RESEARCH ARTICLE

Life-Style and Genome Structure of Marine *Pseudoalteromonas* Siphovirus B8b Isolated from the Northwestern Mediterranean Sea

Elena Lara¹, Karin Holmfeldt²*, Natalie Solonenko², Elisabet Laia Sà¹, J. Cesar Ignacio-Espinoza³, Francisco M. Cornejo-Castillo¹, Nathan C. Verberkmoes⁴, Dolors Vaqué¹, Matthew B. Sullivan²,³, Silvia G. Acinas¹*

¹ Department of Marine Biology and Oceanography, Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta, 37–49, 08003 Barcelona, Spain, ² University of Arizona, Department of Ecology and Evolutionary Biology, 1007 E. Lowell St., Tucson, AZ, United States of America, ³ University of Arizona, Department of Molecular and Cellular Biology, 1007 E. Lowell St., Tucson, AZ, United States of America, ⁴ Chemical Science Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States of America

* Current address: School of Natural Sciences, Linnaeus University, 39182 Kalmar, Sweden
  * sacinas@icm.csic.es

Abstract

Marine viruses (phages) alter bacterial diversity and evolution with impacts on marine biogeochemical cycles, and yet few well-developed model systems limit opportunities for hypothesis testing. Here we isolate phage B8b from the Mediterranean Sea using *Pseudoalteromonas* sp. QC-44 as a host and characterize it using myriad techniques. Morphologically, phage B8b was classified as a member of the *Siphoviridae* family. One-step growth analyses showed that this siphovirus had a latent period of 70 min and released 172 new viral particles per cell. Host range analysis against 89 bacterial host strains revealed that phage B8b infected 3 *Pseudoalteromonas* strains (52 tested, >99.9% 16S rRNA gene nucleotide identity) and 1 non-*Pseudoalteromonas* strain belonging to *Alteromonas* sp. (37 strains from 6 genera tested), which helps bound the phylogenetic distance possible in a phage-mediated horizontal gene transfer event. The *Pseudoalteromonas* phage B8b genome size was 42.7 kb, with clear structural and replication modules where the former were delineated leveraging identification of 16 structural genes by virion structural proteomics, only 4 of which had any similarity to known structural proteins. In nature, this phage was common in coastal marine environments in both photic and aphotic layers (found in 26.5% of available viral metagenomes), but not abundant in any sample (average per sample abundance was 0.65% of the reads). Together these data improve our understanding of siphoviruses in nature, and provide foundational information for a new ‘rare virosphere’ phage–host model system.
Introduction

In marine environments, phages influence global biogeochemical cycles by lysing bacterial cells which alters nutrient and organic matter fluxes, as well as the dynamics and diversity of microbial communities [1–9]. Additionally, marine phages help drive microbial evolution through phage-mediated gene transfer [10]. Despite their relevance, viral diversity is hard to measure because (i) viruses lack a universally conserved gene marker (e.g., ribosomal RNA genes in cellular organisms) [11], and (ii) most (>99%) bacteria in nature are resistant to cultivation using standard techniques [12] which limits the hosts available for virus isolation efforts [13]. Even when it is possible to grow the host organism in the lab, not all phages produce identifiable plaques [6, 14]. To circumvent these limitations, viral community diversity has been analyzed by culture-independent approaches including (i) Pulse-Field Gel Electrophoresis (PFGE) which discriminates viruses by genome size [15, 16], (ii) Randomly Amplified Polymorphic DNA PCR (RAPD) which provides a genetic fingerprint for the whole viral community [17–19] and most recently (iii) viral metagenomics (viromics) which, currently, provides fragmented sequence data for the whole double-stranded DNA (dsDNA) viral community [20–28].

Culture-independent and metagenomic methods are powerful, but each approach has its own limitations. Although PFGE is often used to estimate the size of individual phage genomes, it does not efficiently discriminate among natural viral populations with the same or similar genome size. RAPD-PCR is a valid alternative although it may under- or overestimate viral richness if genetically different DNA templates produce PCR amplicons of the same size or if a single viral genome contains more than one priming site resulting in multiple bands from the same virus in the final banding pattern. Finally, metagenomic approaches are severely database limited due to the lack of sequenced viral genomes. For example, the majority (>70%) of the predicted viral open reading frames (ORFs) in metagenomes have no similarity to previously described sequences [20, 21, 26, 29, 30]. While informatic advances are eliminating some of these issues (e.g., protein cluster organization [26] and kmer-based ecological modeling [27]), the viral metagenomes themselves, while now quantitative for dsDNA viruses [31–36] are currently not capturing RNA viruses, ssDNA viruses, and giant viruses [37–41]. Thus, new methods are needed to capture RNA and ssDNA viral sequence space, and relevant and representative isolate-based genomes are essential to better map dsDNA viral sequence space and virus–host interactions in nature.

Most sequenced marine phage genomes belong to cyanophages [42], with recent addition of phages infecting other ecologically important marine heterotrophic bacteria [13, 43, 44]. Within heterotrophic bacteria, Pseudoalteromonas sp. strains are members of Gammaproteobacteria, and this class of Proteobacteria may comprise up to 30% of total marine bacterioplankton with a 20 to 80% of them taking up 3H-leucine [45], reflecting active members of the microbial loop. Moreover, it has been shown that members of the Gammaproteobacteria bacterial group show the highest growth rates among their oceanic counterparts and they are subjected to higher viral pressure than other groups in the NW Mediterranean Sea [46]. Also, Pseudoalteromonas sp. are in many cases associated to particle attached bacterial assemblages, wherein it has been shown that Gammaproteobacteria abundance reached maximum peaks in the range of 24 to 60% of the total OTUs studied using pyrosequencing [47]. Several studies have reported the ecological and evolutionary importance of the Pseudoalteromonas phages [48–51]. For instance, the ecogenomic analysis of the marine Pseudoalteromonas phage H105/1 revealed the presence of several genes in estuarine samples and this phage showed evolutionary relationships with its host in some proteins and functional modules [49]. Moreover, some of the sequenced Pseudoalteromonas bacterial genomes showed genes indicative of integrated prophages [49, 52, 53]. However, there are only four marine Pseudoalteromonas phage
genomes sequences available in public datasets: *Pseudoalteromonas* phage PM2 (*Corticoviridae*), *Pseudoalteromonas* phage H105/1 (*Siphoviridae*), *Pseudoalteromonas* phage RIO-1 (*Podoviridae*) and *Pseudoalteromonas* phage pYD6-A (*Podoviridae*) [49, 54, 55]. Despite infecting bacteria of the same genus, these phages do not share any genes and even belong to different phage families. While PM2 is the only sequenced corticovirus, H105/1 has functional organization similar to λ-like siphoviruses [49] and RIO-1 and pYD6-A are distantly related to T7-like and N4-like podoviruses, respectively [55]. This suggests high phage diversity and the importance of phage pressure in this bacterial group.

Thus, to better expand our understanding of *Pseudoalteromonas* phage diversity, phage–host interactions in the marine environment and the genomic features of marine phages, we isolate and characterize the *Pseudoalteromonas* phage B8b.

**Material and Methods**

**Phage isolation**

*Pseudoalteromonas* phage B8b was obtained from Blanes Bay Microbial Observatory (BBMO, 41°40’13.5"N 2°48’00.6"E http://www.icm.csic.es/bio/projects/icmicrobis/bbmo), a surface coastal site in the NW Mediterranean Sea, in winter 2009. No specific permissions to sample were required for this location. Four liters of surface seawater was collected and after a 0.22 µm prefiltration (Millipore, Whatmann), phages were concentrated by tangential flow filtration (30KDa VIVAFLOW cartridge, Sartorius) to a final volume of 20 ml. Phages were isolated using liquid enrichment cultures and plaque assays [56]. The host strain was *Pseudoalteromonas* sp. QC-44 (accession number: KM609273); which was isolated from the same marine site in 2009 using Zobell medium (1.0 g yeast extract, 5 g peptone, 15 g agar and 250 ml MQ water and 750 ml 30 kDa filtered seawater). This isolate was chosen because it was highly retrieved from our marine site. In the enrichment assay, 1 ml of viral concentrate was added to 3 ml *Pseudoalteromonas* sp. QC-44 exponentially growing in liquid Zobell medium (1.0 g yeast extract, 5 g peptone, 250 ml MQ water and 750 ml 30 kDa filtered seawater). After 24h of incubation in the dark, the mixture was centrifuged (5,000 x g, 10 min) and the supernatant was filtered through a 0.22 µm filter to remove any remaining bacterial cells. Phage enrichment was confirmed by plaque assay, in which 100 µl phage sample from 10x dilution series was combined with 400 µl of liquid bacterial culture (~10⁸ cells) and plated using the agar overlay technique by adding 3.5 ml of molten soft agar (0.5% agar in Zobell; 50°C). After plating, a well-resolved plaque was picked from the lawn of host cells and eluted with MSM buffer (450 mM NaCl, 50 mM MgSO₄ x 7H₂O, 50 mM Tris base, pH 8). To ensure clonal phage isolates, each isolate was plaque purified three times. After purification, high titer phage stocks were prepared by adding 5 ml of MSM to fully lysed plates. The plates were incubated on a shaker (110 RPM) for 40 min and the phage-MSM solution was transferred to a sterile tube and centrifuged at 5,000 x g for 10 min where after the supernatants were 0.22 µm filtered and stored at 4°C in the dark.

**CsCl purification**

Phages for transmission electron microscopy and virion structural proteome analysis were further purified by CsCl centrifugation [57]. Briefly, phage lysate from ~20 fully lysed plates was concentrated using polyethylene glycol (PEG). Here, 3.25 g NaCl was added to 50 ml of filtered phage lysate. The mixture was incubated 1 h at 4°C in the dark followed by centrifugation at 11,000 x g, 10 min. The pellet was discarded and PEG 8000 (10%) was added to the supernatant. After an incubation of 1 h at 4°C in the dark, it was centrifuged (10,000 x g, 10 min). The supernatant was discarded and the pellet was resuspended with MSM buffer. The centrifuge
tube (Ultra-Clear, Beckman, Fullerton, CA, USA) was layered with 1.125 ml each of (1) 1.7 g CsCl ml\(^{-1}\), (2) 1.5 g CsCl ml\(^{-1}\), (3) 1.45 g CsCl ml\(^{-1}\) and (4) 1.2 g CsCl ml\(^{-1}\), and finally topped with the viral concentrate and centrifuged (102,000 \(\times\) g, 4 h). A turbid white line containing the phages was removed with a syringe (2 ml total volume) and dialyzed (Slide-A-Lyzer Dyalisis Cassette G2 10 K MWCO, Rockford, IL, USA) three times in 1 liter buffer for at least 1 hour, (1 M Tris-HCl pH 8, 10 mM MgCl\(_2\)) containing three sequentially decreasing NaCl concentrations at each buffer change (3 M NaCl; 1.8 M NaCl; 0.6 M NaCl).

Electron microscopy of *Pseudoalteromonas* phage B8b

Transmission electron microscopy grids were prepared by placing 10 \(\mu\)l of CsCl-purified lysate (see above) onto 200 mesh formvar-coated copper grids (Ted Pella) for 5 min. The solution was subsequently removed with filter paper and grids were negatively stained with 2% uranyl acetate solution by rinsing the grids with 2 drops of the solution and staining for 45 s with a third drop. The grids were examined using a Philips CM12 microscope with an accelerating voltage of 80 kV. Viral capsid diameter and tail length were determined based on an average of several images and they were measured using ImageJ software (US National Institutes of Health, Bethesda, MD, USA; [37, 58].

One-step growth experiments

The burst sizes and one-step growth curves were determined as described by Weiss et al. [59], with minor modifications. One milliliter of *Pseudoalteromonas* sp. QC44 overnight culture was transferred to 10 ml of fresh 20% Zobell media and incubated with shaking (120 RPM) for about 20 min, until the \(A_{600}\) was \(\sim 0.02\) (mid-log phase), which was equivalent to a viable cell count of around \(10^8\) cells/ml. The concentration of bacterial cells at \(A_{600} \sim 0.02\) was verified by flow cytometry [60]. One milliliter of the bacterial culture was then transferred to an eppendorf tube and mixed with phage at a multiplicity of infection of 0.1. The mixture was incubated at room temperature for 15 min to allow phage adsorption. After this adsorption, the mixture was diluted to \(10^{-2}\) in 20 ml of 20% Zobell media to prevent further adsorption of phage. Samples were removed to enumerate total and free phage concentration. In order to detect the free phages, samples were 0.22 \(\mu\)m filtrated before plating. The number of phages in both cases was determined, in duplicate, using the double-agar-layer method as described above. Finally, burst size was calculated as described in [61]. Briefly, burst size was measured as the ratio of the final count of liberated phage particles to the initial count of infected bacterial cells during the latent period.

Phage specificity

To determine phage host range and bacterial susceptibility, a cross infectivity test was done where plaque assays for each virus–bacteria combination were performed with phage B8b on 52 strains of *Pseudoalteromonas* spp. as well as 34 strains of *Alteromonas* spp., *Marinobacter* spp., *Vibrio* spp., Bacteroidetes, *Nereida* spp., and *Erytrobacter* spp. (S1 Table) using 100 \(\mu\)l of two different phage stock dilutions (\(10^{-5}\) and \(10^{-8}\)). Lysis was evaluated after overnight incubation in the dark. Once the bacterial strains showed phage susceptibility in the first test, a more thorough analysis was performed to determine the efficiency of infection on each strain. Here, plaque assays were performed with a range of 10x diluted phage stock and plaques were enumerated after 1 and 2 days incubation. Efficiency of infection was expressed in relative PFU (Plaque Forming Units), where the highest was set to 100%.
Pulsed-field gel electrophoresis

Phage genome size was determined by pulsed-field gel electrophoresis (PFGE) [15]. For this procedure phage lysate was concentrated by Amicon Ultra-15 centrifugal filter units (Millipore) from 5 ml to a final volume of 400 μl. Of this, equal amounts (400 μl) were mixed with melted 1.6% low-melting-point agarose (Pronadisa), transferred to plugs molds, left to solidify at room temperature for a few minutes and then kept for 15 minutes at 4°C. Plugs were incubated overnight at 50°C in ESP (0.5 M EDTA, pH 9, 0.1% N-laurylsarcosine and 1 mg ml⁻¹ proteinase K) and stored at 4°C until further analysis. PFGE was performed on a CHEF-DR III system (Bio-Rad) using 1% agarose gel (LE agarose SeaKem n.50005 BERLABO S.A.). The gel was run for 22h in 0.5X TBE buffer (1X TBE is 89 M Tris, 2 mM EDTA, and 89 mM boric acid, pH 8.3) at a 5.0-15.0 seconds switch time, 6V cm⁻¹ and an included angle of 120 degrees. After electrophoresis, the gel was stained with SYBR Gold (Molecular probes, 10.000X) diluted to 10⁻⁴ in 150 ml of TBE for 15 min and washed with MQ water for 15 min. Lambda Low Range (New England Biolabs) was used as molecular size marker. We did three replicates of the PFGE and all of them give us the same genome size estimation.

Viral DNA purification and genome sequencing

Viral DNA was obtained using the Lambda Wizard DNA kit (Promega Corp. Madison, WI) [62, 63]. Phage lysate from ~15 fully lysed plates were concentrated using polyethylene glycol as described earlier (CsCl purification section). One ml of Purification Resin (Promega, product A7181 Madison WI) was added to 1.5 ml of phages (the PEG pellet resuspended with MSM) and mixed gently by inverting the tube. The mixture was loaded onto a mini-column (Promega, product A7211 Madison WI) through a 5 ml syringe attached to the column, pushing the mixture through with the syringe plunger. The column was then washed with 2 ml 80% isopropanol, the syringe was removed and the mini-column placed into a 1.5 ml eppendorf tube and centrifuged (10,000 x g, 2 min, room temperature) to remove any remaining liquid. Phage DNA was eluted from the column by adding 100 ml TE buffer (80°C), and the DNA was recovered in a 1.5 ml eppendorf tube through centrifugation (10,000 x g, 30 s, room temperature). Phage DNA was stored at-20°C. The genome was sequenced by the Life sequencing company (Valencia, Spain) using the standard shotgun sequencing reagents and a 454 GS FLX Titanium Sequencing System (Roche), according to the manufacturer’s instructions.

Genome assembly and annotation

B8b phage genome sequences were assembled into 4 contigs using Newbler (Roche). In the absence of complete genome coverage, attempts were made to close the gaps using PCR and by direct Sanger sequencing. Forward and reverse primers were designed for every contig using PRIMER3 VERSION 0.4.0 [64], producing a 300–400 bp overlap among the different contigs (see S2 Table). Unfortunately, we failed to close the genome since we could not get PCR products derived from any primer and contig combination. Moreover, we did not obtain any good enough sequence from direct sequencing using any of the designed primers.

ORFs were predicted using a pseudo-automated pipeline where the ORFs were assigned by GeneMark Heuristic [65] followed by refinement through synteny and maximizing ORF size where alternative start sites were present. Gene identification and annotation was done using the BLASTP program against the NCBI non-redundant (nr) database (e-value cut off <0.001, August 2013).

Accession number of the B8b phage genes was deposited into NCBI under the following accession numbers: KJ944830, KM000061, KM000062 and KM000063.
Proteome analysis

Phages were harvested with MSM from fully lysed plates and CsCl purified as described above. The purified phage particles were prepared prior to 2d-LC-MS/MS analyses using an optimization of the FASP kit (Protein Discovery, Knoxville, TN) [66]. All reagents were provided for in the kit. Briefly, purified phages were re-suspended in 8M Urea/10mM DTT, denatured and passed over the 30kDa filter, then washed with 8M Urea and treated with Iodoacetamide (IAM) to label cysteine residues. IAA was washed away with 8M Urea and then 50mM Ammonium Biocarbonate. Sequencing grade trypsin was then added and digestion processed overnight. The next day peptides were eluted from the 30kDa filter via Ammonium Biocarbonate buffer, NaCl buffer and water/0.1% Formic acid. Three aliquots were prepared per sample and frozen at-80°C until 2d-LC-MS/MS analyses. The FASP prepared peptides (>500 ng) were loaded onto the back column of a split phase 2D column (~3–5cmSCX and 3–5cm C-18) (all packing materials purchased from Phenomenex, Torrance, CA). The column was loaded to the HPLC and washed with 100% aqueous solution for 5 min, followed by a ramp from 100% aqueous to 100% organic solution for 10 min. The column [66] was connected to a front column (RP C-18, 15cm) with a nanospray source on LTQVelos and run for 5–12 h two dimensional separation of increasing salt pulses (ammonium acetate) followed by water to organic gradients (see [66]). All instrument were run in a data-dependent manner as previous described [67, 68]. To recruit peptides to the phage genomes, the resulting MS/MS spectra were searched against a database consisting of annotated phage proteins, all phage ORFs > 30 amino acids (aa) (to identify ORFs possibly missed through the annotation), and proteins from sequenced Pseudoalteromonas bacteria (Pseudoalteromonas haloplanktis TAC125, Pseudoalteromonas sp. TW-7, Pseudoalteromonas atlantica T6c, Pseudoalteromonas tunicata D2) and eukaryotic organisms (human and mouse) to use as indicator for false positives. Data analyses were performed using SEQUEST and filtered with DTA Select with conservative filters [67]. For proteomics, databases, peptide and protein results, MS/MS spectra and supplementary tables are archived and available at https://maple.lsd.ornl.gov/mspipeline/sullivan/, while MS.raw files or other extracted formats are available upon request.

Phylogenetic analysis

DNA polymerase, phage portal protein, and phage large terminase amino acid sequences of known bacteriophages (S3, S4, S5 Tables) were used to investigate the phage B8b phylogeny. Multiple sequence alignment was automatically performed using the program ClustalW (default parameters) [69]. Maximum likelihood trees were built using the JTT model [70] with bootstrap analysis (1000 replicates) using MEGA version 5.1 [71].

Fragment recruitment analysis of B8b phage on Pacific Ocean Viral metagenomics (POV)

Fragment recruitment analyses (FRA) were performed to get a sense of the phage B8b relative abundance in the 32 marine viral metagenomes from the Pacific Ocean Virome [26] (available at CAMERA (http://camera.calit2.net) under the following project accessions: CAM_P_0000914 and CAM_P_0000915). We used the Reciprocal Best Blast approach (RBB) [72] applying the same rationale to that employed elsewhere [43]. Briefly, individual metagenomic samples are made into a BLAST database, and then the predicted ORFs of the phage B8b are searched against it using TBLASTn. After this initial blast, hits to the POV database are extracted and become the query for a second BLAST search (BLASTx) against an internal protein genome reference database with a total size of 8,512,217 ORFs that included: (i) protein
viral genomes (Refseq Release 60; 4958 genomes and 163,830 ORFs), (ii) bacterial genomes (RefSeq Release 60; 197,527 contigs and 8,348,231 ORFs) and (iii) the *Pseudoalteromonas* phage B8b (4 Contigs, 55 ORFs). Only those metagenomic sequences that returned as a best hit a sequence from the genome of the *Pseudoalteromonas* phage B8b were extracted from the database and counted as hits for subsequent step. Finally, to calculate the relative abundance of B8b phage and two other phages used as reference genomes (the abundant Pelagiphage HTVOC10P (KC465898) and the non marine Enterobacteriaphage T4 (NC_000866)) in the POV dataset, we normalized the number of hits to: 1) protein length, 2) sequencing depth and 3) mean abundances across the 32 POV metagenomes. This was calculated by dividing the number of hits (H) by the total number of sequences (N) and the amino acid length of the hit protein (L). Finally, to avoid large numbers of significant figures, the abundances were rescaled to the mean abundances (mean normalization) across all samples where the numerator is calculated from individual samples and the denominator is calculated from all the samples.

\[ A_{\text{rel}} = \frac{(H \times N^{-1} \times L^{-1})}{(H \times N^{-1} \times L^{-1})} \]

**Results and Discussion**

Morphology and biology characterization of *Pseudoalteromonas* phage B8b

Phage B8b was isolated from Blanes Bay Microbial Observatory (BBMO), an oligotrophic surface coastal site in the NW Mediterranean Sea, and it formed clear, round plaques when grown on its host of isolation, *Pseudoalteromonas* sp. QC-44. Morphological examination showed that phage B8b belonged to the *Siphoviridae* family based on ICTV rules of nomenclature [73] and had an isometric capsid of 49.8 ± 1.6 nm in diameter connected to a long and flexible tail of 175.5 ± 3.2 nm in length (Fig. 1).

The one-step growth curve of phage B8b showed a latent period of 70 min and approximately 172 new viral particles were released from each infected *Pseudoalteromonas* sp. QC-44 cell (Fig. 2). These values differed from the marine *Pseudoalteromonas* phage PM2, which produced 300 viral particles per infected cell about 70–90 min after infection [74], as well as other marine siphoviruses, e.g. *Vibrio* phage SIO-2, which had a latent period of 45–60 min and an average burst size of 60 [75] or the cyanosiphovirus S-BBS1, which had a 540 min (9 h) of latent period and approximately 250 progeny viruses were produced per infected host cell [76]. However, this is not surprising as burst size and latent period is known to vary between phages, but also depend on which host they infect [61], nutrient availability, specific growth rate of the

![Figure 1. Transmission electron micrograph showing negatively stained *Pseudoalteromonas* phage B8b.](https://doi.org/10.1371/journal.pone.0114829.g001)
host, and temperature [77]. Additionally, marine bacteria thrive under lower nutrient concentrations than provided in the lab and, consequently, *in situ* burst size is likely smaller than the values we measured [77].

To examine the host range of the isolated phage, infectivity was tested on 52 *Pseudoalteromonas* sp., 15 *Alteromonas* sp., 8 *Vibrio* sp. strains, 3 *Marinobacterium*, sp., all those belonging to Gammaproteobacteria class plus 5 Bacteroidetes (Flavobacteria) and 6 Alphaproteobacteria (Rhodobacterales and Sphingomonadales) (S1 Table). All tested bacterial strains were isolated from the same BBMO marine station as the phage. Phage B8b only infected 3 of 52 *Pseudoalteromonas* spp. strains (Fig. 3) and the phage’s efficiency of infection range between 67–100% on the 3 different *Pseudoalteromonas* strains (Fig. 3). These narrow host range findings agree with previous *Pseudoalteromonas* phage host ranges—PM2 infected 2 of 13 *Pseudoalteromonas* strains [74], H105/5 infected 3 of 52 *Pseudoalteromonas* strains [50], and RIO-1 infected 4 of 11 *Pseudoalteromonas* strains [55]. The use of a single-host enrichment method in this study might bias the results towards a narrow host range phage [78]. However, this narrow siphovirus host range is consistent with previous findings on *Pseudoalteromonas* phages and cyanophages [50, 79] and compared with extended myovirus host range, but contrasts observations in the *Cellulophaga* phages [13, 80].

Of the 37 non-*Pseudoalteromonas* bacterial strains tested, phage B8b was only able to infect a single *Alteromonas* strain. This strain belongs to a different family (Alteromonadaceae) than the phage B8b original host (Pseudoalteromonadaceae) with only 86% nucleotide identity at the 16S rRNA locus between the two bacterial strains (Fig. 3). Also, a lower efficiency of infection (58%) was observed on *Alteromonas* sp. compared to the infection on the host of isolation (Fig. 3). Previously, phages infecting across genera boundaries have been reported, but this is commonly among large myoviruses, like cyanophages infecting *Prochlorococcus* [79], enterophage LG1 and AR1 [81], and vibriophage KVP40 [82], and the two genera do not represent different families of host microbes. Among siphoviruses, one isolate has been reported to infect two bacterial strains of different genera in wastewater [83, 84], but no such cross-genera

![Figure 2. One-step growth curve of *Pseudoalteromonas* phage B8b on *Pseudoalteromonas* sp. QC-44 strain (●, total PFU; O free PFU).](https://doi.org/10.1371/journal.pone.0114829.g002)
infections have been reported for siphoviruses from the marine environment. The fact that this phage may infect over genus boundaries highlights the potential of this phage for mediating transduction, and thereby increasing microdiversity, not only among closely related bacterial strains but also across larger taxonomic space [85].

Figure 3. Phylogenetic analyses of the bacterial hosts used to test the Pseudoalteromonas phage B8b phage host range. Bacterial strains infected by siphovirus B8b are labeled in black and the efficiency of phage B8b in hosts infected is indicated. Efficiency is expressed in relative PFU, where the highest was set to 100% and the same phage titer dilution was used for all the bacterial strains (10⁶). Names in brackets are strain designations (See S1 Table for more information).
Structure and general properties of the siphovirus B8 genome

While the PFGE analyses predicted that *Pseudoalteromonas* phage B8b had a genome size of 46 kb (S1 Fig.), the combined length of the 4 sequenced contigs was 42,700 bp. These represented two major contigs (20,209 and 19,353 bp) and two short contigs (2,155 and 1,012 bp). Although these contigs could not be closed, probably due to existence of host DNA in the phage DNA sample, the PFGE sizing as compared to the summed contig lengths suggests that about 90% of the phage genome was sequenced. Moreover, the obtained banding pattern in the PFGE gel (S1 Fig.) suggests that the phage had a concatemeric genome where multiple copies of the original DNA were linked in a continuous series of multiples of the predicted genome size. This can be produced by rolling circle replication and/or recombination and is a common replication strategy for dsDNA bacteriophages genomes [86]. The 4 genomic contigs had an average G+C content of 50% and contained 58 predicted open reading frames (ORFs; Table 1). Thirty of these ORFs had significant sequence similarity to proteins in GenBank, but only 12 could be annotated to a function (Table 1), which is similar to other previously sequenced marine *Pseudoalteromonas* phages [49, 54, 55] and siphoviruses [75, 87–89]. Among the genes with detected similarity, 40% were most similar to viruses, 27% to prophages and 33% showed similarity to genes detected in bacterial genomes (Table 1). *Pseudoalteromonas* phage B8b displayed two distinctive functional modules (Fig. 4). A replication module was found in contig 1, which had several genes bioinformatically identified as involved in DNA replication and nucleotide metabolism, such as DNA primase (Contig1_ORF10), helicase (Contig1_ORF21) and DNA polymerase (Contig1_ORF23). Furthermore, the majority of ORFs with the highest similarity to phages (9 of 12) were detected in contig 1, being 7 of them most similar to siphoviruses (Table 1). A packaging/structural module was observed in contig 2 and contained genes that encoded proteins including phage terminases (Contig2_ORF2 and ORF4), phage portal protein (Contig2_ORF6), prohead peptidase (Contig2_ORF14), and tail tape measure protein (Contig2_ORF22). No genes involved for transcription regulatory functions were identified.

Proteomic analysis

Given that only 5 viral structural proteins were identified by sequence similarity, we performed virion structural proteomic analyses to experimentally confirm identified structural genes and determine the remaining unknown structural proteins. The portal protein, prohead peptidase, tail tape measure protein as well as 8 ORFs of unknown function in contig 2 were detected as part of the phage particle (Table 1). Further, the 2 ORFs of unknown function in contig 3 are part of the phage structural particle, as well as two proteins of unknown function in contig 1. Three spectra also matched against the DNA polymerase gene, however, they were considered false positives as the total peptides detected covered <4% of the gene.

Phylogenetic relationships

In order to get insights into the phylogenetic relatedness of phage B8b compared to other phages, three relevant genes were compared phylogenetically to similar genes: the B8b DNA polymerase, the phage large terminase, and phage portal protein (S2, S3, S4 Figs. and S3, S4, S5 Tables).

DNA polymerase genes are crucial in genomic replication and mutagenic repair and it has been used to define phylogenetic relationships for novel isolated phages [75, 90, 91]. Surprisingly, the B8b DNA polymerase clustered together with several myoviruses (S2 Fig.). Two of them were isolated from marine bacteria (*Edwardsiella* phage MSW-3 and *Klebsiella* phage JDOO1) [92, 93] and most of them were lytic phages, except for *Vibrio* phage CP-T1 that is known to be temperate (i.e., capable of forming a lysogen; [94]). Although DNA polymerases
| Contig.ORF | Nucleotide start position | Nucleotide end position | Strand | Product length (aa) | % aa ID | Predicted identity or function of product | Strain with closest hit (E-value) | Accession number | Taxonomy | Sequence count | Spectral count | Sequence coverage (%) |
|-----------|---------------------------|-------------------------|--------|---------------------|--------|------------------------------------------|---------------------------------|-----------------|----------|----------------|-------------|---------------------|
| Contig1_ORF1 | 7                         | 630                     | +      | 208                 | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF2 | 676                       | 1221                    | +      | 182                 | 42.0   | dUTPase                                  | Lactococcus phage Q33 (8.0E-13) | AFV51054.1 | Siphoviridae, Caudovirales | | | |
| Contig1_ORF3 | 1218                      | 1625                    | +      | 136                 | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF4 | 1622                      | 2128                    | +      | 169                 | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF5 | 2121                      | 2456                    | +      | 112                 | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF6 | 2536                      | 3297                    | +      | 254                 | 37.0   | DNA binding protein                      | Salmonella phage E1 (4.0E-45)   | WP_003849806.1 | Siphoviridae, Caudovirales | | | |
| Contig1_ORF7 | 3282                      | 3500                    | +      | 73                  | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF8 | 3503                      | 3760                    | +      | 86                  | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF9 | 3753                      | 4376                    | +      | 208                 | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF10 | 6914                     | 4560                    | -      | 785                 | 35.0   | DNA primase                              | Salmonella phage FSL SP-062 (1.0E-72) | AGF89287.1 | Siphoviridae, Caudovirales | | | |
| Contig1_ORF11 | 7155                     | 6919                    | -      | 79                  | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF12 | 7580                     | 7152                    | -      | 143                 | 47.0   | Conserved hypothetical phage protein     | Edwardsiella phage MSW-3 (6.0E-101) | YP_007348969 | Myoviridae, Caudovirales | | | |
| Contig1_ORF13 | 8746                     | 7580                    | -      | 389                 | **| Hypothetical phage protein                | Non-significant                   | |          |              |            |                     |
| Contig1_ORF14 | 9873                     | 8749                    | -      | 375                 | 33.0   | Conserved hypothetical phage protein     | Salmonella phage FSL SP-062 (2.0E-33) | AGF89282.1 | Siphoviridae, Caudovirales | 3 | 8 | 10.9 |

(Continued)
| Contig1_ORF   | Nucleotide start position | Nucleotide end position | Strand | Product length (aa) | % aa ID | Predicted identity or function of product | Strain with closest hit (Evalue) | Accession number | Taxonomy                          | Sequence count | Spectral count | Sequence coverage (%) |
|--------------|---------------------------|-------------------------|--------|---------------------|--------|--------------------------------------------|--------------------------------|-----------------|----------------------------------|----------------|---------------|------------------------|
| Contig1_ORF15 | 10783                     | -                       | 302    | 42.0                | RecT protein | *Marichromatium purpuratum* 984 (1.0E-4) | WP_005220619 | Gammaproteobacteria, Chromatiales |
| Contig1_ORF16 | 11037                     | -                       | 75     | 51.0                | Conserved hypothetical phage protein | *Acinetobacter* phage Ac42 (2.0E-20) | YP_004009376 | Myoviridae, Caudovirales          |
| Contig1_ORF17 | 11117                     | +                       | 55     | HNPhage1 | Hypothetical phage protein | Non-significant |
| Contig1_ORF18 | 11286                     | +                       | 88     | HNPhage2 | Hypothetical phage protein | Non-significant |
| Contig1_ORF19 | 11546                     | +                       | 66     | HNPhage3 | Hypothetical phage protein | Non-significant |
| Contig1_ORF20 | 11709                     | +                       | 61     | HNPhage4 | Hypothetical phage protein | Non-significant |
| Contig1_ORF21 | 11958                     | +                       | 557    | 35.0                | Helicase | *Salmonella* phage FSL SP-062 (3.0E-92) | AGF89284.1 | Siphoviridae, Caudovirales         |
| Contig1_ORF22 | 13621                     | +                       | 79     | HNPhage5 | Hypothetical phage protein | Non-significant |
| Contig1_ORF23 | 13847                     | +                       | 737    | 39.0                | DNA polymerase | *Salmonella* phage FSL SP-062 (9.0E-145) | AGF89344.1 | Siphoviridae, Caudovirales         |
| Contig1_ORF24 | 16103                     | +                       | 201    | HNPhage6 | Hypothetical phage protein | Non-significant |
| Contig1_ORF25 | 16705                     | +                       | 78     | HNPhage7 | Hypothetical phage protein | Non-significant |
| Contig1_ORF26 | 17471                     | -                       | 157    | 40.0                | Conserved hypothetical phage protein | *Klebsiella* phage phiKO2 (2.0E-17) | YP_006634.1 | Siphoviridae, Caudovirales         |
| Contig1_ORF27 | 17695                     | -                       | 76     | 41.0                | Conserved hypothetical phage protein | *Alishewanella jeotgali* KCTC 22429 (7.0E-11) | WP_008951684 | Gammaproteobacteria, Alteromonadales |
| Genomic data | Proteomic data |
|--------------|---------------|
| Contig1_ORF28 17688 19877 - 720 30.0 Conserved | Marinobacterium phage MarM11 | 4.0E-28 |
| Contig1_ORF29 20170 19874 - 99 33.0 Conserved | Hypothetical phage protein | 7.0E-5 |
| Contig2_ORF1 256 486 + 77 | Hypothetical phage protein | Non-significant |
| Contig2_ORF2 410 1006 + 199 23.0 Small terminase subunit | Escherichia phage vB_EcoM_ECO1230 –10 | 7.12E-7 |
| Contig2_ORF3 890 1902 + 171 25.0 Small terminase subunit | Hypothetical phage protein | Non-significant |
| Contig2_ORF4 1519 3518 + 66 59.0 Phage large terminase subunit | Hypothetical phage protein | Non-significant |
| Contig2_ORF5 3514 3514 + 72 | Hypothetical phage protein | Non-significant |
| Contig2_ORF6 2323 2323 + 143 | Hypothetical phage protein | Non-significant |
| Contig2_ORF7 5660 5218 + 149 | Hypothetical phage protein | Non-significant |
| Contig2_ORF8 6234 5952 + 149 | Hypothetical phage protein | Non-significant |
| Contig2_ORF9 6283 6283 + 77 | Hypothetical phage protein | Non-significant |
| Contig2_ORF10 6224 6224 + 67 | Hypothetical phage protein | Non-significant |
| Contig2_ORF11 6225 6225 - 98 | Hypothetical phage protein | Non-significant |

(Continued)
Table 1. (Continued)

| Contig2 ORF | Genomic data | Proteomic data |
|------------|--------------|----------------|
|            | Nucleotide start position | Nucleotide end position | Strand | Product length (aa) | % aa ID | Predicted identity or function of product | Strain with closest hit (Evalue) | Accession number | Taxonomy | Sequence count | Spectral count | Sequence coverage (%) |
| Contig2 ORF12 | 7423 | - | 207 | 40.0 | Conserved | Hypothetical | Phage protein | Pseudoalteromonas phage RIO-1 (2.0E-6) | YP_008051111.1 | Podoviridae, Caudovirales | 6 | 10 | 37.30 |
| Contig2 ORF13 | 7800 | - | 126 | Hypothetical | Phage protein | Non-significant | 6 | 22 | 57.90 |
| Contig2 ORF14 | 7912 | + | 685 | 44.0 | Peptidase | U35 phage prohead HK97 | Marinobacterium stanierii S30 (3.0E-168) | WP_010322158 | Gammaproteobacteria, Alteromonadales (Prophage) | 28 | 937 | 42.70 |
| Contig2 ORF15 | 10022 | + | 113 | 48.0 | Conserved | Hypothetical | Phage protein | Marinobacterium stanierii S30 (1.0E-17) | WP_010322157 | Gammaproteobacteria, Alteromonadales (Prophage) | 7 | 67 | 43.00 |
| Contig2 ORF16 | 10341 | + | 114 | Hypothetical | Phage protein | Non-significant | 3 | 3 | 33.60 |
| Contig2 ORF17 | 10675 | + | 208 | 41.0 | Conserved | Hypothetical | Phage protein | Vibrio crassostreae (3.0E-44) | WP_017059000 | Gammaproteobacteria, Vibrionales | 67 | 3 | 43.00 |
| Contig2 ORF18 | 11295 | + | 159 | 30.0 | Conserved | Hypothetical | Phage protein | Marinobacterium stanierii S30 (4.0E-4) | WP_010322154 | Gammaproteobacteria, Alteromonadales (Prophage) | 3 | 24.40 |
| Contig2 ORF19 | 11774 | + | 125 | 35.0 | Chaperone | GroES | Pseudoalteromonas tunicata (1.0E-4) | WP_009840504 | Gammaproteobacteria, Alteromonadales | 3 | 24.40 |
| Contig2 ORF20 | 12148 | + | 57 | Hypothetical | Phage protein | Non-significant | 57 | 3 | 33.60 |
| Contig2 ORF21 | 12318 | + | 254 | 40.0 | Conserved | Hypothetical | Phage protein | Marinobacterium stanierii S30 (8.01E-39) | WP_010322152 | Gammaproteobacteria, Alteromonadales (Prophage) | 9 | 290 | 54.90 |
| Contig2 ORF22 | 13148 | + | 1403 | 32.0 | Phage tail measure protein | TP901, core region | Marinobacterium stanierii S30 (1.29E-161) | WP_010322151 | Gammaproteobacteria, Alteromonadales (Prophage) | 46 | 74 | 43.50 |

(Continued)
Table 1. (Continued)

| Contig.ORF | Nucleotide start position | Nucleotide end position | Strand | Product length (aa) | % aa ID | Predicted identity or function of product | Strain with closest hit (Evalue) | Accession number | Taxonomy | Sequence count | Spectral count | Sequence coverage (%) |
|------------|--------------------------|-------------------------|--------|---------------------|---------|------------------------------------------|---------------------------------|-----------------|----------|-----------------|---------------|----------------------|
| Contig2.ORF23 | 17359 | 17769 | + | 137 | 39.0 | Conserved hypothetical phage protein | Pseudoaltermonas sp. S9 (8.0E-17) | WP_01049077 | Gammaproteobacteria, Alteromonadales |
| Contig2.ORF24 | 17769 | 19343 | + | 525 | 35.0 | Conserved hypothetical phage protein | Pseudomonas aeruginosa (7.0E-73) | WP_019396974.1 | Gammaproteobacteria, Pseudomonadales | 12 | 21 | 36.19 |
| Contig3.ORF1 | 9 | 1694 | + | 562 | 38.0 | Conserved hypothetical phage protein | Pseudomonas aeruginosa (5.0E-82) | WP_019396974.1 | Gammaproteobacteria, Pseudomonadales | 12 | 38 | 27.63 |
| Contig3.ORF2 | 1694 | 2154 | + | 153 | Hypothetical phage protein | Non-significant | | | 3 | 3 | 30.10 |
| Contig4.ORF1 | 12 | 350 | + | 113 | 29.0 | Conserved hypothetical phage protein | Klebsiella oxytoca (9.0E-5) | WP_004131755 | Gammaproteobacteria, Enterobacteriales |
| Contig4.ORF2 | 343 | 609 | + | 89 | Hypothetical phage protein | Non-significant | | | | |
| Contig4.ORF3 | 824 | 699 | - | 42 | Hypothetical phage protein | Non-significant | | | | |
have been suggested to be good phylogenetic markers for investigating viral phylogeny since they offer the greatest number of viral homologs [95], our results showed that this gene’s phylogeny may be incongruent with electron microscopy- and genome-based taxonomy.

The phage large terminase and phage portal proteins are commonly highly conserved among phage genes, possibly due to their specific enzymatic functions [96], and phage phylogeny has been investigated using these genes in several other studies [89, 94, 96–98]. The phage terminases are DNA packaging enzymes that contain the ATPase activity that powers DNA translocation and most terminases also contain an endonuclease that during DNA packaging cuts concatemeric DNA into genome lengths. Terminases must also recognize viral DNA in a pool that may include host DNA [86, 99]. Phage portal proteins, one the other hand, are structurally associated with the phage capsid and facilitate DNA packaging during head assembly [86]. Phylogenetically, both B8b terminase and portal protein were most closely related to Stenotrophomonas phage S1 (S3, S4 Figs.), a temperate siphovirus isolated from sewage [100].
They also clustered together with the putative temperate siphoviruses *Synechococcus* S-CBS1 and S-CBS3 (terminase) [98], as well as several temperate myoviruses, like *Acidithiobacillus* phage AcaML (terminase and portal) [101], *Halomonas* phage phiHAP-1 (portal) [102], and *Vibrio* phage VP882 (portal) [103]. Again, these single marker gene based findings are inconsistent; while some are congruent with the morphological observations, some are not.

Together, these results from three phage gene markers suggest the rampant mosacism posited for siphoviruses [104] may also be true in marine siphoviruses since it has been detected not only in phage B8b but also in other marine siphoviruses [49, 89, 98]. In contrast, other phage groups (e.g., T4-like myoviruses) appear to have clear signals of vertical descent, particularly in their core gene sets as observed in isolates [105, 106] and large-scale analyses of field populations [107].

**Distinctive genes in *Pseudoalteromonas* phage B8b**

The B8b genome encoded a RecT protein (Contig1_ORF15), which is involved in homologous recombination of importance to a variety of cellular processes, including the maintenance of genomic integrity [108]. It provides means for repair of DNA double-stranded breaks, which can arise during DNA replication as well as after damage by external factors such as irradiation [109]. As a ssDNA-binding protein, RecT promotes ssDNA annealing, strand transfer, and strand invasion *in vitro* [110]. In *Escherichia coli*, homologous recombination is mediated by bacteriophage RecT protein that permits efficient DNA engineering in various *E. coli* hosts [111]. Although integrase or excisionase genes were not identified in our genome, the RecT gene encoded might facilitate the integration of the phage B8b genome into the bacterial hosts genome, opening up for the potential of phage B8b to act as a prophage.

The presence of chaperone GroES (also called chaperonin 10; Contig2_ORF19) in B8b is unique as it is the first time GroES been reported in a siphovirus, while it has previously been detected in myoviruses and podoviruses [13]. Chaperonins are known to promote the correct folding of newly synthesized polypeptides and to prevent aggregation of proteins denatured under stress [112]. In *E. coli*, the genes that encode for GroES/GroEL chaperonin system were first identified as host factors required for bacteriophage morphogenesis and subsequent work established that the GroES and GroEL proteins were essential for the correct assembly of λ pro-heads and T5 tails [113, 114]. The presence of this gene in phage B8b might point out that possibly could have a more complex viral capsid or tail structure than other siphoviruses, which requires that it provides its own chaperonin.

**Possibility of lysogenic replication strategy**

Phage B8b was isolated as a lytic phage and its lytic nature was confirmed by the one-step growth curve (Fig. 2). However, from our phylogenetic analyses and the presence of the RecT protein, phage B8b was closely related to several phages that are known to be able to perform lysogeny. Also, a large number of phage B8b’s structural proteins had their closest blast hits to proteins from the *Marinobacterium stanieri S30* microbial genome (see Table 1), which begged its comparison (Fig. 4). The synteny between phage B8b and this bacterial contig suggests that a prophage is present in this microbial genome, although it could be a relic or defective prophage, which would represent the closest available genome representative to phage B8b. This highlights the possibility that B8b could be a temperate phage and also, it is possible that lysogenic replication of phage B8b might be detected if different host strains are infected [61], other growth conditions, which might promote lysogeny, are used [115], or under changed phage-host density ratios [116, 117]. Lysogeny would be an attractive lifestyle in oligotrophic marine environment, such as the NW Mediterranean Sea source waters, as lysogeny is a...
survival strategy during conditions of low host cell encounter rates [118–120]. Moreover, the presence of a prophage may be advantageous for the bacterial host. Homomimunty protects lysogens from infection by closely related phages [121] and it has been also proposed that marine prophages may contribute to host survival in unfavorable environments through the suppression of unnecessary metabolic activities [122].

Marine Gammaproteobacteria host genes
Given that cyanophage genomes commonly contain "host genes" (e.g., [10, 63, 123] and recent metagenomic findings that such viral-encoded host genes cover broad metabolic categories [124] including nearly all of central carbon metabolism [24], we wondered whether such Auxiliary Metabolic Genes (AMGs, sensu [125]) existed in this new phage B8b genome. Of the 58 phage B8b’s ORFs, 10 were bacterial and 8 prophage related, and all eight ORFs were related to Alteromonadales (Gammaproteobacteria). In fact, Alteromonas sp. MED111 was the only non Pseudoalteromonas strain that could be infected by phage B8b in the host-range assay. If phages can act as vectors to genetically transfer DNA across bacterial taxa through lateral gene transfer (LGT) [85] one would expect to find host genes within phages that infect similar hosts. Lateral gene transfer has been previously observed in cyanophages [10, 126] and Pseudoalteromonas phages [49]. Genetic interaction of phage and bacterial genomes has been predicted to be highly specific such in co-evolutionary models [127] although it is well know that phages can infect hosts from different species and even genera [79]. Emerging phage-bacteria interactions are now being viewed as networks rather than coupled simplistic interactions [128]. The genus-crossing host range detected in phage B8b and the fact that many of the genes found in our B8 genomes were related to a prophage, from a different host specifically to the genus Marinobacter spp. within Gammaproteobacteria stressing that possibility of genetic exchange between different host genera.

Relative abundance of phage B8b in Pacific Ocean Viral metagenomes
Given the recent availability of a large-scale viral metagenomic dataset (32 Pacific Ocean Viromes, [26]) that was consistently prepared using extensively well-documented quantitative methods [31–36], we wondered whether this relatively novel phage B8b genome was observed in other marine systems and if so how abundant it was. The normalized relative abundance showed that phage B8b was mainly present in the surface, coastal waters with 1.15% assigned reads to phage B8b (Fig. 5). However, an average of 0.46% of the metagenomic reads from deeper, aphotic samples were also recruited to these genomes, which might reflect that a similar host are consistently present through the entire water column since this phage was isolated from surface waters in NW Mediterranean Sea. For environmental phages, these numbers are low when compared to phages for abundant hosts. For example, phages for SAR11, Synechococcus and Prochlorococcus represented closer to 58.7%, 21.6% and 12.4%, respectively, in diverse ocean viral metagenomes [43]. However, these Pseudoalteromonas B8b phage abundances are similar to environmental phages for less abundant hosts—e.g., Cellulophaga phages—considered to be representatives of the ‘rare virosphere’ [13]. The percentage of the genome covered by the metagenomics reads in POV database was on average 24.2%, although only 0.65% was exclusive to phage B8b. This suggests that many phage B8b ORFs are likely conserved across a diversity of phages (Fig. 5). Consistent with this hypothesis, the amino acid percentage identity of the predicted proteins for phage B8b genes (24.2%) were similar to that observed for non-marine T4-like phages (29%), but contrasted the identity for pelagiphages (81.4%). Such identities aid in discriminating between whether a new reference genome is itself being observed or is instead the best recruit for reads that likely derive from a more divergent
group of phages (see Fig. 5 in [13]). Together we interpret these findings to suggest that this phage is ubiquitous phage in surface, coastal marine waters, but likely another member of the ‘rare virosphere’.

**Conclusions**

The *Pseudoalteromonas* phage B8b genome adds a new siphovirus genome for marine Gamma-proteobacteria phages. This phage shares many features with available marine siphoviruses and likely represents another member of a ubiquitous class of phages in the ‘rare virosphere’. Its cross-genera host range revealed infection across the genus level and hints of its genome structure suggest that phage B8b may have also a lysogenic lifestyle. Future experimental tests based on phageFISH [129] may allow us to dig into the temperate phage biology within this novel model system.

**Supporting Information**

S1 Fig. Genome size of phage B8b analyzed by Pulse Field Gel Electrophoresis (PFGE). (TIF)
S2 Fig. Phylogenetic relationships of the DNA polymerase across diverse bacteriophages. In green are represented the *Myoviridae* phages, in black the *Siphoviridae* and in blue the *Podoviridae*. Phage B8b is represented in red.
(TIF)

S3 Fig. Phylogenetic relationships of the phage large terminase across diverse bacteriophages. In green are represented the *Myoviridae* phages, in black the *Siphoviridae* and in blue the *Podoviridae*. Phage B8b is represented in red.
(TIF)

S4 Fig. Phylogenetic relationships of the phage portal protein across diverse bacteriophages. In green are represented the *Myoviridae* phages, in black the *Siphoviridae* and in blue the *Podoviridae*. Phage B8b is represented in red.
(TIF)

S1 Table. Bacterial hosts used to test the *Pseudoalteromonas* phage B8b phage host range. Bacterial strain from the phage was isolated is labeled in black. Bacterial strains infected by B8b siphovirus are labeled in red.
(DOCX)

S2 Table. Set of designed primers used in the PCR and direct sequencing in order to close the phage B8b genome.
(DOCX)

S3 Table. Phage DNA polymerase gene sequences used for phylogenetic analysis.
(DOCX)

S4 Table. Phage large terminase gene sequences used for phylogenetic analysis.
(DOCX)

S5 Table. Portal protein gene sequences used for phylogenetic analysis.
(DOCX)

Acknowledgments

We thank Bonnie Poulos, Cristina Howard and the Tucson Marine Phage Lab for support during E.Lara stay in Tucson, Arizona. We also thank Daniel J. Nasko for his help in genome assembly. EL was a recipient of the FPI-predoc fellowship from the Spanish Ministry of Science and Innovation (MICINN). SGA was supported by a Ramon y Cajal contract from MICINN and by Microb3 (FP7-OCEAN-2011). KH was supported by a postdoctoral fellowship from the Swedish Research Council (623–2010–6548). This work was supported by grants MICROVIS (CTM2007–62140) and MEFISTO (CTM2013–43767-P) to DV, from the MICINN and MINECO, MICRODIVERSITY (CGL2008–00762/BOS) and PANGENOMICS (CGL2011–26848/BOS) to SGA from the MICINN, and a Gordon and Betty Moore Foundation (grant #2631) to MBS.

Author Contributions

Conceived and designed the experiments: EL KH NS ELS MBS SGA. Performed the experiments: EL ELS KH. Analyzed the data: EL KH NS JCIE FMCC NCV. Contributed reagents/materials/analysis tools: DV MBS SGA. Wrote the paper: EL KH NS ELS FMCC DV MBS SGA.
References

1. Danovaro R, Corinaldesi C, Dell’Anno A, Fuhrman JA, Middelburg JJ, et al. (2011) Marine viruses and global climate change. FEMS Microbiol Rev 35: 993–1034. doi: 10.1111/j.1574-6976.2010.00298.x PMID: 21204862

2. Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399: 541–548. doi: 10.1038/21119 PMID: 10376593

3. Suttle CA (2007) Marine viruses—major players in the global ecosystem. Nat Rev Microbiol 5: 801–812. doi: 10.1038/nrmicro1750 PMID: 17853907

4. Suttle CA (2005) Viruses in the sea. Nature 437: 356–361. doi: 10.1038/nature04160 PMID: 16163346

5. Brussaard CPD, Wilhelm SW, Thingstad F, Weinbauer MG, Bratbak G, et al. (2008) Global-scale processes with a nanoscale drive: the role of marine viruses. ISME J 2: 575–581. doi: 10.1038/ismej.2008.31 PMID: 18385772

6. Breitbart M (2012) Marine Viruses: Truth or Dare. Ann Rev Mar Sci 4: 425–448. doi: 10.1146/annurev-marine-120709-142805 PMID: 22457982

7. Wommack KE, Colwell RR (2000) Virioplankton: Viruses in aquatic ecosystems. Microbiol Mol Biol Rev 64: 69–114. doi: 10.1128/MMBR.64.1.69-114.2000

8. Weinbauer MG (2004) Ecology of prokaryotic viruses. FEMS Microbiol Rev 28: 127–181. doi: 10.1016/j.femsre.2003.08.001 PMID: 15109783

9. Sullivan MB, Lindell D, Lee JA, Thompson LR, Bielawski JP, et al. (2006) Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. PLoS Biol 4: 1344–1357. doi: 10.1371/journal.pbio.0040234

10. Rohwer F, Thurber RV (2009) Viruses manipulate the marine environment. Nature 459: 207–212. doi: 10.1038/nature09060 PMID: 19444207

11. Edwards RA, Rohwer F (2005) Viral metagenomics. Nat Rev Microbiol 3: 504–510. doi: 10.1038/nrmicro1163 PMID: 15866693

12. Rappé MS, Giovannoni SJ (2003) The uncultured microbial majority. Ann Rev Microbiol 57: 369–394. doi: 10.1146/annurev.micro.57.030502.090759

13. Holmfeldt K, Solonenko N, Shah M, Corrier K, Riemann L, et al. (2013) Twelve previously unknown phage genera are ubiquitous in global oceans. Proc Nat Acad Sci 110: 12798–12803. doi: 10.1073/pnas.1305956110 PMID: 23858439

14. Seguritan V, Feng IW, Rohwer F, Swift M, Segall AM (2003) Genome sequences of two closely related Vibrio parahaemolyticus phages, VP16T and VP16C. J Bacteriol 185: 6434–6447. doi: 10.1128/JB.185.21.6434-6447.2003 PMID: 14563879

15. Steward GF (2001) Fingerprinting viral assemblages by pulsed field gel electrophoresis (PFGE). Method Microbiol 30: 85–103. doi: 10.1016/S0580-9517(01)30041-7

16. Steward GF, Montiel JL, Azam F (2000) Genome size distributions indicate variability and similarities among marine viral assemblages from diverse environments. Limnol Oceanogr 45: 1697–1706. doi: 10.4319/lo.2000.45.8.1697

17. Winget DM, Wommack KE (2008) Randomly amplified polymorphic DNA PCR as a tool for assessment of marine viral richness. Appl Environ Microbiol 74: 2612–2618. doi: 10.1128/AEM.02829-07 PMID: 18344351

18. Comeau AM, Short S, Suttle CA (2004) The use of degenerate-primed random amplification of polymorphic DNA (DP-RAPD) for strain-typing and inferring the genetic similarity among closely related viruses. J Virol Methods 118: 95–100. doi: 10.1016/j.jviromet.2004.01.020 PMID: 15081604

19. Comeau AM, Chan AM, Suttle CA (2006) Genetic richness of vibriophages isolated in a coastal environment. Environ Microbiol 8: 1164–1176. doi: 10.1111/j.1462-2920.2006.00106.x PMID: 16817925

20. Angly FE, Felts B, Breitbart M, Salamon P, Edwards RA, et al. (2006) The marine viromes of four oceanic regions. PLoS Biol 4: 2121–2131. doi: 10.1371/journal.pbio.0040368

21. Breitbart M, Salamon P, Andresen B, Mahaffy JM, Segall AM, et al. (2002) Genomic analysis of uncultured marine viral communities. Proc Nat Acad Sci 99: 14250–14255. doi: 10.1073/pnas.202488399 PMID: 12384570

22. Rohwer F (2003) Global phage diversity. Cell 113: 141–141. doi: 10.1016/S0092-8674(03)00276-9 PMID: 12705861

23. Rodriguez-Brito B, Li LL, Weygley L, Furlan M, Angly F, et al. (2010) Viral and microbial community dynamics in four aquatic environments. ISME J 4: 739–751. doi: 10.1038/ismej.2010.1 PMID: 20147985
24. Hurwitz B, Hallam S, Sullivan MB (2013) Metabolic reprogramming by viruses in the sunlit and dark ocean. Genome Biol 14: R123. doi: 10.1186/gb-2013-14-11-r123 PMID: 24200126

25. Hurwitz BL, Brum JR, Sullivan MB (2014) Depth-stratified functional and taxonomic niche specialization in the ‘core’ and ‘flexible’ Pacific Ocean Virome. ISME J. doi: 10.1038/ismej.2014.143 PMID: 25093636

26. Hurwitz BL, Sullivan MB (2013) The Pacific Ocean Virome (POV): A marine viral metagenomic dataset and associated protein clusters for quantitative viral ecology. PLoS One 8(2):e57355. doi: 10.1371/journal.pone.0057355 PMID: 23468974

27. Hurwitz BL, Westveld AH, Brum JR, Sullivan MB (2014) Modeling ecological drivers in marine viral communities using comparative metagenomics and network analyses. Proc Nat Acad Sci 111: 10714–10719. doi: 10.1073/pnas.1319778111 PMID: 25002514

28. Roux S, Hawley AK, Torres Beltran M, Scofield M, Schwientek P, et. al. (2014) Ecology and evolution of viruses infecting uncultivated SUP05 bacteria as revealed by single-cell- and meta- genomics. eLife. doi: 10.7554/eLife.03125 PMID: 25171894

29. Williamson SJ, Rusch DB, Yooseph S, Halpern AL, Heidelberg KB, et al. (2008) The Sorcerer II global ocean sampling expedition: Metagenomic characterization of viruses within aquatic microbial samples. PLoS One 3: e1456. doi:10.1371/journal.pone.0001456 PMID: 18213365

30. Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, et al. (2008) Functional metagenomic profiling of nine biomes. Nature 452: 629–632. doi:10.1038/nature06810 PMID: 18337718

31. Duhaime MB, Deng L, Poulos BT, Sullivan MB (2012) Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: a rigorous assessment and optimization of the linker amplification method. Environ Microbiol: 14: 2526–2537. doi: 10.1111/j.1462-2920.2012.02791.x

32. Duhaime MB, Sullivan MB (2012) Ocean viruses: rigorously evaluating the metagenomic sample-to-sequence pipeline. Virology 434: 181–186. doi: 10.1016/j.virology.2012.09.036 PMID: 23084423

33. John S, Mendez C, Deng L, Poulos B, Kauffman A, et al. (2011) A simple and efficient method for concentration of ocean viruses by chemical flocculation. Environ Microbiol Rep 3: 195–202. doi:10.1111/j.1758-2229.2010.00208.x PMID: 21572525

34. Solonenko SA, Ignacio-Espinoza JC, Alberti A, Cruaud C, Hallam S, et al. (2013) Sequencing platform and library preparation choices impact viral metagenomes. BMC Genomics 14: 320. doi:10.1186/1471-2164-14-320 PMID: 23663384

35. Solonenko SA, Sullivan MB (2013) Preparation of metagenomic libraries from naturally occurring marine viruses. Method Enzymol. Microbial Metagenomics, Metatranscriptomics and Metaproteomics edition 531: 143–165. doi:10.1016/B978-0-12-407863-5.00008-3

36. Hurwitz BL, Deng L, Poulos BT, Sullivan MB (2013) Evaluation of methods to concentrate and purify ocean virus communities through comparative, replicated metagenomics. Environ Microbiol 15: 1428–1440. doi: 10.1111/j.1462-2920.2012.02836.x PMID: 22845467

37. Brum JR, Schenck RO, Sullivan MB (2013) Global morphological analysis of marine viruses shows minimal regional variation and dominance of non-tailed viruses. ISME J 7: 1738–1751. doi:10.1038/ismej.2013.67 PMID: 2365867

38. Steward GF, Culley AI, Mueller JA, Wood-Charlson EM, Belcaid M, et al. (2013) Are we missing half of the viruses in the ocean? ISME J 7: 672–679. doi:10.1038/ismej.2012.121 PMID: 23151645

39. Labonté JM, Suttle CA (2013) Previously unknown and highly divergent ssDNA viruses populate the oceans. ISME J 7: 2169–2177. doi:10.1038/ismej.2013.110 PMID: 23842650

40. Hingamp P, Grimsley N, Acinas SG, Clerissi C, Subirana L, et al. (2013) Exploring nucleo-cytoplasmic large DNA viruses in Tara Oceans microbial metagenomes. The ISME J 7: 1678–1695. doi:10.1038/ismej.2013.59

41. Hopkins M, Kailasan S, Cohen A, Roux S, Tucker KP, et al. (2014) Diversity of environmental single-stranded DNA phages revealed by PCR amplification of the partial major capsid protein. ISME J 8: 2093–2103. doi: 10.1038/ismej.2014.43 PMID: 24694711

42. Paul JH, Sullivan MB (2005) Marine phage genomics: what have we learned? Curr Opin Biotech 16: 299–307. doi:10.1016/j.copbio.2005.03.007 PMID: 15961031

43. Zhao YL, Temperton B, Thrash JC, Schwabach MS, Vergin KL, et al. (2013) Abundant SAR11 viruses in the ocean. Nature 494: 357–360. doi:10.1038/nature11921 PMID: 23407494

44. Kang I, Oh H-M, Kang D, Cho J-C (2013) Genome of a SAR116 bacteriophage shows the prevalence of this phage type in the oceans. Proc Nat Acad Sci 110: 12343–12348. doi: 10.1073/pnas.1219930110 PMID: 23798439
45. Ruiz-González C, Gali M, Lefort T, Cardelús C, Simó R, et al. (2012) Annual variability in light modulation of bacterial heterotrophic activity in surface northwestern Mediterranean waters. Limnol Oceanogr 57: 1376–1388. doi: 10.4319/lo.2012.57.5.1376

46. Ferrera I, Gasol JM, Sebastián M, Hojerová E, Kobízeck M (2011) Comparison of growth rates of Aerobic Anoxicogenic Phototrophic bacteria and other bacterioplankton groups in coastal Mediterranean waters. Appl Environ Microbiol 77: 7451–7458. doi: 10.1128/AEM.00208-11 PMID: 21724878

47. Crespo BG, Pommier T, Fernández-Gómez B, Pedrós-Alió C (2013) Taxonomic composition of the particle-attached and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA. MicrobiologyOpen 2: 541–552. doi: 10.1002/mbo3.92 PMID: 23723056

48. Moebus K (1992) Further investigations on the concentration of marine bacteriophages in the water around Helgoland, with reference to the phase-host systems encountered. Helgoländer Meeresunters. 46: 275–292. doi: 10.1007/BF02367099

49. Duhaime MB, Wichels A, Waldmann J, Teeling H, Glockner FO (2011) Ecogeonomics and genome landscapes of marine Pseudoalteromonas phage H105/1. ISME J 5: 107–121. doi: 10.1038/ismej.2010.94 PMID: 20613791

50. Wichels A, Biel SS, Gelderblom HR, Brinkhoff T, Muyzer G, et al. (1998) Bacteriophage diversity in the North Sea. Appl Environ Microbiol 64: 4128–4133. PMID: 9797256

51. Wichels A, Gerds G, Schutt C (2002) Pseudoalteromonas spp. phages, a significant group of marine bacteriophages in the North Sea. Aquat Microb Ecol 27: 233–239. doi: 10.3354/ame027233

52. Thomas T, Evans FF, Schiebeck D, Mai-Prochnow A, Burke C, et al. (2008) Analysis of the Pseudoalteromonas tunicata Genome Reveals Properties of a Surface-Associated Life Style in the Marine Environment. PLoS One 3: e3252. doi: 10.1371/journal.pone.0003252 PMID: 18813346

53. Xie BB, Shu YL, Qin QL, Rong JC, Zhang XY, et al. (2012) Genome Sequences of Seven Species of the Marine Bacterium Pseudoalteromonas. J Bacteriol 194: 2746–2747. doi: 10.1128/JB.00265-12 PMID: 22535931

54. Männistö RH, Kivelä HM, Paulin L, Bamford DH, Bamford JKH (1999) The complete genome sequence of PM2, the first lipid-containing bacterial virus to be isolated. Virology 262: 355–363. doi: 10.1006/viro.1999.9837 PMID: 10502514

55. Hardies SC, Hwang YJ, Hwang YC, Jang GI, Cho BC (2013) Morphology, Physiological Characteristics and Complete Sequence of Marine Bacteriophage spRIO-1 Infecting Pseudoalteromonas marina. J Virol 87: 9189–9198. doi: 10.1128/JVI.01521-13 PMID: 23760254

56. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. 2nd ed, Vol 1. USA: Cold Spring Harbor Laboratory.

57. Sambrook J, Russell DW (2000) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory.

58. Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics Int 11: 36–42.

59. Weiss BD, Capage MA, Kessel M, Benson SA (1994) Isolation and characterization of a generalized transducing phage for Xanthomonas campestris pv. campestris. J Bacteriol 176: 3354–3359.

60. Gasol JM, Del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Sci Mar 64: 197–224.

61. Holmfield K, Howard-Varona C, Solonenko N, Sullivan MB (2014) Contrasting genomic patterns and infection strategies of two co-existing Bacteroidetes podovirus genera. Environ Microbiol 16:2501–2513. doi: 10.1111/1462-2920.12391 PMID: 24428166

62. Henn MR, Sullivan MB, Stange-Thomann N, Osburne MS, Berlin AM, et al. (2010) Analysis of High-Throughput Sequencing and Annotation Strategies for Phage Genomes. PLoS One 5: e9083. doi: 10.1371/journal.pone.0009083 PMID: 20140207

63. Sullivan MB, Huang KH, Iggo-Espinoza JC, Berlin AM, Kelly L, et al. (2010) Genomic analysis of oceanic cyanobacterial myoviruses compared with T4-like myoviruses from diverse hosts and environments. Environ Microbiol 12: 3035–3056. doi: 10.1111/j.1462-2920.2010.02280.x PMID: 20662890

64. Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18: 117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x

65. Besemer J, Borodovsky M (1999) Heuristic approach to deriving models for gene finding. Nucleic Acids Res 27: 3911–3920. doi: 10.1093/nar/27.19.3911 PMID: 10481031

66. Wisniewski JR, Zougman A, Mann M (2009) Combination of FASP and StageTip-based fractionation allows in-depth analysis of the hippocampal membrane proteome. J Proteome Res 8: 5674–5678. doi: 10.1021/pr900748n PMID: 19948406
67. Verberkmoes NC, Russell AL, Shah M, Godzik A, Rosenquist M, et al. (2008) Shotgun metaproteomics of the human distal gut microbiota. ISME J 3: 179–189. doi: 10.1038/ismej.2008.108 PMID: 18971961
68. Erickson AR, Cantarel BL, Lamendella R, Darzi Y, Mongodin EF, et al. (2012) Integrated Metagenomics/Metaproteomics Reveals Human Host-Microbiota Signatures of Crohn’s Disease. PLoS ONE 7: e49138. doi: 10.1371/journal.pone.0049138 PMID: 23209564
69. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948. doi: 10.1093/bioinformatics/btm404 PMID: 17846036
70. Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci 8: 275–282. PMID: 1633570
71. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol 28: 2731–2739. doi: 10.1093/molbev/msr121 PMID: 21546353
72. Baudoux AC, Hendrix RW, Lander GC, Bailly X, Podell S, et al. (2012) Genomic and functional analysis of two coliphages. Appl Environ Microbiol 69: 5364–5371. doi: 10.1128/AEM.02504-10 PMID: 21257810
73. Kivelä HM, Männistö RH, Kalkkinen N, Bamford DH (1999) Purification and protein composition of PM2, the first lipid-containing bacterial virus to be isolated. Virology 262: 364–374. doi: 10.1006/viro.1999.9838 PMID: 10502515
74. Baudoux AC, Hendrix RW, Lander GC, Bailly X, Podell S, et al. (2012) Genomic and functional analysis of Vibrio phage SIO-2 reveals novel insights into ecology and evolution of marine siphoviruses. Environ Microbiol 14: 2071–2086. doi: 10.1111/j.1462-2920.2011.02685.x PMID: 22225728
75. Suttle CA, Chan AM (1993) Marine cyanophages infecting oceanic and coastal strains of Synechococcus abundance, morphology, cross-infectivity and growth-characteristics. Mar Ecol Prog Ser 92: 99–109. doi: 10.3354/meps092099
76. Hadas H, Einav M, Fishov I, Zaitisky A (1997) Bacteriophage T4 development depends on the physiology of its host Escherichia coli. Microbiology 143: 179–185. doi: 10.1099/0221287-143-1-179 PMID: 9025929
77. Jensen EC, Schrader HS, Rieland B, Thompson TL, Lee KW, et al. (1998) Prevalence of broad-host-range lytic bacteriophages of Synechococcus elongatus, Escherichia coli, and Pseudomonas aeruginosa. Appl Environ Microbiol 64: 575–580. PMID: 9464396
78. Sullivan MB, Waterbury JB, Chisholm SW (2003) Cyanophages infecting the oceanic cyanobacterium Prochlorococcus. Nature 424: 1047–1051. doi: 10.1038/nature01929 PMID: 12944965
79. Holmfeldt K, Middelboe M, Nybroe O, Riemann L (1999) Large Variabilities in host strain susceptibility and phage host range govern interactions between lytic marine phages and their Flavobactetium hosts. Appl Environ Microbiol 73: 6730–6739. doi: 10.1128/AEM.73.11.6730-6739.1999 PMID: 10776444
80. Goodridge L, Gallaccio A, Griffiths MW (2003) Morphological, host range, and genetic characterization of two coliphages. Appl Environ Microbiol 69: 5364–5371. doi: 10.1128/AEM.69.9.5364-5371.2003 PMID: 12957924
81. Matsuzaki S, Tanaka S, Koga T, Kawata T (1992) A broad host-range vibriophage, KVP40, isolated from sea water. Microbiol Immunol 36: 93–97. doi: 10.1111/j.1348-0427.1992.tb01645.x PMID: 1584076
82. Chang HW, Kim KH (2011) Comparative genomic analysis of bacteriophage EP23 infecting Shigella sonnei and Escherichia coli. J Microbiol 49: 927–934. doi: 10.1007/s12275-011-1577-0 PMID: 22203555
83. Kim M, Ryu S (2011) Characterization of a T5-Like Coliphage, SPC35, and Differential Development of Resistance to SPC35 in Salmonella enterica Serovar Typhimurium and Escherichia coli. Appl Environ Microbiol 77: 2042–2050. doi: 10.1128/AEM.02504-10 PMID: 21257810
84. Jiang SC, Paul JH (1998) Gene transfer by transduction in the marine environment. Appl Environ Microbiol 64: 2780–2787. PMID: 9687430
85. Rao VB, Feiss M (2008) The Bacteriophage DNA Packaging Motor. Ann rev Genet 42: 647–681. doi: 10.1146/annurev.genet.42.110807.091545 PMID: 18687038
86. Lohr JE, Chen F, Hill RT (2005) Characterization of a T5-Like Coliphage, Phi HSIC, a Pseudotemperate marine phage of Listonella pelagia. Appl Environ Microbiol 71: 3311–3320. doi: 10.1128/AEM.71.6.3311-3320.2005 PMID: 15933034
89. Sullivan M, Krastins B, Hughes J, Kelly L, Chase M, et al. (2009) The genome and structural proteome of an ocean siphovirus: a new window into the cyanobacterial ‘mobilome’. Environ Microbiol 11: 2935–2951. doi: 10.1111/j.1462-2930.2009.01810.x PMID: 19841000

90. Angly F, Youle M, Nosrat B, Srinagesh S, Rodriguez-Brito B, et al. (2009) Genomic analysis of multiple Roseophage SJ101 strains. Environ Microbiol 11: 2863–2873. doi: 10.1111/j.1462-2930.2009.01813.x PMID: 19659499

91. Chen F, Suttle CA (1996) Evolutionary relationships among large double-stranded DNA viruses that infect microalgae and other organisms as inferred from DNA polymerase genes. Virology 219: 170–178. doi: 10.1006/viro.1996.0234 PMID: 8623526

92. Yasuike M, Sugaya E, Nakamura Y, Shigenobu Y, Kawato Y, et al. (2013) Complete genome sequence of a novel myovirus which infects atypical strains of Edwardsiella tarda. Genome annouc 1:00248–12. doi: 10.1128/genomeA.00248-12

93. Cui Z, Shen W, Wang Z, Zhang H, Me R, et al. (2012) Complete Genome Sequence of Klebsiella pneumoniae Phage JD001. J Virol 86: 13843–13843. doi: 10.1128/JVI.02435-12 PMID: 23166250

94. Comeau AM, Tremblay D, Moineau S, Rattei T, Kushkina AI, et al. (2012) Phage morphology recapitulates phylogeny: The comparative genomics of a new group of myoviruses. PLoS One 7: e40102. doi: 10.1371/journal.pone.0040102 PMID: 22792219

95. Fileé J, Forterre P, Sen-Lin T, Laurent J (2002) Evolution of DNA polymerase families: Evidences for multiple gene exchange between cellular and viral proteins. J Mol Evol 54: 763–773. doi: 10.1007/s00239-001-0078-x PMID: 12029358

96. Casjens SR (2005) Comparative genomics and evolution of the tailed-bacteriophages. Curr Opin Virol 3:566 doi: 10.1016/j.coviro.2004.08.021 PMID: 15518819

97. Serwer P, Hayes SJ, Zaman S, Lieman K, Rolando M, et al. (2004) Improved isolation of under-sampled bacteriophages: finding of distant terminase genes. Virology 329: 412–424. doi: 10.1016/j.viro.2004.08.021 PMID: 15158819

98. Huang S, Wang K, Jiao N, Chen F (2012) Genome sequences of siphoviruses infecting marine Synechococcus unveil a diverse cyanophage group and extensive phage-host genetic exchanges. Environ Microbiol 14: 540–558. doi: 10.1111/j.1462-2930.2011.02667.x PMID: 22188618

99. Catalano CE, Cue D, Feiss M (1995) Virus DNA packaging: the strategy used by phage lambda. Mol Microbiol 16: 1075–1086. doi: 10.1111/j.1365-2958.1995.tb02333.x PMID: 8577244

100. Garcia P, Monjardin C, Martin R, Madera C, Soberon N, et al. (2008) Isolation of new Stenotrophomonas bacteriophages and genomic characterization of temperate phage S1. Appl Environ Microbiol 74: 7552–7560. doi: 10.1128/AEM.01709-08 PMID: 18952876

101. Tapia P, Flores FM, Covarrubias PC, Acuna LG, Holmes DS, et al. (2012) Complete genome sequence of temperate bacteriophage AcaML1 from the extreme acidophile Acidithiobacillus caldus ATCC 51756. J Virol 86: 12452–12453. doi: 10.1128/JVI.02261-12 PMID: 23087115

102. Mobberley JM, Authement RN, Segall AM, Paul JH (2008) The temperate marine phage Phi HAP-1 of Halomonas aquamarina possesses a linear plasmid-like prophage genome. J Virol 82: 6618–6630. doi: 10.1128/JVI.00140-08 PMID: 18448537

103. Lan SF, Huang CH, Chang CH, Liao WC, Lin IH, et al. (2009) Characterization of a new plasmid-like prophage in a pandemic Vibrio parahaemolyticus O3:K6 Strain. Appl Environ Microbiol 75: 2659–2667. doi: 10.1128/AEM.02483-08 PMID: 19286788

104. Hendrix RW, Smith MCM, Burns RN, Ford ME, Hatfull GF (1999) Evolutionary relationships among diverse bacteriophages and prophages: All the world’s a phage. Proc Nat Acad Sci 96: 2192–2197. doi: 10.1073/pnas.96.5.2192 PMID: 10051617

105. Ignacio-Espinoza JC, Solonenko SA, Sullivan MB (2013) The global virome: not as big as we thought? Curr Opin Virol 3:566–571. doi: 10.1016/j.coiviro.2013.07.004 PMID: 23962729

106. Ignacio-Espinoza JC, Sullivan MB (2012) Phylogenomics of T4 cyanophages: lateral gene transfer in the ‘core’ and origins of host genes. Environ Microbiol 14: 2113–2126. doi: 10.1111/j.1462-2930.2012.02704.x PMID: 22348436

107. Deng L, Ignacio-Espinoza JC, Gregory AC, Poulos BT, Weitz JS, et al. (2014) Viral tagging reveals discrete populations in Synechococcus viral genome sequence space. Nature 513: 242–245. doi: 10.1038/nature13459 PMID: 25043051

108. Kogoma T (1996) Recombination by replication. Cell 85: 625–627. doi: 10.1016/S0092-8674(96)81229-5 PMID: 8646771

109. Haber JE (1999) DNA repair—Gatekeepers of recombination. Nature 398: 665–6. doi: 10.1038/19423 PMID: 10227286

110. Hall SD, Kolodner RD (1994) Homologous pairing and strand exchange promoted by the Escherichia coli RecT protein. Proc Nat Acad Sci 91: 3205–3209. doi: 10.1073/pnas.91.8.3205 PMID: 8159725
111. Zhang YM, Buchholz F, Muyrers JPP, Stewart AF (1998) A new logic for DNA engineering using recombination in Escherichia coli. Nature Genet 20: 123–128. doi: 10.1038/2417 PMID: 9771703

112. Kurochkina LP, Semenyuk PI, Orlov VN, Robben J, Sykilinda NN, et al. (2012) Expression and functional characterization of the first bacteriophage-encoded chaperonin. J Virol 86: 10103–10111. doi: 10.1128/JVI.00940-12 PMID: 22787217

113. Georgopoulos C, Hendrix RW, Casjens SR, Kaiser AD (1973) Host participation in bacteriophage λ head assembly. J Mol Biol 76: 45–60. doi: 10.1016/0022-2836(73)90080-6 PMID: 4578100

114. Keppel F, Rychner M, Georgopoulos C (2002) Bacteriophage-encoded cochaperonins can substitute for Escherichia coli’s essential GroES protein. EMBO Rep 3: 893–898. doi: 10.1093/embo-reports/kvf176 PMID: 12189177

115. St-Pierre F, Endy D (2008) Determination of cell fate selection during phage lambda infection. Proc Nat Acad Sci 105: 20705–20710. doi: 10.1073/pnas.080831105 PMID: 19098103

116. Joh R, Weitz JS (2011) To Lyse or Not to Lyse: To Lyse or Not to Lyse: Transient-Mediated Stochastic Fate Determination in Cells Infected by Bacteriophages. PLoS Comput Biol 7: e1002006. doi: 10.1371/journal.pcbi.1002006 PMID: 21423715

117. Kourilsk P (1973) Lysogenization by bacteriophage lambda. I. Multiple infection and lysogenic response. Mol Gen Genet 122: 183–195. doi: 10.1007/BF00435190

118. Stewart FM, Levin BR (1984) The population biology of bacterial viruses: why be temperate? Theor Popul Biol 26: 93–117. doi: 10.10160040-5809(84)90026-1 PMID: 6484871

119. Weinbauer MG, Brettar I, Hofle MG (2003) Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. Limnol Oceanogr 48: 1457–1465. doi: 10.4319/lo.2003.48.4.1457

120. Boras JA, Sala MM, Vázquez-Domínguez E, Weinbauer MG, Vaqué D (2009) Annual changes of bacterial mortality due to viruses and protists in an oligotrophic coastal environment (NW Mediterranean). Environ Microbiol 11: 1181–1193. doi: 10.1111/j.1462-2920.2008.01849.x PMID: 19207563

121. Brüssow H, Canchaya C, Hardt WD (2004) Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol Mol Biol Rev 68: 560–602. doi: 10.1128/MMBR.68.3.560-602.2004

122. Paul JH (2008) Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? ISME J 2: 579–589. doi: 10.1038/ismej.2008.35 PMID: 18521076

123. Mann N, Cook A, Millard A, Bailey S, Clokie M (2003) Marine ecosystems: Bacterial photosynthesis genes in a virus. Nature 424: 741–741. doi: 10.1038/424741a PMID: 12917674

124. Sharon I, Battchikova N, Aro E, Giglione C, Meinnel T, et al. (2011) Comparative metagenomics of microbial traits within oceanic viral communities. ISME J 5: 1178–1190. doi: 10.1038/ismej.2011.2 PMID: 21307954

125. Böhringer M, Schaper L, Suttle C, Sullivan M (2007) Exploring the vast diversity of marine viruses. Oceanography 20: 135–139. doi: 10.5670/oceanog.2007.58

126. Sullivan MB, Coleman ML, Weigele P, Rohwer F, Chisholm SW (2005) Three Prochlorococcus cyanophage genomes: signature features and ecological interpretations. PLoS Biol 3: e144. doi: 10.1371/journal.pbio.0030144 PMID: 15928858

127. Desiere F, McShan WM, van Sinderen D, Ferretti JJ, Brüssow H (2001) Comparative genomics reveals close genetic relationships between phages from dairy bacteria and pathogenic streptococci: Evolutionary implications for prophage-host interactions. Virolology 286: 325–341. doi: 10.1006/viro.2001.1085 PMID: 11601904

128. Weitz JS, Poisot T, Meyer JR, Flores CO, Valverde S, et al. (2013) Phage-bacteria infection networks. Trends Microbiol 21: 82–91. doi: 10.1016/j.tim.2012.11.003 PMID: 23245704

129. Allers E, Moraru C, Duhairme MB, Beneze E, Solonenko N, et al. (2013) Single-cell and population level viral infection dynamics revealed by phageFISH, a method to visualize intracellular and free viruses. Environ Microbiol 15: 2306–2318. doi: 10.1111/1462-2920.12100 PMID: 23489642