonly used name Arctic cod for B. saida is valid for Arctogadus glacialis (Peters, 1872) (Froese and Pauly, 2013); average standard length 14.4 cm.

Other host: unknown.

Type locality: Greenland Sea, part of the Billefjorden, Isfjorden, Petunia Bay in the central part of Svalbard archipelago (78° 69' N, 16° 53' E).

Other localities: none.

Description of sporogonic stages: plasmodia mostly di-, rarely polysporic; round to oval in shape; clear differentiation between smooth ectoplasm and granular endoplasm; for dimensions see Table 1.

Description of myxospores: shape of spores considerably variable, from spores with one side vaulted appearing almost rounded triangular to spores of ellipsoidal shape; suture line irregularly oblique, two shell valves completely asymmetrical; subspherical to spherical PCs located in the spore ends and discharging to opposite sides parallel with axis of the spore from the apical view, 7 coils of polar filament; for dimensions see Table 1.

Description of myxospores: shape and size of spores considerably variable, from spores with one side vaulted appearing almost rounded triangular to spores of ellipsoidal shape; suture line irregularly oblique, two shell valves completely asymmetrical; subspherical to spherical PCs located in the spore ends and discharging to opposite sides parallel with axis of the spore from the apical view, 7 coils of polar filament; for dimensions see Table 1.

Localization of sporogonic stages: coelozoic, renal tubules, urinary bladder.

Prevalence: 44% (4 of 9 urinary bladders; 2 samples co-infected with Schulmania aenigmatosa).

Pathology: no material available for evaluation the species pathogenicity.

Materials deposited: DNA sample (nr. 1376) stored in –80°C in the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR; SSU (GenBank accession No. KF874229) and LSU (GenBank accession No. KF874226) DNA sequences.

Remark: Kabata (1962) described Sphaerospora irregularis from American plaice in northern North Sea. This species was later assigned to other genera: Myxoproteus (Gaevskaya and Kovaleva, 1984), and Ortholinea (Arthur and Lom, 1985). After re-examination, Keie et al. (2007b) suggested S. irregularis may belong to Parvicapsula. Despite S. irregularis was reported from another host, Pleuronectes platessa (MacKenzie et al., 1976), this report most probably corresponds to Parvicapsula bicornis later described from this host (Keie et al., 2007b). Unfortunately, the report of “S. irregularis” by MacKenzie et al. (1976) lacked sufficient morphological documentation and comparison with similar species. Therefore, P. bicornis from P. platessa was regarded as syn. part. of S. irregularis (Keie et al., 2007b). Since this species is now re-examined and molecularly characterised we claim that P. bicornis and the re-described P. irregularis are two morphological and molecularly different species. S. testicularis as the closest relative of P. irregularis has a wider and thicker spore.
Materials deposited: DNA sample (nr. 1608) stored in –80 °C and blocks in resin nrs. 541a and 543a in the Institute of Parasitology, Laboratory of Fish Protistology, BC ASCR; SSU rDNA sequence (GenBank accession No. KF874231).

Etymology: The species name of *Z. hildae*, type species of the genus *Zschokkella*, refers to Hilda (a shorten version of the German name) used by author Auerbach (1910) in honour of his wife. We name *Z. siegfriedi* n. sp. according to the German heroic poem “The Song of Nibelungs” with the lovers Siegfried and Kriemhilda (Hilda) reflecting the close phylogenetic relationship between *Z. hildae* and our new species.

Remarks: We found *Zschokkella siegfriedi* from the kidney of polar cod to be genetically distinct (2.8% of dissimilarity) (Supplementary Table 1) from *Z. hildae* SSU rDNA sequence from *G.*

Fig. 3. Histology of *Schulmania aenigmatosa* infection. (A-C) *Schulmania aenigmatosa* infection in excretory system of *Hippoglossoides platessoides*. (A) Early plasmodial stages localised in ureter as seen in histological section stained with HE. (B) Advanced plasmodial stages filling urinary bladder. Giemsa stained stage (inserted). (C) Semithin section stained with toluidine blue documents numerous plasmodial stages attached to the wall of urinary bladder. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
morhua. Zschokkella hildae, the type species of the genus Zschokke-la, typically infects fish from the family Gadidae and was previ-
ously reported from B. saida without providing any molecular
data (Aseeva, 2002; Køie, 2009). In the light of the new data, we
suggest B. saida was most likely either infected with Z. siegfriedi
in the report of Aseeva (2002) or this host is susceptible for both
Z. hildae and Z. siegfriedi species. The spores of Z. hildae
possess
some degree of pleiomorphy during maturation; morphologically,
Z. hildae and Z. siegfriedi are indistinguishable. However, Z. hildae
was found to infect the host’s urinary bladder and collecting duct
of the kidney, unlike Z. siegfriedi which develops in the upper
excretory system and the renal tubules. Nevertheless, we expect
Z. siegfriedi to infect also urinary bladder as reported for
Z. hildae since we were not able to cheque the urinary bladder of B. saida.
We determined that Z. siegfriedi is a distinct species based on biol-
ogy and genetics; biologically, Z. siegfriedi has (i) significant genetic
difference based on SSU rDNA; (ii) localization of sporogonic stages
in renal tubules vs collecting duct; (iii) different but very closely
related host species to that of Z. hildae.

Parvicapsula petuniae n. sp. (Fig. 1D, Fig. 7).
Family Parvicapsulidae Shulman, 1953.
Genus Parvicapsula Shulman, 1953.
Type host: G. tricuspis (Reinhardt, 1830), Arctic staghorn sculpin;
average standard length 13.9 cm.
Other hosts: unknown.
Type locality: Greenland Sea, part of the Billefjorden, Isfjorden,
Petunia Bay in the central part of Svalbard archipelago (78° 69’
N, 16° 53’ E).
Other localities: none.
Description of sporogonic stages: disporic plasmodia, early plas-
modia subspherical to oval, sometimes with filopodial projections;
plasmodia located in renal tubules; for dimensions see Table 1.
Description of myxospores: spores asymmetrical with somewhat
curved and wavy suture line, ellipsoidal in frontal view; two pyri-
form PCs of equal size; closely apposed, discharging in the same
apical direction, 8 coils of polar filament; single distinct binucleate
sporoplasm; measurements see Table 1.
Localization of sporogonic stages: coelozoic, renal tubules, uri-
nary bladder.
Prevalence: 9% (2 of 22 kidney samples and of 17 urinary
bladders).
Pathology: No material was available for evaluation the species
pathogenicity.
Materials deposited: DNA sample (nr. 1423) stored in \(-80^\circ\text{C}\) in the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR; SSU (GenBank accession No. KF874230) and LSU (GenBank accession No. KF874223) rDNA sequences.

Etymology: specific name refers to the type locality Petunia Bay.

Remarks: This is the first report of a Parvicapsula species from *G. tricuspis*. The shape and size of *P. petuniae* spores are similar to the asymmetrical spores of *P. hoffmani* infecting the intestinal epithelium of mullet (India) (Dorothy and Kalavati, 1993) and even more similar to *P. karenii* infecting the urinary bladder of a flatfish from the Yellow Sea (Zhao et al., 2000). Nevertheless, the above mentioned morphologically similar Parvicapsula species differ in their host species preference and with different distributions from *P. petuniae* thus considering it to be a distinct species.

**Sinuolinea arctica** n. sp. (Fig. 1G, H, Fig. 8).

Family Sinuolineidae.

Genus *Sinuolinea* Davis, 1917.

*Type host*: *M. scorpius* (Linnaeus, 1758), Shorthorn sculpin; average standard length 18.7 cm.

*Other hosts*: unknown.

*Type locality*: Greenland Sea, part of the Billefjorden, Isfjorden, Petunia Bay in the central part of Svalbard archipelago (78° 69' N, 16° 53' E).

*Other localities*: none.

*Description of sporogonic stages*: mostly disporic, rarely polysporic spherical plasmodia; freely floating in urine; spores maturing either inside the plasmodium or in pansporoblasts which are as a whole separated from the plasmodium and where spores
subsequently undergo complete maturation; for dimensions see Table 1.

Description of myxospores: spores spherical with protrusive sinuous suture line twisted in its axis; valves with smooth surface; two spherical PCs of equal size, separated from one another and discharging sideways, 7 coils of polar filament; spores with a single distinct sporoplasm; for dimensions see Table 1.

Localization of sporogonic stages: coelozoic; urinary bladder.

Prevalence: 10% (5 of 48 urinary bladders).

Pathology: unknown.

Materials deposited: DNA sample (nr. 1317) stored in –80 °C in the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR; SSU (GenBank accession No. KF874232) and LSU (GenBank accession No. KF874227) rDNA sequences.

Etymology: species name refers to the geographic origin in Arctic.

Remarks: Sinuolinea arctica is the first Sinuolinea species described from M. scorpius. Its size and morphology are similar to those of the type species S. dimorpha, but the spore of S. arctica is slightly bigger (14.8–15 μm vs. 15.4–16 μm) (Dyková et al., 2013). Sinuolinea sp. from urine of M. scorpius was previously reported by Lom (1984) and has identical morphology to S. arctica. However, dimensions of Sinuolinea sp. are significantly larger (L 22.9 μm and W 20.7 μm) than of S. arctica (L 15.7 ± 0.9 and W 15.4 ± 0.8). Assigning taxonomic status of Sinuolinea sp. would require molecular characterisation. Myxoproteus myxoxocephali Fantham, 1940 (family Sinuolineidae) was described from gall bladder of M. scorpius. However, infecting gall bladder, which is not a typical site of infection of sinuolineid species, and poor morphological description of M. myxoxocephali puts doubt on correct systematic position of this species.

3.2.3. Characterization of new organism

Latyspora-like organism (Fig. 11–N, Figs. 9 and 10).

Family Sinuolineidae Shulman, 1959.

Genus Latyspora Bartošová, Freeman, Yokoyama, Caffara and Fiala, 2010.

Type host: C. harengus Linnaeus, 1758, Atlantic herring; average standard length 20.9 cm.

Other hosts: unknown.

Type locality: Greenland Sea, part of the Billefjorden, Isfjorden, Petunia Bay in the central part of Svalbard archipelago (78° 69’ N, 16° 53’ E).

Other localities: none.

Description of sporogonic stages: disporic plasmodia globular in shape containing numerous refractile granules; plasmodia developing in renal tubules (attached to the epithelium and sometimes invading into epithelium); for dimensions see Table 1.

Description of myxospores: spores bean-shaped or trapezoidal from frontal view, oval from the apical view; both valves smooth with rounded shape; spore folds formed by the shell valve at its posterior pole; straight sutural line running perpendicularly between two spherical PCs of equal size, PCs located close together at anterior pole and oriented in the same direction, discharging sideways, PCs with a straight central shaft of the filament, 6–7 coils of polar filament; single sporoplasm with two nuclei; for dimensions see Table 1.

Localization of sporogonic stages: coelozoic; renal tubules.

Prevalence: 14% (9 of 66 kidney samples).

Pathology: advanced infection associated with alteration to the epithelium of renal tubules either by atrophy of epithelial cells and pyknosis of cell nuclei or complete loss of integrity of epithelium due to necrotic changes; hypertrophy of renal corpuscles caused by foreign material accumulated in dilated Bowman’s spaces not possible to unambiguously associate with infection (Fig. 10).

Materials deposited: DNA sample (nr. 1365) stored at –80 °C and paraffin blocks nrs. 695/09, 700/09, 704/09 stored in the Laboratory of Fish Protistology, Institute of Parasitology, BC ASCR; SSU (GenBank accession No. KF874234) and LSU (GenBank accession No. KF874225) rDNAs sequences.

Remarks: Classification of this species near the genus Latyspora is based on the current state of Latyspora taxonomy (Bartošová et al., 2011). The taxonomic status of Latyspora-like organism will be emended in the future when myxozoan taxonomy and in particular the genus Latyspora is revis ed. Latyspora-like organism differs morphologically from the genus Latyspora in one morphological characteristic: the suture line is straight in Latyspora-like Fig. 9. Line drawing of Latyspora-like organism, sutural view. Scale bar = 10 μm.
organism vs. sinuous in the type species Latyspora scomberomori. Other morphological and biological characteristics e.g. localization in the fish host fully correspond to Latyspora and phylogenetically, the genus type species L. scomberomori and Latyspora-like organism are distantly related.

3.3. Phylogenetic analyses

Seven newly molecularly characterised myxosporeans clustered within the marine myxosporean lineage in the rDNA-based phylogenies (Fig. 11, Supplementary Fig. 1A). Phylogenetic tree based on five new LSU rDNA and concatenated analysis based on SSU + LSU rDNA shows the marine urinary clade monophyletic, however subclades are not well resolved (Supplementary Fig. 1A, B).

All species, except Ceratomyxa porrecta, clustered within the clade of marine myxosporeans mainly infecting the urinary bladder of fish i.e. the marine urinary clade according to Bartošová et al. (2011). Ceratomyxa porrecta branched in the Ceratomyxa clade (Supplementary Fig. 2B) with a close relationship to C. auerbachi described from the North Sea and Norwegian Sea region. SSU rDNA sequences of M. gadi and M. finnmarchicum, obtained in this study, clustered with sequences of these species currently available in GenBank (Supplementary Fig. 2A). Their sequence similarities within the species were higher than 98% (Supplementary Table 2).

The analysis of the marine Myxidium clade confirmed the sister relationship of M. gadi and M. bergense and revealed the position of M. finnmarchicum as an early branching species closely related to S. phyllopteryxa and M. incurvatum (Supplementary Fig. 2A).

The marine urinary clade was enlarged by the addition of six newly sequenced species (Fig. 11). Parvicapsula irregularis was very closely related to pathogenic P. bicornis in both SSU and SSU + LSU rDNA-based trees with maximum bootstrap support (Fig. 11 and Supplementary Fig. 1A) and with a high sequence similarity of 98.1% (Supplementary Table 1). Parvicapsula petuniae clustered with S. testicularis with high nodal support in the SSU rDNA-based ML and BI (MP bootstrap support was low; Fig. 11) and their sequence similarity was 85.9% (Supplementary Table 1). Both newly obtained Parvicapsula sequences branched within the Parvicapsula subclade of the marine urinary clade with high nodal support in the SSU rDNA-based tree (Fig. 11). The Zschokkella subclade is enriched almost 1.5 times. Latyspora-like organism was the most basal species of the Zschokkella subclade and did not cluster with the type species L. scomberomori in any analyses (Fig. 11, Supplementary Fig. 1A).

S. arctica and Zschokkella siegfriedi clustered together with both Z. hildae and Sinuolinea sp. with high nodal support in the SSU and SSU + LSU rDNA-based phylogenies (Fig. 11 and Supplementary Fig. 1A). The clade of the four aforementioned species was characterised by the long branch in the phylogenetic trees (Fig. 11, Supplementary Fig. 1A), Zschokkella siegfriedi and Z. hildae were closely related (Fig. 11, Supplementary Fig. 1A) with relatively high sequence similarity of 97.2% (Supplementary Table 1). Schulmania aenigmatosa was revealed as the sister taxon to the abovementioned long-branching group with low nodal support in the SSU rDNA tree (Fig. 11). Concatenated analysis of SSU + LSU rDNA data supported the relationship of S. aenigmatosa with the group containing L. scomberomori and S. dimorpha (99% bootstrap support in ML) and revealed the long-branching group of two Zschokkella spp. and two Sinuolinea spp. inside the Parvicapsula subclade (Supplementary Fig. 1A). In addition, the topology within the marine urinary clade was identical after changing the outgroup (three Ceratomyxa species instead of three freshwater myxosporeans) in the analysis testing the influence of the selected outgroup on the resulting topology (tree not shown). Based on the three main alignments focused on the Myxosporea infecting urinary...
bladder, the marine urinary clade was a well resolved group with nodal supports (ML/MP/BI) of 87/76/1.00 in the SSU rDNA-based tree, 60/80/1.00 in the LSU rDNA-based tree and 100/99/0.92 in the SSU + LSU rDNA-based tree, respectively (Fig. 11, Supplementary Fig. 1A, B).

4. Discussion

All our findings of myxosporean species in this study are the most Northern records of the Myxosporea. *Myxidium gadi* has an extended geographic distribution around the north part of the Northern hemisphere. Polar cod, host of *Zschokkella siegfriedi*, is one of the most northerly distributed gadid fish, has a circumpolar distribution (Froese and Pauly, 2013). We can assume that most of the myxosporeans described from Svalbard have a wide distribution not limited by the Svalbard archipelago but not exceeding the range of their intermediate fish hosts.

Differences in prevalence and infection intensity of Myxosporea were detected in the fish hosts. The highest myxosporean prevalence and infection intensity was observed in benthopelagic fish infected with *H. platessoides* (43%) and in benthic fish (44%). Generally, the prevalence of all four myxosporeans found in benthic fish was low, however, this host was infected with the highest number of myxosporean species (Table 1). The rich myxosporean fauna of *M. scorpius* was low, however, this host was infected with the highest number of myxosporean species (Table 1). The rich myxosporean fauna of *M. scorpius* was revealed during sequencing of selected microscopically myxosporean positive samples. Hidden mixed infections of *Myxidium finnmarchicum* and *M. gadi* revealed by
PCR suggest the presence of pre sporogonic or sporogonic stages with low infection intensity, which can be easily overlooked or misidentified with stages of belonging to myxosporean species with high prevalence. The differences in infection intensity and parasite abundance between hosts may be explained by competition or other negative interactions among parasites in the fish host (Seppala et al., 2009).

Myxosporeans have not been reported on Svalbard or the surrounding marine environment so far except the finding of Z. hildae from B. saïda (Kaie, 2009). Therefore, we can only provide a comparison of myxosporean parasitofauna with geographic regions close to the Arctic. We chose the ratio of total number of myxosporean species found/number of dissected fish species as a measure to determine and compare the biodiversity among the regions. The ratio in our study (1.3) was very similar to the ratio (1.5) obtained in the study of gadid fish in the North Sea and Norwegian waters (Kalavati and Mackenzie, 1999). However, a much lower ratio (0.5) was recorded in 28 mero- and bathypelagic fish species from the continental shelf of Newfoundland and Labrador (water depth from 200 to 1000 m) (Khan et al., 1986). On the other side, one parasite per fish species in average (ratio 1.0) was revealed in mesopelagic fish in the North Atlantic (Yoshino and Noble, 1973). Therefore, it seems sea water depth rather than geographic distribution is an important factor influencing myxosporean fauna. Deep water fish (except bentipelagic) had the lowest ratio of myxosporeans per fish, which corresponds to observation of low parasite richness by Klimpel et al. (2006) in different meso- and bathypelagic fish. In contrast to high ratio of myxospore infections observed in epi- and mesopelagic gadid fish (Kalavati and Mackenzie, 1999) which is one of the most dominant Arctic fish families. Although we did not dissect any Atlantic cod, whose parasitofauna has been well studied and includes a total 11 myxosporean species, we did examine fishes from the same depth range with similar parasite/host ratios. Heteroxenous parasite expansion is dependent also on the other host involved in the life cycle. It means that the myxosporean distribution area is restricted not only by fish abundance but also by the particular definitive host.

The morphologically simplified body organisation of the Myxozoa together with ancestral polymorphism and convergent evolution limit the number of characteristic features important for the classification of myxozoan genera (Avise, 2004). Moreover, myxosporeans often possess a certain degree of spore plasticity within evolutionary closely related species, especially within species clustering in the marine urinary clade (Fiala and Bartošová, 2010). Phylogenetic positions of myxosporeans obtained in this study strengthened the typical myxosporean discrepancies between taxonomy based on the morphological similarities and the observed phylogenetic relationships. This is evident in the close relationship of P. petuniae with S. testicularis and unrelated phylogenetic positions of the Latyspora-like organism and L. scomberomori as well as Simulinaea arctica and S. dimorpha.

The myxospore shape of species from the marine urinary clade is very variable in comparison with shapes shared among species in other e.g. Ceratomyxa and Kudoa clades. Variability of the myxospore morphology can be seen in the position of PCs, twisting of the suture line around the valves and by alterations of the overall spore shape e.g. prolongation and broadening of the spore. Bartošová et al. (2011) investigated the evolution of the suture line in the marine urinary clade. They found the character of the suture line to be a typical homoplastic feature. Phylogenetic positions of the myxosporeans reported from Svalbard represented by the genera Zschokkella, Parvicapsula, Simulineaea, Latyspora (all with curved or sinuous suture line) and Schulmania (straight suture line) supported the homoplasy of this feature. Moreover, Bartošová et al. (2011) traced the evolutionary character of the suture line i.e. sinuous or curved vs. straight on the SSU rDNA-based phylogeny. They found that an ancestor of the marine urinary clade possessed the curved suture line. Latyspora-like organism as the basal species of the Zschokkella subclade, has a remarkably straight suture line. Therefore, the evolutionary history of this feature would be different if we again trace this character on the tree which is in congruence with the statement of Bartošová et al. (2011) that poor taxon sampling influences the tracing of character evolution.

Latyspora-like organism is a problematic species, a taxonomic “hard nut to crack”, detailed in the description above. It has the straight suture line and differences in PC discharge and its phylogenetic position from the type species thus not allowing us to assign it to the genus Latyspora. The genera Latyspora and Ceratomyxa have very similar types of spores, nevertheless characters of suture line and position of PCs distinguish these two genera (Bartošová et al., 2011). The appropriate focus plane is crucial for the correct characterisation of the suture line as seen in the picture of Latyspora-like organism in Fig. 1N. We assume that the documentation of sinuous suture line of L. scomberomori (Bartošová et al., 2011) is questionable in that halo effect around the PCs, may have resulted in misinterpretation of the character of suture line. In any case, these two species are not phylogenetically closely related and thus Latyspora-like organism should not be assigned to the genus Latyspora which would make this genus polyphyletic. However, Latyspora-like organism may be representative of another so far undescribed genus.

Variability of the myxospore morphology was also studied at the level of a single species e.g. Zschokkella pleomorpha and Bipteria formosa during spore development. It was documented that the maturation process changes the shape and dimensions of the myxospore (Lom and Dyková, 1995) or formation of lateral wings (Karshak and Kaie, 2009). We assume that the lateral wings of Schulmania aenigmatosa undergo similar maturation changes as those in B. formosa. In these cases, it is important to provide morphometric data from the completely mature spores to avoid obtaining of misleading spore dimensions.

Speciation is not always accompanied by morphological change and many species remain undescribed (Bickford et al., 2007). Research on cryptic species has increased since molecular tools helped to distinguish closely related and morphologically similar or identical species. In our study, two species of the genus Myxidium, M. finnmaricum and M. gadi, were hard to distinguish based on the morphology of the spores, which is a tool of classic myxosporean taxonomy. Both species occurred in the same host species and were present in low prevalence. The presence of these two different species was uncovered based on SSU rDNA screening of the sample and supported by a detailed morphometric analysis. Another example is a cryptic myxosporean species found in Polar cod kidney tubules. Aseeva (2002) observed this myxosporean in Polar cod and classified it as Zschokkella hildae based on identical morphological and biological features. However, we revealed this myxosporean to be a cryptic species based on the genetic differences in the SSU rDNA and we named it as Zschokkella siegfriedi. Zschokkella hildae has been recorded in nine gadid fish including Arctic cod from Arctic region of Greenland (Kaie et al., 2008b). Up to now SSU rDNA data of Z. hildae are available from the Atlantic cod only (Holzer et al., 2010). Hypothetically, more species can be revealed from the family Gadidae by molecular characterisation and they can represent hidden or misidentified species as in the case of Z. siegfriedi from Polar cod. The type host of Z. hildae, the Greater forkbeard Physico blemnoides, phylogenetically clusters apart from the other reported hosts of Z. hildae (Møller et al., 2002; Teletchea et al., 2006; Ros-Varon and Orti, 2009). This may suggest that Z. hildae from the type host may not correspond to the myxosporean described (and sequenced) from Atlantic cod. More information about the Zschokkella subclade including increased taxon sampling effort together with providing biological characters
from life cycles, development, ecology of definitive host etc. may lead to the radical taxonomic changes. Pleomorphic myxospores resembling Zschokkella morphotype and presence of the Zschokkella type species in the Zschokkella subclade may provoke assignment of all members of this subclade to the genus Zschokkella.

Discovery of Z. siegfriedi, morphologically identical species with Z. hildae, based on SSU rDNA sequence divergence underlines the importance of molecular data for species description and for parasite new host records. However, the level of myxosporean genetic interspecific dissimilarity is fluctuating, which do not allow simple use of arbitrary chosen level of genetic dissimilarity to discriminate between species. For example, members of the genus Ceratomyxa have much lower sequence difference up to 0.4% (Gunter and Adlard, 2009), which is in contrast to Chloromyxum leydi with 1.8% intraspecific variation (Gleson and Adlard, 2012). Similarly in this study, Myxidium gadi a generalist parasite of gadid fish has 1.2% of intraspecific variation and, on the other hand, sequence dissimilarity between Parvicapsula limandae and P. asymmertica is 0.9%, and among Ellipsomyxa spp, is even about 0.5%. As already investigated in Gunter and Adlard (2009), the level of DNA sequence difference must be assessed on a case to case basis using a whole evidence approach.

Marine myxosporean life cycles are poorly resolved with only few described ones for specifically Ceratomyxa auberbachii, Gadimyxa atlantica, Sigmomyxa sphaerica, two species of Parvicapsula and two species of Ellipsomyxa. All of them have a polychaete definitive host in their life cycle (Kaie et al., 2004; Kaie et al., 2007a; Kaie et al., 2008a; Rangel et al., 2009; Karlshakk and Kaie, 2012; Kaie et al., 2013). Lower levels of species richness may give polar regions an advantage for studying myxozoan life cycles compared to species-rich subtropical or tropical regions. Therefore, the Svalbard coast may be a suitable area for life cycle studies, supported by preliminary data on the life cycle of Gadimyxa sphaerica (results will be published elsewhere). We may hypothesise a polychaete worm as a host for P. petuniae, since the closely related P. minibiocoris uses a freshwater polychaete, Manayunkia speciosa as a host (Bartholomew et al., 2006). Nevertheless, the elucidation of the life cycles of myxosporeans from Svalbard region is a task for future studies.

Except of the universal SSU rDNA marker, we also sequenced LSU rDNA of Myxosporea in order to add more molecular data to our analyses. Nevertheless, the single LSU rDNA analysis of the marine urinary clade contained significantly less taxa compared with the SSU rDNA analysis. This discrepancy in amount of the characters for particular taxa may cause the different topological pattern of SSU vs SSU + LSU rDNA analyses. Moreover, LSU rDNA has higher phylogenetic signal and may suppress the signal of SSU rDNA leading to different topology (Bartošová et al., 2009).

Our research indicates that increased taxon sampling effort is needed to elucidate myxosporean relationships, mainly of species from the urinary system clustering in the marine urinary clade. This clade accommodates many diverse myxosporean morphotypes and therefore, new molecular data for species from urinary systems of marine fish, especially those classified to genera with missing molecular data, are needed. There is also an obvious importance of studying parasites from the Arctic as a region most influenced by climate change (Post et al., 2009) in order to monitor its changing parasitofauna. New phylogenetic data from species infecting urinary tract contribute to the knowledge of evolution of the marine myxosporeans.

5. Conclusions

Our focus on myxosporeans of benthic and pelagic fish collected in the central part of Svalbard revealed the presence of several new myxosporean species. Results of the present study increase the species richness of myxosporeans in a polar region as well broaden the spectrum of their hosts and their distribution in the studied area. We mostly found myxosporean species infecting the urinary tract that are distinguished by the morphologically variable spores and classified to five myxosporean genera. These species clustered together based on shared tissue tropism rather than their myxospore morphology. Based on adequate taxon sampling and SSU and LSU rDNA-based phylogeny, we discussed evolutionary trends within the marine urinary clade.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpaw.2014.02.001.

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