Novel Abundant Oceanic Viruses of Uncultured Marine Group II Euryarchaeota Identified by Genome-Centric Metagenomics

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Abstract

Marine Group II Euryarchaeota (MGII) are among the most abundant microbes in the oceanic surface waters. So far, however, representatives of MGII have not been cultivated, and no viruses infecting these organisms have been described. Here we present complete genomes for 3 distinct groups of viruses assembled from metagenomic sequence datasets highly enriched for MGII. These novel viruses, which we denote Magroviruses, possess double-stranded DNA genomes of 65 to 100 kilobase in size that encode a structural module characteristic of head-tailed viruses and, unusually for archaeal and bacterial viruses, a nearly complete replication apparatus of apparent archaeal origin. The newly identified Magroviruses are widespread and abundant, and therefore are likely to be major ecological agents.

Euryarchaeal Marine group II (MGII) members are highly abundant in the

30   photic zone of oligotrophic oceans (*1-3*). This group has been reported to show

seasonal variation (*4*) and to comprise up to 90% of the total archaea and one third

of total microbial cells during spring blooms in the Atlantic Ocean (*5*). Metagenomic

studies have demonstrated higher abundance of MGII in surface waters, mixed layer

and halocline in the Arctic Ocean (*6, 7*), the Gulf of Mexico (*8*) and the Pacific Ocean

35   (*9*). The MGII has been divided into two distinct sub-lineages, II.a and II.b (*10*).

Metatranscriptomic analyses shows that the MGII archaea are among the most

transcriptionally active microbial groups in the coastal Pacific Ocean, with

transcription levels and patterns similar to those of *Pelagibacter ubique* and SAR86

(*11, 12*).

40   Despite their high abundance and transcription activity, to date, not a single

representative of MGII has been cultured. Nevertheless, Iverson et al. (2012) (*13*)

were able to assemble a complete MG II.a genome from metagenomic sequences.

The analysis of this genome suggests that group MG II.a are photoheterotrophic

(using proteorhodopsins) and motile (*14*). A representative genome from MG II.b

45   (*Thalassoarcahea*) has been recently assembled from fosmid libraries derived from

Deep Chlorophyll Maximum samples in the Mediterranean (*15*). Metagenomic

fragment recruitment analysis indicates that MG II.a are more abundant in costal and

estuarine habitats whereas MG II.b are more abundant in oligotrophic ocean habitats

(*15*).

50   To gain further insight into the diel activity of MGII archaea in the Red Sea, we

examined metagenomic bins containing MGII contigs. These bins were constructed

from contigs retrieved from a cross assembly of microbial, viral and transcriptomic

samples collected at four time points during a single day in the Gulf of Aqaba in the

Red-Sea (ENA project PRJEB19060). Close manual inspection of these sequences

55 showed that one bin (169) contained, along with contigs of MGII, a Metagenome-

Assembled Genome (MAG) (156409) carrying hallmark viral genes including

predicted Major Capsid Protein (MCP), Portal protein, and large subunit of the

Terminase, as well as DNA Polymerase of the B family (DNAP). This contig was

found to contain overlapping terminal regions suggesting that it represents a

60 complete, terminally redundant viral genome (Supplementary Fig. S1).

Euryarchaeota comprise a large, diverse archaeal phylum that, in addition to

MGII, includes thermophiles, halophiles and methanogens (*16, 17*). Viruses have

been previously isolated from members of different euryarchaeal groups. The

morphologies of euryarchaeal dsDNA viruses are diverse and include spindle–

65 shaped, unclassified icosahedral, pleomorphic and head-tailed viruses. The latter

group resembles the bacterial head-tailed phages (order *Caudovirales*), both in

morphology and genome organization, and is currently classified into the families

Myoviridae, *Podoviridae* and *Siphoviridae*, each of which contains bacterial and

archaeal viruses (*18-20*). Head-tailed archaeal viruses so far have been found to

70 infect only Euryarchaea, including mainly halophiles and methanogens (*21-23*). No

viruses infecting MGII have been reported so far (*18*), and despite the increasing

amount of viral metagenomic data released in recent years (*24, 25*), candidate viral

contigs from the MGII group have not been reported either.

The protein sequence of the predicted MCP encoded in MAG 156409 showed

75 significant, albeit moderate (33% identity), similarity solely to the MCPs of

haloarchaeal siphoviruses (haloviruses) (Fig. 1A). Three additional proteins encoded

in MAG 156409, namely, primase (Fig. 1C), portal protein and prohead protease

(Supplementary Fig. S2 and S3), showed comparable levels of similarity to

homologs from haloviruses, indicating a relatively distant evolutionary link. We used

80 these protein sequences as queries in BLASTP searches against our entire Red Sea

assembly dataset, in an attempt to expand the repertoire of virus-related sequences

for subsequent, comprehensive comparative genomic analysis. This search resulted

in additional 9 MAGs (Supplementary Table 1), all showing the same pattern of
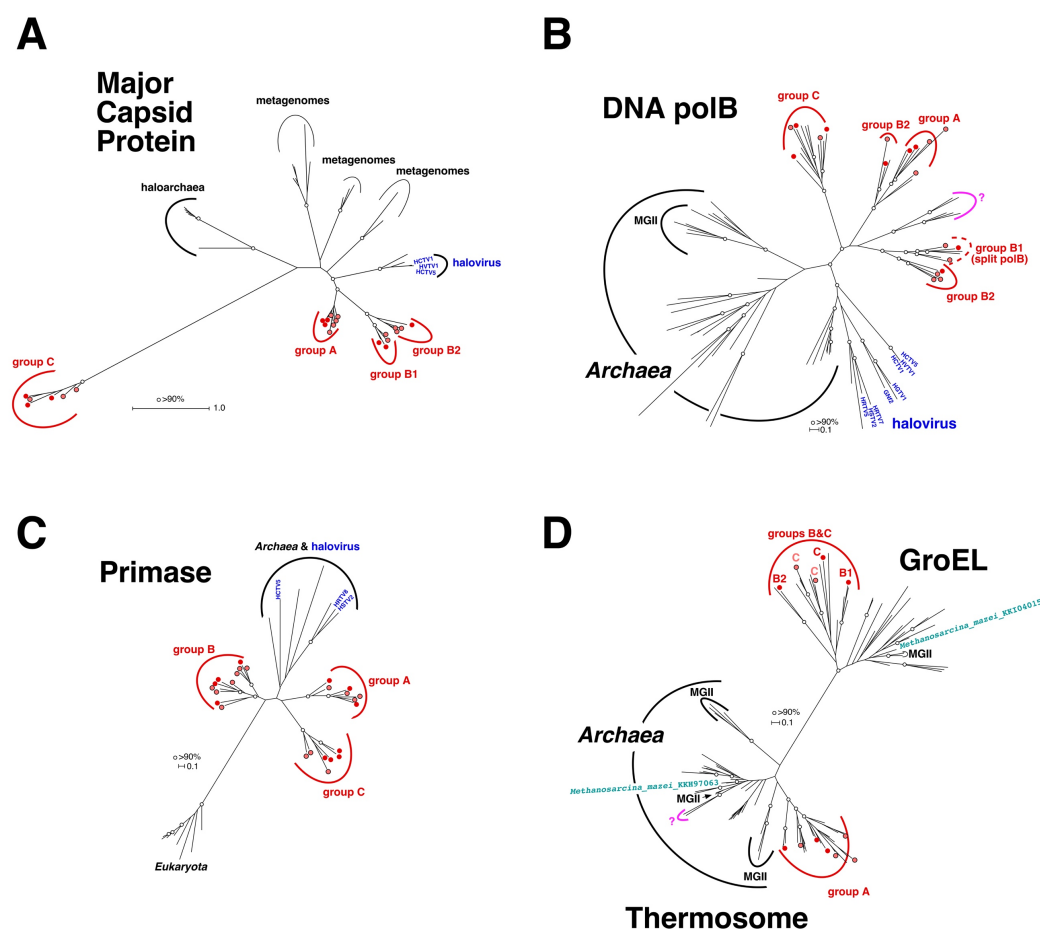
homology to haloviruses for all tested genes.

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Figure 1: Unrooted Maximum likelihood phylogenetic trees of conserved Magrovirus genes.
(**A**) Major Capsid Protein (MCP); (**B**) DNA Polymerase B (DNAP); (**C**) archaeo-eukaryotic Primase
(AEP); (**D**) Chaperonins (thermosome subunit and GroEL). Metagenome-assembled complete or
90 nearly complete genomes (MAGs) from the Red Sea and *Tara* Oceans metagenomes are marked
with red and light red circles, respectively. Bootstrap support values greater than 90 are marked with
white circles.

To validate and expand our observations on Red Sea metagenomes, we used

95   the same query sequences in a BLAST search against both the original *Tara* Oceans

assembly datasets (*26, 27*) and a reassembly of the raw data from this project

(Sharon, in prep. see methods). This *Tara* Oceans search yielded 15 additional

putative viral genomes related to the Red Sea MAGs (Supplementary Table 1). All

together, we identified 26 putative viral MAGs that were assembled from two

100   independent metagenomic projects (Red Sea and *Tara* Oceans).

In the phylogenetic tree of the euryarchaeal virus and provirus MCPs, and

their environmental homologs (Fig. 1A), the MAG proteins split into 3 distinct groups,

two of which (A, B) join in a clade affiliated with the halovirus MCPs, whereas the

third group (C) forms a long branch with an uncertain affiliation. A similar

105   phylogenetic pattern was observed for other hallmark caudoviral genes of the MAGs,

namely prohead protease (Supplementary Fig. S3), portal protein (Supplementary

Fig. S2), and large subunit of the terminase (Supplementary Fig. S4). Whereas

groups A and B cluster together in all these phylogenetic trees, the position of group

C changed from tree to tree, suggesting rapid evolution. These findings, along with

110   the fact that 11 MAGs are terminally redundant linear genomes of about 100 kbp in

size (Supplementary Table 1, Supplementary Fig S1), suggest that these MAGs

represent a novel family of head-tailed archaeal viruses. Because, as we show

below, these MAGs appear to be strongly associated with MGII, we provisionally

denote them Magroviruses (MArine GROup II viruses).

115   Having tentatively delineated 3 distinct Magrovirus groups from the hallmark

gene phylogenies, we compared their gene complements and genome organizations

in detail. Although, as with many other archaeal viruses (*28*), most of the Magrovirus

genes encode proteins without significant similarity to any sequences in current

databases, the genomes include two readily identifiable, compact gene blocks, the

120 structural-morphogenetic module and the replicative module (Figure 2A). The

structural module consists of the genes encoding major capsid protein, portal

protein, prohead protease, terminase, and closely resembles the corresponding

genomic module of haloviruses (Figure 2B). The distinctive feature of Magroviruses

is the presence of a suit of genes for proteins involved in the genome replication

125 (Figure 2B). All 26 genomes encode DNAP, sliding clamp, clamp loader ATPase

(replication factor C), archaeo-eukaryotic primase (AEP), replicative helicase (MCM

protein), RadA-like ATPase, single-stranded DNA-binding protein (ssb), and several

nucleases; all viruses except for Group C also encode one or two ATP-dependent

DNA ligases (Figure 2B). Taken together, these proteins could comprise a nearly

130 complete archaeal-type replisome (*29*), which is unprecedented among currently

known viruses of archaea and bacteria. In particular, haloviruses that share the

morphogenetic gene block with the Magroviruses encompass a smaller complement

of replicative genes (Fig. 2 and Supplementary Fig. S5). The gene order within both

the replicative and the structural modules of Magroviruses is highly conserved, with

135 limited rearrangements, largely in group C (Figure 2B), possibly, because operonic

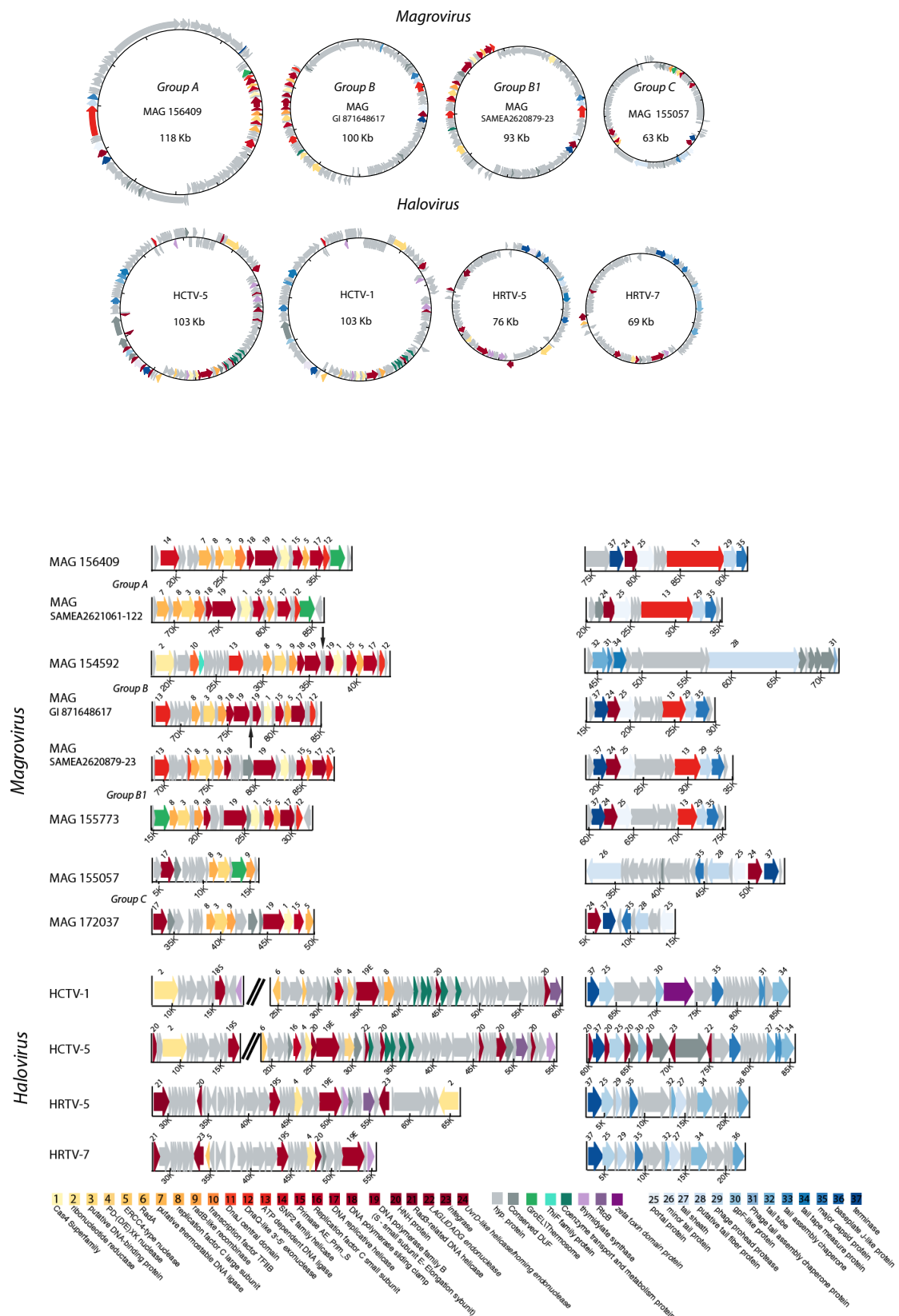organization of functionally linked genes is important for virus reproduction.

Figure 2: Genome organization in different groups of Magroviruses and haloviruses. For each group, the overall genome organization is shown (**A**) followed by detailed schemes (**B**) of the replicative gene block (left, yellow to red) and the structural gene block (right, different shades of blue). Homologous genes with predicted functions are shown using color code (see key in the bottom). Green arrows indicate thermosome genes and gray arrows indicate hypothetical proteins. Split DNAP genes from group B1 are marked with a narrow black arrow pointing to the regions between the split genes. See Supplementary Fig. S5 for greater details.

With the exception of the DNAP, AEP and ligases, the replicative proteins of Magroviruses show low sequence similarity to their homologs from cellular organisms that could be detected only through sensitive profile-profile searches. Nevertheless, most of these proteins show signs of archaeal origin as indicated by the provenance of the closest homologs (Supplementary File S1). Due to the low sequence conservation, informative phylogenetic analysis was feasible only for DNAP and AEP. In the phylogenetic tree of DNAPs, the Magrovirus polymerases cluster with the halovirus DNAPs (*23, 30*), and together, these viral polymerases are related to archaeal DNApolB III that is involved in lagging strand replication (Figure 1B). This gene is apparently subject to frequent horizontal transfer among archaea, so that its phylogeny does not follow the archaeal evolutionary tree. It appears likely that the transfers of the PolB III genes were at least partially mediated by viruses (*31*). Groups A and C that were delineated by analysis of morphogenetic genes retain monophyly in the DNAP tree (Figure 1B). In contrast, group B splits between two branches one of which is affiliated with group A (Figure 1B). This clear discrepancy between the MCP and DNAP phylogenies suggests genetic exchange among diverse Magroviruses. In addition to groups A, B, and C, a putative new group (labeled "?" in Fig. 1B & D) was observed in the DNAP tree. The MAGs encoding this group of DNAPs (tentatively, Group D) are not represented in the Red Sea metagenomes but are present among scaffolds originating from the *Tara*

Oceans project. Group D genomes contain genes for DNAP (Fig. 1B), thermosome

170 (Fig. 1C), resolvase, and DNA-dependent RNA poymerase A', all with the highest

similarity to archaeal homologs (Supplementary File S2). So far no closed genomes

of this group were assembled, and a morphogenetic gene block was not identified.

Therefore, although a connection to Magroviruses is apparent, the true nature of

group D (virus, provirus, mobile element, megaplasmid, or uncultured archaea)

175 remains to be determined once complete genomes become available.

Notably, 7 group B Magroviruses possess a split DNAP gene whereas in all

other viral MAGs the DNAP gene is uninterrupted (Fig 2B and Supplementary

Figuers S5 and S6). The split in the group B DNAP gene is located within the

sequence encoding the catalytic domain, similarly to the DNAP genes of the

180 cyanophage P-SSP7 (*32, 33*) and *Methanobacterium thermoautotrophicum*. In the

latter case, it has been directly shown that the two portions of the split DNAP are

expressed and interact to form the active polymerase (*34*). Interrupted DNAP genes

are also found in other Euryarchaeota in which the inserts consist of post-

transcriptionally excised inteins (*35-37*). The positions of the split in these archaeal

185 polymerase and the group B Margovirus DNAP are similar but not identical,

suggesting independent, convergent evolutionary events resulting in gene

fragmentation. Regardless of the exact evolutionary scenario, the split DNAP is so

far unique among archaeal viruses and supports the monophyly of Magrovirus Group

B.

190 The phylogenetic tree of the AEP supports the monophyly of all 3 groups of

Magroviruses as well as the common origin of primases in Magroviruses and

haloviruses; in this case, however, Magrovirus groups A and C cluster with

haloviruses, suggesting the possibility of gene exchange between these archaeal viruses (Fig. 1C).

195   Except for Group C, all Magroviruses encode a DnaQ-like exonuclease that can be implicated in proofreading during viral genome replication. Unlike most of the other Magrovirus genes, this nuclease shows significant sequence similarity only to bacterial homologs (Supplementary Fig. S6). Thus, somewhat unexpectedly, Magroviruses appear to have acquired genes not only from archaea but also from

200   bacteria.

A notable feature of Magroviruses is the presence of two genes encoding distinct ATP-dependent DNA ligases genes in groups A and B, one located within the replicative module and the other one, unexpectedly, embedded in the structural module (Figure 2B). The provenances of these ligases are quite different as

205   indicated by phylogenetic analysis: the ligase encoded within the structural module represents a distinct family that along with the Magrovirus proteins includes ligases from uncharacterized bacteria; the ligase in the replicative block of Group B is a typical archaeal variety, whereas the one in Group A replicative block belongs to the distinct family known as 'thermostable ligases' (Supplementary Fig. S5). Thus,

210   apparently, ligases have been acquired by Magroviruses on 3 independent occasions.

The structural modules of all Magroviruses also contain another inserted gene that in different groups encodes distinct nucleases (Figure 2B). In groups A, B, and D, this is a homing endonuclease (LAGLI-DADG family) homologous to nucleases of

215   mobile elements, such as Group I self-splicing introns and inteins, which are also present in many bacteriophages. In contrast, in Group C, the inserted gene encodes a homolog of the exonuclease subunit of the archaeal DNA polymerase D (*38*). An

intriguing possibility is that the two nucleases play analogous roles in Magroviruses replication. In addition to the conserved replicative genes, several genes implicated

220 in replication are found in individual groups of Magroviruses, *e.g.* ribonucleotide reductase in Group B and SNF2 family helicase in Group A.

Apart from the replicative and structural-morphogenetic proteins, 12 of the 26 Magroviruses encode either a bacterial-type chaperonin GroEL (Groups B, B1 and C) or a thermosome subunit, the archaeal homolog of GroEL in Groups A and D

225 (Fig. 2B). Unlike the replicative genes, these Magrovirus proteins are highly similar to the archaeal and bacterial homologs. Phylogenetic analysis confirmed the sharp split between GroEL and thermosome subunits (Fig. 1D). The thermosome subunits of Magroviruses group with homologs from MGII, which is compatible with relatively recent acquisition of these genes by the viruses. A subset of MGII archaea encode

230 GroEL instead of the thermosome subunit, conceivably owing to displacement of the ancestral archaeal chaperonin by a bacterial homolog. Although the Magrovirus GroEL do not group with those of MGII in the phylogenetic tree, this could be due to the accelerated evolution in the viruses; acquisition of this gene from MGII archaea remains a likely scenario. Comparison of the topology of the chaperonin tree with

235 those of the MCP, DNAP and AEP trees suggests that the common ancestor of the Magroviruses acquired a GroEL gene that was replaced by the thermosome subunit in Group A. Chaperonins are not encoded by any known archaeal viruses but have been detected in several bacteriophages. Notably, these phages do not encode the co-chaperonin GroES, and GroES is not required for the phage chaperonin activity

240 (*39*). This is likely to be the case for the Magrovirus chaperonins as well. Given that, in Magroviruses, the chaperonin genes reside in the replicative gene cluster, it

seems plausible that the chaperonins facilitate folding of the replicative proteins and replisome assembly.

Mapping reads from the Red Sea and from the *Tara* Oceans on the 26

245 Magrovirus genomes indicate that these viruses are globally widespread, with higher abundance in the Indian Ocean, the Red Sea and the South Pacific and Atlantic Oceans (Fig 3B). Furthermore, Magroviruses are highly abundant in the marine environment, third only to SAR11 phages and cyanophages (*40*). Surprisingly, the overall abundance of Magroviruses was found to be higher than that of SAR116

250 phages, previously reported to be the second most abundant phage group in the oceans (*41*) (Fig. 3A and Supplementary Fig. S9).
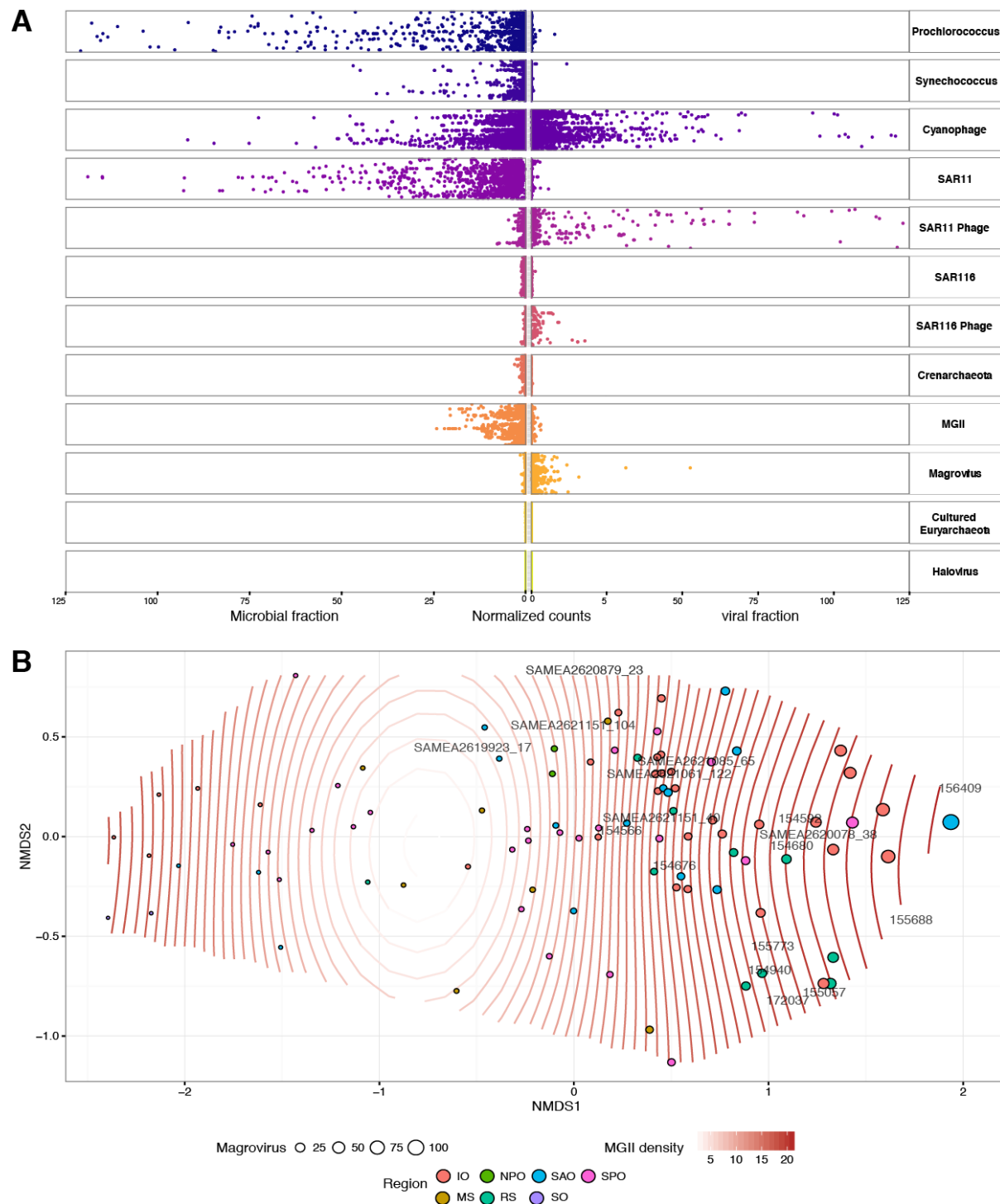
Figure 3: A. The global spread of Magroviruses. The abundance of the Magrovirus reads in Red-Sea samples and all *Tara* Oceans microbiome (*27*) and virome (*24*) is shown along with the abundances of the putative host MGII; other major archaeal groups; Cyanobacteria *Synechococcus* and *Prochlorococcus* and their phages; SAR11 bacteria and their phages; SAR116 and their phages. Horizontal axis: the normalized count (genome fragments per kilobase reference sequence per million library reads (GFPM), see methods.); vertical axis: the sampling stations. **B. NMDS Plot of sampling**

sites Magrovirus abundance. circle diameter indicates Magrovirus abundance at a specific site. MGII abundance was fitted using *Ordisurf* function in Vegan package in R (*44*) and is represented by white to red contour lines. A linear response between the Magrovirus abundance ordination and the MGII abundance variable is represented by fitted contours that are equally spaced parallel lines perpendicular to the MGII abundance vector (R-sq.(adj) = 0.432 Deviance explained = 48%, p-value = 4.82e-10). Additional NMDS plots with smooth fitted surface for Cyanobacteria, Euryarachaeota and are shown in Supplementary Fig. 11. Region abbreviations: IO – Indian Ocean; NAO – North Atlantic Ocean; RS – Red Sea; SO – Southern Ocean; MS – Mediterranean Sea; NPO – North Pacific Ocean; SAO – South Atlantic Ocean; SPO – South Pacific Ocean.

So far, based on microscopic measurements (*30*), head-tailed viruses appeared to be the least abundant morphotype of archaeal viruses in the environment. However, our abundance estimates for Magroviruses suggest that these previously uncharacterized head-tailed viruses dominate the archaeal virome in surface marine environments. In contrast to the cyanophages, and to a lesser extent the SAR11 phages, the normalized counts of Magroviruses are almost negligible in the microbial fraction (larger than 0.2µm) of the samples but high in the virus-enriched fraction (<0.2 µm) (Fig. 3A). Thus, the principal source of the Magrovirus reads appear to be free virus particles rather than cell-associated viruses, proviruses or megaplasmids (Supplementary Fig. S8).

Despite the high abundance of MGII in the oceans and their apparent ecological importance, no viruses infecting these organisms have been identified so far. Here, using metagenomic approaches, we describe 3 distinct groups of viruses associated with MGII. Unequivocal demonstration of the virus-host relationship between magroviruses and MGII is currently unfeasible due to the lack of cultivable MGII representatives. Nevertheless, several lines of evidence strongly suggest that MGII archaea are indeed the hosts of Magroviruses. First, our abundance estimates show that MGII is the dominant archaeal group in the samples from which the Magrovirus genomes were assembled. Moreover, our Red-Sea assembly dataset

290   consisted almost exclusively of MGII and Magrovirus contigs (Fig 3A). Second, most

of the replicative genes of Magroviruses and especially the viral chaperonins show

clear signs of archaeal provenance, and in some cases, a specific connection with

homologs from MGII. Furthermore, Group B Magroviruses encode two tRNAs

(tRNA^{Leu} and tRNA^{Arg}; Supplementary Fig. S10) that are most similar to the

295   respective tRNAs of MGII archaea.

       The discovery of the Magroviruses is part of the growing trend in virology

whereby viruses are recognized solely from metagenomics sequence analysis, a

practice that has been recently formalized by the International Committee for

Taxonomy of Viruses (*42*). Genome analysis of the Magroviruses is consistent with

300   modular evolution of viruses whereby the structural and replicative modules have

distinct origins. Magroviruses are unusual among viruses of bacteria and archaea in

that they encode an elaborate, apparently (almost) self-sufficient replication

apparatus. The closest analogy are the large and giant viruses of eukaryotes in the

proposed order "Megavirales" that replicate in the cytoplasm of eukaryotic cells (*43*).

305   Given the high abundance of both MGII archaea and Magroviruses, the latter are

likely to be important ecological agents, similar to cyanophages and members of the

"Megavirales" that infect abundant unicellular algae.

ACKNOWLEDGMENTS

References

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1. R. Massana, A. E. Murray, C. M. Preston, E. D. DeLong, Vertical distribution and phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara channel. *Appl Environ Microbiol* **63**, 50-56 (1997).
2. R. Massana, E. F. DeLong, C. Pedrós-Alió, A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. *Appl Environ Microbiol* **66**, 1777-1787. (2000).
3. G. J. Herndl *et al.*, Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. *Appl Environ Microbiol* **71**, 2303-2309 (2005).
4. A. E. Murray *et al.*, A timeseries assessment of planktonic archaeal variability in the Santa Barbara Channel. *Aquat. Microb. Ecol.* **20**, 129-145 (1999).
5. A. Pernthaler, C. M. Preston, J. Pernthaler, E. F. DeLong, R. Amann, Comparison of fluorescently labeled oligonucleotide and polynucleotide probes for the detection of pelagic marine bacteria and archaea. *Appl Environ Microbiol* **68**, 661-667. (2002).
6. N. Bano, S. Ruffin, B. Ransom, J. T. Hollibaugh, Phylogenetic composition of Arctic Ocean Archaeal assemblages and comparison with Antarctic assemblages. *Appl Environ Microbiol* **70**, 781-789 (2004).
7. P. E. Galand, E. O. Casamayor, D. L. Kirchman, M. Potvin, C. Lovejoy, Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing. *ISME J* **3**, 860-869 (2009).
8. B. Liu *et al.*, Community structure of archaea in the water column above gas hydrates in the Gulf of Mexico. *Geomicrobiol J* **26**, 363-369 (2009).
9. S. A. Lincoln *et al.*, Planktonic Euryarchaeota are a significant source of archaeal tetraether lipids in the ocean. *Proc Natl Acad Sci U S A* **111**, 9858-9863 (2014).
10. P. E. Galand, C. Gutiérrez-Provecho, R. Massana, J. M. Gasol, E. O. Casamayor, Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay Microbial Observatory). *Limnol Oceanogr* **55**, 2117-2125 (2010).
11. E. A. Ottesen *et al.*, Pattern and synchrony of gene expression among sympatric marine microbial populations. *Proc Natl Acad Sci U S A* **110**, E488-497 (2013).
12. F. O. Aylward *et al.*, Microbial community transcriptional networks are conserved in three domains at ocean basin scales. *Proc Natl Acad Sci U S A* **112**, 5443-5448 (2015).
13. V. Iverson *et al.*, Untangling genomes from metagenomes: revealing an uncultured class of marine Euryarchaeota. *Science* **335**, 587-590 (2012).
14. W. D. Orsi *et al.*, Ecophysiology of uncultivated marine euryarchaea is linked to particulate organic matter. *ISME J* **9**, 1747-1763 (2015).
15. A.-B. Martin-Cuadrado *et al.*, A new class of marine Euryarchaeota group II from the Mediterranean deep chlorophyll maximum. *ISME J* **9**, 1619-1634 (2015).
16. R. Cavicchioli, Archaea--timeline of the third domain. *Nature reviews* **9**, 51-61 (2011).
17. C. L. Zhang, W. Xie, A.-B. Martin-Cuadrado, F. Rodriguez-Valera, Marine Group II Archaea, potentially important players in the global ocean carbon cycle. *Front Microbiol* **6**, 1108 (2015).
18. D. Prangishvili, The wonderful world of archaeal viruses. *Annual review of microbiology* **67**, 565-585 (2013).

19. M. Krupovic, D. Prangishvili, R. W. Hendrix, D. H. Bamford, Genomics of bacterial and archaeal viruses: dynamics within the prokaryotic virosphere. *Microbiol Mol Biol Rev* **75**, 610-635 (2011).

20. K. Porter, B. E. Russ, M. L. Dyall-Smith, Virus-host interactions in salt lakes. *Current opinion in microbiology* **10**, 418-424 (2007).

21. M. Pina, A. Bize, P. Forterre, D. Prangishvili, The archeoviruses. *FEMS Microbiol Rev* **35**, 1035-1054 (2011).

22. N. S. Atanasova, E. Roine, A. Oren, D. H. Bamford, H. M. Oksanen, Global network of specific virus-host interactions in hypersaline environments. *Environ Microbiol* **14**, 426-440 (2012).

23. M. K. Pietilä *et al.*, Insights into head-tailed viruses infecting extremely halophilic archaea. *Journal of virology* **87**, 3248-3260 (2013).

24. J. Brum *et al.*, Patterns and ecological drivers of ocean viral communities. *Science* **348**, 1261498 (2015).

25. D. Paez-Espino *et al.*, Uncovering Earth's Virome. *Nature* **536**, 425-430 (2016).

26. S. Pesant *et al.*, Open science resources for the discovery and analysis of Tara Oceans data. *Scientific data* **2**, 150023 (2015).

27. S. Sunagawa *et al.*, Structure and function of the global ocean microbiome. *Science* **348**, 1261359 (2015).

28. J. Iranzo, E. V. Koonin, D. Prangishvili, M. Krupovic, Bipartite Network Analysis of the Archaeal Virosphere: Evolutionary Connections between Viruses and Capsidless Mobile Elements. *Journal of virology* **90**, 11043-11055 (2016).

29. K. S. Makarova, E. V. Koonin, Archaeology of eukaryotic DNA replication. *Cold Spring Harb Perspect Biol* **5**, a012963 (2013).

30. A. W. Luk, T. J. Williams, S. Erdmann, R. T. Papke, R. Cavicchioli, Viruses of haloarchaea. *Life (Basel)* **4**, 681-715 (2014).

31. K. S. Makarova, M. Krupovic, E. V. Koonin, Evolution of replicative DNA polymerases in archaea and their contributions to the eukaryotic replication machinery. *Front Microbiol* **5**, 354 (2014).

32. M. B. Sullivan, M. L. Coleman, P. Weigele, F. Rohwer, S. W. Chisholm, Three *Prochlorococcus* cyanophage genomes: Signature features and ecological interpretations. *PLoS Biol* **3**, e144 (2005).

33. G. Sabehi, D. Lindell, The P-SSP7 cyanophage has a linear genome with direct terminal repeats. *PLoS ONE* **7**, e36710 (2012).

34. Z. Kelman, S. Pietrokovski, J. Hurwitz, Isolation and characterization of a split B-type DNA polymerase from the archaeon *Methanobacterium thermoautotrophicum* deltaH. *The Journal of biological chemistry* **274**, 28751-28761 (1999).

35. D. R. Smith *et al.*, Complete genome sequence of *Methanobacterium thermoautotrophicum* deltaH: functional analysis and comparative genomics. *Journal of bacteriology* **179**, 7135-7155 (1997).

36. H. P. Klenk *et al.*, The complete genome sequence of the hyperthermophilic, sulphate- reducing archaeon *Archaeoglobus fulgidus*. *Nature* **390**, 364-370 (1997).

37. Y. Kawarabayasi *et al.*, Complete sequence and gene organization of the genome of a hyper- thermophilic archaebacterium, *Pyrococcus horikoshii* OT3. *DNA Res* **5**, 55-76 (1998).

38. M. Jokela, A. Eskelinen, H. Pospiech, J. Rouvinen, J. E. Syvaoja, Characterization of the 3' exonuclease subunit DP1 of *Methanococcus jannaschii* replicative DNA polymerase D. *Nucleic acids research* **32**, 2430-2440 (2004).

39. P. I. Semenyuk, V. N. Orlov, O. S. Sokolova, L. P. Kurochkina, New GroEL-like chaperonin of bacteriophage OBP *Pseudomonas fluorescens* suppresses thermal protein aggregation in an ATP-dependent manner. *Biochem J* **473**, 2383-2393 (2016).

40. Y. Zhao *et al.*, Abundant SAR11 viruses in the ocean. *Nature* **494**, 357-360 (2013).

41. I. Kang, H.-M. Oh, D. Kang, J.-C. Cho, Genome of a SAR116 bacteriophage shows the prevalence of this phage type in the oceans. *Proc Natl Acad Sci U S A* **110**, 12343-12348 (2013).

42. P. Simmonds *et al.*, Consensus statement: Virus taxonomy in the age of metagenomics. *Nature reviews* **advance online publication**, (2017).

43. P. Colson *et al.*, "Megavirales", a proposed new order for eukaryotic nucleocytoplasmic large DNA viruses. *Arch Virol* **158**, 2517-2521 (2013).

44. J. Oksanen *et al.*, vegan: Community Ecology Package. R package version 2.4-0. https://CRAN.R-project.org/package=vegan. 2016.