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REGULAR PAPER

Novel marine diatom ssRNA virus NitRevRNAV infecting *Nitzschia reversa*

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Background and aims – Diatoms are one of the most species rich groups of organisms on Earth. They are fundamental in supporting the biomass of the natural environment. The presence of marine viruses can greatly influence diatom diversity in their natural environment and has attracted interest from multi-disciplinary research teams after a diatom infected virus was reported for the first time in the 21st century. As initial research in this field demonstrated, for the acquisition of a new virus it is important to learn about their infect hosts. Therefore, we have been searching for new viruses that infect diatoms.
Methods – A clonal host species *Nitzschia reversa* was isolated from natural sea water. Aliquots of the filtrates obtained from this sea water were inoculated to yield the exponentially-growing isolated host species. The resultant lysate was used as a clonal lysate and treated as a clonal virus suspension. The suspension was then used for further analysis of various biochemical studies.
Key results – We discovered and isolated a new virus that infected the pennate diatom *Nitzschia reversa*. Since this newly discovered virus was a single strand RNA virus, it has capsid proteins with 30 nm size icosahedron without an envelope. The species epithet is NitRevRNAV.
Conclusions – In this study, we described the morphology, genome type and partial sequences, as well as lytic activities, of the new species NitRevRNAV. Of note, the virus harbours a positive-sense single-stranded RNA genome. These features were highly similar to those of previously known diatom RNA viruses therefore NitRevRNAV is a new member of the genus Bacillarnavirus in the Order Picornavirales.

Key words – Marine virus, algal virus, new species, *Nitzschia*, Bacillariophyta, diatom.

INTRODUCTION

The ecosystems of the oceans, lakes and rivers are excellently placed for crucial research topics in both basic and applied science. A range of interdisciplinary research projects, involving fields such as geography, chemistry, biology, etc., are needed to address both the patterns of life and the processes affecting the organisms that inhabit aquatic sites (e.g. Carpenter et al. 1999). Diatoms are one such group of or-

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ganisms. They are highly diverse with more than 10 000 species already described and they are responsible for over 40 percent of the photosynthesis in the world's oceans (Nelson et al. 1995, Kooistra et al. 2007). Consequently, they contribute up to 20% of annual global CO_2 fixation (Matsuda et al. 2017). Further, because of their contributions to aquatic productivity, many other organisms depend on them either directly or indirectly for their survival (Mann 1999, Tréguer et al. 2017).

More than two hundred million virus particles per millilitre of natural seawater were reported by a Norwegian research group at the end of the 1980s (Bergh et al. 1989). From this simple observation the new field of marine virology was born. It has been estimated that there may be more than 30 million viruses that infect algal species (Norton et al. 1996), yet their existence was not discovered until the end of the last century.

Suttle (2007) suggested that by affecting the composition of marine communities, marine viruses have a major influence on biogeochemical cycles and have important roles in relation to microbial mortality (e.g. Brum & Sullivan 2015). Some of the marine virus component is composed of those that infect eukaryotic algae, which boast an overwhelming biomass and species diversity in marine ecosystems (Nelson et al. 1995). From the enormous genetic information and structural proteins they possess, new discoveries are made. For example, Yolken et al. (2014) identified DNA sequences homologous to virus (Chlorovirus) ATCV-1, an algal virus not previously known to infect humans, in healthy human oropharyngeal samples, and Tomaru et al. (2007) showed that in natural environments HcRNAV (a *Heterocapsa circularisquama* RNA virus) has a significant effect on populations of *H. circularisquama* in terms of biomass and composition. These reports suggested that algal viruses in the environment can have tremendous biological effects.

Among the various studies of marine viruses, viral genome particles are currently one of the most advanced fields of research in viral metagenomic analysis. Various studies have reported on marine viruses, such as metagenomic and metatranscriptomic surveys of marine RNA viruses, including picornaviruses, which have explored the diversity of marine virus communities, etc. (e.g. Culley et al. 2003, Moniruzzaman et al. 2017, Allen et al. 2017, Urayama et al. 2018). Observations of many other phenomena, from a scientific standpoint, have only scratched the surface of the significance of viruses, and there is still a great need to conduct further studies in order to know more about marine viruses. Several dozen species infecting raphidophytes and dinoflagellates have been confirmed (Nagasaki et al. 2005), and for diatoms, more than a dozen viruses have been isolated and characterized (Tomaru et al. 2015). However, although it is expected that there are a large number of unknown viruses that infect diatoms, it has not yet been established what their relationship is to the host in nature. Hence, more study of novel viruses is needed, along with study of infected host organisms.

The possibility of establishing some estimate of the diversity of genetic resources in natural waters exists thanks to metagenomic analyses. However, in order to clarify in detail how each virus exists in nature, how it infects the hosts, proliferates and declines, and whether it affects the evolution of both host and virus, a different approach and methods are required. Therefore, one must examine both host and virus together.

In this study, we isolated a novel single-stranded RNA virus, named NitRevRNAV, that infects the pennate diatom *Nitzschia reversa* W.Sm. As far as we know, this is the first report characterizing both the morphologic and genomic details of a diatom virus that infects species in the genus *Nitzschia*.

MATERIALS AND METHODS

Host culture

A clonal host species *Nitzschia reversa* (fig. 1A), strain number KT30, was isolated from the sea surface using a plankton filter in Inagenohama, Chiba, Tokyo Bay (35°61′N, 140°06′E) in May 2010. Cells were isolated into clonal monoculture and grown in modified F2 medium solution (Guillard & Ryther 1962, Guillard 1975) at half strength, enriched with Gel Culture (Daiichi Seimo, Japan), Gamborg's vitamin solution (Sigma–Aldrich, Japan) and 2nM Na_2SeO_3 (e.g. Imai et al. 1996) under a 12/12h light–dark cycle using white fluorescent illumination at 17.5°C. Cultures such as phage may contaminate any new viruses, thus for aseptic manipulation of stable growth condition cells, the strains were sterilized using AM9 solution (Provasoil et al. 1959) with low concentration penicillin to remove bacteria and phage.

To search and isolate a novel virus from natural sea water

We conducted viral isolations following the method of Nagasaki & Bratbak (2010), partially modified as follows. Surface seawater samples (with 0–1 cm depth seafloor sand) were collected from a sandy beach at Sagami bay, Japan (34°80′N, 139°06′E), in November 2010. The samples were delivered to the laboratory without fixation and stored at 15°C in the dark for three days. The supernatants of the samples were passed through 0.2 μm Dismic-25cs filters to remove bacteria. Aliquots (0.2 mL) of the filtrates obtained from the samples were inoculated into exponentially-growing *N. reversa* strain KT30. Aliquots (0.2 mL) of the filtrates obtained from sediment samples were inoculated into exponentially-growing host culture, followed by incubation at 15°C using the light/dark cycle conditions described above for host culture. Reproducibility of the strain was again confirmed for that in which cell burst was confirmed in the assumed period of about 10 to 14 days. From the cultures that showed an apparent burst in host cells after inoculation of the filtrates (e.g. fig. 1B), the responsible pathogens were cloned through two extinction–dilution cycles (Suttle 1993, Tomaru et al. 2004). The lysate was diluted in the modified medium in a series of 10-fold dilution steps; aliquots (500 μL) of each dilution step were added to 8 wells in cell culture plates with 48 flat-bottom culture wells containing 600 μL of an exponentially growing host culture. Then, the lysate in the most diluted well of the first assay was carried over to the second extinction dilution procedure. Finally, the resultant lysate in

the final end-point dilution was used as a clonal lysate, and established a mass culture system for that system (fig. 1C).

Virus purification

Several strains of a 100-mL exponentially growing culture were inoculated with 1 mL of the virus suspensions. The lysate was passed through 0.2 µm pore size polycarbonate membrane filters to remove cellular debris. After ultracentrifugation at 150,000 × *g* at 4°C for 4 h, the pellet was washed with 10 mM phosphate buffer (pH 7.5). This procedure was repeated, concentrating multiple sets into one pellet. The resultant viral pellets were used for genome analysis, in viral protein analysis and negative staining observations under transmission electron microscopy (TEM). The resultant axenic lysate was treated as a clonal virus suspension and used for further analysis.

TEM observations

An exponentially growing culture of *N. reversa* KT30 was inoculated with the virus suspension. For a control, a *N. reversa* culture was inoculated with autoclaved half-strength F2 medium. Virus particles negatively stained with uranyl acetate were observed using transmission electron microscopy (TEM). Briefly, a drop of purified virus suspension was mounted on a grid (PVF-C15 STEM Cu150 Grid (Okenshoji, Tokyo, Japan)) for 30 s, excess water was removed using filter paper. After 4% uranyl acetate was applied for 10 s, excess dye was removed using filter paper. The negatively stained virus particles were observed using TEM (JEM-1010 electron microscope: JEOL, Tokyo, Japan) at 80 kV. Particle diameters were estimated using the negatively stained images. As for ultrathin sections of the cells, an aliquot of the cell suspension was sampled at 36 h post-inoculation, and fixed with 2% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.5) for 2 h at 4°C. Then, cells were collected by centrifugation and washed twice with 0.1 M phosphate-buffered saline (pH 7.5). Washed samples were post-fixed with 2% OsO_4 in 0.1 M phosphate-buffered saline (pH 7.5). After washing with buffer, the samples were dehydrated in a graded acetone series and then embedded in Spurr's epoxy resin. Samples were polymerized for 12 h at 70°C. 70-nm thin sections were cut using a diamond knife on a Reichert Ultracut S microtome (Leica, Wetzlar, Germany), and mounted on formvar-coated oneslot grids. Sections were stained with 4% uranyl acetate and 3% lead citrate and observed with a JEM-1010 electron microscope.

Viral proteins

Aliquots (5 µL) of the virus suspension were mixed with four volumes of denaturing sample buffer (62.5 mM Tris-HCl [pH 6.8], 5% 2-mercaptoethanol, 2% sodium dodecyl sulfate [SDS], 20% glycerol, and 0.005% bromophenol blue) and boiled for 5 min. The proteins were then separated by SDS-polyacrylamide gel electrophoresis (90×78×1 mm, 3–10% gradient polyacrylamide, 150 V) using the Polyacrylamide Gel Electrophoresis System (ATTO, Tokyo, Japan). Proteins were visualized using Oriole Fluorescent Gen Stain (Bio-Rad Laboratories, Inc., California, USA). Protein Ladder One (Nacalai tesque, Inc., Tokyo, Japan), ranging from 10 to 250 kDa, were used for size calibration.

Genome sequence

The sequencing of a partial viral genome was analysed. In brief, based on the viral particles pelleted by the ultra-centrifuge from the mass viral culture, viral genome (ssRNA) was extracted using RNeasy Plant Mini Kit (Qiagen, Tokyo, Japan) and from a random part of viral RNA double-stranded cDNA was synthesized using PrimeScript™ Double Strand cDNA Synthesis Kit (Takara Bio Inc., Shiga, Japan), which

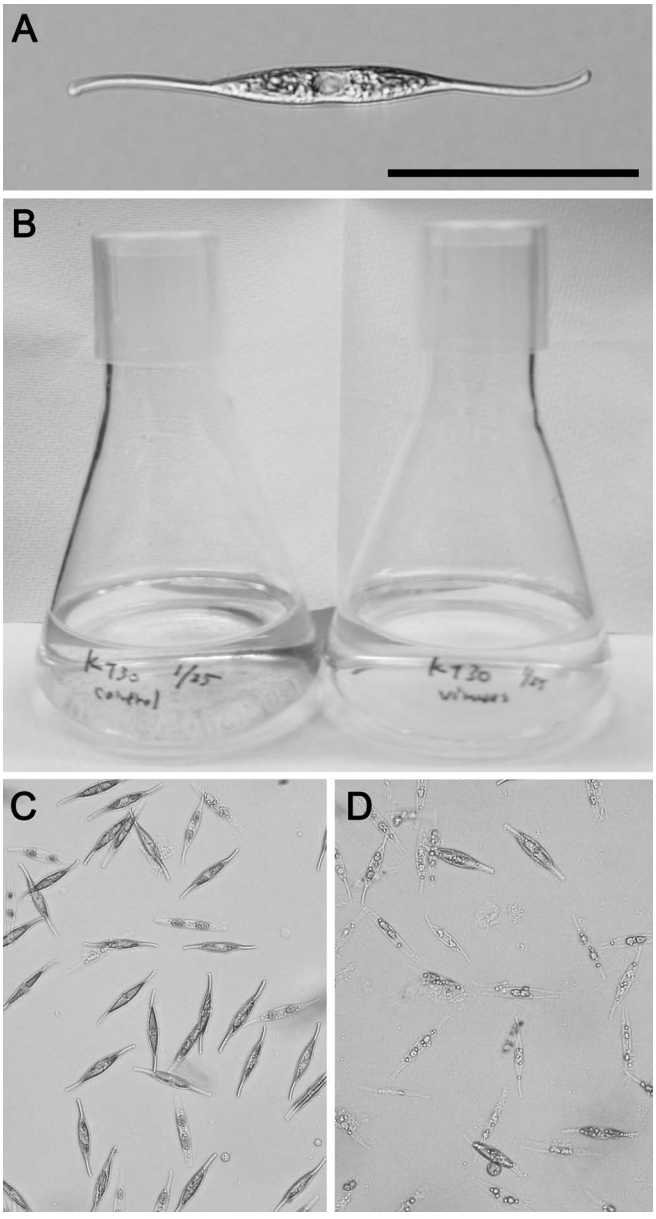

Figure 1 – *Nitzschia reversa* strain KT30: A, Light Microscope (LM) view of *Nitzschia reversa*; B, *N. reversa* mass cultures of control (left) and added NitRevRNAV (right), 7 days post-inoculation; C, LM view of intact cells; D, LM view of NitRevRNAV-infected cells at 60 h post-inoculation. Scale bar: A = 50 µm.

was used with random primer and oligo(dT)18 primer. The synthesized cDNA were amplified by chemical TA-cloning methods (Mighty TA-cloning Kit: Takara Bio, Shiga, Japan) with *Escherichia coli* DH5α competent cells. Partial nucleotide sequences of the virus, by cycle sequence method, were analysed from the obtained plasmid. The resultant fragment sequences were reassembled using DNASTAR (DNASTAR Inc., WI, USA). For determination of the viral RNA gene sense strand, negative or positive, specific primers pair, "5'-TCAGAGTAATCTGACTTAGGTGGAA-3'" and "5'-TTCCACCTAAGTCAGATTACTCTGA-3'", which works on the site around 100 base upstream of poly-A tail, also "5'-ATGCCTCATCTTGCACATTCA-3'" and "5'-TGAATGTGCAAGATGAGGCAT-3'", which works on NitRevRNAV Sequence 2, were designed from the obtained base sequence of the virus, and it was judged whether cDNA were synthesized or not. Each RNA sequence was translated to its amino acid sequence with all reading frames and homology of all translated sequences searched against protein sequence database of diatom infecting viruses. Subsequently, the predicted amino acid sequences and capsid proteins were aligned with those of other diatom-infecting ssRNA viruses by using Clustal W program (Thompson et al. 1994), and the partial genome map of NitRevRNAV which referred the aligned diatom ssRNA genome map was constructed. Especially, partial amino acid sequences of RNA-dependent-RNA-polymerase (RdRp) protein (NitRevRNAV Sequence 2) was annotated by the HHpred program (https://toolkit.tuebingen.mpg.de/#/tools/hhpred).

Phylogenetic tree of virus

Partial viral RNA sequences were uploaded to the data base of DNA Data Bank of Japan (DDBJ); registered names with the database accession numbers are as follows: NitRevRNAV_Sequence1, LC466844; NitRevRNAV_Sequence2, LC466845; NitRevRNAV_Sequence3, LC466846; NitRevRNAV_Sequence4, LC466847. Partial amino acid sequences of RdRp protein (NitRevRNAV_Sequence2) detected in this work were compared with RdRp protein sequence dataset of several ssRNA viruses. Partial sequences of capsid protein (NitRevRNAV_Sequence4) detected in the above experiment were also merged with the dataset of the capsid protein sequence of ssRNA viruses. Each merged dataset was automatically aligned using the MAFFT program with default parameters built into GENETIX-MAC version 19 (https://www.genetyx.co.jp) and partial Clustal W alignment program built into MEGA7, respectively (Kumar et al. 2016, Thompson et al. 1994). Finally, the amino acid sequence datasets were manually aligned. Phylogenetic trees based on amino acid sequences of the RdRp and capsid protein were constructed using the maximum likelihood (ML) method with the Jones-Taylor-Thornton matrix (JTT model) packaged in the MEGA7. Bootstrap consensus trees were constructed using x1000 analysis.

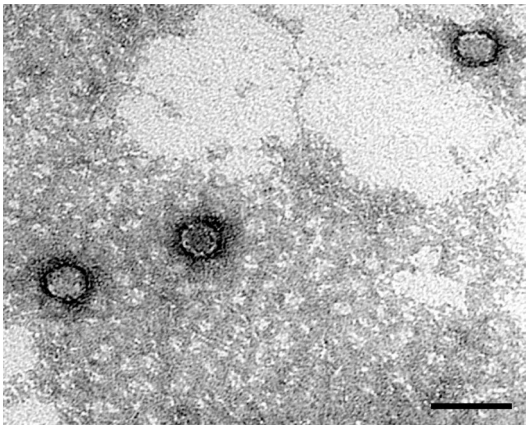

Figure 2 – Negatively stained NitRevRNAV particles. Scale bar = 50 nm.

RESULTS AND DISCUSSION

Isolation of viral pathogens and determination of their host range

Host specificity of the virus was revealed by inoculating various diatom species, such as *Nitzschia* spp., *Cylindrotheca* spp., *Chaetoceros* spp., *Skeletonema* spp., *Achnanthes* spp. etc. (data not shown), with virus suspensions. The virus was lytic to its original host *Nitzschia reversa* strain KT30, but to no other microalgal species tested. These results indicated the high species-specific infection of this virus, which is a general feature of microalgal viruses (e.g. Nagasaki et al.

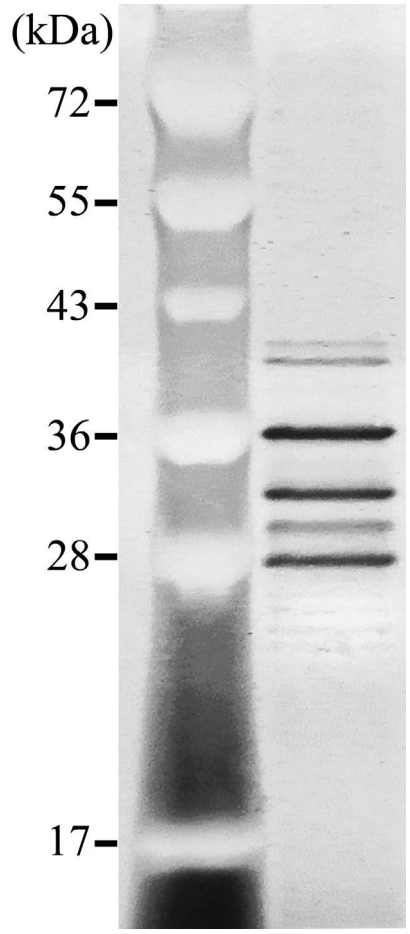

Figure 3 – Major structural proteins of NitRevRNAV visualized by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

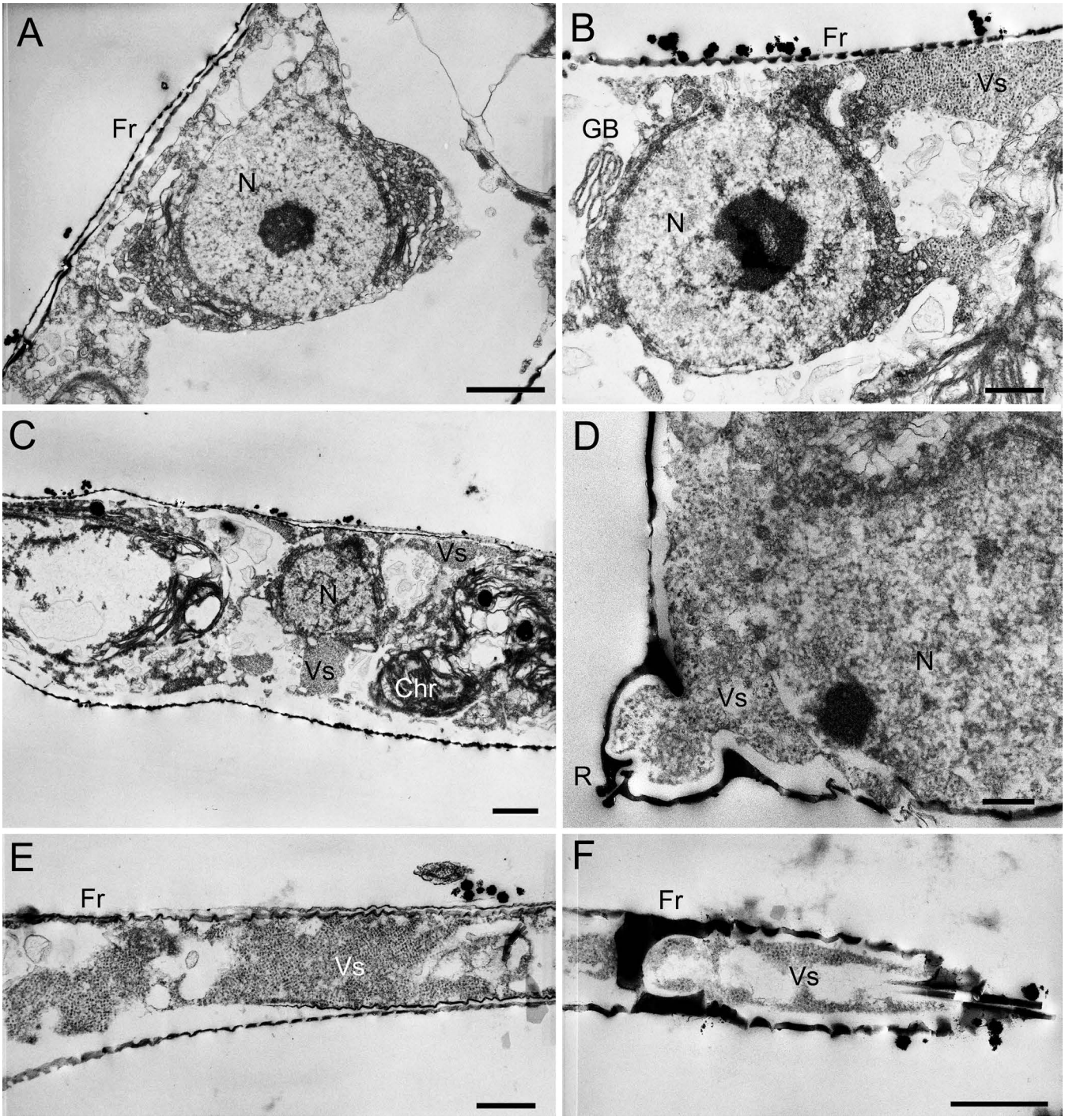

Figure 4 – Transmission electron micrographs of ultrathin sections of *Nitzschia reversa* strain KT30: A, healthy cell; B–F, infected cell with NitRevRNAV at 48h post-inoculation; B & C, around nucleus, showing nucleus undamaged and virus particles confirmed outside of the nucleus; D, expanded view of raphe side, showing viral particles growing clustered on the inside of the raphe; E & F, virus particles accumulated up to the tip of the frustule; particles were replicated abundantly. Scale bars: A & C = 1 μm; B, E & F = 500 nm; D = 200 nm. Chr: chloroplast; GB: golgi body; Fr: frustule; N: nucleus; R: raphe; Vs: virus-like particles.

2004, Tomaru et al. 2009). In culture strains ingesting the virus, the cultured cells gradually die (burst) around 36 hours later, so that more than half of the individuals have burst after 48 hours (fig. 1C right), and within 7 days after virus inoculation, all of the cultured cells in the strains have burst.

Morphological features

The size of virus particles, in diameter, was found to be 30 ± 1 nm (n = 17) by TEM observation of negative staining (fig. 2). The size of each individual of the observed virus was approximately the same, and it appeared as a hexagon in two-dimensional representations. As with many other viruses, which include viruses that infect organisms other than diatoms, the result indicates that the capsid forming the particle of the virus is a regular icosahedral conformation. As for virion diameter, this was almost the same as in already-known diatom viruses such as AglaRNAV and Csp05DNAV (e.g. Tomaru et al. 2012, Toyoda et al. 2012). Among the viruses that infect algae, the capsid may or may not be surrounded by an envelope (Mackinder et al. 2009); NitRevRNAV does not possess envelopes. This shows that the virus particles are formed only from capsid proteins and genes. In the experiment using the biochemical method, the size numbers of the structural proteins of the virus particles were examined by SDS-Page, which revealed four major polypeptides at 36k, 32k, 30k and 28kDa (fig. 3). The result was similar to that for other diatom infectious ssRNA viruses such as Csp03RNAV, RsetRNAV, CtenRNAV, and CsfrRNAV (Nagasaki et al. 2004, Shirai et al. 2008, Tomaru et al. 2009, Tomaru et al. 2013).

From TEM observations of ultrathin sections of *N. reversa* infected with NitRevRNAV strain KT30, it was found that, as predicted, the viral particles made many copies of themselves in the infected cells. It extends to the tip of frustule and grows throughout the cell (fig. 4B–F). However, virus particles were not observed inside the nucleus, and it seems that the nuclei were not damaged. Accumulations of isolated bacillarnaviruses (a genus of RNA viruses affecting diatoms) (e.g. Tomaru & Nagasaki 2011) have been observed in their host's cytoplasm, not in the nucleus such as in diatom DNA viruses (e.g. Tomaru et al. 2012, 2013). The replication of NitRevRNAV might occur in the cytoplasm.

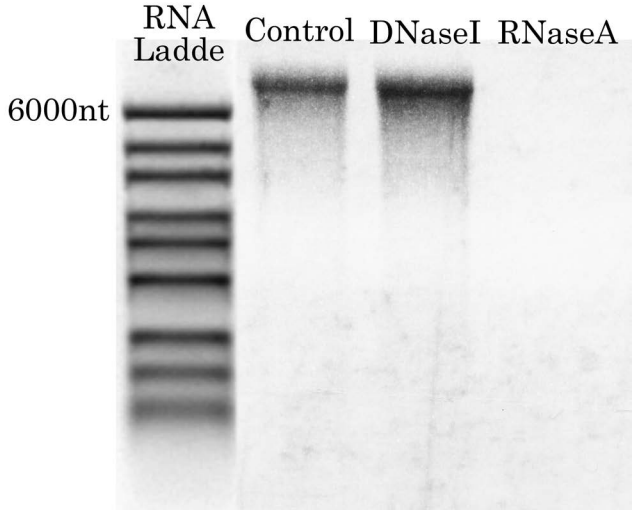

Figure 5 – Intact nucleic acids of NitRevRNAV, treatment with DNase I, RNase A. Samples electrophoresed in an agarose gel.

Viral genome, proteins, and phylogeny of NitRevRNAV

From viral genome characterization, denaturing gel electrophoresis revealed that NitRevRNAV has a single molecule of nucleic acid that is approximately 9 kb long and is sensitive to RNase A (fig. 5, lane 3) but is resistant to DNase I (fig. 5, lane 4). These data indicate that the viral genome is a single stranded RNA. RNA analysis revealed that the genomic RNA of the virus is a positive-sense strand and has a poly-A tail at the 3' end of the molecule. This was supported from the results of analysis of 3' end terminal molecules using oligo(dT) primer and cDNA synthesis experiment using specific primer pairs by the inverse PCR method.

Four amino acid sequences detected in this work aligned with the protein sequences of other ssRNA viruses. NitRevRNAV_Sequence1, 2, 3 were similar with open reading frame 1, which codes the gene of replication related to poly protein (fig. 6A); NitRevRNAV Sequence4 is similar with a viral capsid protein of diatom ssRNA viruses (fig. 6B). Finally, NitRevRNAV_Sequence2 is similar with RNA-dependent RNA polymerase (RdRp) protein of Norwalk Virus of e-value of 3.3e-29 (see table 1).

In previous reports, diatom viruses are divided into two groups based on genome conformation: single-stranded RNA (ssRNA) (Nagasaki 2004, Shirai et al. 2008, Kimura & Tomaru 2015, Arsenieff et al. 2019) or single-stranded DNA (ssDNA) (Tomaru et al. 2011, Toyoda et al. 2012). Phylogenetic analysis of ssRNA viruses using deduced amino acid sequences of RNA-dependent RNA polymerase (RdRp) shows that diatom RNA viruses, including NitRevRNAV, are

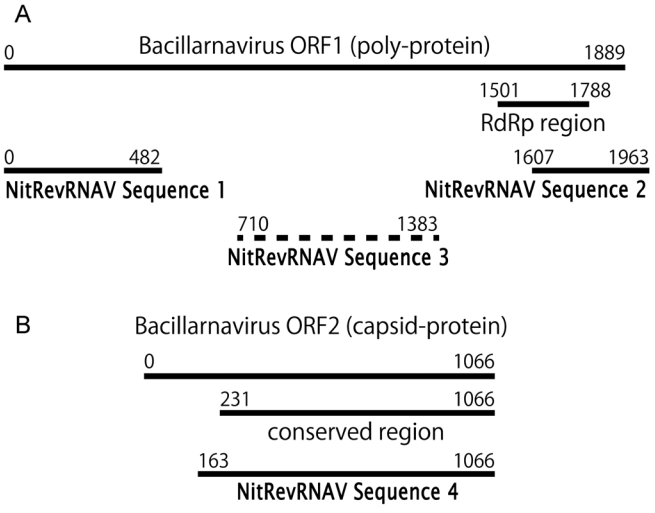

Figure 6 – Genome map of NitRevRNAV based on the aligned database of open reading frames constructed using other diatom ssRNA virus genomes: Continuous lines of NitRevRNAV sequence indicate that they have high consensus sequence with other diatom virus genomes. Broken line of NitRevRNAV shows low similarity with other diatom virus genomes.

monophyletic; this result is supported by a high bootstrap value (97%) (fig. 7A). Furthermore, structural proteins of NitRevRNAV and other diatom RNA viruses also reveal a monophyletic relationship with a bootstrap value of 97% (fig. 7B). Considering these results, it is appropriate to consider NitRevRNAV as a new member of the genus *Bacillarnavirus*.

Concluding remarks

In this study, we have documented the morphology, genome type and partial sequences of NitRevRNAV, as well as its lytic activities. These features were highly similar to those of previously known diatom RNA viruses. Therefore, our conclusions are that NitRevRNAV is a new member of the genus *Bacillarnavirus* in the Order Picornavirales, harbouring a positive-sense single-stranded RNA genome.

A recent study showed diatom-virus dynamics in nature and suggested their ecological relevance, based on several years of field surveys (Tomaru et al. 2011, Yau et al. 2011). For example, Tomaru et al. (2011) showed that virus-infected diatom cells occurred throughout the year in sediments derived from water-column populations. As for the specificity of the host, Kimura & Tomaru (2015) demonstrated that CtenRNAV type-II can infect multiple diatom host species.

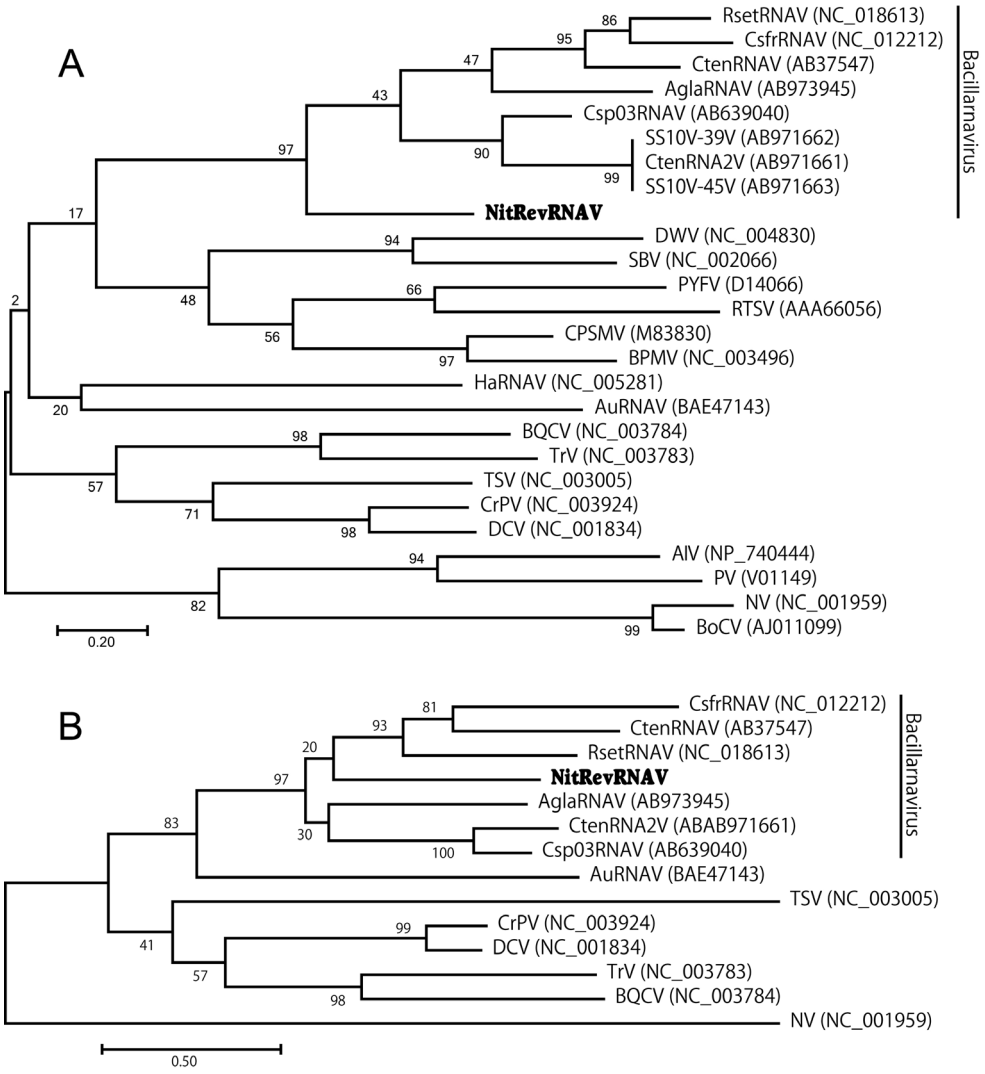

Figure 7 – Phylogenetic analysis of (A) RNA-dependent-RNA-polymerase and (B) capsid protein of ssRNA viruses: Each tree was constructed using the maximum likelihood (ML) method. Bootstrap values (%) from 1,000 samples are shown at the nodes. ML distance scale bars are shown under each ML tree. Virus names; AglaRNAV: *Asterionellopsis glacialis* RNA virus, AIV: aichi virus, AuRNAV: *Aurantiochytrium* single-stranded RNA virus, BoCV: bovine enteric calicivirus, BPMV: bean pod mottle virus, BQCV: black queen cell virus, CPSMV: cowpea severe mosaic virus, CrPV: cricket paralysis virus, CsfrRNAV: *Chaetoceros socialis* f. *radians* RNA virus, Csp03RNAV: *Chaetoceros* sp. strain SS08-C03 RNA virus, CtenRNAV: *Chaetoceros tenuissimus* RNA virus, CtenRNA2V: *Chaetoceros tenuissimus* RNA virus Type-II, SS10-39V: *Chaetoceros tenuissimus* RNA virus strain SS10-39, SS10-45V: *Chaetoceros tenuissimus* RNA virus strain SS10-45. DCV: drosophila C virus, DWV: deformed wing virus, HaRNAV: *Heterosigma akashiwo* RNA virus, NV: Norwalk virus, PV: Human poliovirus 1 Mahoney, PYFV: Parsnip yellow fleck virus, RsetRNAV: *Rhizosolenia setigera* RNA virus, RTSV: Rice turgo spherical virus, SBV: sacbrood virus, TrV: Triatoma virus, TSV: taura syndrome virus.

Table 1 – Viral protein annotation.

			Best hit HHPred comparison					
Virus isolate	**ORF_AA**	**Hit**	**Hypothetical function of the gene product**	**Organism**	**Probability**	**E-value**	**Score**	**Identities (%)**
NrevRNAV_Sequence2	IRLCNYEPEDIAIMNAMTADICY AYIAFNGDLVSLTEGSHISGNSL TVIINGICGSLNLRCKFYSLHPY TNFDERKKFRDYVALMTYGDD NIGSTTKDSGFTIKGASEFLKEY GQVYTMXDKESELTDYLPKDD IEFLKRKSVYHPKLGVNIGALA DKSIFKSLHCYLRGKGRPHTPE MASAINIDGAMREFFCHGEEVY NKRQSQMREIAERAGIAHMCE GIERTYDDRVNEWKVTYDESY RPYEKVDYWIDDYNTSDLA*VS KRRTPYDQWGFMYIVE*	3BSO_A	RNA dependent RNA polymerase	Norwalk virus	99.95	3.30E-29	238.42	19

This ssRNA virus lysed four different diatom species within the genus *Chaetoceros* in addition to its original host. Therefore, diatom viruses may have an impact on the population dynamics not only of the original host population, but the diatom community. However, except for CtenRNAV and CtenDNAV which infect the same host *Chaetoceros tenuissimus*, all the other isolated diatom viruses that have ever been reported, specifically lyse their specific host diatom species; therefore, they are thought to be an important factor in controlling the dynamics of their respective host populations (Nagasaki et al. 2004, Tomaru et al. 2008, Toyoda et al. 2012). Our study on NitRevRNA shows infections for in only one strain, KT30. Further study is needed to know what kinds of phenomena are involved in the specificity for this infected host. This may also bring a new perspective on diatom classification, which have been based on the frustule morphology and some rDNA sequences.

Traditionally, various environmental factors, such as water temperature, salinity and nutrients, have been considered to affect diatom dynamics (e.g. Sarthou et al. 2005). However, recent studies have shown the potential significance of viruses in controlling the population dynamics of diatoms in natural environments (Tomaru et al. 2011, Arsenieff et al. 2019), just as between other eukaryotic microorganisms and their viruses (Mackinder et al. 2009, Moniruzzaman et al. 2017, Urayama et al. 2018). However, to understand the role of diatom viruses in the regulation of host populations, more knowledge of different diatom virus species is needed.

This study is the first report on a virus that infects *Nitzschia reversa*. This study will be linked to new methods for screening viral infections between viruses and their hosts in natural environment.

Using these new methods, progress in this field of research will allow the acquisition of important data to demonstrate some further relationships, such as the pathways of infection and disease especially with respect to the dynamics of the interactions between algae and viruses.

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