Biogeography of marine giant viruses reveals their interplay with eukaryotes and ecological functions

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Abstract

Nucleocytoplasmic large DNA viruses (NCLDVs) are ubiquitous in marine environments and infect diverse eukaryotes. However, little is known about their biogeography and ecology in the ocean. By leveraging the *Tara* Oceans pole-to-pole metagenomic data set, we investigated the distribution of NCLDVs across size fractions, depths and biomes, and their associations with eukaryotic communities. Our analyses revealed a heterogeneous distribution of NCLDVs across oceans, with an elevated uniqueness in polar biomes. The community structures of NCLDV families were correlated with specific eukaryotic lineages including many photosynthetic groups. NCDLV communities were generally distinct between surface and mesopelagic zones, but at some locations, they exhibited a high similarity between the two depths. This vertical similarity was correlated to surface phytoplankton biomass but not to physical mixing processes, suggesting the potential role of vertical export in structuring mesopelagic NCLDV communities. These results underscore the importance of the coupling between NCLDVs and eukaryotes in biogeochemical processes in the ocean.

Introduction

The photic zone is the most productive layer of the ocean, containing a wide variety of microorganisms such as bacteria, autotrophic and heterotrophic protists and multicellular organisms. The population dynamics of these organisms determine the flows of energy and materials thorough marine food webs, playing a fundamental role in ecosystem functioning and biogeochemical cycles in the ocean[1,2]. Viruses exert a top-down control on marine organisms and release material to the pools of particulate and dissolved organic matter[3]. This material and remineralized inorganic nutrients are utilized by autotrophic and mixotrophic phytoplankton[4]. The recycling of nutrients in the surface layer potentially reduces the transfer of fixed organic carbon to higher trophic levels and the deep sea[5,6]. However, it is also possible that viruses enhance downward carbon flux by facilitating cell aggregation and producing carbon-enriched materials from infected cells [7-9].

Nucleocytoplasmic large DNA viruses (NCLDVs or so-called "giant viruses") represent a monophyletic group of viruses that infect a variety of eukaryotic lineages[10-12]. Studies focusing on conserved marker genes such as family B DNA polymerase (*polB*) have revealed that NCLDVs are highly diverse and abundant in aquatic environments[13-16]. The diversity of a family of NCLDVs, namely the *Megaviridae*, exceeds that of bacteria and archaea in the ocean[17] and their richness in a few liters of seawater can reach more than 5,000 operational taxonomic units[18]. More recently, several thousand draft genomes (i.e. metagenome-assembled genomes, MAGs) of NCLDVs were constructed from environmental sequences, thanks to the development of high-throughput sequencing and bioinformatics technologies[19,20]. However, the global biogeography of marine NCLDVs still remains under-explored.

A growing number of marine eukaryotes have been reported as host organisms of NCLDVs, particularly phytoplankton groups such as haptophytes, chlorophytes and dinoflagellates[21-23]. Other eukaryotic lineages, including non-photosynthetic organisms such as bicosoecids and choanoflagellates, have also been reported as host organisms of

NCLDVs in marine environments[24,25]. These studies collectively suggest the ecological importance of NCLDVs in the ocean via top-down effects on eukaryotic communities. However, our knowledge of NCLDV-host relationships is highly limited, given the large phylogenetic diversities of NCLDVs and microeukaryotes.

Here we reveal patterns in the global biogeography of NCLDVs using the metagenomic data from the *Tara* Oceans project. The metagenomic data cover varying geographic regions including polar and deep-sea ecosystems, in which NCLDVs are under-researched[26-28]. We constructed NCLDV taxonomic abundance profiles for 283 samples, representing two viral size fractions, three ocean depth ranges (surface, deep chlorophyll maximum and mesopelagic), and four biomes (coastal, trades, westerlies and polar). The global biogeography of NCLDVs derived from these data reveals strong associations between NCLDVs and eukaryotic microorganisms. Furthermore, vertical connectivity of NCLDV communities indicates a possible mechanism for how mesopelagic NCLDV communities are structured with respect to ocean biogeochemical processes.

Results

NCLDV phylotypes detected in *Tara* Oceans metagenomes

We detected 6,818 PolBs affiliated with NCLDVs in the second version of the Ocean Microbial Reference Gene Catalog (OM-RGC.v2)[28] using the pplacer phylogenetic placement method[29] (see methods for details). The OM-RGC.v2 was built based on 370 *Tara* Oceans metagenomes from femto- (<0.2 μm; 151 samples), pico- (0.22–1.6 or 0.22–3.0 μm; 180 samples) and other (39 samples) size fractions. After removing 39 samples from non-target size fractions and depths and 48 samples with a low NCLDV frequency, the remaining 283 samples contained 6,783 NCLDV PolB sequences. The pplacer classified these PolBs into nine NCLDV families/lineages. The number of phylotypes (distinct *polB* at 95% nucleotide sequence identity) was most diverse in the *Megaviridae* (5,091 phylotypes), followed by the *Phycodnaviridae* (981 phylotypes). The number of

phylotypes taxonomically assigned to the *Iridoviridae*, *Medusavirus* and *Asfarviridae*, were 239, 120 and 109, respectively. We also detected PolBs assigned to the *Pithoviridae* (93), *Ascoviridae* (78), *Poxviridae* (51) and *Marseilleviridae* (21). However, the *Poxviridae* was omitted from our discussion as the environmental gene sequences were distantly related to known *Poxviridae*. Rarefaction analysis showed that, at the end of sampling, the number of NCLDV phylotypes increased by less than 0.01% per sample for all samples, and ranged from 0.02% to 0.32% when samples were divided into different size fractions, depths and biomes (Supplementary Fig. 1).

To examine phylogenetic affiliations among the observed NCLDVs, we constructed a phylogenetic tree using selected PolB sequences (Fig. 4, see also Supplementary Figs. 2–4 for details). Among the *Megaviridae* family, genes closely related to the algal-infecting subfamily, recently proposed as "Mesomimivirinae" (e.g. AaV, CeV, pkV, PgV, PoV and TetV)[30], which infect pelagophytes (the genus *Aureococcus*), haptophytes (the genera *Haptolina*, *Prymnesium* and *Phaeocystis*), and chlorophytes (the genera *Pyramimonas* and *Tetraselmis*), were relatively abundant. Among the *Phycodnaviridae*, the genus *Prasinovirus* (e.g. BpV, MpV, OtV and OlV), which infect chlorophyte genera such as *Bathycoccus*, *Micromonas* and *Ostreococcus*, was the most abundant in terms of richness.

Heterogeneity in NCLDV community structure across size, depth and biomes

The dominant NCLDV taxa detected from all sample locations and depths in the pico-size fraction were the *Megaviridae* and *Phycodnaviridae*, with average contributions of 64.6% and 25.4%, respectively (Fig. 1A). The dominant groups of NCLDVs varied widely among sites and depths in samples from the femto-size fraction (Fig. 1B). In this fraction, *Phycodnaviridae* and *Asfarviridae* had relatively high contributions to the total NCLDVs with the mean values of 29.7% and 19.9%, respectively. The *Megaviridae* and *Ascoviridae* were also important contributors with mean values of 12.2% and 11.1%, respectively.

A non-metric multidimensional scaling (NMDS) analysis showed that NCLDV

assemblages clustered according to size fraction, depth and biome (Fig. 2A–2C). Significant differences in NCLDV community composition were detected among all categories (PERMANOVA, p <0.01), and size fraction, depth and biome explained 5.5%, 4.3% and 10.9% of the total variance, respectively.

Taxonomic richness and the Shannon diversity index were used to investigate variation in NCLDV community diversity. In this study, we analyzed the samples from all depths and size fractions to compare diversity differences among depth ranges, although latitudinal trend in Shannon's diversity for pico-sized communities from the surface was reported previously[31]. In the pico-size fraction, mean values for NCLDV richness at the surface and in the DCM layer were about 1.7 times higher than that in the mesopelagic layer (Kruskal-Wallis and Dunn's post hoc test, p <0.01) (Supplementary Fig. 5A). In the femto-size fraction, NCLDV richness was significantly higher at the surface and MES layer than in the DCM layer (Dunn's test, p = 0.04–0.05), although the differences were small and not consistent with the pico-size fraction.

High uniqueness of NCLDV phylotypes in the Arctic Ocean

We analyzed the overlap and uniqueness of NCLDV phylotypes across different ecological zones (i.e. size fraction, depth and biome) to evaluate their ability to disperse across different environments. Each ecological category was divided into two major groups (i.e. pico- and femto-sizes, euphotic and mesopelagic zones, and polar and non-polar biomes), because the NCLDV community in mesopelagic zone or polar biome was separated most significantly from other depths or biomes (Fig. 2). There were 4,003 (59.0% to the total NCLDVs) shared NCLDV phylotypes across size fractions, 4,737 (69.8%) shared phylotypes across depth ranges, and 1,950 (28.7%) shared phylotypes across biomes (Fig. 3A). Only twelve unique phylotypes were detected in the femto-size fraction, whereas 2,768 unique phylotypes were identified in the pico-size fraction. The euphotic zone (surface and DCM) harbored 1,986 unique phylotypes, whereas the aphotic mesopelagic zone had only 60 unique phylotypes. The polar biome (the Arctic and the

166 Southern Ocean) included 620 unique NCLDV phylotypes, whereas 4,213 unique
167 NCLDVs were detected in non-polar biomes (i.e. trades, westerlies and coastal).
168 To further characterize regional differences in the NCLDV community, we
169 investigated the total and unique NCLDV phylotypes observed in nine geographic regions
170 and the phylotypes shared among regions. The total number of phylotypes was relatively
171 high in the Atlantic, Pacific and Indian Oceans and in the Mediterranean Sea, with values
172 of between 3,665 and 4,685 (Fig. 3B). Lower numbers of NCLDV phylotypes were
173 identified from the Red Sea (2,653) and the Arctic Ocean (2,467). The Southern Ocean
174 presented the lowest number of NCLDV phylotypes (561), although this was based on
175 only 5 samples. The Arctic Ocean samples displayed a high number of unique NCLDV
176 phylotypes (551), which corresponded to 22.3% of the total phylotypes detected in this
177 region. In contrast, the number of unique phylotypes from other regions ranged from 0 to
178 134 (0.0% to 3.4%).
179 There was no linear or saturation trend in the number of total or unique NCLDV
180 phylotypes with increasing sample size (Fig. 3C). The high proportion of unique
181 phylotypes in the Arctic Ocean was not a function of sample size, although the number
182 of total phylotypes detected in the Southern Ocean may be limited by the low number of
183 samples. The phylogenetic positions of unique NCLDVs from the polar biome were
184 dispersed across most of the NCLDV families (Fig. 4)
185
186 **NCLDV distributions correlate with eukaryotic communities**
187 A partial Mantel test was conducted to assess community associations among the
188 NCLDV families/lineages or environmental variables (a matrix combining temperature,
189 salinity, nitrate, phosphate and silicate) and major eukaryotic lineages. The pairwise
190 partial correlation coefficients (Spearman's ρ) varied from –0.17 to 0.76 (Fig. 5A), and
191 93.5% of the examined pairs (249 out of 260 for the pico-size fraction and 237 out of 260
192 for the femto-size fraction) showed statistically significant differences (p <0.01) after
193 false discovery rate (FDR) correction. Pairs from pico-sized NCLDV communities with

194 a significant coefficient ≥0.53 were considered to represent strong positive associations,
195 because 8 out of 9 known marine virus-host lineage associations were recovered by this
196 criterion, with little increase in unknown associations (Figs. 5A and 5B). Using this
197 threshold, 30 out of 234 NCLDV-eukaryote lineage pairs and 3 out of 26 environment-
198 eukaryote pairs were found to have strong linkages (Fig. 5C). The NCLDV
199 families/lineages were generally highly correlated with the known host groups among
200 autotrophic and mixotrophic microalgae (haptophytes, chlorophytes, dinophytes,
201 pelagophytes and raphidophytes) (ρ = 0.54–0.67). Interestingly, the *Megaviridae* was
202 strongly correlated with chrysophyte microalgae (ρ = 0.65), which are not currently
203 known as NCLDV hosts. Other than algal lineages, a strong positive correlation was
204 found between the *Megaviridae* and heterotrophic eukaryote choanoflagellates (ρ = 0.76),
205 which are a known lineage of *Megaviridae*. A group of non-photosynthetic heterokonts
206 bicosoecids are also a known host of the *Megaviridae* species *Cafeteria roenbergensis*
207 *virus* in marine environments, but this group was not highly correlated with *Megaviridae*
208 (ρ = 0.30).
209
210 **Potential chrysophyte viruses constitute novel branches of the *Megaviridae***
211 To explore possible associations between NCLDVs and chrysophytes as indicated by
212 the Mantel's regression analysis (Fig. 5C), we tested for chrysophyte-derived genes in
213 the metagenome-assembled genomes (MAGs) of NCLDVs generated by Moniruzzaman
214 et al. (2019)[19] and Schultz et al. (2020)[20]. Results showed that 89 out of 2,263 MAGs
215 contained genes closely related to the transcripts of the chrysophytes (Supplementary
216 Data 1). Comparisons between PolB sequences revealed 27 PolBs from the OM-RGC.v2
217 that were closely related to the NCLDV MAGs with chrysophyte homologs. Most of these
218 PolBs constituted novel branches within the *Megaviridae* (Fig. 4; Supplementary Fig. 4).
219
220 **Vertical connectivity of NCLDV communities**
221 The vertical connectivity of NCLDV communities was investigated using Bray-Curtis

community similarity measures to compare between epipelagic (surface or DCM) and mesopelagic samples at individual sampling locations. The Bray-Curtis similarities were less than 0.10 for about half of the tested locations (20 out of 36 surface sites and 13 out of 26 DCM sites; Fig. 6A; Supplementary Fig. 6A). All sites in the Arctic Ocean and several sites in tropical and subtropical regions showed relatively high similarities between the two depth (0.15 to 0.60). The NCLDV community similarity value was positively correlated with the chlorophyll *a* concentration in the epipelagic layer ($\rho = 0.52$, $p < 0.01$, n = 36 for surface; $\rho = 0.44$, $p = 0.02$, n = 25 for DCM) and NCLDV richness in the mesopelagic layer ($\rho = 0.82$, $p < 0.01$, n = 36 for surface; $\rho = 0.70$, $p < 0.01$, n = 26 for DCM) (Figs. 6B and 6C; Supplementary Figs. 6B and 6C). We also evaluated relationships between NCLDV vertical similarity and physical environmental factors including: the sampling depth of mesopelagic water, the mixed layer depth, and the temperature difference between epipelagic and mesopelagic waters. No significant correlations were detected among these parameters ($p > 0.05$, n = 32–36 for surface samples and n = 25–26 for DCM samples) (Figs. 6D–F; Supplementary Figs. 6D–F).

We plotted correlations among the relative contributions of NCLDV phylotypes between the euphotic and aphotic zones at all sampling locations (Supplementary Figs. 7 and 8). Where there was a strong similarity in the NCLDV community found at different depths, the *Phycodnaviridae* generally contributed highly to samples from the Arctic Ocean (e.g. stations 158, 201 and 209), and both the *Megaviridae* and *Phycodnaviridae* contributed strongly in tropical and subtropical regions (e.g. stations 72, 110 and 122).

Discussion

We investigated the diversity and community structure of NCLDVs based on metagenomic PolB sequences collected from the world oceans. NCLDV communities differed substantially between pico- and femto- size fractions (Fig. 1). NCLDV communities in the pico-size fractions were dominated by the *Megaviridae* and *Phycodnaviridae*, regardless of sampling location or depth (Fig. 1A). In marine

environments, species from the haptophytes (the genera *Prymnesium*, *Haptolina*, and *Phaeocystis*), chlorophytes (*Pyramimonas*), pelagophytes (*Aureococcus*), bicosoecids (*Cafeteria*) and choanoflagellates (*Bicosta*) are known hosts of the *Megaviridae*, while species of haptophytes (*Emiliania*), chlorophytes (*Ostreococcus*, *Micromonas* and *Bathycoccus*) and raphidophytes (*Heterosigma*) have been reported as *Phycodnaviridae* hosts (Virus-Host DB)[32]. Although the dominance of the *Megaviridae* and *Phycodnaviridae* have been reported in previous studies, mainly from coastal seawater[13,14], our results demonstrate the ubiquitous nature of these protist-infecting viruses across world ocean biomes. A proportion of the NCLDVs in the pico-size fraction were present within infected cells, because cell sizes of some host species such as *Aureococcus anophagefferens* and *Micromonas pusilla* are less than 3 μm. Thus, the abundance of these lineages in the pico-size fraction may be partly enriched by the viruses replicating inside their hosts.

In addition to the *Phycodnaviridae* and *Megaviridae*, the *Asfarviridae* also contribute an important proportion of NCLDVs in the femto-size fraction of most euphotic zones (Fig. 1B). Although very limited information is available regarding the natural hosts for this group, a representative *Asfarviridae*-like species in marine environments is *Heterocapsa circularisquama DNA virus* (HcDNAV), which infects the red-tide-forming dinoflagellate *H. circularisquama*[33]. In the terrestrial ecosystem, this viral family is known to infect a wide variety of organisms such as amoebozoa, arthropods and mammals[32,34]. Given the broad range of host species for this viral lineage, there may be an unknown but wide-spread host taxa for *Asfarviridae* in the ocean.

Our study revealed a heterogeneous pattern in the distribution of NCLDVs across the oceans of the world (Fig. 2C). Although there are limited studies available on the factors controlling the large-scale distribution of viruses, it is widely accepted that both deterministic (environmental factors and inter-specific interactions) and stochastic processes (e.g. immigration and speciation) are important in making up microbial assemblages[35-37]. The distribution and diversity of viruses would not be directly affected

278 by environmental variables such as temperature and nutrient availability, but is directly
279 influenced by the geographic ranges of their host species[3,38]. Recent work with
280 cyanophages demonstrated that a significant number of free-living viruses are locally
281 produced through active infection rather than from migration[39]. Therefore, we expect that
282 viral community structure will reflect host distribution as well as infectious activity.
283 Despite significant differences in community composition across oceanic biomes, we
284 found that most NCLDV phylotypes are dispersed throughout tropical and temperate
285 regions (Figs. 3A and 3B), presumably following their host community composition,
286 which is primarily determined by temperature[40]. However, the polar biome (mainly the
287 Arctic Ocean) constitutes a "hotspot" of unique NCLDV phylotypes from a wide variety
288 of families, despite having a low total richness in comparison to other regions (Figs. 3B
289 and 3C). We revealed that NCLDVs unique to non-polar biome were also abundant (Fig.
290 4), indicating a strong separation of NCLDV communities between polar and non-polar
291 biomes. A geographical barrier and steep environmental gradients may underlie this
292 distinct ecosystem structure (i.e. different host community and their productivity) in the
293 Arctic Ocean[27,28,31]. Moreover, the Arctic Ocean is characterized by high amount of river
294 discharge, contributing more than 10% to global runoff flux[41]. Consequently, biological
295 processes in the Arctic may be influenced by river inputs from terrestrial ecosystems.
296 These factors may collectively contribute to the remarkable number of unique NCLDV
297 phylotypes found in the Arctic, that were undetectable in other regions. The biogeography
298 of NCLDVs on a global scale implies a tight link between the NCLDVs and the
299 distribution of their hosts, which is strongly influenced by physicochemical and
300 biological factors.
301 Tight coupling between NCLDVs and their hosts was further corroborated by our
302 partial Mantel statistics, which described both known virus-host interactions and
303 additional but currently unrecognized associations between viruses and eukaryotic
304 lineages at the community level. Using the pico-sized NCLDV community, we detected
305 almost all known virus-host interactions, except for those against bicoecea (Fig. 5C). This

306 demonstrates that distance-based correlation analysis using global ocean samples is
307 useful for detecting virus-host interplay in natural environments. Strong positive
308 relationships between NCLDVs and eukaryotes involved many phytoplankton lineages
309 including haptophytes, chlorophytes, dinophytes, pelagophytes and raphidophytes, all of
310 which include known host lineages of NCLDVs (Fig. 5C). Strong correlations were also
311 detected with heterotrophic choanoflagellates, which have recently been identified as a
312 novel host of the *Megaviridae*[25]. Some NCLDVs, especially *Megaviridae*, had strong
313 correlations with chrysophytes, although no host species have yet been reported for this
314 lineage. Many environmental NCLDV genomes were found to encode genes that are
315 likely to be derived from marine chrysophytes (Supplementary Data 1–3). Phylogenetic
316 analysis suggests that most of these phylotypes represent previously unknown branches
317 of the *Megaviridae* tree (Supplementary Fig. 4), suggesting that chrysophytes may be an
318 important host lineage of *Megaviridae* in the ocean.
319 The global distribution of NCLDVs are determined by the geographic ranges of their
320 host organisms. Therefore, the virus-eukaryote associations that we detected likely arose
321 under these constraints. On the other hand, it is expected that NCLDVs influence the
322 abundance of eukaryotes at a local scale. Previous studies show that bacterial viruses have
323 an important role in determining bacterial mortality, because they substantially
324 outnumber their hosts and have highly specific infection mechanisms[42]. Similarly,
325 NCLDVs are reported to be more abundant than their host cells and have high infection
326 specificity[11,14,43]. For example, *Emiliania huxleyi viruses* (EhVs) of the *Phycodnaviridae*
327 family are responsible for almost all of the mortality of the haptophyte *E. huxleyi* during
328 blooms[22,44,45]. Another field study suggests that viral lysis can explain a greater proportion
329 of phytoplankton mortality than grazing by zooplankton[6]. These studies, combined with
330 the global associations that were detected in this study, emphasize the potential
331 importance of NCLDVs in structuring eukaryotic communities.
332 Our results indicate that marine phytoplankton lineages could represent one of the
333 most important host groups of NCLDVs. Therefore, NCLDVs could be involved in the

regulation of biogeochemical processes mediated by phytoplankton. We investigated this by assessing the vertical connectivity of viral communities. The NMDS analysis showed clear differences between the NCLDV community composition of epipelagic (euphotic) and mesopelagic (aphotic) zones at most sampling sites (Fig. 2B). The vertical separation of viral communities may be caused by the stable stratification below the mixed layers (typically above 200 m depth), which severely inhibits vertical water exchange. Despite this limitation, mesopelagic ecosystems shared a significant number (98.7%) of NCLDV phylotypes with the upper epipelagic layers (Fig. 3A), suggesting vertical transport of NCLDV communities. Indeed, some mesopelagic NCLDV communities were very similar to surface communities (Fig. 6A and Supplementary Fig. 6A). This implies that the surface and mesopelagic NCLDV communities may be connected at some locations. The major source of energy and materials in the mesopelagic layer is the gravitational export of organic particles from the surface layer (i.e. the biological carbon pump)[46-48]. Therefore, some surface viruses may be exported to mesopelagic layers with sinking aggregated phytoplankton cells[49-51].

A significant positive correlation existed between surface phytoplankton biomass and NCLDV community similarity across depths (Fig. 6B and Supplementary Fig. 6B), indicating that highly productive areas are likely to have a greater flux of settling particles to the deeper layers. High vertical connectivity was consistently associated with an increase in NCLDV richness in the mesopelagic zone (Fig. 6C and Supplementary Fig. 6C). Numerous studies based on sediment trap measurement have shown that the larger phytoplankton, such as diatoms, contribute strongly to vertical flux because of their high sinking velocities[52,53]. However, recent studies show that smaller phytoplankton including haptophytes and chlorophytes, known hosts of marine NCLDVs, also contribute greatly to downward carbon export[8,9,54]. The high vertical connectivity of NCLDVs was not affected by the extent of the depth range nor by proxies for vertical mixing (Figs. 6D–F and Supplementary Figs. 6D–F), indicating that the migration of NCLDVs occurred regardless of physical processes such as upwelling, turbulent mixing,

and convection. This result suggests that sinking export is a major source of NCLDVs to deeper waters, which originally had low NCLDV diversity. A recent study revealed that some *Phycodnaviridae* and *Megaviridae* potentially accelerate biological carbon export from the productive surface layer to deep layers, presumably by promoting cell death and aggregation of their host species[55]. The *Phycodnaviridae* and *Megaviridae* also contributed strongly to high vertical connectivity in our study (Supplementary Figs. 7 and 8). The infection of the coccolithophore by the *Phycodnaviridae* EhV was observed to facilitate the sinking of host cells, likely by enhancing the production of transparent exopolymer particles and subsequent aggregation[9]. Therefore, the high vertical connectivity of NCLDVs detected in our analysis may be associated with enhanced vertical export of their infected hosts.

The present study expands our knowledge of marine NCLDV biogeography. Most NCLDV phylotypes are ubiquitously distributed over the oceans of the globe, although a high proportion of unique NCLDVs were detected in the Arctic Ocean. Our comparison of community distribution patterns highlighted the tight interplay between NCLDVs and microeukaryotes. As marine ecological and biogeochemical processes are governed primarily by microbes, NCLDVs would have an important influence on the dynamics of marine systems. We also identified unexpected similarity of NCLDV communities between surface and deep waters at some location. This supports the idea that viral activity may be related to the strength of the biological carbon pump, because the efficiency and sinking rate of export production depends largely on surface phytoplankton composition and their infection status[8,9,53,56]. Our findings underscore the importance of NCLDVs as a component of marine microbial communities, and contribute to refine our knowledge of marine ecosystems, a key regulator of the Earth's climate.

Methods

Sample collection

Metagenomic datasets were generated from samples collected by the *Tara* Oceans

expeditions from 2009 to 2013[26-28,31,57]. The second version of the Ocean Microbial Reference Gene Catalog (OM-RGC.v2) is a non-redundant gene catalog constructed from 370 metagenomic samples from the *Tara* Oceans project[28] (https://www.ocean-microbiome.org). The catalog includes 46,775,154 genes in total, and the gene abundance profiles are expressed as the sum of within-reads aligned base pairs normalized by gene length, in *Tara* Oceans samples[28].

Recruitment of NCLDV marker genes from the OM-RGC.v2

To assess the community composition of NCLDVs, we used family B DNA polymerase (*polB*) as a marker gene of NCLDVs. Initially, amino acid sequences of the OM-RGC.v2 were searched against an in-house profile hidden Markov model (HMM) of NCLDV PolB sequences using the software HMMER, hmmsearch (version 3.1)[58] with a threshold E-value $<1\times10^{-5}$. Consequently, 29,315 PolB sequences were obtained from the OM-RGC.v2. To classify the taxonomic identity of each sequence, phylogenetic mapping was performed within known PolB sequences. A maximum-likelihood (ML) reference phylogenetic tree was built based on 211 PolB reference protein sequences from eukaryotes, bacteria, archaea and NCLDVs. These sequences were aligned using the default settings of the multiple sequence alignment program MAFFT-linsi (version 7)[59] and ML tree was constructed with the use of randomized axelerated maximum likelihood (RAxML) program (version 7.2.8)[60]. In the reference trees, we included sequences from eight proposed families of NCLDVs[61]: *Megaviridae* (synonymous with *Mimiviridae*), *Phycodnaviridae*, *Pithoviridae*, *Marseilleviridae*, *Ascoviridae*, *Iridoviridae*, *Asfarviridae*, and *Poxviridae* (Supplementary Figs. 2–4). A sequence from a novel NCLDV clade *Medusavirus* was also included as a reference[62]. Query sequences were aligned against the reference alignment using the MAFFT 'addfragments' option, and then mapped onto the reference tree using the software program pplacer[29].

Abundance profiling of NCLDVs

We used the abundance profile of NCLDV genes from the OM-RGC.v2 to evaluate the relative frequency and diversity of NCLDVs. In the abundance matrix, we only included samples from the pico-size (0.22–1.6 or 0.22–3.0 μm) and femto-size (<0.22 μm) fractions. Samples used in the analysis were from three depth ranges: the surface (2–9 m), the deep chlorophyll maximum (DCM, 15–180 m) and the mesopelagic (MES, 250–1,000 m). Samples for which the sum of length-normalized PolB abundance was less than 50 (set at a proxy for low NCLDV frequency) were removed from the analysis. The abundance matrix was then standardized by the sample with the lowest sum of length-normalized PolB abundance value. The minimum value of PolB abundance among NCLDV phylotypes in the sample having the lowest sum of length normalized PolB was set as the cutoff threshold. For each sample, NCLDV phylotypes with a length-normalized abundance of less than this threshold were treated as zero values. A sample of a femto-size fraction of surface water from station 155 was also removed, because it contained only one NCLDV PolB after standardization. Consequently, our dataset was comprised of 283 samples (172 pico-fraction samples and 111 femto-fraction samples), covering 88 sampling sites. These sites were categorized into four biomes (coastal, trades, westerlies and polar biomes) according to latitude or distance from the shore, and nine oceanic regions, as defined by Longhurst[63] (Supplementary Table 1).

Phylogenetic tree construction

To construct a phylogenetic tree, the NCLDV-derived PolB sequences obtained from the OM-RGC.v2 were filtered by length (≥700 amino acid sequences). Amino acid sequences from the resulting 911 genes were aligned with reference sequences using the *linsi* option from the MAFFT. The ML tree was constructed using RAxML with the use of a reference sequence tree as a backbone constraint. We confirmed the validity of the pplacer family assignment for 905 out of 911 selected sequences. The remaining six sequences that were incorrectly placed within the phylogenetic tree were removed. The ML tree was visualized using the program iTOL[64].

446

447 **Prediction of potential chrysophyte viruses using metagenomic assembled genomes**

448 To explore the genomic contents of environmental NCLDVs, we made use of two sets
449 of metagenome-assembled genomes (MAGs) of NCLDVs (MoMAGs, 2019[19]; GVMAGs
450 high and medium quality[20]), which were generated from environmental metagenomic
451 datasets collected on global scales. Gene prediction was made for all MAGs using the
452 program GeneMarkS[65], then the predicted genes were searched using BLASTP against a
453 database that combines the NCBI Reference Sequence database (RefSeq release 90) and
454 the marine microbial eukaryote transcriptomes project (MMETSP) database[66]. We
455 identified MAGs whose genes exhibited the best hit to transcripts of chrysophytes with
456 >50% amino acid identity and >100 alignment length (Supplementary Data 1). These
457 genes were considered potential candidates for horizontal gene transfer between
458 chrysophytes and NCLDVs, and were BLASTP searched against the RefSeq database for
459 additional functional annotation (Supplementary Data 2). We then extracted PolB
460 sequences from the NCLDV MAGs which had a chrysophyte-related gene using the
461 HMMER hmmsearch program. These PolBs were BLASTP searched against the NCLDV
462 PolBs from the OM-RGC.v2. MAG-derived PolBs aligned with over 700 amino acid
463 sequences with >90% identity were assigned to the PolB phylotypes derived from the
464 OM-RGC.v2 (Supplementary Data 3). Phylogenetic affiliations of PolB from the
465 chrysophyte-related MAGs were confirmed using a phylogenetic tree.

466

467 **Diversity analyses**

468 Diversity and multivariate analyses were performed using the statistical software R
469 (version 3.6.2) (https://www.r-project.org/). To evaluate the diversity of each sample, the
470 number of NCLDVs (richness) and Shannon's entropy were assessed by the package
471 'vegan' (https://cran.r-project.org/web/packages/vegan). NCLDV richness among sizes
472 and depths were compared using a Kruskal-Wallis test followed by Dunn's multiple
473 comparison. Compositional variation among samples was assessed with a non-metric

multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarity. Statistical significance of differences among the sample groups (size, depth and biomes) was tested using a permutational multivariate analysis of variance (PERMANOVA)[67] with 9,999 permutations.

Partial Mantel test

A partial Mantel test was performed to assess the correlation between two multivariate matrices while controlling the potential effects of geographic distance (spatial autocorrelation) using the R package 'vegan'. Abundance matrices for the NCLDV and eukaryotic lineages were constructed from the integrated abundance tables, and the total abundance at each site was normalized to 1. The eukaryote abundance table was constructed based on 18S rRNA gene metabarcoding[68]. Data for NLCDVs were obtained from pico- (0.22–1.6/3.0 μm) or femto-size (<0.2 μm) fractions and for the eukaryotic community from the pico- to meso-size fraction (0.8–2,000 μm). There were 84 overlapping sampling events between pico-size NCLDVs and eukaryotic communities and 55 overlapping sampling events between femto-size NCLDVs and eukaryotic communities. All overlapping samples were derived from the surface or DCM depth layers. Values of environmental variables (temperature, salinity, nitrate, phosphate and silicate) were standardized to the range 0–1, because the matrix existed as a mixture of heterogeneous variables. Distance matrices for viruses, eukaryotes and environmental data were calculated using the Bray-Curtis measure. Geographic distances among sample sites were also measured using Haversine distance and were used as a third distance matrix. Partial Mantel correlations were computed between all pairs of distance matrices of eukaryotic communities and NCLDVs or environmental variables with 9,999 permutations for each comparison. The false discovery rate (FDR) was computed using the Benjamini-Hochberg method[69].

Statistical test

Two-sided test was applied for all statistical tests.

Data availability

The complete sequence data of the OM-RGC.v2 and the abundance profile can be downloaded from https://www.ocean-microbiome.org. All sequences of 18S rRNA gene metabarcoding have been deposited at European Nucleotide Archive (ENA) under the BioProject ID PRJEB6610 and PRJEB9737. Environmental metadata are archived at https://doi.pangaea.de/10.1594/PANGAEA.875582. Files used for recruiting NCLDV PolB genes as well as processed abundance profiles of eukaryotes and NCLDVs with corresponding environmental data are available at the GenomNet FTP: ftp://ftp.genome.jp/pub/db/community/tara/Biogeography/.

Code availability

Computer codes are available from the corresponding author upon request.

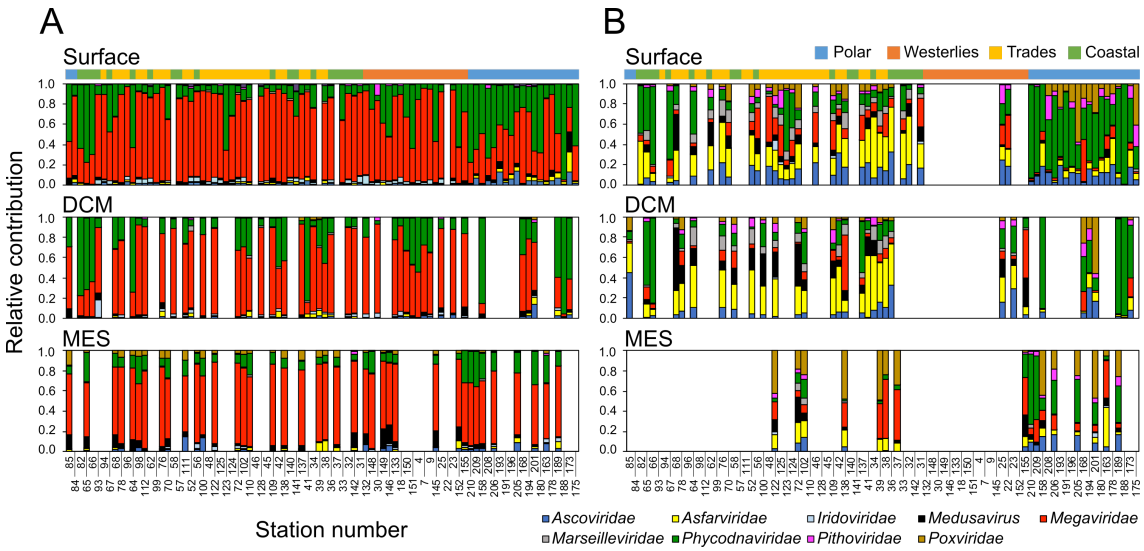


Figure 1 Latitudinal patterns in NCLDV community composition. Relative contributions of NCLDV families at each depth range of (A) pico- and (B) femto-size fractions. Sampling stations were arranged in rows from south to north, and color-coded based on biome.

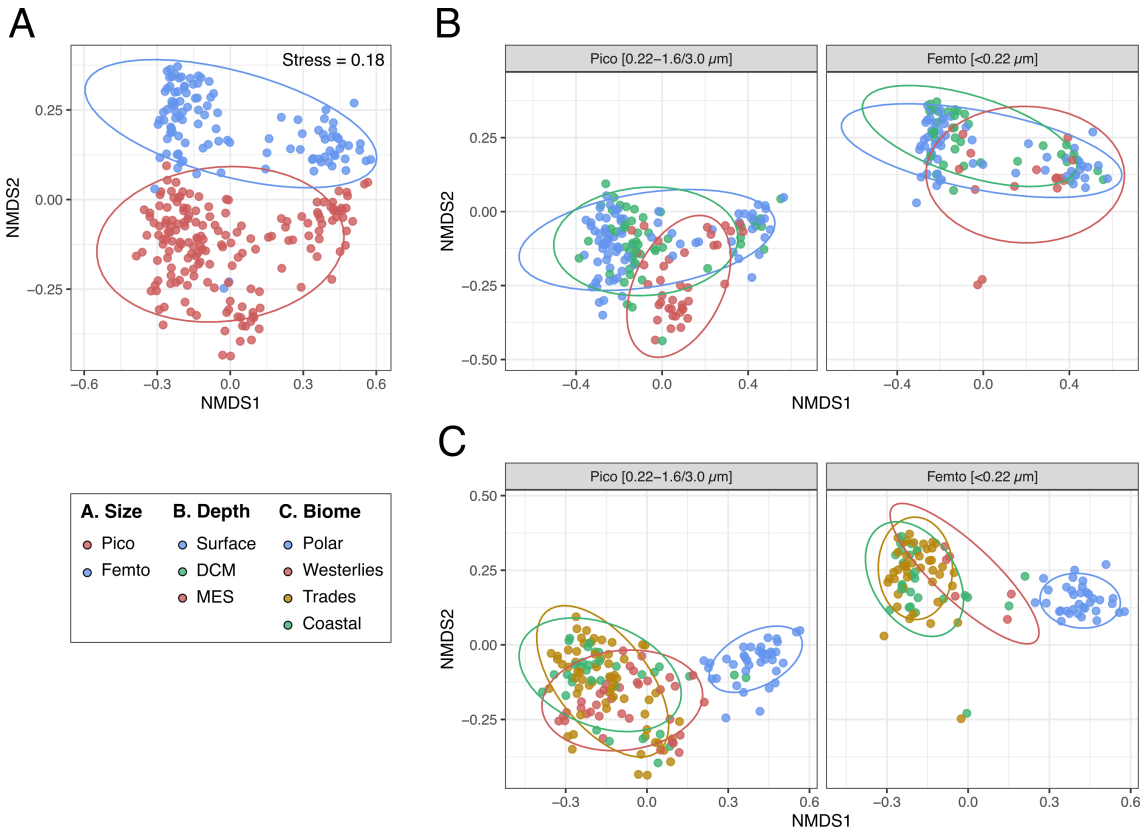


Figure 2 Community characteristics of NCLDVs. Non-metric multidimensional scaling (NMDS) ordination based on the NCLDV community showing results for all samples (A) and separately for pico- and femto-size fractions (B and C). Sample groups are color-coded by size fraction (A), depth (B) and biome (C). Ellipses represent 90% confidence levels for each group. All group categories are significantly different from each other as analyzed using PERMANOVA (p <0.01).

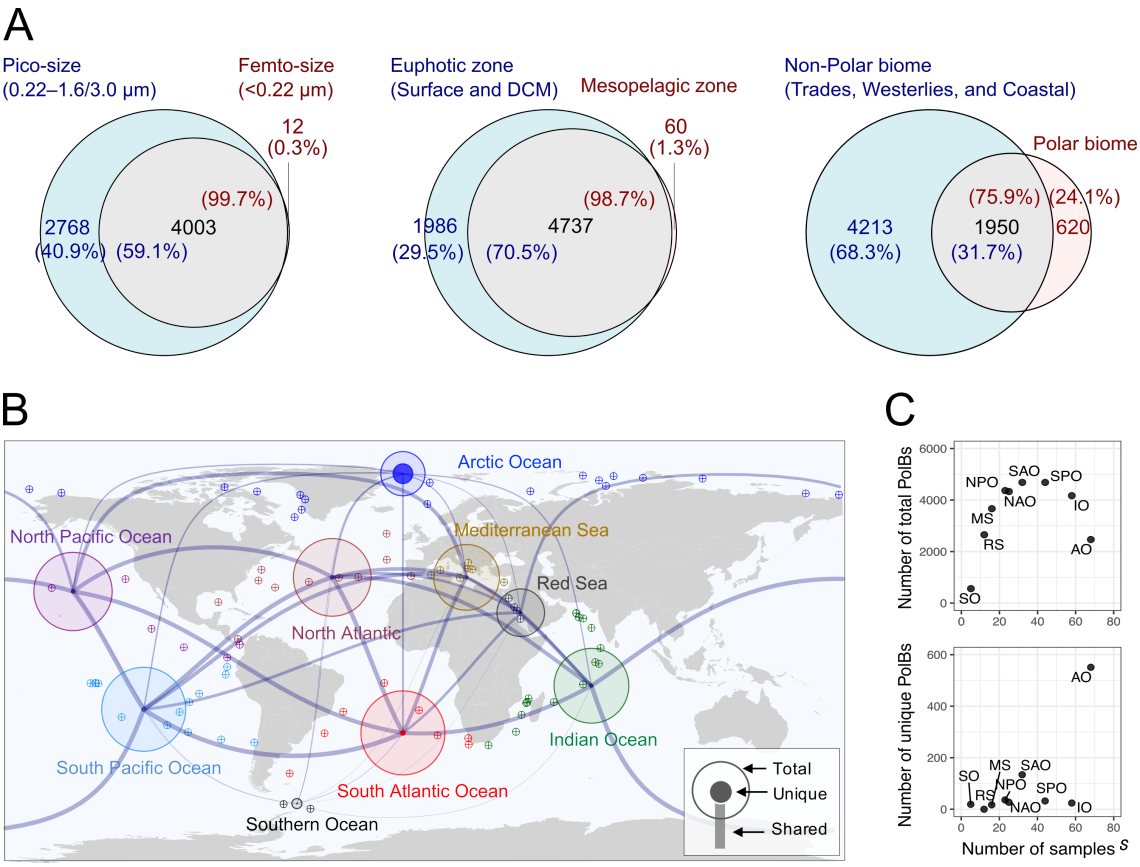


Figure 3 Structural differentiation of NLCDV community across ecological zones. (A) Venn diagrams showing the numbers of shared or unique NLCDVs phylotypes across size fractions (left), depths (center) and biomes (right). (B) Map showing the number of total, unique and shared NCLDVs across nine oceanic regions. (C) Relationships among sample size and total or unique NCLDVs detected in each region (Abbreviations as in Supplementary Table 1).

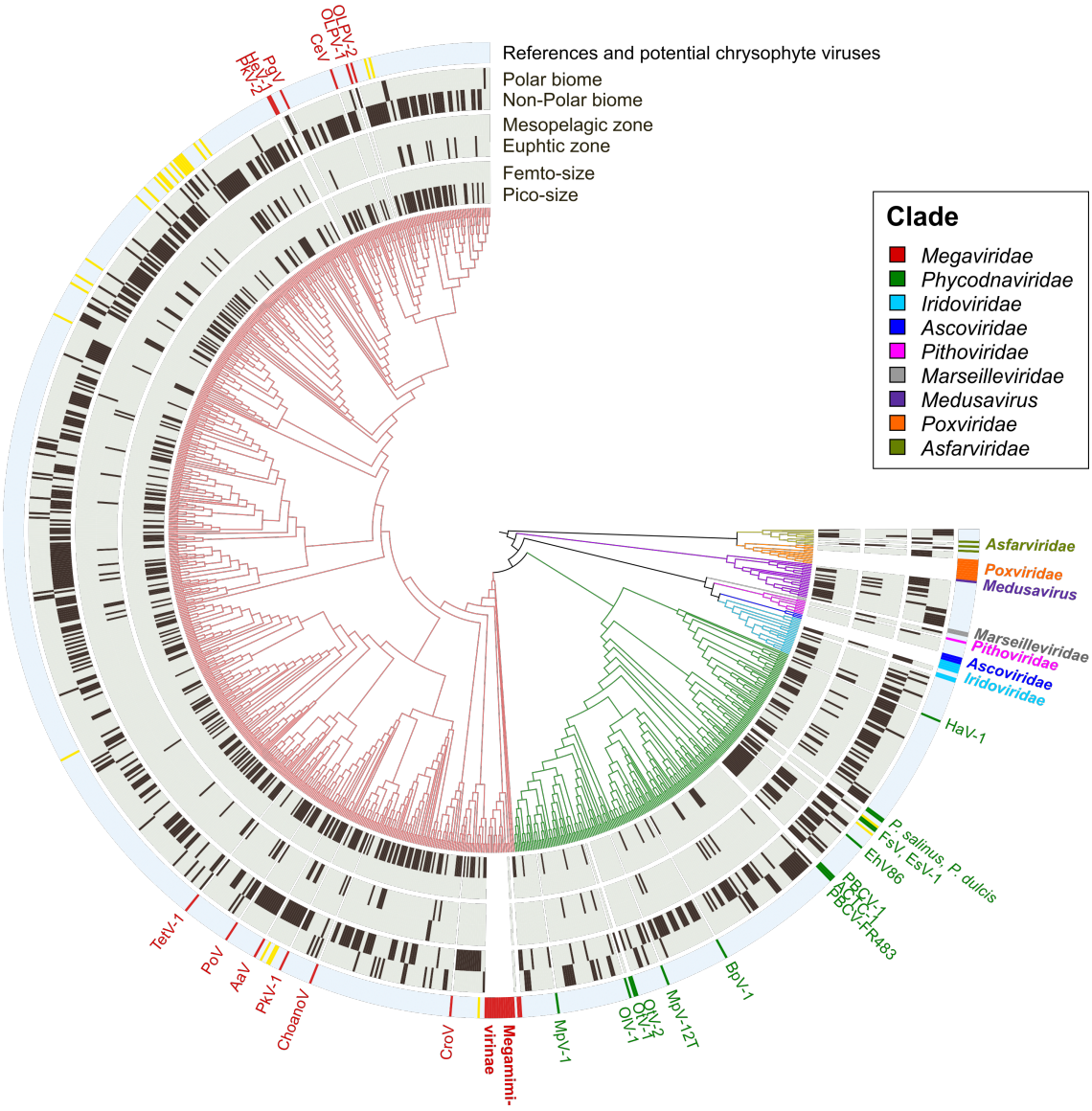


Figure 4 Phylogenetic affiliations of environmental NCLDVs and their dispersal characteristics. Phylogenetic tree constructed from 905 long (≥700 amino acid) PolB sequences from the OM-RGC.v2 and 67 reference sequences (see also Supplementary Figs. 2–4 for details). The first six layers indicate the occurrence of NCLDVs unique to each size fraction, depth and biome. The outside layer denotes phylogenetic positions of reference sequences (color code as in the legend) and the phylotypes closely related (>90% amino acid identity) to those of NCLDV MAGs having chrysophyte homologs (indicated in yellow). Abbreviations: OLPV-2: *Organic Lake phycodnavirus* 2; OLPV-1: *Organic Lake phycodnavirus* 1; CeV: *Chrysochromulina ericina virus* 1; PgV: *Phaeocystis globosa virus* 16T; HeV: *Haptolina ericina virus* RF02; PkV-2; *Prymnesium kappa virus* RF02; TetV-1: *Tetraselmis virus* 1; PoV:

Pyramimonas orientalis virus 1; AaV: *Aureococcus anophagefferens virus* BtV-01; PkV-1; *Prymnesium kappa virus* RF01; ChoanoV: ChoanoVirus; CoV: *Cafeteria roenbergensis virus* BV-PW1; MpV-1: *Micromonas* sp. RCC1109 virus MpV1; OlV-1: *Ostreococcus lucimarinus virus* 1; Otv-1: *Ostreococcus tauri virus* 1; Otv-2: *Ostreococcus tauri virus* 2; MpV-12T: *Micromonas pusilla virus* 12T: BpV-1: *Bathycoccus* sp. RCC1105 *virus*; BCV-FR483: *Paramecium bursaria Chlorella virus* FR-483; ACTV-1: *Acanthocystis turfacea Chlorella virus* 1; PBCV-1: *Paramecium bursaria Chlorella virus* 1; EhV-86: *Emiliania huxleyi virus* 86; FsV: *Feldmannia species virus*; EsV-1: *Ectocampus siliculou virus* 1; *P. salinus*: *Pandoravirus salinus*; *P. dulcis*: *Pandoravirus dulcis*; HaV-1: *Heterosigma akashiwo virus* 1.

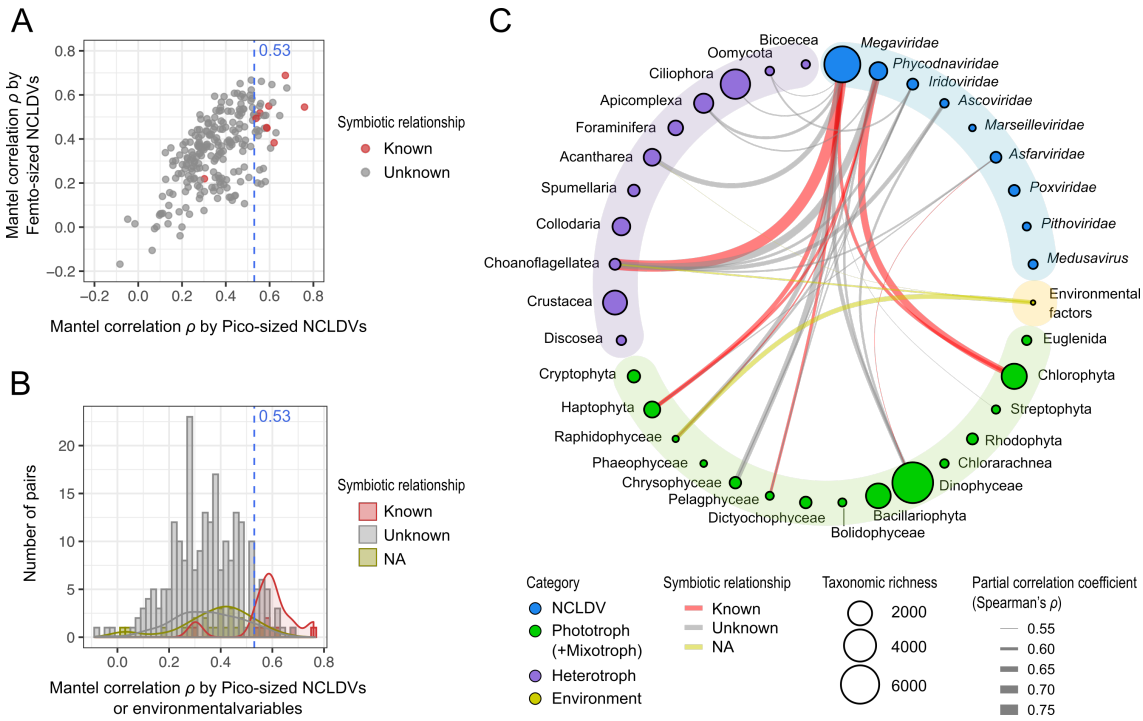
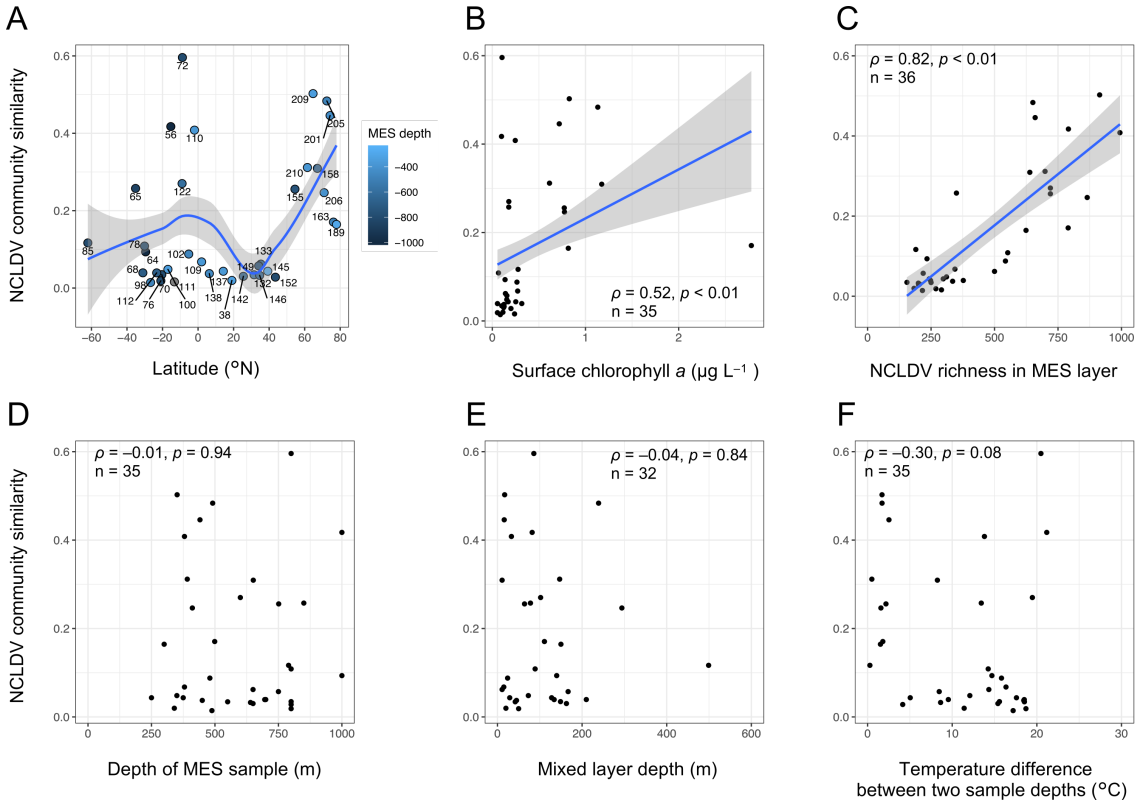


Figure 5 Associations between NCLDVs and eukaryotic communities. (A) Partial Mantel correlation coefficients (Spearman's ρ) between NCLDVs and eukaryotic communities. Each plot shows the value of ρ computed based on pico- (x-axis) and femto-sized (y-axis) NCLDV communities. Known virus-host associations are shown as red dots. (B) Histogram and density estimates showing the distribution of ρ values in known (red), unknown (gray) and environmental (NA, yellow) pairs. (C) Pairwise comparisons of the partial Mantel correlation coefficients between NCLDV and eukaryotic lineages. Correlation coefficients ρ >0.53 based on pico-size NCLDV communities are drawn as edges. Known virus-host associations are shown in red, whereas unknown associations are shown in gray. Correlations between environmental variables and eukaryotic lineages are shown in yellow.



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582

583 **Figure 6 Vertical linkage of NCLDV communities between the surface and**
584 **mesopelagic layers.** (A) Latitudinal trend in NCLDV community similarity
585 between two depths (with the station numbers). Relationship between NCLDV
586 vertical similarity and (B) the surface chlorophyll *a* biomass, (C) NCLDV
587 richness in the mesopelagic layer, (D) sampling depth of mesopelagic seawater,
588 (E) the mixed layer depth and (F) temperature difference between epipelagic
589 and mesopelagic samples. All NCLDV data were generated based on the pico-
590 size fraction.

591

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Author contributions

HE and HO designed the study. HE performed most of the bioinformatics analysis. RBM, YL contributed to the bioinformatics analysis. GS, NH, KL, CdV, MBS, CB, PW, LKB, and SS contributed to the generation of primary data. CdV, MBS, CB, PW, LKB, SS, and HO coordinated *Tara* Oceans. All authors contributed to the writing of the manuscript.

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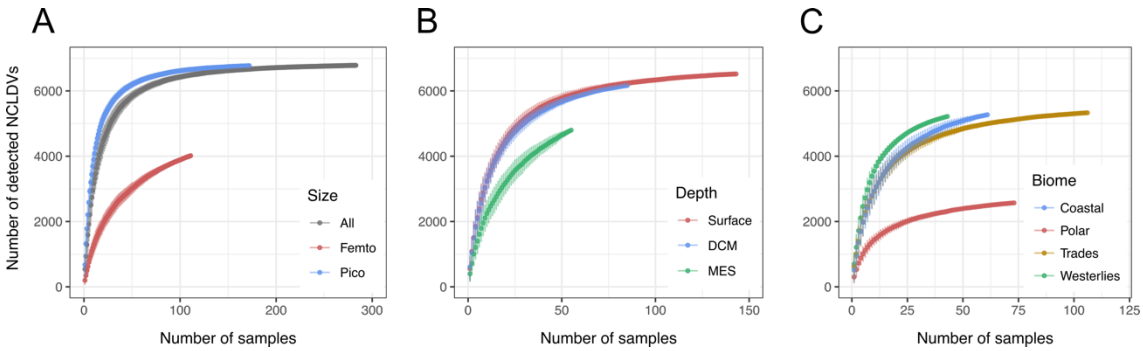
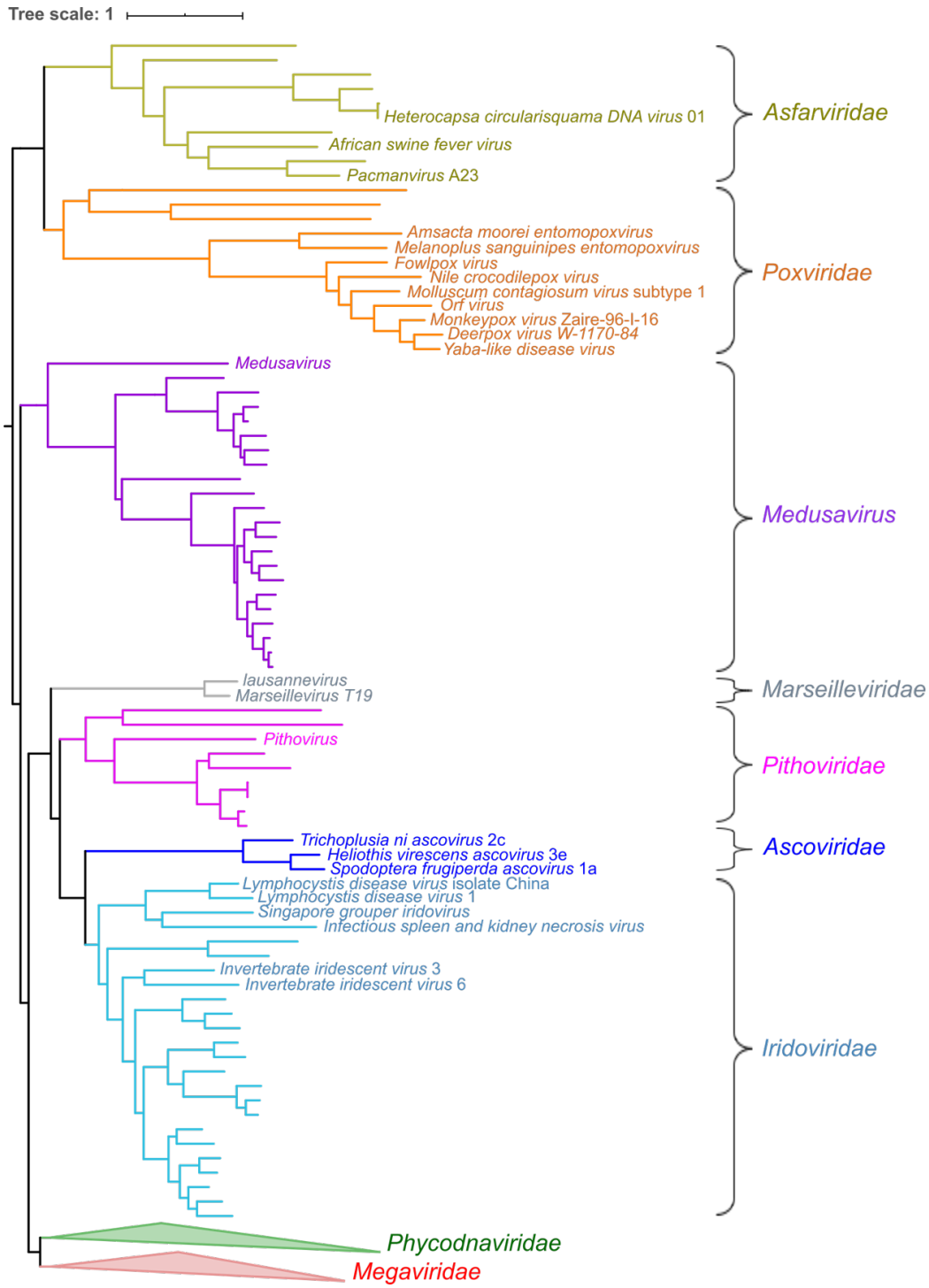
Competing financial interests

The authors declare no competing financial interests.

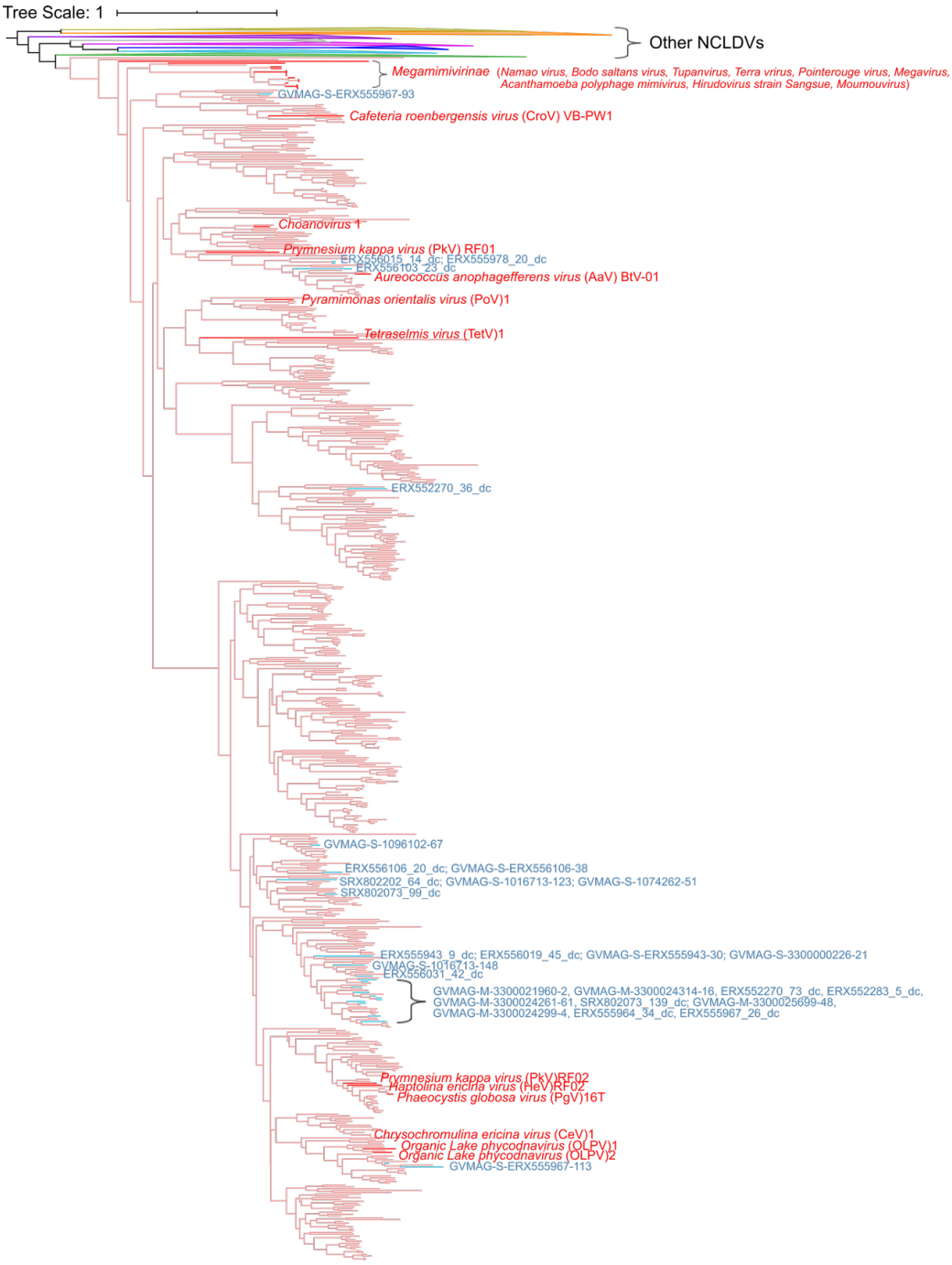


Supplementary Figure 1 Sample-size dependence of the observed NCLDVs. Sample-based rarefaction curves showing accumulated richness of NCLDV PolB genes detected in different (A) sizes fractions, (B) depths and (C) biomes. Error bars indicate ±1 standard deviation.

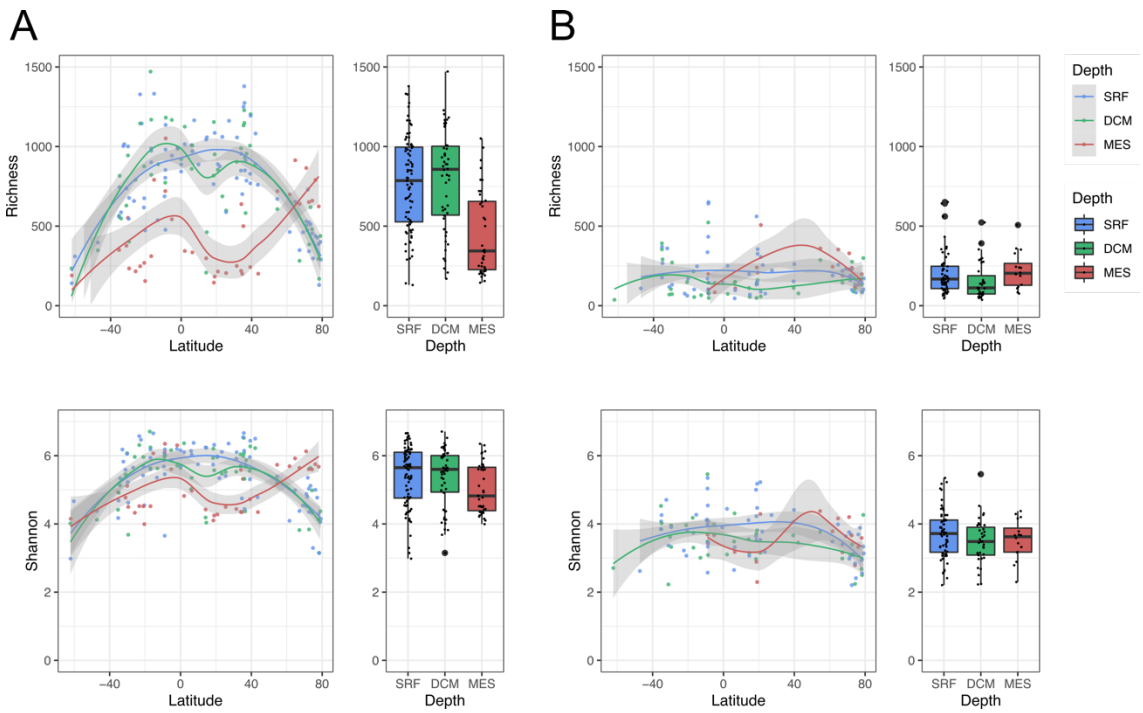


Supplementary Figure 2 Phylogenetic affiliations of environmental NCLDVs. Phylogenetic tree constructed from 905 long (≥700 amino acid) PolB sequences from the OM-RGC.v2 and 68 reference sequences. Sequences branches of the *Megaviridae* and *Phycodnaviridae* were collapsed in the tree. Reference sequences and the environmental gene sequences were mapped and color-coded according to family-level classification.

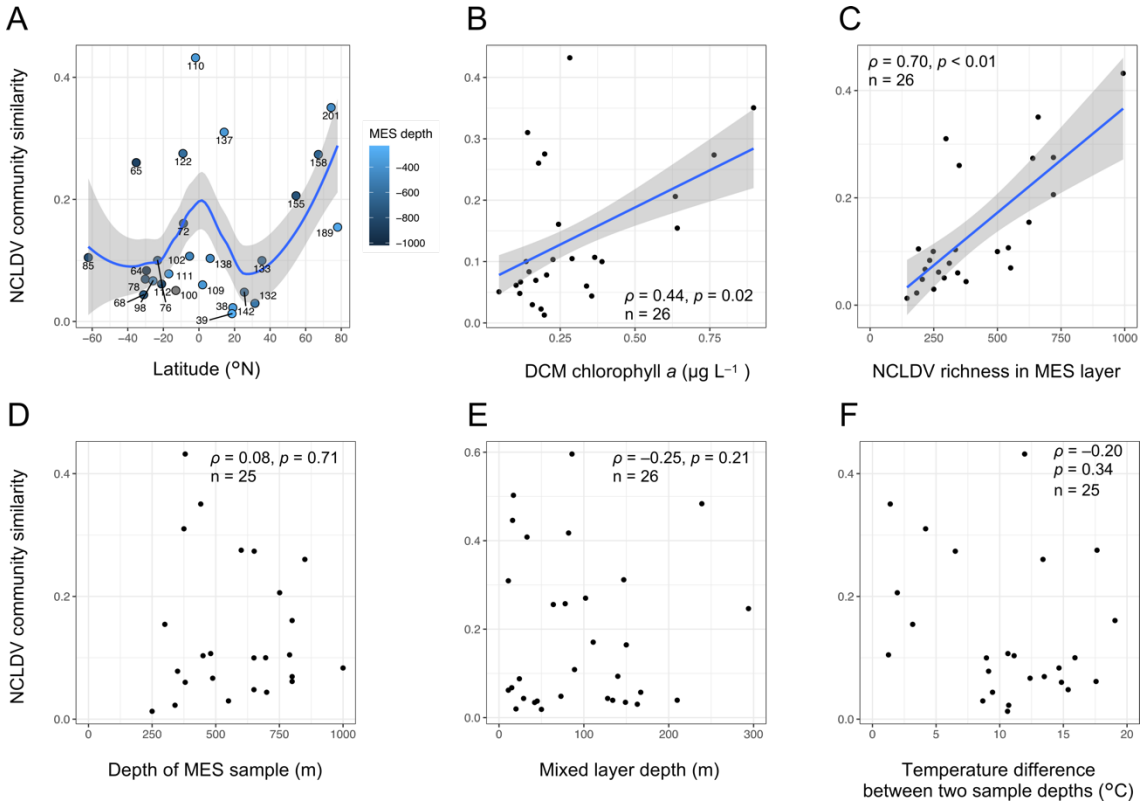
Supplementary Figure 3Phylogenetic affiliations of environmental *Phycodnaviridae*. Phylogenetic tree constructed from 905 long (≥700 amino acid) PolB sequences from the OM-RGC.v2 and the 68 reference sequences. Branches other than the *Phycodnaviridae* were collapsed in the tree. Reference sequences and the environmental gene sequences were mapped and color-coded according to family-level classification. Blue branches indicate phylotypes closely related (>90% amino acid identity) to those of NCLDV MAGs having chrysophyte homologs.



Supplementary Figure 4 Phylogenetic affiliations of environmental *Megaviridae*. Phylogenetic tree constructed from 905 long (≥700 amino acid) PolB sequences from the OM-RGC.v2 and 68 reference sequences. Branches other than the *Megaviridae* were collapsed in the tree. Reference sequences and the environmental gene sequences were and color-coded according to the family-level classification. Blue branches indicate phylotypes closely related (>90% amino acid identity) to those of NCLDV MAGs having chrysophyte homologs.



Supplementary Figure 5 Latitudinal patterns in NCLDV diversity from pole to pole. Latitudinal variations in richness and Shannon diversity index at each depth for (A) pico- and (B) femto-size fractions (left panels). Box plots are shown (right panels) to summarize variation in richness and Shannon's index across sampling depths (center line, median; box limits, 25%–75% quantiles; whiskers, 1.5× interquartile range).



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859
860 **Supplementary Figure 6 Vertical linkage of NCLDV communities between the DCM**
861 **and mesopelagic layers.** (A) Latitudinal trend in NCLDV community similarity between
862 two depths (with the station numbers). (B–F) Relationship between vertical similarity and
863 (B) the surface chlorophyll *a* biomass, (C) NCLDV richness in the mesopelagic layer, (D)
864 sampling depth of mesopelagic seawater, (E) the mixed layer depth and (F) temperature
865 difference between epipelagic and mesopelagic samples. All NCLDV data were
866 generated using the pico-size NCLDV fraction.
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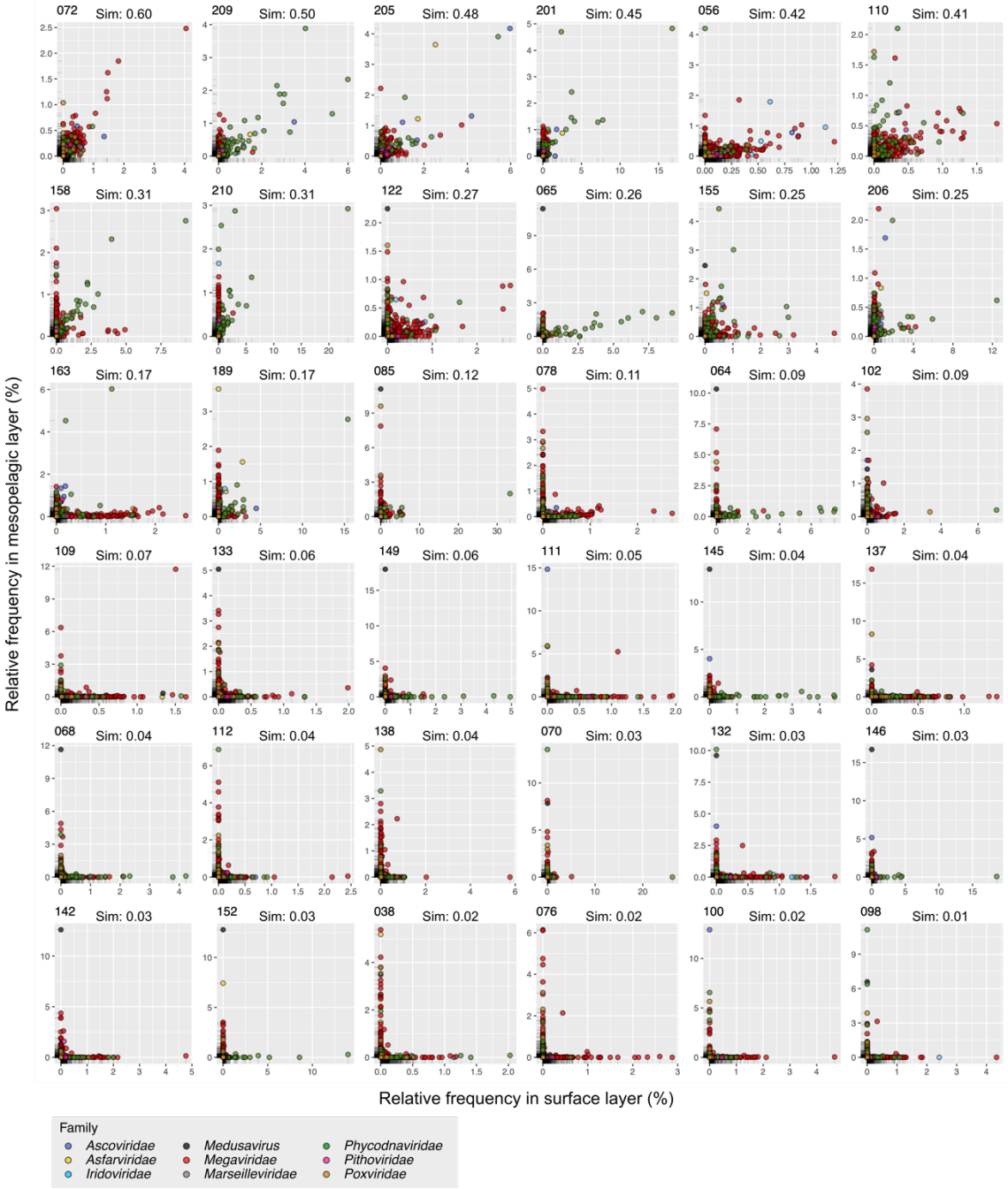
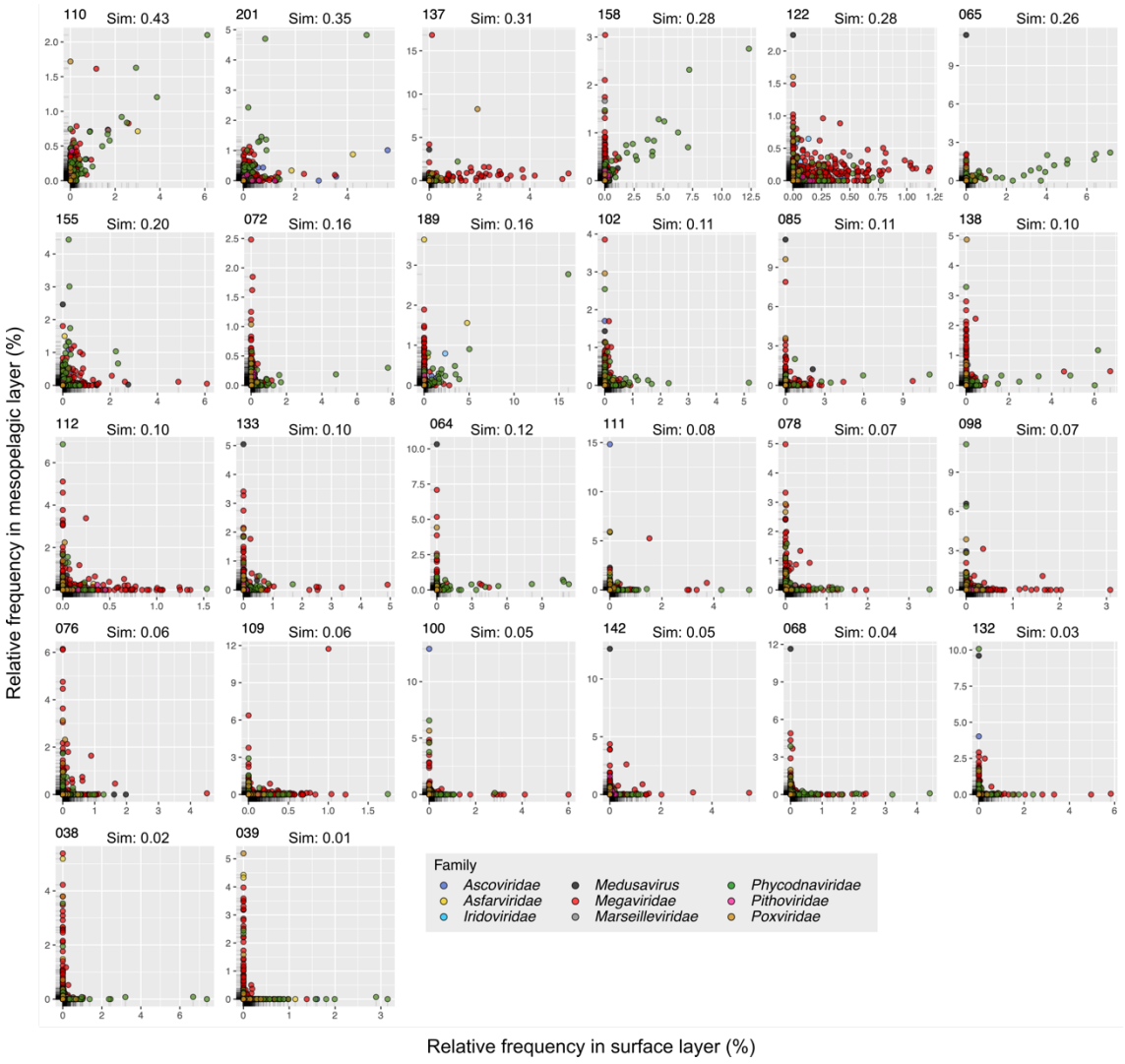


Supplementary Figure 7 Comparisons of relative frequencies in NCLDV phylotypes between the surface and mesopelagic layers. Station number and the Bray-Curtis similarities between two depths are indicated above each plot. Samples are presented in ascending order of the similarity. Each dot is color-coded according to family-level classification.



Supplementary Figure 8 Comparisons of relative frequencies in NCLDV phylotypes between the DCM and mesopelagic layers. Station number and the Bray-Curtis similarities between two depths are indicated above each plot. Samples are presented in ascending order of the similarity. Each dot is color-coded according to family-level classification.

Supplementary Table 1 The number of samples representing each biome, oceanic region, depth and size fraction.

	Ecozone	Abbreviation	Pico-size (0.22–1.6/3 μm)				Femto-size (<0.22 μm)			
			SRF	DCM	MES	Total	SRF	DCM	MES	Total
Biome	Coastal Biome	Coastal	18	11	6	35	16	9	1	26
	Trade winds Biome	Trades	27	19	16	62	23	15	6	44
	Westerlies Biome	Westerlies	17	12	8	37	2	3	1	6
	Polar Biome	Polar	21	8	9	38	19	8	8	35
Oceanic region	North Atlantic Sea	NAO	11	5	7	23	0	1	1	2
	Mediterranean Sea	MS	6	6	0	12	2	2	0	4
	Red Sea	RS	4	2	0	6	5	1	0	6
	Indian Ocean	IO	12	9	6	27	17	11	3	31
	South Atlantic Ocean	SAO	8	6	5	19	7	5	1	13
	Southern Ocean	SO	2	1	1	4	0	1	0	1
	South Pacific Ocean	SPO	15	9	7	31	7	4	2	13
	North Pacific Ocean	NPO	6	5	5	16	3	3	1	7
	Arctic Ocean	AO	19	7	8	34	19	7	8	34
	Total		83	50	39	172	60	35	16	111