A new family of “megaphages” abundant in the marine environment

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Megaphages, bacteriophages harbouring extremely large genomes, have recently been found to be ubiquitous, being described from a variety of microbiomes ranging from the animal gut to soil and freshwater systems. However, no complete marine megaphage has been identified to date. Here, using both short and long read sequencing, we assembled >900 high-quality draft viral genomes from water in the English Channel. One of these genomes included a novel megaphage, Mar_Mega_1 at >650 Kb, making it one of the largest phage genomes assembled to date. Utilising phylogenetic and network approaches, we found this phage represents a new family of megaphages. Genomic analysis showed Mar_Mega_1 shares relatively few homologues with its closest relatives, but, as with other megaphages Mar_Mega_1 contained a variety of auxiliary metabolic genes responsible for carbon metabolism and nucleotide biosynthesis, including a NADP-dependent isocitrate dehydrogenase [ldh] and nicotinamide-nucleotide amidohydrolase [PncC], which have not previously been identified in megaphages. Mar_Mega_1 was abundant in a marine virome sample and related phages are widely prevalent in the oceans.

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each phage suggesting these phages do not form a single family based on current definitions [8]. Phages LR756502 and LR745206 share nearly twice as many genes with each other (30.6–31.5%) than either phage does with Mar_Mega_1 (14.4–17.5%). This suggests Mar_Mega_1 represents a new family of phages in the megaphage size range based on current standards [8] (Fig. S2).

Having established Mar_Mega_1 as the first representative of a new family, we sought to establish its distribution in the marine environment. Although Mar_Mega_1 was present only in the samples taken from Plymouth Sound (Table S1), we have estimated that it is as abundant as cultivated phages that infect marine bacteria such as *Pelagibacter* and *Synechococcus* (e.g. *Synechococcus* phage S-SKS1, *Pelagibacter* phage HTVC115P and Lentibacter phage vB_LenP_ICBM2) through read mapping (Fig. 2). However, the abundance of Mar_Mega_1 might be underestimated as the majority of virions larger than 0.22 μm should have been removed during the filtration step. As no megaphages have been cultured to date their virion size remains unknown, but it is probable they have larger capsids. Using the TerL sequence from Mar_Mega_1 to query the TARA contigs via BlastP, phylogenetic analysis revealed a further nine phages that are sister to the group containing Mar_Mega_1, suggesting closely related phages are present in the marine environment. Moreover, CheckV analysis of the genome fragments supports the hypothesis they are fragments of much larger phages (Table S4). Furthermore, using contigs on which the TerL homologues were identified with a read mapping approach the prevalence of Mar_Mega_1-like phages in TARA and GOV2.0 viromes was investigated (Fig. 2B, Table S5) [9, 10]. Despite collectively several thousands of reads mapping to Mar_Mega_1, no single sample passed the accepted threshold of >1x coverage across 70% of the genome [11]. In contrast, we found contigs carrying TerL homologues to be widely distributed across the 162 marine stations (Fig. 2B, Table S5). Thus, whilst a marine origin of this megaphage family is likely, because of the abundance of Mar_Mega_1 only in our Plymouth Sound sample we cannot rule out a freshwater source for this phage given the close proximity of this site to a river estuary.

Several methods were tested to determine a putative host for Mar_Mega_1 (see Supplementary Methods). However, no host could be predicted with a high degree of certainty. Genomic analysis of Mar_Mega_1 identified several proteins detected in other megaphages including phage structural proteins and phage replication proteins (Table S2). Unlike sporadically identified megaphages no CRISPR-cas system was identified [3]. However, a range of auxiliary metabolic genes (AMGs) were detected, homologues of which have not yet been identified in other megaphages (Table S2). These included putative nicotinamide-nucleotide amidohydrolase (PncC), NADP-dependent isocitrate
dehydrogenase [Idh] and patatin-like phospholipase (PLP) [12] enzymes. In addition, Mar_Mega_1 possessed a putative TonB-dependent receptor (SusC) which was also present in LR745206, as well as AMGs encoding putative dihydrofolate reductase, phosphoesterase and peptidase enzymes which were also found in megaphages LR745206, LR756501, LR756502, LR756503 and LR756504 (Table S2).

The presence of AMGs potentially involved in carbon metabolism in Mar_Mega_1 is consistent with previous research indicating the prevalence of AMGs responsible for carbohydrate and amino acid uptake and metabolism in model marine phage systems and viral metagenomes [13]. For example, the TonB-dependent receptor SusC might be responsible for increasing carbohydrate uptake during infection [14], whereas the NADP-dependent isocitrate dehydrogenase [Idh], an AMG which was previously detected in marine viromes [15] carries out the oxidative decarboxylation of isocitrate to α-ketoglutarate (αKG). αKG is a rate-determining intermediate in the tricarboxylic acid cycle and crucial for both cellular energy metabolism and as a source of glutamate and glutamine. As such, it is a central regulator affecting numerous metabolic pathways through its role in bridging carbon and nitrogen metabolism, as well as being a key signalling molecule of cellular nutrient status [16]. Thus this enzyme potentially plays an important role during the infection process. Furthermore, AMGs responsible for pyridine nucleotide synthesis such as nicotinamide-nucleotide amidohydrolase (PncC) whilst new to megaphages, have previously been found in other phages such as Vibrio phage KVP40 which encodes its own NAD⁺ salvage pathway [17]. Moreover, the Mar_Mega_1 phage encoded dihydrofolate reductase could act two-fold by increasing the host’s capacity to convert dihydrofolate into tetrahydrofolate which is essential for purine nucleotide biosynthesis or, due to similarity with a putative dfrA3 antibiotic resistance gene, confer protection against diaminopyrimidine antibiotics, which are one of the most common antibiotic pollutants in marine environments [18]. This is the first time a patatin-like phospholipase (PLP) was identified within a phage genome. Although the function of PLPs is currently not clear, a role in bacterial pathogen–eukaryotic host interactions was suggested [19]. We have since been able to identify a homologue of PLP in other phages (acc: LR745206), suggesting that megaphages might increase the virulence of their putative bacterial hosts.

CONCLUSIONS
We identified the largest marine megaphage to date. Using phylogenetic and genomic analyses it is distinctly related to megaphages found in other environments. Analysis of marine viromes suggests Mar_Mega_1-like phages are abundant and widely distributed in the marine environment.

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AUTHOR CONTRIBUTIONS
SM, AM and DJS conceived the work. WHW, SM and AM collected material and data. SM, AM, BR and RC analysed the data. AM, DJS and MJ supervised the project. SM, DJS and AM drafted the manuscript. All authors contributed to revising and approval of the submitted work.

COMPETING INTERESTS
The authors declare no competing interests.

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