

## Supplementary Materials for

### **Virus-host coexistence in phytoplankton through the genomic lens**

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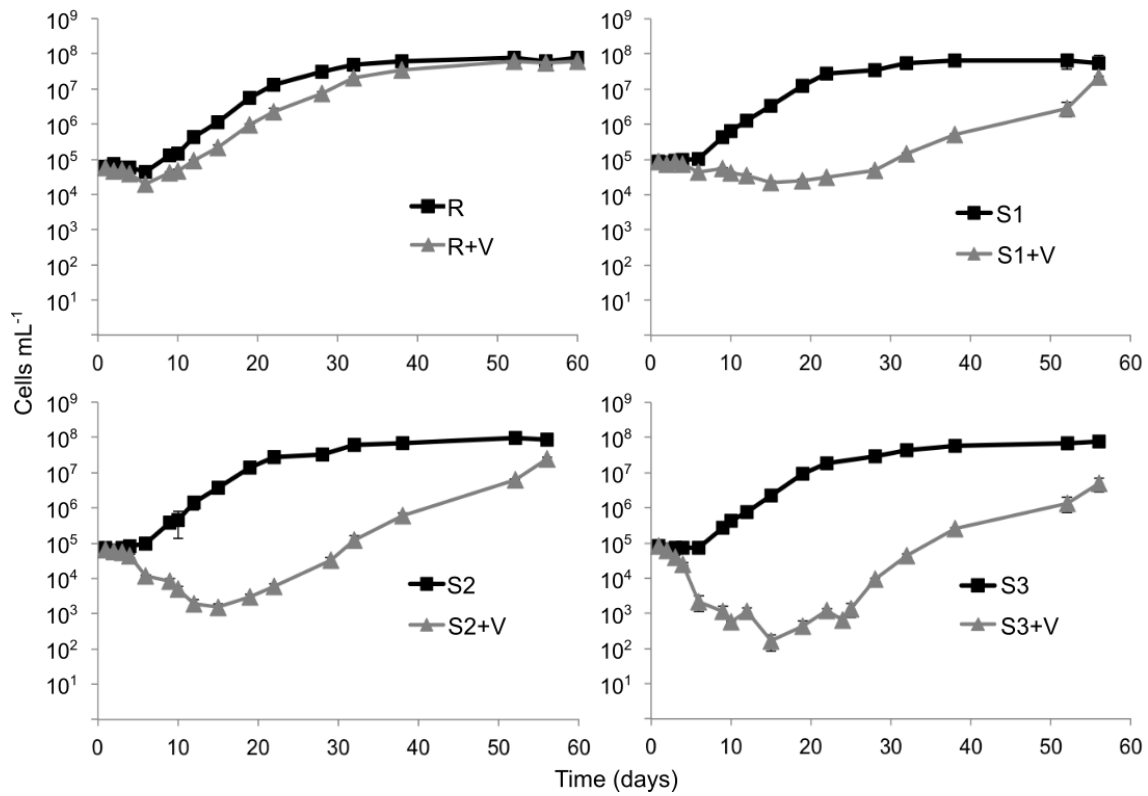
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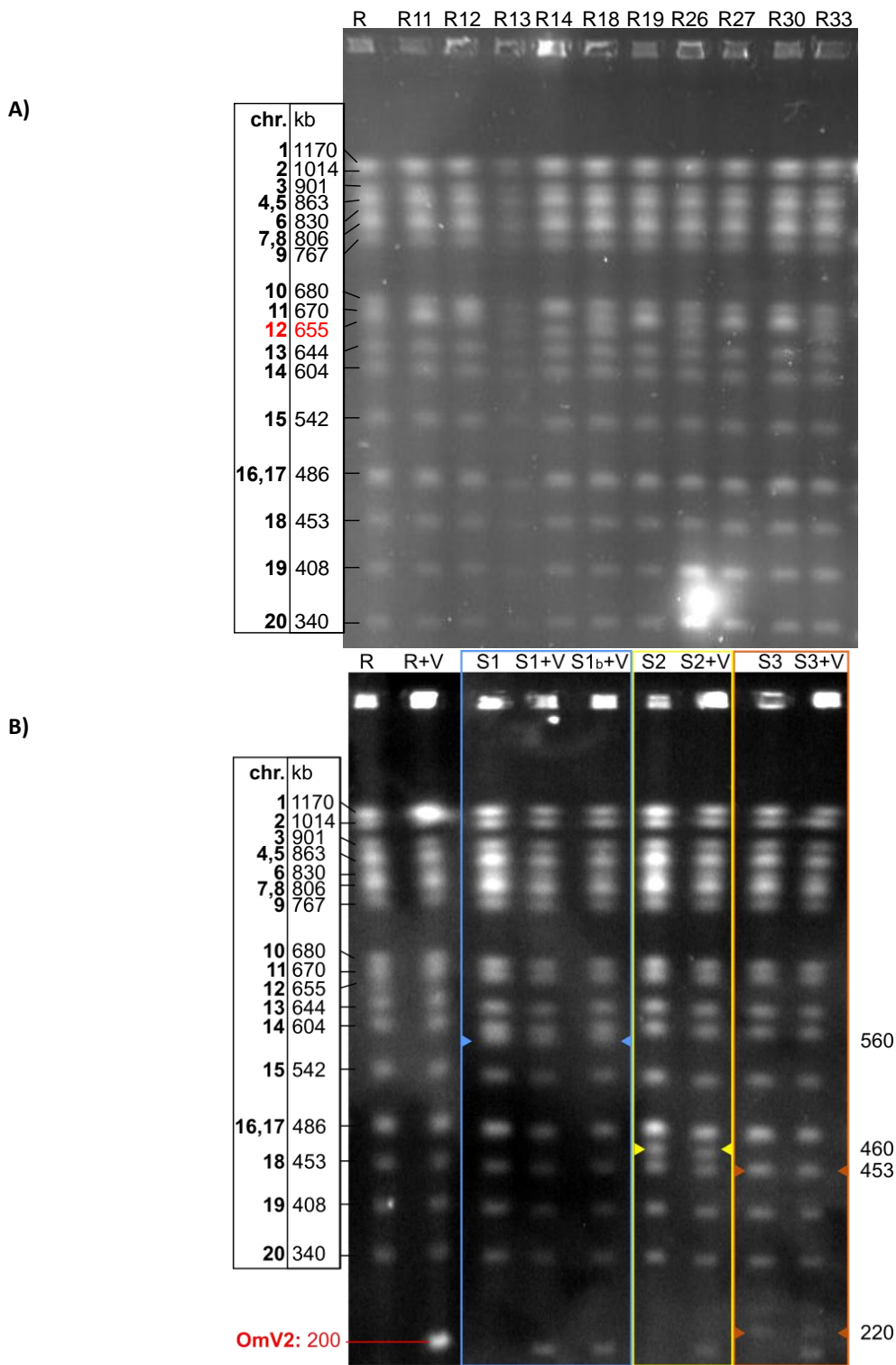
DOI: [10.1126/sciadv.aay2587](https://doi.org/10.1126/sciadv.aay2587)

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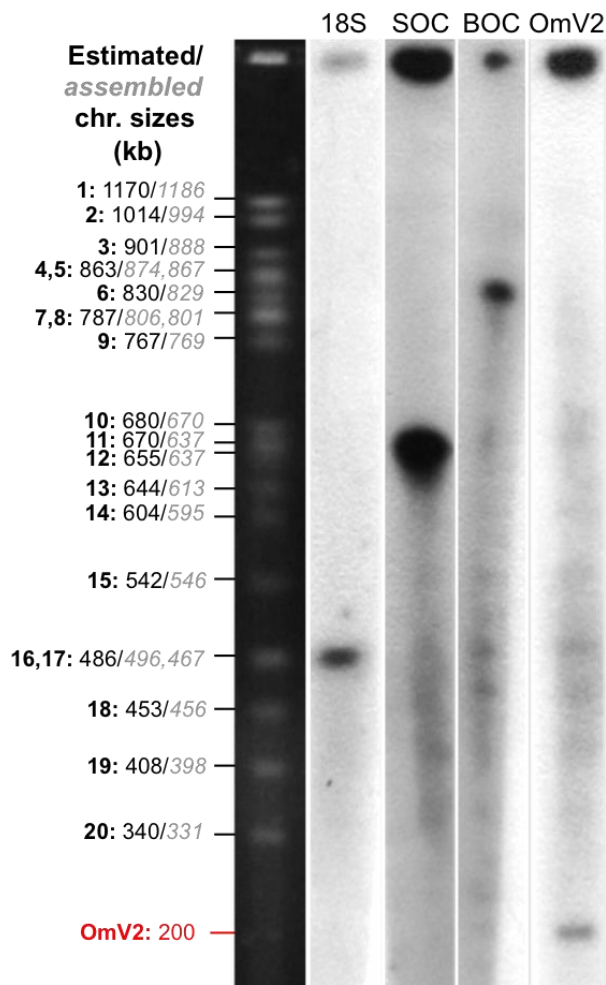
Figs. S1 to S4  
Text S1  
Tables S1 to S4  
References



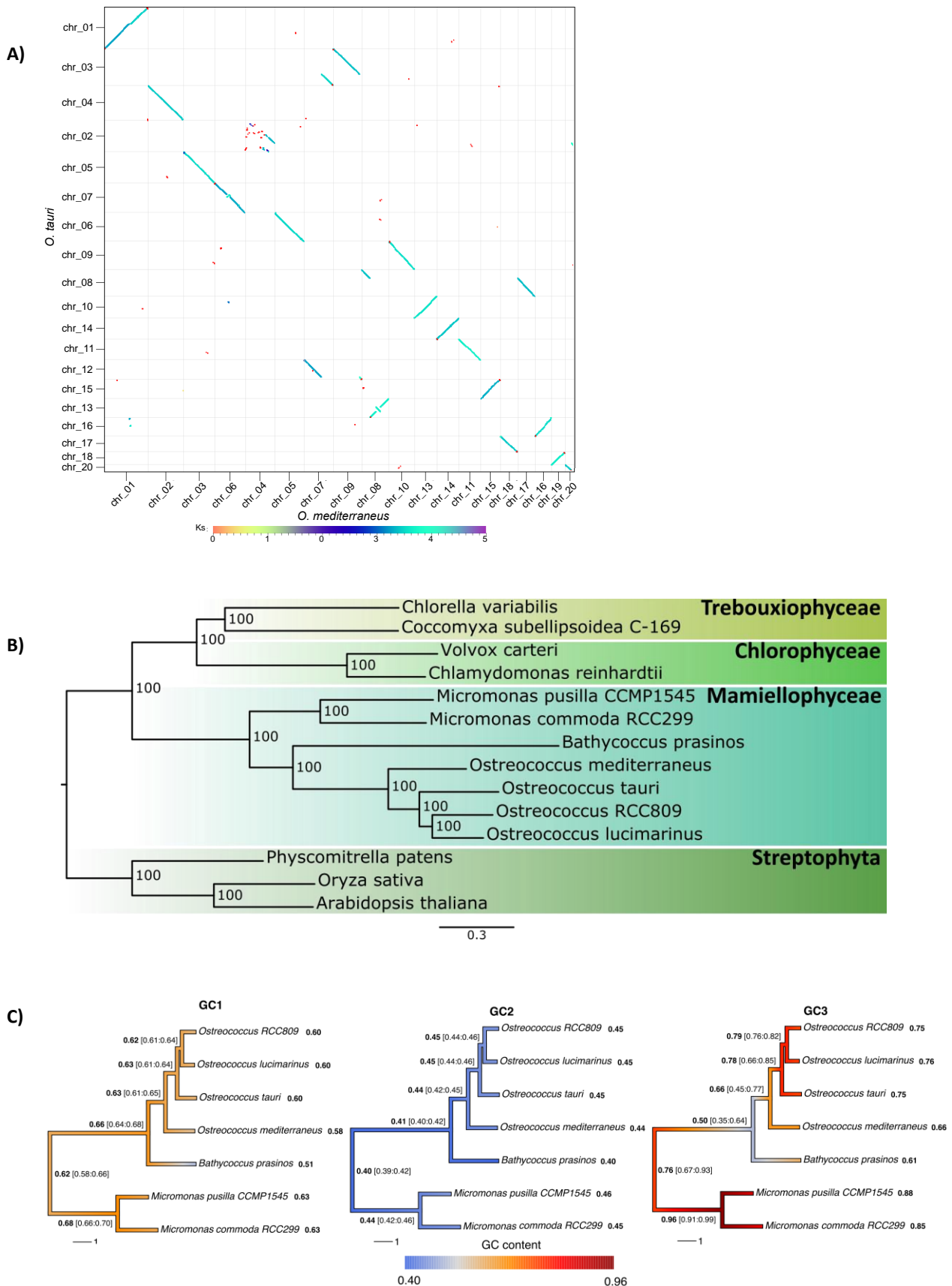
**Supplementary Figure S1. Growth curves of uninfected control and virus infected *O. mediterraneus* culture lines.** Cell concentrations are shown in the ordinate and time on the abscissa. Black squares – uninfected controls, Grey triangles – OmV2 infected. Error bars show the standard deviation between three replicate culture flasks. R is a virus-free OmV2 resistant line, S1, S2 and S3 are OmV2-susceptible lines.



**Supplementary Figure S2. (A) PFGE of the chromosomes of *O. mediterraneus* RCC2590 OmV2-resistant lines.** R is the parental line from which the numbered R lines were cloned. Chromosome 12 (red) is the SOC of the parental line which shows minor variation in size among the R lines. The estimated chromosome number and sizes are inferred from band intensity and mobility, respectively. The white spot in the lane of R26 between chromosomes 19 and 20 is an artefact from ethidium bromide staining. **(B) PFGE comparing the chromosome sizes of OmV-2 susceptible *O. mediterraneus* lines after addition of OmV2 virus and development of resistance to viral lysis.** Cultures were from the growth curves shown in Supplementary Figure 1. +V indicates OmV2 had been added to the culture and it had regrown. R is the OmV2-resistant parental line. OmV2-susceptible culture lines = S1, S2 and S3. S1b is a biological replicate of S1 and so only the culture with added virus is shown. The estimated chromosome number and sizes are shown for the control R line on the left. The estimated prasinovirus OmV2 size is shown in red. S1, S2 and S3 lines are boxed in blue, yellow and orange, respectively. The bands corresponding to the SOC in the S lines are flanked by triangles of the same color and their estimated sizes shown on the right.



**Supplementary Figure S3. PFGE of the chromosomes of *O. mediterraneus* RCC2590.** The estimated chromosome number and sizes are inferred from band intensity and mobility, respectively, and chromosome sizes from the assembled genome are indicated left black background. Hybridizations to the PFGE with radioactive probes specific to the 18S rRNA gene, SOC, BOC and OmV2 regions are indicated on the right in a white background. Estimated prasinovirus OmV2 size is shown in bold red. The topmost band present in all lanes is non-specific hybridization to unmigrated DNA remaining in the loading wells. Probes were generated by PCR using primer pairs listed in Supplementary Table 3.



**Supplementary Figure S4. (A) Colinearity between *O. mediterraneus* RCC2590 (x-axis) and *O. tauri* RCC4221 (y-axis) chromosomes.** Note: The small outlier chromosomes (chromosome 19 of *O. tauri* and chromosome 12 of *O. mediterraneus*) do not share colinearity and are not shown.

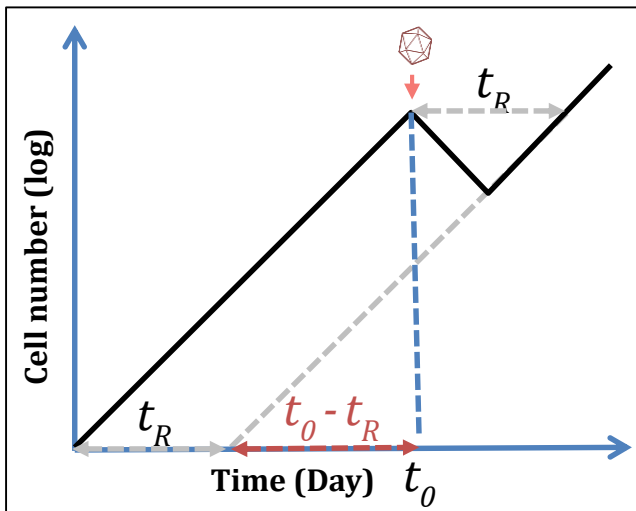
**(B) Phylogenomic analysis of *O. mediterraneus*.** Tree generated using RaxML (model PROTGAMMAWAG, 100 bootstraps) from the concatenated amino acid sequences of 188 single copy gene families shared in the Chlorophyta.

**(C) Reconstructed ancestral GC contents from first codon (left), second codon (middle) and third codon (right) positions** are shown on the same scale with position specific GC contents of extant genomes shown after the species names in bold and the ancestral estimates shown at the nodes with ranges in square brackets.

**Supplemental Text S1.** Likelihood of the origin of resistance in the microalgae from spontaneous mutations as a consequence of random errors of the replication machinery.

### 1. Estimation of the number of mutations in the culture upon viral infection in an *Ostreococcus* infection experiment.

Let us estimate the number of spontaneous mutations segregating in the culture at the time the virus is introduced. If  $t_R$  is the time required for recovery to pre-infection population density, in number of days, and provided post-infection growth rate can be approximated by pre-infection growth rate, the mutation leading to the resistant population appeared  $t_R$  days after starting the culture from one single cell. Given the typical growth rate of one division per day, the number of divisions in the culture when this mutation occurred can thus be approximated by  $2^{t_R-1}$ . The rate of spontaneous mutations per genome,  $U_G$ , in Mamiellophyceae has been estimated between  $1 \times 10^{-2}$  and  $4 \times 10^{-3}$  mutations per cell division (Krasovec et al. 2017). The expected number of mutations at  $t_R$  in the microalga population is thus given by  $U_G * 2^{t_R-1}$ , and the maximal estimate obtained with the largest  $t_R$  (7 days) and  $U_G$  observed is 0.64. It is thus very likely that one (0.64) genome carrying one mutation was present in the culture at the time viruses were introduced.



### 2. Confronting expected number of spontaneous mutations to prevalence of acquisition of resistance in the microalgae.

The combination of these two observations enables to estimate that at the time of infection, there was one (0.64) cell on average bearing a mutation in each culture. Therefore, it is very likely that one genome carrying the mutation was present in the culture at the time viruses were introduced. However, *this* spontaneous mutation had to confer viral resistance, which implies that 100% of spontaneous mutations confer resistance to viruses. Furthermore, a previous study on the evolution of viral resistance in the *O. tauri* and the prasinovirus OtV5 found all 38 independently infected cell lines became resistant to OtV5 (Yau et al. 2016). We have now repeated this experiment on 24 OmV2 susceptible *O. mediterraneus* cell lines isolated from the S3 culture. This experiment confirmed that all independently infected lines evolved resistance within seven days in *O. mediterraneus* too.

In conclusion, precise calculation of the probability of a random spontaneous mutation producing a resistant phenotype, based on the knowledge we have on spontaneous mutation rate in these microalgae, enables us to reject spontaneous point mutations generated by random replication errors as the cause of the acquisition of resistance.

## Supplementary Tables

**Supplementary Table S1.** OmV2 predicted protein coding genes with available functional annotation. BBH : Best Blast Hit.

CDS	Gene names and genomic features	BBH	CDS	Gene names and genomic features	BBH
<b>DNA, RNA, replication, recombination and repair</b>			<b>Lipid/Fatty acid metabolism</b>		
orf00008	Exonuclease	OmV1	orf00172	Patatin-like phospholipase	OtV1
orf00020	ATPase	OmV1	<b>Protein synthesis, modification and degradation</b>		
orf00075	Poxvirus A22 protein	OIV4	orf00009	Zinc knuckle	OmV1
orf00077	Thymidylate synthase complementing protein	OmV1	orf00018	Peptidase family M41	OIV7
orf00151	Proliferating cell nuclear antigen, N-terminal domain	OtV5	orf00052	DegT/DnrJ/EryC1/StrS aminotransferase family	OIV2
orf00163	NTPase/helicase	OtV2	orf00087	NAD(P)-binding Rossmann-like domain	OmV1
orf00166	NTPase/helicase	OtV2	orf00128	WLM domain	OIV2
orf00183	YqaJ-like viral recombinase domain	OIV1	orf00252	Clp protease	OtV2
orf00228	D5 N terminal like	OtV1	orf00266	Proline dehydrogenase	OIV1
orf00235	RNase H	OtV5	<b>Signaling</b>		
orf00243	Eukaryotic DNA topoisomerase I	OmV1	orf00032	Ring finger domain	OIV5
orf00251	ATP dependent DNA ligase domain	OtV2	orf00209	Protein kinase domain	OtV5
orf00253	RsgA GTPase	OtV5	orf00295	Rhodanese-like domain	OIV3
orf00304	DNA polymerase family B	OtV5	<b>Structural</b>		
<b>DNA methylation and site-specific endonucleases</b>			orf00091	Large eukaryotic DNA virus major capsid protein	OmV1
orf00090	Type III restriction enzyme, res subunit	OIV1	orf00101	Major capsid protein N-terminus	OtV1
orf00131	D12 class N6 adenine-specific DNA methyltransferase	OtV1	orf00114	Large eukaryotic DNA virus major capsid protein	OtV2
orf00160	C-5 cytosine-specific DNA methylase	OIV4	orf00133	Major capsid protein N-terminus	OIV2
orf00180	SWIB/MDM2 domain	OtV5	orf00233	Large eukaryotic DNA virus major capsid protein	OIV1
<b>Sugar manipulation enzymes</b>			orf00302	Large eukaryotic DNA virus major capsid protein	OtV5
orf00015	GDP-mannose 4,6 dehydratase	OtV1	orf00307	Large eukaryotic DNA virus major capsid protein	OIV2
orf00048	Glycosyl transferase family 2	OtV1	<b>Miscellaneous</b>		
orf00053	GDP-mannose 4,6 dehydratase	OIV3	orf00010	T5orf172 domain	OIV1
orf00241	Phosphofruktokinase	OIV4	orf00021	cell division protein	OIV4
orf00246	Glycosyltransferase family 25 (LPS biosynthesis protein)	OtV1	orf00023	Methyltransferase domain	OIV1
<b>Transcription</b>			orf00044	PhoH-like protein	OtV1
orf00036	Transcription factor S-II (TFIIS)	OtV1	orf00049	Thiamine pyrophosphate enzyme, N-terminal TPP binding domain	OtV2
orf00126	mRNA capping enzyme	OIV3	orf00071	Baculoviridae p74 conserved region	No hit
orf00186	SNF2 family N-terminal domain	OtV5	orf00073	Chaperone of endosomalidase	No hit
orf00154	Poxvirus Late Transcription Factor VLTF3 like	OIV2	orf00079	ABC1 family	OIV1
orf00201	Transcription factor TFIID (or TATA-binding protein, TBP)	OtV1	orf00111	Chaperone of endosomalidase	OIV7
orf00217	Transcription factor TFIIB repeat	OtV1	orf00118	NUDIX domain	OtV5
orf00220	mRNA capping enzyme, catalytic domain	OmV1	orf00159	Cytidyltransferase-like	OtV2
orf00221	mRNA capping enzyme, beta chain	OtV2	orf00162	2OG-Fe(II) oxygenase superfamily	OtV1
<b>Nucleotide metabolism</b>			orf00171	viralA-type inclusion protein	OIV3
orf00182	Ribonuclease-III-like	OtV2	orf00195	MazG nucleotide pyrophosphohydrolase domain	OIV4
orf00191	Ribonucleotide reductase, barrel domain	OmV1	orf00237	2OG-Fe(II) oxygenase superfamily	OmV1
orf00215	Ribonucleotide reductase, small chain	OmV1	orf00240	Phytanoyl-CoA dioxygenase (PhyH)	OtV6
orf00250	5' nucleotidase, deoxy (Pyrimidine), cytosolic type C protein (NT5C)	OIV4	orf00244	A nuclease family of the HNH/ENDO VII superfamily with conserved AHH	OtV5
orf00275	Thymidine kinase	OIV7	orf00248	PQ loop repeat	OtV6
orf00284	dUTPase	OIV1	orf00287	2OG-Fe(II) oxygenase superfamily	OIV1
			orf00289	Domain of unknown function (DUF814)	OtV6
			orf00291	2OG-Fe(III) oxvoenase superfamily	OtV2

**Supplementary Table S2.** *O. mediterraneus* strains and genomic and transcriptomic datasets used in this study. RCC2590 is genomic sequence data and strains with an MMETSP identifier are transcriptome sequences. Abbreviations: N/A, non-applicable; PE, paired-end; MP, mate-paired; RCC, Roscoff Culture Collection; S1, S2 and S3 (Mutation Accumulation lines derived from RCC2590); MMETSP, Marine Microbial Eukaryotic Transcriptome Sequencing Project; assem., nuclear genome assembly; aln reads, read fragments re-aligned to genome; ID, identifier. \*percentage of properly aligned PE reads to the reference RCC2590 genome.

Strain ID	MMETSP ID	isolation date	site	coordinates	reads	assem. (Mb)	aln reads* (%)
RCC2590	N/A	23 Mar. 2009	Thau	43°24'N 3°36'E	10,257,955 PE 13,542,963 MP 80× PACBIO	13.86	85
S1	N/A	N/A	N/A	N/A	747,558 PE 80× PACBIO	13.81	75
S2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
S3	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RCC2572	0929	16 Jun. 2006	Bages	43°03'14"N 2°59'54"E	43,616,308 PE	11.6	83
RCC1621	0930	2 Jun. 2003	SOLA	42°29'18"N 3°08'42"E	29,821,828 PE	12.4	89
RCC2596	0932	23 Mar. 2009	Thau	43°24'N 3°36'E	27,255,407 PE	14.0	95
RCC2573	0936	18 Jan. 2008	Leucate	42°48'24"N 3°01'27"E	28,940,482 PE	12.2	89
RCC2593	0937	23 Mar. 2009	Thau	43°24'N 3°36'E	25,147,103 PE	11.5	84
RCC1107	0938	1 Jan. 2006	Bages	43°03'14"N 2°59'54"E	27,277,118 PE	12.9	88



**Supplementary Table S3.** Primers used in this study on *O. mediterraneus* RCC2590 and OmV2. Abbreviations: Seq. F, forward primer sequence; Seq. R, reverse primer sequence; SOC, small outlier chromosome; BOC, big outlier chromosome; DNApol, viral DNA polymerase B; MCP, viral Major Capsid Protein.

Primer ID	Seq. F. (5'–3')	Seq. R. (5'–3')	Size (bp)	Target	Reference
SY1F/R	TCGAACACGAGGACTTA GCG	ATCAGCAGGGTTGTCATCC G	448	SOC	This study
SY2F/R	CACTACGTCACCGCCGAT AA	CTTCACACTTTGTGCCCGTG	517	SOC	This study
SY3F/R	CATCATGTGCGCCTTTCT CG	GAAACAAGCACAACGTCCC C	642	SOC	This study
SY4F/R	ATGGATTCCGAGGAACC GTG	GAACGCACCCGATCCAGTT A	640	OmV2	This study
SY5F/R	CCTCCACGACGTTTCTTC A	CGCACTGTAATACGCACAC G	603	BOC	This study
SY6F/R	GCGTGAACGCATCGACA ATC	CCGGATACCCAAACCGTTG A	534	SOC	This study
SY7F/R	ATCAATGCGATTCGTTGC GG	ATTGCCGAGAGTGATGCCA A	654	OmV2	This study
18SF/R	ACCTGGTTGATCCTGCCA G	TGATCCTCCGCAGGTTAC	1765	18S rRNA	(46)
VpolAS4/ VpolAAS1	GARGGIGCIACIGTIYTNG A	CCIGTRAAICCRTAIACISWRT TCAT	320	DNApol	(47)
VmcpAS3/ VmcpAAS1	GGIGGICARMGIRTIGAYA A	TGIACYTGYTCDATIARRTAY TCRTG	350	MCP	(47)

**Supplementary Table S4.** Species list and genome versions used for annotation and comparative genomics analysis. For species denoted with asterisk functional annotations (GO annotations and InterPro domains) were retrieved using the Uniprot Gene Association File (downloaded 10/09/2015). For all other species InterPro was ran (January 2016) and mapped to GO terms.

Species	Source	PubmedID
<i>Aureococcus anophagefferens</i>	JGI 1.0	21368207
<i>Asterochloris</i> sp. Cgr/DA1pho v2.0	JGI 7.45.13	/
<i>Auxenochlorella protothecoides</i>	Beijing Genomics Institute 1.0	25012212
<i>Arabidopsis thaliana</i> *	TAIR10	11130711
<i>Amborella trichopoda</i> *	Amborella v1	24357323
<i>Bathycoccus prasinus</i>	Ghent University	22925495
<i>Chondrus crispus</i>	ENSEMBL protists release 28	23536846
<i>Caenorhabditis elegans</i> *	ENSEMBL release 81	9851916
<i>Cyanidioschyzon merolae</i>	Tokyo University	15071595
<i>Chlorella variabilis</i> NC64A	JGI 1.0	20852019
<i>Chlamydomonas reinhardtii</i>	JGI 5.5 (Phytozome 10.2)	17932292
<i>Coccomyxa subellipsoidea</i> C-169	JGI 2.0 (Phytozome 10.2)	22630137
<i>Dictyostelium discoideum</i> *	ENSEMBL protist release 28	15875012
<i>Drosophila melanogaster</i> *	ENSEMBL release 81	10731132
<i>Emiliana huxleyi</i> *	ENSEMBL protist release 28	23760476
<i>Ectocarpus siliculosus</i>	Ghent University	20520714
<i>Fragilariopsis cylindrus</i>	JGI 1.0	28092920
<i>Galdieria sulphuraria</i>	ENSEMBL protists release 28	23471408
<i>Homo sapiens</i> *	ENSEMBL release 81	11181995
<i>Helicosporidium</i> sp.	Illinois University 1.0	24809511
<i>Micromonas pusilla</i> strain CCMP1545	Ghent University	24273312
<i>Micromonas</i> sp RCC299	JGI 3.0	19359590
<i>Mus musculus</i> *	ENSEMBL release 81	12466850
<i>Nannochloropsis gaditana</i> *	ENSEMBL protist release 28	23966634
<i>Ostreococcus lucimarinus</i>	JGI 2.0	17460045
<i>Ostreococcus</i> sp RCC809	JGI 2.0	/
<i>Oryza sativa</i> *	MSU RGAP 7	16100779
<i>Ostreococcus tauri</i>	Ghent University v2.0	25494611
<i>Phytophthora sojae</i> *	ENSEMBL protist release 28	16946065
<i>Physcomitrella patens</i>	Phytozome 9.1 (v1.6)	18079367
<i>Picochlorum costavermella</i>	This study	/
<i>Picochlorum</i> sp. SENEW3 (SE3)	Rutgers University 1.0	24965277
<i>Paramecium tetraurelia</i> *	ENSEMBL protist release 28	17086204
<i>Phaeodactylum tricorutum</i> *	ASM15095v2	18923393
<i>Saccharomyces cerevisiae</i> strain S288C *	ENSEMBL release 81	8849441
<i>Schizosaccharomyces pombe</i> *	ENSEMBL fungi release 28	11859360
<i>Thalassiosira pseudonana</i>	JGI 3.0	15459382
<i>Volvox carteri</i>	JGI 2.0 (Phytozome 10.2)	20616280

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