Viral assemblage variation in an Arctic shelf seafloor

Running Title: Viromes of Barents Sea sediment

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ASTRACT: Spatial differences in microbial communities are observable even in habitats with moderate environmental variation, such as within the pelagic zone or seafloor of geographically finite regions of the oceans. Here we explore if biogeographical variations are manifested at this level also in the structure of viral assemblages by comparing DNA viromes from the Barents Sea upper seafloor, collected at five geographically separated locations. Twenty-seven to forty-four percent of the open reading frames showed significant similarity to genes of viral genomes in the Refseq database. The majority of the identified open reading frames, i.e. 86 to 95%, were affiliated with sequences of single-stranded DNA (ssDNA) viruses, but the ssDNA virus genetic material was likely strongly overrepresented due to the use of phi29 DNA polymerase for amplifying viral DNA. The majority of ssDNA virus sequences originated from the *Microviridae* family of phages and the eukaryotic Circular Rep-encoding ssDNA (CRESS-DNA) viruses. The sediment virus assemblages showed higher overall similarity to counterparts from deep sea sediment of the Pacific Ocean than to

e.g. Arctic Ocean pelagic viromes, supporting the presence of common compositional features in sediment viral assemblages across continental-scale geographical separations. The Barents Sea viromes grouped biogeographically in accordance with the south-north environmental division of this Arctic sea by the oceanic polar front, thereby mirroring a corresponding 16S rRNA gene based biogeographical division of the bacterial communities. However, compositional differences in the eukaryotic virus assemblages rather than the bacteriophages appeared as the primary basis for this spatial separation.

KEYWORDS: viruses; ssDNA virus; Barents Sea; marine sediments; biogeography

INTRODUCTION

Viruses and virus-like particles are the most abundant biological entities in marine ecosystems and they have an impact on processes like horizontal gene transfer, microbial community structuring and biogeochemical cycling (Bergh et al. 1989, Fuhrman 1999, Weinbauer 2004, Weinbauer & Rassoulzadegan 2004, Suttle 2005, Bouvier & del Giorgio 2007, Yang et al. 2010). Their genomes may have double-stranded (ds) or single-stranded (ss) DNA or ds or ss RNA. Traditionally the dsDNA group has been considered the most abundant in marine environments (Wommack & Colwell 2000, Breitbart et al. 2002, Breitbart et al. 2004, Steward & Preston 2011, Fancello et al. 2013). In recent years, high abundancies of ssDNA viruses have also been reported in various aquatic environments, including marine waters (Angly et al. 2006, Tucker et al. 2011, Labonté & Suttle 2013a, b), fresh water (Lopez-Bueno et al. 2009, Roux et al. 2012), deep seafloor sediments (Yoshida et al. 2013) and methane seep sediments (Bryson et al. 2015). However, it has become increasingly clear that the high prevalence of ssDNA viruses to a large extent is a consequence of amplification bias caused by the use of phi29 DNA polymerase based multiple-displacement DNA amplification (MDA) to obtain sufficient metagenomic material for high-throughput sequencing (Kim & Bae 2011). While the majority of marine dsDNA viral genomes range from 25 to 70 kilobases (kb) in size (Steward et al. 2000), circular ssDNA viruses are small both with respect to physical and genomic size, as the latter largely vary in the range from 1 to 9 kb (Fauquet et al. 2005). Subsequently, the ssDNA viruses are not easily enumerated by epifluorescent microscopy or flow cytometry viral counts because of weak fluorescence signal (Tomaru & Nagasaki 2007, Holmfeldt et al. 2012).

Temporal and geographical variations in aquatic viral assemblages are well-documented (Bergh et al. 1989, Wommack & Colwell 2000, Short & Suttle 2003, Clasen et al. 2008, Danovaro et al. 2008, Yang et al. 2010, Helton et al. 2012, Parsons et al. 2012,

64 Hurwitz et al. 2014) and these variations are at some level presupposed to be related to
65 concomitant variations in the host communities although these relationships may be complex.
66 Uncertainty factors include e.g. lytic versus temperate strategies of the viruses and r versus K
67 type strategies of the hosts (Suttle 2007). Conceptual models like “kill the winner”, implying
68 that the fastest growing host populations in a community are the ones most susceptible to viral
69 infections (Thingstad 2000), have been fruitful in explaining the viral contribution to
70 maintenance of high host diversities in natural environments.
71 While there has been a vast increase in the available genomic information from marine
72 microbiotas in the last decade owing to the implementation of next-generation sequencing
73 technologies, the number of isolated and characterized virus-host systems is still limited. Due
74 to their ecological importance marine primary producers, including the ubiquitous
75 *Prochlorococcus* and *Synechococcus* among the prokaryotes and diatoms and dinoflagellates
76 among the eukaryotes, have been principal targets of such studies (Sullivan et al. 2003,
77 Nagasaki 2008, Avrani et al. 2011, Kimura & Tomaru 2015). Among heterotrophic bacteria,
78 phages infecting the *Bacteroidetes* species *Cellulophaga baltica* has been thoroughly
79 characterized by Holmfeldt et al. (2007) and later infection studies of the ubiquitous, but hard-
80 to-cultivate pelagic groups SAR11 and SAR116 have been added to the list (Kang et al. 2013,
81 Zhao et al. 2013). To evade the limitations caused by the consistent lack of cultivability of the
82 large majority of marine prokaryotes, single-cell genomics based approaches have been
83 introduced to identify and characterize individual bacterioplankton-virus systems (Roux et al.
84 2014b, Labonté et al. 2015). To our knowledge, no isolated virus-host system from marine
85 sediments has been characterized.
86 Although a large fraction of the individual or contiguous gene sequences resulting
87 from metagenomic sequencing projects on aquatic viromes have been classified as having
88 unknown function and taxonomic affiliation (Breitbart et al. 2004, Edwards & Rohwer 2005,

Angly et al. 2006, Desnues et al. 2008, Lopez-Bueno et al. 2009, Roux et al. 2012, Fancello et al. 2013, Yoshida et al. 2013), such projects have still given valuable insights into compositional and biogeographical variations. The pioneering shotgun cloning based study by Breitbart et al. (2004) indicated a higher viral diversity in Californian coastal sediment than in the adjacent pelagic water masses. The two assemblage types were also phylogenetically distinct. Yoshida et al. (2013) have compared pyrosequence data from three deep-sea sediment viromes and conclude they show marked similarities that distinguish them from e.g. sea water viromes, despite spatial separations by more than 1000 km and marked host community and environmental differences.

The objective of the present study was to complement the hitherto fragmentary knowledge on diversity variations in seafloor viromes by adding data from an oceanic area that deviate both geographically and environmentally from the ones addressed in preceding studies in the field. Sampling at different sites, separated by up to 640 km, in the Barents Sea shelf seafloor opened for elucidation of viral biogeographical variations within a coherent and environmentally moderately variable shelf sediment area. In a recent study based on the same sediment material, 16S rRNA gene based prokaryotic community variation was linked to the environmental and geographic separation of the Barents Sea by the oceanic polar front (Nguyen & Landfald 2015). Hence, the present analysis opened the possibility of elucidating if coincident biogeographical patterns could be observed for bacterial and bacteriophage assemblages originating from the same material.

MATERIALS AND METHODS

Sampling

Sediment samples were collected by Van Veen grab within three days (20 - 23 of May 2009) from five locations separated by up to 640 km (Fig. 1) in the western part of the Barents Sea. The three southern sampling stations (1 - 3) were south of the polar front, hence dominated by relatively warm and saline Atlantic water (temperature 2.5 - 2.7ºC at seafloor at time of sampling), while the northern stations 4 and 5 were dominated by colder Arctic water (temperature 0.9 - 1.2ºC). Furthermore, ocean depth was largest at the southern stations (442 - 474 m versus 230 - 290 m at the northern ones), while other recorded physicochemical variables (salinity, grain size distribution, organic carbon content) showed minimal or inconsistent differences between sampling sites. Additional details of sampling, locations and sediment characteristics are given in Nguyen & Landfald (2015). The present stations 1 to 5 correspond to stations 1, 3, 4, 7 and 10 in that study.

Purification and isolation of viral DNA

Viral purification was performed with modifications of the procedure of Thurber et al. (2009). Three mL sediment was suspended in 3 mL of 2% (v/w) tetrasodium pyrophosphate and incubated for 15 min in the dark at 4ºC. To ensure release of the viral particles from the sediment samples, the suspension was sonicated on ice for 3 x 45 sec with a Branson 3210 sonicator (Triad Scientific, Inc. Manasquan, USA), and centrifuged at 1000 x g for 5 min. Thereafter, the sediment pellets were twice re-suspended in 3 mL of 2% (v/w) NaCl and centrifuged at 2500 x g for 5 min. The supernatants were pooled and successively passed slowly through 0.45 µm and 0.2 µm Whatman filters to remove residual cells. The filtrate was concentrated by using a 30 kDa NMWCO centrifugal ultrafiltration device (Ultracel PL-30

membrane, Millipore). The concentrate was treated with DNase I (2.5 U ml^{-1}) and incubated for 1 hour at 37ºC to remove free DNA. To check for purity of the viral preparation, each sample was stained with SYBRGold (Invitrogen) and inspected under an epifluorescence microscope (Leica DM6000 B). If no contamination by prokaryotic cells was revealed, the viral nucleic acids were extracted using the formamide and phenol/chloroform/isoamyl alcohol procedure (Thurber et al. 2009). After the extraction, the absence of detectable quantities of bacterial DNA was confirmed by subjecting the DNA preparation to PCR amplification with universal bacterial 16S rRNA gene primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACCTTGTTACGACTT-3′). The PCR master mix contained 2.5 µl of 10X PCR buffer (Invitrogen, Waltham, MA, USA), 2.0 µl of 0.2 mM dNTPs (Invitrogen), 2.5 µl of 0.5 µM of each primer (Eurofins MWG, Ebersberg, Germany), 1.25 U of Taq polymerase (Invitrogen), 10 ng of genomic DNA template, and Milli-Q water to a total volume of 25 µl. The thermocycler (Applied Biosystems) conditions were initial denaturation step at 95ºC for 5 min; 30 cycles at 95ºC for 30 s, 55ºC for 30 s, and 72ºC for 1 min; a final extension at 72ºC for 5 min. Only preparations where no bacterial cells were observed and no bacterial PCR products were generated from the DNA extracts were used for subsequent viral amplification.

Amplification of viral DNA and sequencing

Viral genomic DNA (10 ng) was amplified using the Genomiphi V2 DNA Amplication kit (GE Healthcare Life Sciences, Freiburg, Germany) according to the manufacturer's instructions. After amplification, amplicon DNA was purified on silica columns (Qiagen Dnase Kit), precipitated with ethanol and resuspended in pure water. The concentration and quality of amplified DNA were determined by a NanoDrop ND-1000 spectrophotometer (Wilmington, DE, USA). The amplifications were done in duplicate for each sample to reduce

bias. The samples were subjected to shotgun pyrosequencing (GS FLX Titanium; 454 Life Sciences, Branford, CT, installed at The Norwegian High-Throughput Sequencing Centre, University of Oslo, Norway; http://www.sequencing.uio.no).

Sequence assembly

Flowgram sequence data in sff format from the GS FLX Titanium were assembled using Newbler version 2.5.3 for all five stations. Several runs with varying parameters were tested to produce optimal assemblies based on N50 and total contig yield (bp). Default settings for *de novo* assembly and "autotrimming" were considered the most successful. A minimum contig size threshold of 500 bp was applied to discard the smallest contigs.

Taxonomic classifications

The metagenomic sequence and contig data were analyzed using the Metavir (Roux et al. 2011) and Metavir2 (Roux et al. 2014a) pipeline (http://metavir-med.univbpclermont.fr/) with taxonomic affiliations based on BLASTx queries in the NCBI Refseq complete viral genomes protein sequence database (release of 2016-01-19). BLAST hits with E-values $<10^{-3}$ were considered significant. Sequences affiliated with individual *Circoviridae*, *Geminiviridae* and *Nanoviridae* genomes or metagenomic genomes categorized as Circular Rep-encoding ssDNA (CRESS-DNA) viruses were classified as CRESS-DNA virus sequences in accordance with Rosario et al. (2015). Taxonomic assignments were also generated via MG-RAST (Meta Genome Rapid Annotation using Subsystem Technology, v3.1) (Meyer et al. 2008) based on BLASTx searches against the M5NR non-redundant database. An E-value cutoff of 10^{-3}, a minimum identity cutoff of 60% and minimum alignment length of 15 bp were used as parameters for this analysis.

186

187 **Phylogenetic analyses of marker genes**

188 The protein sequences of the major capsid protein F (Vp1) of the *Microviridae* family
189 (Desnues et al. 2008) and the replication-associated protein (Rep) of the
190 *Circoviridae/Nanoviridae/Geminiviridae* families (Rosario et al. 2009a) were used to infer
191 phylogenetic trees. Representative sequences covering the whole or major parts of the
192 complete gene products from the Barents Sea sediment viromes were aligned with
193 homologous sequences obtained from translated genomic and metagenomic sequences of
194 public data and the Metavir server, by using the ClustalW algorithm in MEGA 6 program
195 package (Tamura et al. 2013). Phylogenetic trees were constructed using the maximum
196 likelihood method, with Jones-Taylor-Thornton substitution model, gamma distribution
197 among sites, partial deletion of missing data and bootstrapping with 100 resamplings, as
198 implemented in the MEGA package.

199

200 **Comparative metagenomics of viral communities**

201 The viral metagenomics data from our study were compared with public virome projects from
202 other environments, available in the MetaVir web server (Roux et al. 2011). The imported
203 data only included studies in which multiple displacement amplification (MDA) had been
204 used to increase DNA quantities and comprised data from deep-sea surface sediment
205 (Yoshida et al. 2013), sea water (Angly et al. 2006, Cassman et al. 2012) and fresh water
206 (Roux et al. 2012, Fancello et al. 2013). In the MetaVir workflow, the similarity of the virus
207 metagenomes were compared and scored as tBLASTx sequence similarity scores. Only
208 viromes that contained more than 50 000 sequences with length exceeding 100 bp on average
209 were used for the comparision. Similarity scores for two by two viromes were computed as
210 the sum of the best BLAST hit scores of a sequence component in one virome community

against its counterpart in the other virome. The score sums for the virome pairs were stored in a matrix format and used to hierarchically cluster the communities in the Metavir web server.

Separate hierarchical clustering trees for the *Microviridae* and the eukaryotic subgroups of virus sequences of the Barents Sea sediment data were inferred by the *pvclust* package with default parameters (Suzuki & Shimodaira 2006) in the R software (http://www.r-project.org). Bootstrap values (1000 resamplings) were calculated.

Bacterial community comparisons. The total DNA was extracted using the PowerSoil™ DNA Isolation kit (Mo Bio Labs, Inc., Carlsbad, CA, USA) according to the manufacturer's instruction. The bacterial 16S rRNA genes were amplified by use the primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 338R (5′-TGCTGCCTCCCGTAGGAGT-3′). Subsequently, the amplicons were sequenced by multiplex pyrosequencing on a 454/Roche GS-FLX Titanium instrument, and the sequences were binned into operational taxonomic units (OTUs) at 97% identity level, as described in Nguyen and Landfald (2015). Sequence-based hierarchical clustering of the bacterial communities was done by average linkage in the R package *pvclust*.

Virus/bacteria community relationships. Correlations between variations in bacterial communities and viral assemblages (complete or subsections) were quantified as Spearman rank correlation coefficients calculated from Bray-Curtis distance matrices. Bacterial distances were based on OTU tables, while viral distances were based on tables of significant BLAST hits ($E < 10^{-3}$). The statistical significance of the correlations were tested by the Mantel test with 10^4 Monte Carlo permutations in the *vegan* package in R.

Virome accession numbers. Viral metagenomes from the five sampling stations are accessible in the Metavir server under the name Barents Sea sediment. In the MG-RAST annotation server, they are available under accession numbers: 4564380.3; 4564381.3; 4564382.3; 4564383.3; 4565015.3. The 14 completed circular genomes are deposited in GenBank under accession numbers KX828610 to KX829623.

RESULTS AND DISCUSSION

The Barents Sea sediment virome

The shotgun pyrosequencing produced a total of 674 115 sequence reads with an average length of 507 bp from the five Barents Sea sediment samples (Table 1). Between 81% and 87% of reads in the different samples were assembled into 17805 contiguous sequences (minimum 500 bp) by the Newbler assembler. The median contig length varied in the range 1131 to 1228 bp, the longest being 5745 bp. A total of 39756 complete or partial open reading frames (ORFs) were identified in the contigs and 37 to 51% of the predicted ORFs in the separate samples showed significant homology to sequences present in the NCBI Refseq database. Fourteen contigs, ranging from 819 to 4217 bp in size, were categorized as circular genomes by employing the Metavir2 pipeline, but only four of them produced significant hits (E-value $<10^{-3}$) to established viral genomes. The largest identified genome (4217 bp) was similar to the one of the complete marine *Gokushovirinae* GOM (KC131021; Labonte & Suttle 2013a) while two genomes of size 2163 bp and 1938 bp, respectively, showed highest similarity to the eukaryotic circular Rep-encoding ssDNA (CRESS-DNA) viruses Avon-

Heathcote Estuary associated circular virus 5 and 6 (KM874301 and KM874301) (Dayaram et al. 2015). The former was categorized as circo-like by encoding both the conserved Rep and a putative capsid (Cap) protein (Rosario et al. 2015) while the latter encoded just the Rep protein, as did the fourth, smallest genome (1265 bp), which was similar to *Circoviridae* 3 LDMD-2013 (KF133810; McDaniel et al. 2014).

Analyses of the individual sequences identified 27 to 44% as having significant similarity (BLASTx, E-values < 10^{-3}) to genes within the complete viral genomes of the Refseq database (Table 1). Hence, both the contiguous and individual sequence data confirmed the consistent pattern from preceding environmental virome studies that the majority of sequences remain unassigned. However, the proportions of virus-affiliated sequences compared favorably with preceding metagenomic studies employing the Refseq database, as 1 - 35% viral genes have been reported at < 10^{-3} significance level (Breitbart et al. 2002, Breitbart et al. 2004, Edwards & Rohwer 2005, Angly et al. 2006, Desnues et al. 2008, Lopez-Bueno et al. 2009, Roux et al. 2012, Fancello et al. 2013, Yoshida et al. 2013). Successful enrichment of viral metagenomic material was also indicated by low proportions of prokaryotic (6.7%) and eukaryotic (0.6%) sequences by search in the M5NR database of MG-RAST (Meyer et al. 2008). The rather straightforward method employed in the present study for isolation of virus particles from sediment therefore appeared efficient for enrichment of viral genomic material.

Further phylogenetic classification showed 86.1 to 94.8% of reads affiliated to viruses from the five sampling stations to be confidently associated with ssDNA virus genes of the Refseq database (E-value < 10^{-3}), while reads of dsDNA virus origin constituted 3.0 to 11.6% (Table 2). In accordance with the pattern for the ssDNA viral groups (see below), both a bacteriophage taxon (*Caudovirales*) and a eukaryote-infecting taxon (*Phycodnaviridae*) were represented among of the most predominant dsDNA groups. Several preceding metagenomic

285 studies have indicated a strong presence of ssDNA viruses in marine environments (Desnues
286 et al. 2008, Kim & Bae 2011, Rosario & Breitbart 2011, Tucker et al. 2011, Yoshida et al.
287 2013). However, a common feature of both the present and the referred studies is the use of
288 MDA with phi29 polymerase for amplification of viral metagenomic DNA. MDA biases the
289 genome type distribution towards ssDNA viruses and, in particular, towards small circular
290 ssDNA viruses. For seawater samples this has been demonstrated by comparing viral DNA
291 amplified by the linker amplified shotgun library (LASL) method of Breitbart et al. (2002)
292 with the same samples amplified by MDA (Kim & Bae 2011), while McDaniel et al. (2014)
293 find large differences in abundances of ssDNA virus genes between MDA and unamplified
294 DNA preparations from seawater. Chromatographic separation of viral groups prior to LASL
295 amplification, as devised by Andrews-Pfannkoch et al. (2010), represents an improvement,
296 but it still remains unclarified to what extent the diversities of the resulting metagenomic data
297 are truly representative of the source material. In our study, the proportion of dsDNA viruses
298 may have been lowered also by the filtration step used for removal of cellular and abiotic
299 particles. Electron micrographs show some tailed or filamentous environmental virions to
300 have physical dimensions that limit their passage through the 0.2 μm pore size filters used in
301 the present study (Bergh et al. 1989, Zhao et al. 2013), while circular ssDNA viruses are less
302 likely hindered due to their overall smaller size. To secure the inclusion of large-size virions
303 in the preparations, some recent virome studies have preferred the use of 0.45 μm pore size
304 filters or no filtration step at all (Lopez-Bueno et al. 2009, Fancello et al. 2013, Martínez
305 Martínez et al. 2014). On the other hand, environmental viral diversity studies have also
306 demonstrated variable proportions of ssDNA virus genes and even dominance of dsDNA
307 virus genes in 0.2 μm filtrates amplified by MDA (Rosario et al. 2009b, Cassman et al. 2012,
308 McDaniel et al. 2014), indicating a relationship between abundances of ssDNA viruses in the
309 source material and ssDNA virus genes in the amplified metagenome. Hence, the apparent

predominance by ssDNA viral material in our amplified virome DNA likely reflects a lower but substantial proportion of this virus group in the source material.

The International Committee on Taxonomy of Viruses has classified the ssDNA viruses into 11 families and one genus not assigned to a family (Krupovic & Forterre 2015, Krupovic et al. 2016). The BLASTx search affiliated the open reading frames of the Barents Sea sediment material with six of these families, i.e. *Microviridae*, *Circoviridae*, *Nanoviridae*, *Geminiviridae*, *Parvoviridae* and *Inoviridae*, among which the *Microviridae* phage family constituted 8 - 44% whereas a larger fraction, 46 - 81%, were assigned to the three eukaryotic families of circular Rep-encoding ssDNA viruses (*Circoviridae*, *Nanoviridae*, *Geminiviridae*), collectively named CRESS-DNA viruses (Rosario et al. 2015). More than half of the CRESS DNA virus sequences were affiliated with the *Circoviridae* family by showing highest similarity to genes in complete genomes carrying both Rep and putative capsid encoding genes.

There are still rather few described examples of ssDNA viruses infecting marine microorganisms, the first one being a novel virus that infects bloom-forming diatoms (Nagasaki et al. 2005). More recently, putative *Microviridae* infecting marine *Bacteroidetes* phylotypes have been reported (Holmfeldt et al. 2012). Identification of the virus-host associations by *in silico* analysis generally requires complete or near-complete genomic information of both virus and host (Roux et al. 2015). This is a challenging task by metagenomic approaches even in rather well-characterized microbiotas like the human gut (Pérez-Brocal et al. 2013), and even more so in highly diverse habitat types like marine environments, which are dominated by hitherto uncultivated and genomically uncharacterized phylogenetic groups. However, Mizuno et al. (2013) were able to assign putative hosts to more than 500 viral conigs in a sea water fosmid library by combining several *in silico* analytical approaches. Potential bacterial and archaeal hosts of viral assemblages in

hydrothermal vent environments have been identified by clustered regularly interspaced short palindromic repeat (CRISPR) analysis (Anderson et al. 2011), while Cassman et al. (2012) were unable to identify virus-host pairs by CRISPR analysis in Pacific sea water material. Recently single-cell genome amplification has been demonstrated as a viable new approach to identify virus-bacteria associations even among the uncultivated majority of bacteria in a pelagic marine system (Roux et al. 2014b).

Marker gene phylogenies

Phylogenetic relationships of the major capsid protein F (Vp1) as marker gene for the *Microviridae* and the replication-associated protein (Rep) as marker gene for the CRESS-DNA virus were inferred by comparing sequences covering conserved regions with corresponding sequences from relevant metagenome and genome studies (Fig. 2A, B). *Gokushovirinae* originating from intracellular parasitic bacteria (*Chlamydiae, Bdellovibrio and Spiroplasma* spp.) (Brentlinger et al. 2002), and *Microvirus* from enterobacterial species (including φX174) were the sources of the most closely related Vp1 genes of established genomic origin. The actual host groups of the Vp1-carrying phages within the Barents Sea sediment communities were not established. A preceding study of bacterial community structure in the same material identified *Gammaproteobacteria* and *Deltaproteobacteria* as predominant groups by jointly constituting nearly two-thirds of the 16S rRNA gene pool (Nguyen & Landfald 2015). Outside the marine realm, the *Microviridae* have been shown to infect a diverse range of hosts, including *Proteobacteria*, *Spiroplasma* and *Chlamydiales* (Read et al. 2000, Brentlinger et al. 2002, Garner et al. 2004) and a recent, comprehensive search for viral genomes in prokaryotic genomic data sets (Roux et al. 2015) also ties the

360 *Microviridae* to *Alpha-*, *Gamma-* and *Deltaproteobacteria* as important hosts classes. It
361 therefore appears likely that the mentioned classes of *Proteobacteria* constitutes substantial
362 parts of the *Microviridae* host range.
363 Different Barents Sea Rep-encoding sequences showed affiliation to both animal-
364 infecting (*Circoviridae*) and plant-infecting (*Nanoviridae*, ssDNA Alphasatellites,
365 *Geminiviridae*) genomic virus sequences and metagenomic sequences from sea water, fresh
366 water and deep-sea sediments were also distributed throughout the Rep gene diversity. No
367 distinct grouping of the picked Vp1 or Rep metagenomic sequences in accordance with the
368 south-north climatic division of the Barents Sea was observed, indicating that important
369 groups of host organisms were abundant throughout the sampling range.
370
371 **Community comparisons**
372 A hierarchical clustering tree, including both the five Barents Sea sampling sites and
373 additional aquatic and sediment viromes (Angly et al. 2006, Cassman et al. 2012, Roux et al.
374 2012, Fancello et al. 2013, Yoshida et al. 2013), was inferred from BLAST-based
375 comparisons (Fig. 3) by tools integrated in the MetaVir server (Roux et al. 2011). The three
376 Pacific Ocean marine sediment viromes (Yoshida et al. 2013) clustered with the Barents Sea
377 samples and the environmentally most comparable location, i.e. pelagic sediment (1181 m
378 water depth) off the Japanese coast, also showed the highest assemblage similarity. Even the
379 viromes from two extremely deep Pacific trenches were more similar to the Barents Sea
380 material than any sea water or freshwater sample, including the ones collected from Arctic
381 regions. These common phylogenetic features in seafloor sediment viromes across large
382 geographical separations and extensive variations in water depth likely reflect corresponding
383 similarities in the prokaryotic and eukaryotic host communities, but the specific basis for the
384 observed commonness remains vague as community data is lacking for the microorganisms.

In the present study, some level of coincidence in bacterial and viral biogeography patterns among the Barents Sea sediment sampling stations was indicated by the hierarchical clustering patterns. A previous comprehensive study including 10 sampling stations, established significant differences among bacterial communities in accordance with the main environmental division of this ocean area into a southern and a northern region by the oceanic polar front (Nguyen & Landfald 2015). Here we reconfirmed this bacterial pattern in a study comprising just the five stations included in the virome analysis. The two northernmost stations 4 and 5 constituted a distinct cluster based on 16S rRNA gene sequence data (Fig. 4A). BLAST based similarity scores indicated the same partition of the viral assemblages if the analysis was based on the complete virome sequence data (Fig. 3). However, if restricting the analysis to the dominant group of bacteria-associated virus in the assemblages, i.e. the *Microviridae* family of phages, the viromes showed no clustering pattern reflecting the one exerted by the bacterial communities (Fig. 4B). On the other hand, the northern sampling stations 4 and 5 showed a distinct grouping of the eukaryotic CRESS-DNA virus assemblages (Fig. 4C), suggesting that these taxa were inclined to stronger host variations along the south-north axis than the bacteria-infecting viruses. The absence of relationship between bacteria and bacteriophage community variations was also confirmed by a Mantel test of correlation between the respective community distance matrices ($P = 0.29$). One factor that may have contributed to this lack of coherence is the aforementioned expected bias towards ssDNA virus in MDA-amplified viromes. This would lead to an overestimation of the importance of the *Microviridae* as infective agents in the bacterial communities. Secondly, the bacterial community profiling was based on ribosomal RNA gene frequency comparisons. This implies that the phylogenetic distribution of actively proliferating bacterial phylotypes, which expectedly are the more influential ones on phage assemblage structuring, may have deviated from the observed total 16S rRNA gene based biogeographical variation. The elevated

relative abundance of eukaryotic virus types in the north (Table 2) coincided with higher levels of chlorophyll *a* and chloroplast 16S rRNA gene frequencies at the same locations, as reported elsewhere (Nguyen & Landfald 2015). Hence, increased seafloor deposition of fresh algal material in the northern region, due to the recent ice margin spring bloom, may have contributed to the observed differences among the eukaryotic virus assemblages.

In conclusion, the study confirmed that virome biogeographical variation within a coherent and environmentally moderately variable shelf seafloor area was small as compared with differences among more distinctly separated ecosystems, such as geographically distant lakes or oceans. It furthermore indicated some degree of commonness in composition of seafloor viral assemblages at a global scale. The observed Barents Sea virome variation was principally associated with changes in relative abundance and composition of eukaryotic ssDNA virus genes, while the established bacterial community variation within the same ocean range was not mirrored in the bacteriophage metagenomic material. Our work underlined the need to complement this kind of community-wide approaches with identification and characterization of representative virus-microorganism associations to get comprehensive pictures of the interactions between the groups in their natural environment.

Acknowledgements. This work was supported by grants from the UiT-The Arctic University of Norway, the Centre for Marine Bioactives and Drug Discovery (MABCENT-SFI) and the program Marine Biotechnology in Northern Norway (MABIT). We thank the crew of F/F Helmer Hansen for their technical and logistic assistance during the sampling cruise.

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614

615 Table 1. Outcome of the virome sequence analyses at the individual sampling sites

Sampling site	Number of reads	Average length (bp)	Assembled reads (% of reads)	Number of contigs (>500bp)	Viral hits[1] (% of reads)
ST.1	118 313	497	81.3	3642	40.5
ST.2	158 119	511	80.1	5110	27.3
ST.3	154 403	516	78.5	4526	40.3
ST.4	111 206	502	79.9	2308	32.2
ST.5	132 074	509	86.6	2219	43.8

616 [1]Significant similarity ($E < 10^{-3}$) to the NCBI Refseq database
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620 Table 2. Phylogenetic affiliations of viral sequences from the five sampling stations

Group	Taxonomic composition	ST.1 (%) (n=47948)	ST.2 (%) (n=43193)	ST.3 (%) (n=62148)	ST.4 (%) (n=36110)	ST.5 (%) (n=57784)
ssDNA	Microviridae	36.0	15.7	43.9	7.9	12.9
	CRESS-DNA virus	57.8	69.7	45.7	81.2	81.4
	Parvoviridae	0.1	0.1	0.1	0.0	0.0
	Inoviridae	0.0	0.1	0.1	0.0	0.0
	Unclassified ssDNA viruses	0.4	0.5	0.5	0.5	0.5
dsDNA	Phycodnaviridae	2.4	6.3	1.9	2.1	1.1
	Caudovirales	0.5	3.4	4.9	4.4	1.2
	Mimiviridae	0.2	0.5	0.2	0.3	0.2
	Poxviridae	0.2	0.5	0.2	0.2	0.1
	Herpesvirales	0.1	0.2	0.2	0.1	0.1
	Iridoviridae	0.1	0.1	0.1	0.0	0.0
	Unclassified dsDNA	0.1	0.6	0.7	0.8	0.3
Satellites		1.7	2.1	1.1	2.1	2.0
ssRNA viruses		0.1	0.0	0.1	0.0	0.0
Unclassified viruses		0.3	0.2	0.3	0.4	0.2
*Chloroplast 16S rRNA genes (%)**		*0.1*	*0.0*	*0.2*	*2.5*	*1.8*

621 *(Nguyen & Landfald 2015); CRESS DNA, Circular Rep-encoding ssDNA

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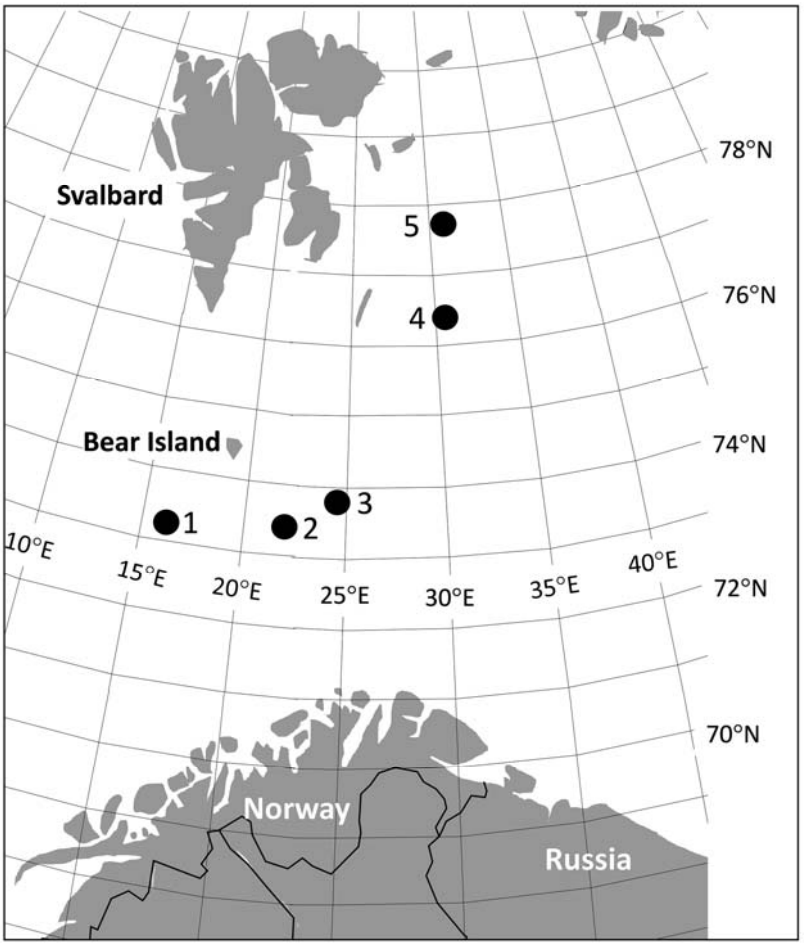
Figure legends

Fig. 1. The western Barents Sea with geographical positions of sampling stations.

Fig. 2. Unrooted maximum likelihood phylogenetic trees computed from the major capsid protein Vp1 of *Microviridae* (A) and the replication-associated protein (Rep) of CRESS-DNA viruses (B). Marker coding: filled circles, sequences of the Barents Sea virome from southern stations 1-3 (red) and northern stations 4-5 (blue); open circles, sequences from other metagenomic projects, including fresh water (FW; Roux et al. 2012), sea water (SW; Labonté & Suttle 2013a, Labonté & Suttle 2013b) and deep-sea sediment (DSS; Yoshida et al. 2013); black triangles, sequences from completed genomes.

Fig. 3. tBLASTx similarity based comparison of environmental virome assemblages. All included sequence data originate from multiple-displacement amplified viral DNA. Sources: sea water (Angly et al. 2006, Cassman et al. 2012), deep-sea surface sediment (Yoshida et al. 2013), and fresh water (Roux et al. 2012, Fancello et al. 2013).

Fig. 4. Hierachical clustering of (A) bacterial communities, (B) *Microviridae* assemblages and (C) CRESS-DNA virus assemblages of the Barents Sea sampling sites. Bacterial 16S rRNA gene sequence data were obtained from Nguyen & Landfald (2015). Multiscale bootstrap values (1000 re-samplings) are indicated at the nodes.


Svalbard
Bear Island
Norway
Russia
1
2
3
4
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78°N
76°N
74°N
72°N
70°N
10°E
15°E
20°E
25°E
30°E
35°E
40°E

Fig.1

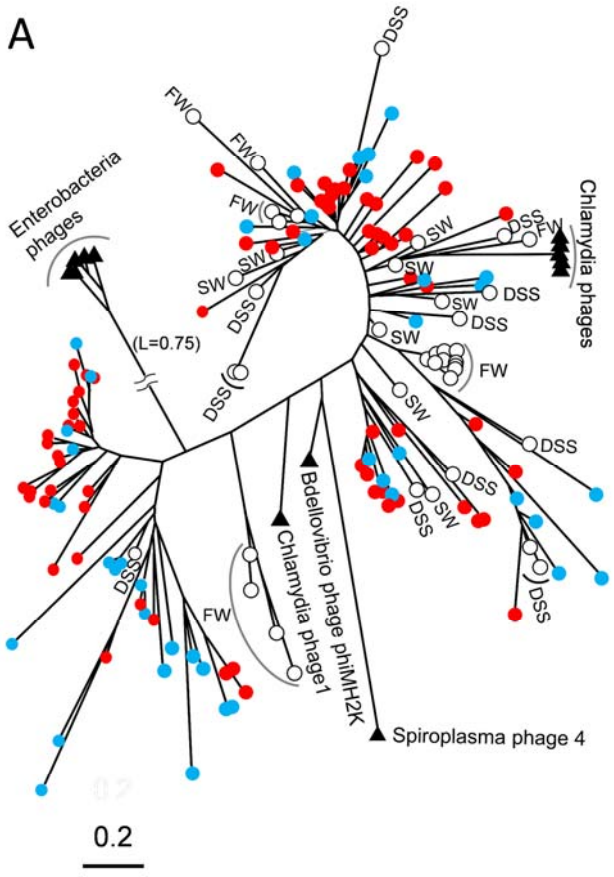

A
Enterobacteria phages
(L=0.75)
Chlamydia phages
Bdellovibrio phage phiMH2K
Chlamydia phage1
Spiroplasma phage 4
FW
SW
DSS
0.2
B
Plant Geminiviridae
Diatom virus isolates
Picobiliphyte nanovirus
Animal circoviruses
Plant nanovirus
Bat circovirus
Plant nanovirus
FW
DSS
0.2

Fig. 2

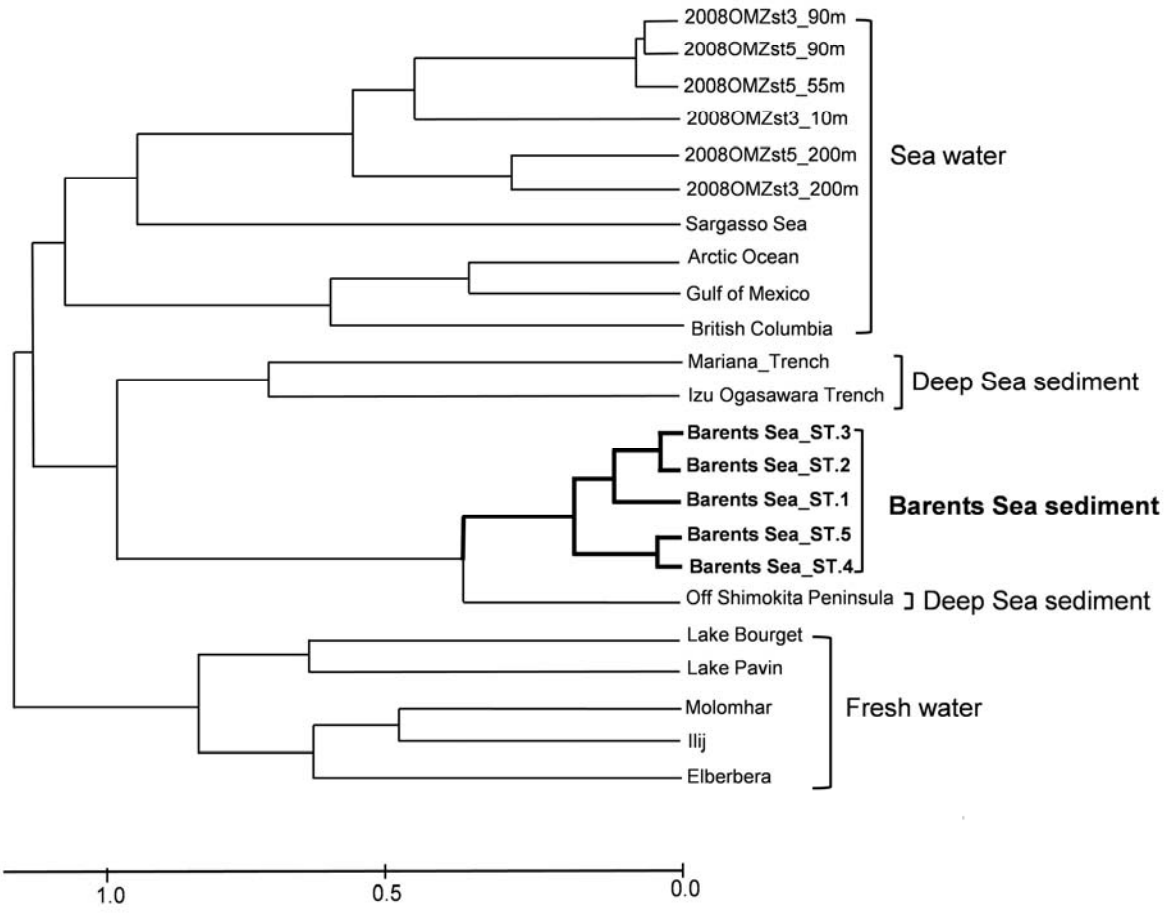
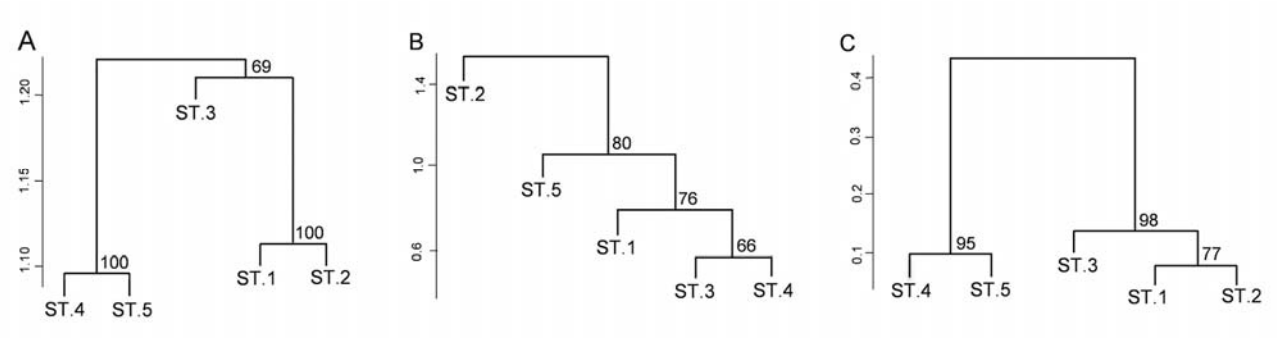

2008OMZst3_90m
2008OMZst5_90m
2008OMZst5_55m
2008OMZst3_10m
2008OMZst5_200m
2008OMZst3_200m
Sargasso Sea
Arctic Ocean
Gulf of Mexico
British Columbia
Sea water
Mariana_Trench
Izu Ogasawara Trench
Deep Sea sediment
Barents Sea_ST.3
Barents Sea_ST.2
Barents Sea_ST.1
Barents Sea_ST.5
Barents Sea_ST.4
Barents Sea sediment
Off Shimokita Peninsula
Deep Sea sediment
Lake Bourget
Lake Pavin
Molomhar
Ilij
Elberbera
Fresh water
1.0
0.5
0.0

Fig.3



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670
671
672 Fig.4
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