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1 **Red Sea SAR11 and *Prochlorococcus* Single-cell Genomes Reflect**
2 **Globally Distributed Pangenomes**

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26 **Abstract**

27 Evidence suggests many marine bacteria are cosmopolitan, with widespread but sparse strains
28 poised to seed abundant populations upon conducive growth conditions. However, studies
29 supporting this “microbial seed bank” hypothesis have analyzed taxonomic marker genes rather
30 than whole genomes/metagenomes, leaving open the possibility that disparate ocean regions
31 harbor endemic gene content. The Red Sea is isolated geographically from the rest of the ocean
32 and has a combination of high irradiance, high temperature, and high salinity that is unique
33 among the ocean; we therefore asked whether it harbors endemic gene content. We sequenced
34 and assembled single-cell genomes of 21 SAR11 (subclades Ia, Ib, Id, II) and 5 *Prochlorococcus*
35 (ecotype HLII) cells from the Red Sea and combined them with globally-sourced reference
36 genomes to cluster genes into ortholog groups (OGs). Ordination of OG composition could
37 distinguish clades, including phylogenetically cryptic *Prochlorococcus* ecotypes LLII and LLIII.
38 Compared with reference genomes, 1% of *Prochlorococcus* and 17% of SAR11 OGs were
39 unique to the Red Sea genomes (RS-OGs). Most (83%) RS-OGs had no annotated function, but
40 65% of RS-OGs were expressed in diel Red Sea metatranscriptomes, suggesting they could be
41 functional. Searching *Tara* Oceans metagenomes, RS-OGs were as likely to be found as non-RS-
42 OGs; nevertheless, Red Sea and other warm samples could be distinguished from cooler samples
43 using the relative abundances of OGs. The results suggest that the prevalence of OGs in these
44 surface ocean bacteria is largely cosmopolitan, with differences in population metagenomes
45 manifested by differences in relative abundance rather than complete presence–absence of OGs.

46 **Importance**

47 Studies have shown that as we sequence seawater from a selected environment deeper and
48 deeper, we approach finding every bacterial taxon known for the ocean as a whole. However,
49 such studies have focused on taxonomic marker genes rather than on whole genomes, raising the
50 possibility that the lack of endemism results from the method of investigation. We took a
51 geographically isolated water body, the Red Sea, and sequenced single cells from it. We
52 compared those single-cell genomes to available genomes from around the ocean, and to ocean-
53 spanning metagenomes. We showed that gene ortholog groups found in Red Sea genomes but
54 not in other genomes are nevertheless common across global ocean metagenomes. These results

55 suggest that Baas Becking’s hypothesis “everything is everywhere, but the environment selects”
56 also applies to gene ortholog groups. This widely dispersed functional diversity may give
57 oceanic microbial communities the functional capacity to respond rapidly to changing
58 conditions.

59 **Introduction**

60 Marine bacteria thrive throughout the surface ocean despite low nutrients, high irradiation, and
61 other physicochemical stressors. Adaptations enabling survival can be at the level of
62 transcriptional, translational, and other methods of cellular regulation that occur at time-scales of
63 minutes to hours (1, 2). Alternatively, microbial genomes can evolve new functions on the scale
64 of thousands to millions of generations (3, 4). Evolution via horizontal gene transfer enables the
65 introduction of entirely new functionality (gene gain) as well as genome streamlining (gene loss)
66 for more efficient resource (e.g., nitrogen, phosphorus) allocation (5). Therefore, it is expected
67 that the genomes of marine bacteria will display differences in gene content correlated with the
68 physicochemical environment in which they live. Indeed, both individual genomes (cultured and
69 single-cell genomes) (6–10) and community genomes (metagenomes) (11, 12) show that bacteria
70 in the oligotrophic (nutrient-poor) surface ocean carry streamlined genomes finely tuned to their
71 environments.

72 Examples of adaptive gene presence–absence patterns are seen in the most numerous groups of
73 bacteria in the oligotrophic tropical and sub-tropical surface ocean, the photoautotrophic
74 picocyanobacteria *Prochlorococcus* and *Synechococcus* and the chemoheterotrophic
75 Alphaproteobacteria SAR11 clade (*Candidatus Pelagibacter ubique*). Genomes of these genera
76 are smaller than their relatives in less nutrient-poor environments (6, 8), suggestive of genome
77 streamlining to conserve resources used for genome replication (3). Consistent with genome
78 streamlining, the genes maintained in *Prochlorococcus* and SAR11 genomes are correlated with
79 physical features in parts of the water column in which they are found, for example, genes for
80 acquisition of nitrite and nitrate in genomes found where those compounds are available (3, 8).
81 Examples revealed through comparative community genomics include an enrichment of
82 phosphorus acquisition gene ortholog groups in the Atlantic relative to the Pacific Ocean (11, 13)

83 and an enrichment in osmolyte oxidation gene ortholog groups in the Mediterranean and Red
84 Seas relative to the Atlantic and Pacific Oceans (12).

85 The Red Sea is an attractive environment for the study of genomic adaptations. Geographically,
86 the Red Sea is largely isolated from the rest of the World Ocean, with only a small sill (the Bab
87 el Mandeb) connecting it to the Indian Ocean (14). Among surface waters catalogued in the
88 World Ocean Database, the Red Sea lies at the high end of the global temperature distribution
89 and is higher than any other sea in the global salinity distribution (Fig. S1). The Red Sea,
90 straddling the Tropic of Cancer, experiences year-round high irradiance, and cloud cover across
91 North Africa and the Arabian Peninsula is among the lowest on the planet (NASA Aqua satellite
92 MODIS sensor). The Red Sea is also oligotrophic, with production thought to be limited by
93 nitrogen (15).

94 Evidence of genomic adaptation to high light and high salinity in the Red Sea has been revealed
95 through comparative metagenomics, showing increased relative abundance of known gene
96 ortholog groups in *Prochlorococcus* and SAR11 (12). Relative to the North Pacific, Sargasso
97 Sea, and Mediterranean Sea, the Red Sea *Prochlorococcus* population had increased frequencies
98 of high-light stress and DNA repair gene ortholog groups (12), the latter likely an adaptation to
99 UV-induced DNA damage. Relative to these same seas, the SAR11 population had increased
100 frequencies of gene ortholog groups for osmolyte degradation (12); osmolytes are important
101 molecules for surviving high salinity in many organisms. Across 45 metagenomes along
102 latitudinal and depth gradients from the surface to 500 m in the Red Sea, temperature explained
103 more variation in gene ortholog groups than any other environmental parameter, and the relative
104 abundance of gene ortholog groups linked to high irradiance, high salinity, and low nutrients
105 were correlated with those parameters (16).

106 The above-mentioned patterns observed in comparative metagenomics studies were all based on
107 relative abundance of known gene ortholog groups, dependent on a reference genome database
108 with no representatives from the Red Sea. Therefore, the question remains if there are gene
109 functions in the *Prochlorococcus* and SAR11 populations in the Red Sea not found in any other
110 *Prochlorococcus* and SAR11 populations in the ocean. Because of its relative geographic
111 isolation, we might expect the Red Sea to be genetically isolated, with endemic genomic
112 adaptations to its unique combination of high solar irradiance, high temperature, high salinity,

113 and low nutrient levels. Newly identified gene ortholog groups could be informative for
114 understanding microbial adaptation and mechanisms of stress tolerance, and have potential
115 biotechnological applications.

116 The question of whether there are genetic functions found in only one sea of the global ocean
117 speaks to theoretical questions of microbial biogeography as well. A prevailing idea in microbial
118 ecology is that most microbial species are found at a given site provided the conditions are
119 conducive for their growth. This is known as the Baas Becking hypothesis: “Everything is
120 everywhere, but the environment selects” (17). Among microbial taxa found in seawater, there is
121 growing evidence for a cosmopolitan distribution of these taxa throughout the global ocean (18,
122 19). Support for the “microbial seed bank” hypothesis has come from deep sequencing of ocean
123 samples, revealing for example that nearly all 16S rRNA operational taxonomic units (OTUs)
124 from a deep-sea hydrothermal vent can be found in the open ocean (19), and that we approach
125 identifying all OTUs in the ocean as sequencing effort increases for a single marine sample (18).
126 Despite this evidence supporting a cosmopolitan distribution of OTUs throughout the ocean,
127 these amplicon sequences (16S rRNA OTUs) are only taxonomic proxies and do not represent
128 the extensive gene-level diversity in microbial genomes. Even if such marker gene sequences are
129 omnipresent across the ocean, genome evolution and diversification, e.g., via horizontal gene
130 transfer, could be occurring that generates gene-level adaptations that are endemic to particular
131 locations. Are microbial gene ortholog groups, defined at the level of genus (SAR11 or
132 *Prochlorococcus*), as widely distributed as microbial 16S rRNA gene sequences?

133 Here, to investigate microbial genomic diversity in SAR11 and *Prochlorococcus*, including
134 possible endemic adaptation in Red Sea populations, we have sequenced single-cell amplified
135 genomes (SAGs) from the Red Sea and compared their gene ortholog group (OG) content to
136 genomes and metagenomes from around the World Ocean. We have quantified expression of
137 OGs in metatranscriptomes from the Red Sea collected over two consecutive 24-hour day–night
138 cycles. This effort has resulted in 21 SAR11 SAGs, including the first genomes from subclades
139 Ib and Id, and 5 *Prochlorococcus* SAGs. Using these Red Sea SAGs and the OGs they contain as
140 queries for genomic and metagenomic analyses, we have analyzed globally-sourced genomes
141 and metagenomes to investigate the extent to which OGs from surface-ocean *Prochlorococcus*
142 and SAR11 are distributed across the World Ocean.

143 **Materials and Methods**

144 **Sample collection**

145 A single seawater sample (100 mL) was collected in a polycarbonate bottle from the surface
146 (depth of 0 m) of an open-ocean site in the east-central Red Sea (19.75 °N, 40.05 °E), near the
147 Farasan Banks region, on June 15, 2010. The sample was preserved with dimethyl sulfoxide (5%
148 final concentration), flash frozen in liquid nitrogen, and stored at –80 °C.

149 Seawater samples for metatranscriptomics were taken March 3–5, 2013, from an open-ocean site
150 in the Red Sea (Kebrit Deep, 24.7244 °N, 36.2785 °E). To obtain broad coverage of the water
151 column by both time of day and water depth, one sample per depth was collected every 4 h over
152 a 48-h period at four depths: surface (10 m), below the mixed layer (40 m; bottom of mixed layer
153 was 35 m), chlorophyll maximum (75 m), and oxygen minimum zone (420 m). For each
154 timepoint and depth, 1 L seawater was filtered using a peristaltic pump with two in-line filters in
155 series: a 1.6- μ m GF/A pre-filter (Whatman), then a 0.22- μ m Sterivex filter (Millipore).
156 RNAlater (QIAGEN) was added immediately to fill the dead space of the Sterivex filter, which
157 was then flash frozen in liquid nitrogen and stored at –80 °C.

158 **Nucleic acid extraction and amplification**

159 Single bacterioplankton cells in the preserved samples were flow-sorted, whole-genome
160 amplified (MDA, multiple displacement amplification), and PCR-screened at the Bigelow
161 Laboratory Single Cell Genomics Center (SCGC, Boothbay Harbor, ME, USA), following
162 previously described protocols (20), with SYTO-13 nucleic acid stain used to stain cells for
163 flow-sorting. SAG identification was carried out with SCGC protocol S-102 for bacteria using
164 16S rRNA primers 27F and 907R (21, 22). A total of 21 and 5 cells were identified from 16S
165 PCR screening and subjected to a second round of MDA before sequencing. The 16S rRNA gene
166 sequences are available from the European Nucleotide Archive with accession numbers
167 LN850141–LN850161.

168 The RNA extraction protocol for metatranscriptomics was adapted from (23–25). After expelling
169 RNAlater from the Sterivex filter, 2 mL lysozyme solution (1 mg/mL in lysis buffer: 40 mM
170 EDTA, 50 mM Tris pH 8.3, 0.73 M sucrose) was added, then filter incubated at 37 °C with

171 rotation for 45 min. Proteinase K solution (50 μ L at 20 mg/mL, QIAGEN/5PRIME) and SDS
172 solution (100 μ L at 20%) were added, then filter incubated at 55 °C with rotation for 2 h. Lysate
173 was expelled to a separate tube; meanwhile, 1 mL lysis buffer was added to the filter to wash at
174 55 °C for 15 min. The two lysates were pooled, to which was added 1.5 mL absolute ethanol.
175 RNA was then extracted from this solution using the RNeasy Protect Bacteria Midi Kit
176 (QIAGEN). RNA was eluted with two volumes of RNase-free water. RNA sample was
177 concentrated using a speed vacuum, from 250 μ L to 60 μ L. To this volume we added DNase (1
178 μ L Ambion TURBO DNA-free, 6 μ L 10x buffer, 60 μ L RNA) and incubated at 37 °C for 30
179 min. This solution was purified using the RNeasy MinElute Cleanup Kit (QIAGEN) and eluted
180 with RNase-free water. Final yield was 1–2 ng total RNA. Total RNA was amplified using the
181 C&E Version ExpressArt Bacterial mRNA Amplification Nano Kit, which preferentially
182 amplifies mRNA (independent of poly-A tail) and selects against rRNAs. A single round of
183 amplification was performed on 2–4 ng of total RNA which yielded about 10 μ g final amplified
184 RNA.

185 **Nucleic acid sequencing**

186 For single-cell genome sequencing, genomic library preparation with Illumina TruSeq and
187 sequencing with Illumina GAIIX and Illumina HiSeq 2000 was done at the KAUST Bioscience
188 Core Laboratory, generating paired 105-bp reads. The assembled contigs (assembly methods
189 below) are available from NCBI with accession numbers PRJEB9287 (BioProject) and
190 SAMEA3368552–SAMEA3368577 (BioSample), and can also be visualized in Integrated
191 Microbial Genomes system (26) under accession numbers 2630968236, 2630968238–
192 2630968254, 2630968277–2630968281, and 2630968285–2630968287.

193 For metatranscriptomics, sequence data were processed as described in (27). Amplified RNA
194 was used to construct sequencing libraries using the TruSeq Stranded RNA LT Sample Prep Kit
195 (Illumina) according to the manufacturer's protocol. Libraries were paired-end sequenced with
196 the Illumina HiSeq 2000 platform (2 \times 100 bp). Raw RNA sequences have been deposited in
197 NCBI GenBank with Bioproject number PRJNA289956. Low-quality reads and sequencing
198 adapters were removed using Trimmomatic v0.32 (28). Sequence reads shorter than 50 bp were
199 discarded. Bowtie 2 v2.2.4 (29) was used to identify and remove PhiX contamination sequences.
200 The remaining sequences were error-corrected using the BayesHammer algorithm (30)

201 implemented in the SPAdes v3.5.0 (31), followed by removal of putative ribosomal RNA
202 (rRNA) gene transcripts with SortMeRNA v2.0 (32).

203 **Genome assembly and annotation**

204 De novo assemblies were generated using CLC Genomics Workbench 4.9. The genomes were
205 assembled independently and, unless otherwise specified, the following applies to all of the
206 SAGs. The reads were first imported and quality trimmed with a limit of 0.01. They were then
207 assembled using CLC's *de novo* assembler with a word size (*k*-mer) of 64 and with the min/max
208 of the insert size set to 100/1000 bp. Only those contigs greater than 200 bp in length were
209 included in downstream analyses. The reads were mapped to the consensus sequence of the
210 assembled contigs using CLC's default parameters but with the length fraction set to 1.0 and the
211 similarity set to 0.95.

212 Assembled SAG contigs were ordered and oriented relative to SAR11 HTCC1062
213 (NC_007205.1) or *Prochlorococcus* MIT 9202 (NZ_DS999537) using ABACAS 1.3.1 (33). The
214 ordered sequences were then imported into GAP4 (34) and additional joins were made between
215 overlapping contigs if conserved synteny supported the arrangement. To identify and remove
216 possible contaminating sequences from the assemblies, each contig was retained only if it met
217 one or both of the following criteria: (i) the contig was binned into a bin annotated as SAR11 or
218 *Prochlorococcus* using Metawatt 3.5 (35), using the "medium" bin level, with a minimum bin
219 size of 50 kbp and minimum contig size of 500 bp; (ii) the contig had a top-10 BLASTN hit
220 against GenBank nt, with E-value $<1e-5$, to SAR11 or *Prochlorococcus*.

221 Prediction of gene open reading frames (ORFs) and functional annotation of SAGs was
222 performed by the RAST web service (36) with FIGfam Release 59.

223 **Ortholog group clustering**

224 Predicted proteins from SAGs were clustered with proteins from published cultured and SAG
225 genomes (supplemental file 1) into ortholog groups (OGs) using OrthoMCL 2.0 (37). OrthoMCL
226 configuration settings were as follows: percentMatchCutoff=50, evaluateExponentCutoff=-5. This
227 yielded 5272 SAR11 OGs and 10439 *Prochlorococcus* OGs. After OrthoMCL clustering, OGs
228 were assigned as core and non-core based on copy number in the non-Red Sea, cultured (non-

229 SAG) genomes: core OGs are those found at least once in each of the non-Red Sea, cultured
230 genomes, and non-core OGs are those not found in at least one of the non-Red Sea, cultured
231 genomes. Among SAR11, there were 683 core OGs and 4589 non-core OGs. Among
232 *Prochlorococcus*, there were 1152 core OGs and 9287 non-core OGs. Protein sequence
233 identifiers and FASTA sequences for each OG have been archived at <https://zenodo.org> with
234 DOI 10.5281/zenodo.2634561.

235 **Estimation of genome completeness**

236 Completeness of SAGs was assessed using two methods. First, completeness was assessed using
237 single-copy ‘core’ OGs, i.e., those OGs found once and only once in each complete genome
238 based on the OrthoMCL clusters (analyzed separately for SAR11 and *Prochlorococcus*).
239 Completeness was calculated as the number of core orthologs present in each SAG out of 649
240 SAR11 or 1144 *Prochlorococcus* single-copy core OGs. Second, genome completeness of the
241 SAGs was assessed using CheckM 1.0.13 (38) using the lineage-specific workflow (lineage_wf)
242 with database file `checkm_data_2015_01_16.tar.gz` downloaded from
243 https://data.ace.uq.edu.au/public/CheckM_databases; CheckM was also used to estimate genome
244 redundancy (called “contamination” in CheckM). For comparison, CheckM completeness and
245 redundancy were calculated for the reference genomes used in this study (Table S1).

246 **Genome taxonomy and phylogenetics**

247 A total of 89 SAR11 and 96 *Prochlorococcus* shared single-copy orthologous genes were
248 identified using the GET_HOMOLOGUES software (39). Amino acid sequences translated from
249 gene sequences were aligned using the MAFFT software (40). These alignments were
250 concatenated, sites with gaps were deleted, and the concatenated data were partitioned using the
251 PartitionFinder software (41) to account for variations of evolutionary processes among gene
252 families. With the Bayesian information criterion (BIC) statistic, a 16-partition framework was
253 chosen to optimally describe the variability, in which the LG rate matrix with Gamma
254 distribution of rate variation (LG+G) was selected for 15 partitions and the VT rate matrix with
255 Gamma distribution of rate variation (VT+G) was selected for the remaining partition. This
256 partition model was used in the maximum-likelihood phylogenomic construction using the
257 RAxML software (42).

258 **Ordination of SAGs and genomes using *k*-mer composition and ortholog**
259 **composition**

260 SAGs and reference genomes (Table S1) were analyzed using principal components analysis
261 (PCA) of nucleotide composition and OG composition. Nucleotide composition of the SAGs and
262 reference genomes (SAR11 and *Prochlorococcus* scaffolds >200 kbp from Integrated Microbial
263 Genomes, <https://img.jgi.doe.gov>) was determined as 6-nucleotide words or *k*-mers (6-mers). *k*-
264 mer frequencies were calculated using Jellyfish 2.2.5; the main command used was jellyfish
265 count -m 6 -t 8 -s 1M. This resulted in a table of 6-mer frequencies in the SAGs and genomes,
266 one table each for SAR11 and *Prochlorococcus*. OG composition was derived from tables of
267 OrthoMCL clusters, which—as the SAGs had variable levels of completeness and gene counts
268 (Table 1)—were subsampled so that all genomes had the same number of gene counts in the
269 table. The number of OG counts subsampled was chosen to balance the number of OG counts
270 with the number of genomes retained (less complete SAGs were excluded): the OG composition
271 tables (with counts of 5272 unique SAR11 OGs and 10439 unique *Prochlorococcus* OGs) were
272 subsampled down to 800 gene counts per SAR11 SAG (keeping 12 of 21 SAGs) and 1400 gene
273 counts per *Prochlorococcus* genome (keeping 5 of 5 SAGs). Prior to PCA, a pseudo-count of 1
274 was added to *k*-mer and OG count tables to account for zero values; *k*-mer counts were then
275 converted to relative abundances for each genome (unnecessary for OG counts because of the
276 subsampling procedure); *k*-mer relative abundances were then standardized to *z*-scores (not done
277 for OG counts because this reduced the resolving power of PCA). PCA was then performed
278 using the Scikit-Learn function `sklearn.decomposition.PCA` (43).

279 **Mapping of metatranscriptomic reads to OGs**

280 The quality-filtered mRNA reads from the 52 samples were mapped against the SAGs using
281 Bowtie 2 (29) with default settings. Each read mapping above the threshold was assigned to
282 exactly one gene in a SAG contig. The resultant read counts were normalized based on the
283 FPKM metric (fragments per kilobase of gene per million mapped reads). Per-sample FPKM
284 counts for each gene were then summed by OGs, resulting in per-sample FPKM counts for each
285 OG. For downstream analysis, counts were converted to a simple presence–absence measure: if

286 any gene belonging to the OG had one or more mapped transcript, that OG was marked as
287 present in that sample.

288 **Detection and rarefaction analysis of OGs in *Tara* Oceans metagenomes**

289 A set of 139 prokaryote-enriched *Tara* Oceans metagenomic gene files (44) was downloaded
290 from the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>, ERZ096909–ERZ097150).
291 Each file contains nucleotide sequences for genes predicted on *Tara* Oceans metagenomic
292 contigs that were assembled from shotgun sequencing reads from individual *Tara* Oceans
293 samples. The prokaryote fraction was 0.22–1.6 μm for stations 004–052 and 0.22–3 μm for
294 stations 056–152; the environmental features of the samples were indicated as “SRF” (surface),
295 “MIX” (mixed layer), “DCM” (deep chlorophyll maximum), and “MES” (mesopelagic zone).
296 The metagenomic gene sequences were queried against a database of translated proteins from the
297 SAGs and genomes with DIAMOND 0.8.26 (45) using the program blastx with parameters $-\text{p } 40$
298 $-\text{k } 25$ $-\text{e } 1\text{e}-3$. The top hit (SAG or genome protein sequence) for each *Tara* gene sequence (E-
299 value $< 1\text{e}-5$) was retained. E-value cutoffs of $1\text{e}-10$ and $1\text{e}-15$ were also tested, which showed
300 the same trends as E-value $< 1\text{e}-5$ but with fewer total OGs identified. Counts of the number of
301 times each protein was a top hit were then summed across each OG. This resulted in a table of
302 OGs by samples where each OG was either present (at least one constituent protein was a top hit
303 at least once) or absent in each sample. These presence–absence tables (one for SAR11, one for
304 *Prochlorococcus*) were used to generate rarefaction curves: samples were added one-by-one
305 randomly (1000 permutations), and the cumulative number of OGs found was recorded.

306 **Ordination of *Tara* Oceans metagenomes by OG composition**

307 OG counts (total, not presence–absence) in *Tara* Oceans surface (SRF) sample metagenomes
308 were used for ordination using PCA. Prior to PCA, a pseudo-count of 1 was added to OG count
309 tables to account for zero values; counts were then converted to relative abundances for each
310 metagenome; OGs with an average relative abundance across all metagenomes less than 0.0001
311 (0.01%) were removed; relative abundances were then standardized to z-scores. PCA was then
312 performed using the Scikit-Learn function `sklearn.decomposition.PCA` (43).

313 **World Ocean temperature and salinity data**

314 Surface temperature and salinity data (WOD13_ALL_SUR_OBS) from the World Ocean
315 Database 2013 (<https://www.nodc.noaa.gov/OC5/WOD13/>) were downloaded from the Research
316 Data Archive at the National Center for Atmospheric Research
317 (<https://rda.ucar.edu/datasets/ds285.0/>).

318 **Results and Discussion**

319 **Single-cell genome properties and taxonomic classification**

320 Following collection of surface seawater from the east-central Red Sea, flow sorting, and
321 amplification, we sequenced and assembled 21 SAR11 and 5 *Prochlorococcus* single-cell
322 amplified genomes (SAGs). These SAGs represent reference genomes in an ocean region with
323 sparse coverage: only one cultured *Prochlorococcus* genome (27) and two cultured SAR11
324 genomes (46) are currently available from the Red Sea. The SAR11 SAGs also represent
325 genomes from clades without other sequenced representatives: two SAGs from subclade Ib and
326 three SAGs from subclade IId (Fig. 1).

327 To account for and remove any possible contaminating DNA sequences, assembled contigs were
328 retained only if they were part of a SAR11 or *Prochlorococcus* Metawatt bin or if they had a top-
329 10 BLASTN hit to a *Prochlorococcus* or SAR11 genome (methods). In Metawatt, assignment to
330 bins is based on tetranucleotide frequency, and the average taxonomy of the bin is determined by
331 BLAST of 500-bp fragments of all the contigs against a prokaryotic database (35). A contig
332 matching the tetranucleotide frequency of a SAR11 or *Prochlorococcus* bin could be retained
333 even if it contained contradictory or missing taxonomic information. However, to check if our
334 secondary, BLASTN-based assignment process could be biased against short contigs, which
335 might lack a neighboring anchor gene, we analyzed the distribution of contig lengths between
336 retained and removed contigs for each SAG. We found that in most cases (20 of 26 SAGs) the
337 median sizes of retained and removed contigs were not different (Fig. S2); in 6 SAGs the
338 retained contigs were larger than the removed contigs (Mann–Whitney U, $p < 0.05$, two-tailed).

339 Genome size and completeness was greater for *Prochlorococcus* SAGs than SAR11 SAGs. Size
340 of *Prochlorococcus* SAGs ranged from from 1.28–1.46 Mbp in 85–221 contigs, containing

341 1428–1710 genes; SAR11 SAGs ranged from 0.29–1.14 Mbp in 55–157 contigs, containing
342 342–1199 genes (Table 1). Completeness was calculated by two methods: fraction of single-copy
343 core genes observed and CheckM completeness score; genome redundancy was calculated by
344 CheckM. Completeness of *Prochlorococcus* SAGs ranged from 85.9–90.3% core completeness
345 and 70.7–78.7% CheckM completeness; SAR11 SAGs ranged from 20.3–90.0% core
346 completeness and 19.1–76.7% CheckM completeness (Table 1). Genome redundancy of
347 *Prochlorococcus* SAGs ranged from 0.1–1.0%, and of SAR11 SAGs ranged from 0.0–1.4%
348 (Table 1). Plotting the number of single-copy core genes as a function of total contig size (Fig.
349 S3) showed a strong correlation between total contig size and number of single-copy core genes;
350 this analysis illustrates the greater completeness of the *Prochlorococcus* SAGs relative to the
351 SAR11 SAGs.

352 Taxonomic assignment of SAGs to clades was done by comparing SAGs against reference
353 genomes using several methods. Phylogenetic analysis was done on concatenated proteins (89
354 SAR11 and 96 *Prochlorococcus* shared single-copy orthologous genes) using the maximum
355 likelihood method (methods). Nucleotide composition (G+C content and *k*-mer composition)
356 was calculated and compared to reference genomes. Ordination using principal components
357 analysis (PCA) of *k*-mer composition and OG composition (presence–absence of each OG in
358 each genome) was used to visualize SAGs in relation to known clades of SAR11 and
359 *Prochlorococcus*.

360 Phylogenetic analysis of concatenated proteins (Fig. 1) revealed that *Prochlorococcus* SAGs
361 were all ecotype HLII (5/5). Surveys of the Red Sea using 16S–23S rRNA internal transcribed
362 spacer (ITS) amplicon sequencing (47), *rpoC1* gene amplicon sequencing (48), and
363 metagenomic sequencing (12) have each shown that HLII is the dominant *Prochlorococcus*
364 ecotype in the surface Red Sea. This pattern is consistent with temperature-driven ecotype
365 distribution patterns of *Prochlorococcus*, where ecotype HLII is predominant in warm/tropical
366 surface waters (and has a higher thermal tolerance in culture) and ecotype HLI is predominant in
367 cool/subtropical surface waters (49). SAR11 SAGs were predominantly subclade Ia (13/21), with
368 the remainder subclades Ib (2/21), Id (3/21), and II (3/21). Placement of the SAR11 SAGs in
369 these respective clades is supported by a previous phylogenetic analysis of 16S rRNA gene
370 sequences that included these SAGs (10). Surveys using amplicon sequencing of the 16S rRNA

371 gene (50) and metagenomic sequencing (12) have both shown that SAR11 subclade Ia dominates
372 the surface Red Sea. Subclade distributions in the 16S survey (50) approximately matched the
373 distribution of the SAG subclades here, suggesting that the SAGs may approximate the natural
374 SAR11 population.

375 DNA G+C content of the *Prochlorococcus* SAGs ranged from 31.0–31.4% (Table 1), which is
376 typical of genomes of *Prochlorococcus* ecotype HLII (51). G+C content of the SAR11 SAGs
377 was lower, ranging from 27.8–30.5% (Table 1). We have previously shown, using the SAR11
378 SAGs and other SAR11 genomes, that the ratio of nonsynonymous to synonymous nucleotide
379 mutations and other genomic evidence in SAR11 genomes is consistent with selection for low
380 nitrogen driving the low G+C content in marine SAR11 (10).

381 Ordination by PCA of genome properties provided visualization and in some cases improved
382 resolution of genome taxonomy relative to tree-based methods. For nucleotide composition
383 analysis, six-nucleotide words (6-mers) were chosen to balance computational time and
384 information content. The distribution of all 4096 possible 6-mers across the genomes was subject
385 to dimensionality reduction using PCA and plotted as the first two principal components (PCs).
386 The first PC explains 27% and 67% of the variation, respectively, for the SAR11 genomes (Fig.
387 2a) and the *Prochlorococcus* genomes (Fig. 2b). The PCA plots show wider spread in the SAR11
388 genomes than in the *Prochlorococcus* genomes; both cluster by clade, but the *Prochlorococcus*
389 genomes are more tightly clustered, with three main clusters (Fig. 2b): HLI nested within HLII
390 and near HLIII/IV (lower-left), then LLI (middle-left) next-closest followed by LLII and LLIII
391 (upper-left), and then LLIV distant from the others and more disperse (lower-right).

392 Ordination by PCA of OG composition was done following subsampling of OG counts down to
393 800 gene counts per SAR11 genome and 1400 gene counts per *Prochlorococcus* genome
394 (methods). This had the effect of dropping 9 SAR11 SAGs, but it allowed the genomes to have
395 even depth of coverage for PCA calculation. PCA ordination revealed patterns of OG
396 composition of SAR11 genomes (Fig. 2c) and *Prochlorococcus* genomes (Fig. 2d). PC1 and PC2
397 each explained 6–9% of the variation for both sets of genomes. For SAR11, ordination of OG
398 composition clustered by clade approximately as well as 6-mer composition. For
399 *Prochlorococcus*, PCA of OG composition provided good separation of the low-light ecotypes

400 (LLI, LLII, LLIII, and LLIV), whereas the high-light ecotypes HLI and HLII formed a single
401 cluster with HLIII/IV nearby.

402 Of particular interest to investigations of the low-light adapted *Prochlorococcus* ecotypes, we
403 note that OG composition clearly distinguished between genomes of ecotypes LLII and LLIII. It
404 has previously been observed that phylogenetic analysis (ITS region) (52, 53) does not resolve
405 ecotypes LLII and LLIII (identified as high B/A II and III by (54)). Similarly, our analysis of 6-
406 mer composition also could not resolve these two low-light ecotypes. Our method of “OG
407 ordination”, however, did distinguish these ecotypes. Thus OG distributions can be a helpful tool
408 to assign genomes to ecotypes that are indistinguishable by other taxonomic or phylogenetic
409 methods. The rich genotypic information provided by OG distribution patterns, combined with
410 an ordination method like PCA, could be applied to other microbial groups for taxonomic
411 classification of closely related genomes.

412 **Gene clustering and identification of Red-Sea-associated ortholog groups**

413 The SAGs described here come from an undersampled region of the ocean (the Red Sea) and in
414 part from undersampled clades of marine bacteria (SAR11 subclades Ib, Id, and II), and therefore
415 provide the opportunity to identify OGs specific for these clades or possibly endemic to this
416 ocean region. To investigate these patterns, we combined the Red Sea SAGs with available
417 cultured genomes and SAGs (separately for *Prochlorococcus* and SAR11), clustered genes into
418 OGs using a Markov clustering algorithm (OrthoMCL, methods), and identified those OGs
419 found only in the Red Sea SAGs and/or only in certain clades.

420 We identified 878 SAR11 OGs and 96 *Prochlorococcus* Red-Sea-associated OGs (RS-OGs), that
421 is, OGs not found (in this analysis) in genomes from other parts of the ocean (supplemental file
422 1). These totals represent 16.7% of all (19.1% of non-core) SAR11 OGs and 0.9% of all (1.0% of
423 non-core) *Prochlorococcus* OGs. Many of the RS-OGs were found only in a single clade: 96 in
424 *Prochlorococcus* ecotype HLII, 484 in SAR11 subclade Ia, 101 in SAR11 subclade Ib, 101 in
425 SAR11 subclade Id, and 132 in SAR11 subclade II. The numerous clade-specific OGs present
426 targets for understanding ecotype-specific physiology.

427 The first pattern of note was that there were more RS-OGs in the SAR11 SAGs than in the
428 *Prochlorococcus* SAGs. This reflects the large contribution of our SAR11 SAGs to the

429 sequenced SAR11 pangenome: the number of SAR11 Red Sea SAGs (=21) was nearly as many
430 as the number of SAR11 reference genomes (=26). In contrast, the number of *Prochlorococcus*
431 Red Sea SAGs (=5) was only 3% of the number of *Prochlorococcus* reference genomes (=140).
432 Emphasizing the effect of the genome reference database on estimates of OG endemicity, after
433 new *Prochlorococcus* genomes (9, 52) were added to the clustering, the number of RS-OGs
434 dropped from 1192 to 96 (Fig. S4). Another explanation for the greater number of new SAR11
435 OGs is that the SAR11 SAGs span previously unsampled or undersampled clades: 334 of the 878
436 Red-Sea-associated SAR11 OGs were found in only one of subclade Ib, Id, or II. Furthermore,
437 SAR11 is a broader phylogenetic group, based on 16S rRNA diversity, than *Prochlorococcus*
438 (55), and therefore its pangenome may be expected to be larger. In summary, we suspect that the
439 large number of new SAR11 OGs (=878), in general, more likely reflects the current dearth of
440 sequence data for SAR11 rather than a significant degree of endemism due to isolation and/or
441 selection.

442 The second pattern we examined was inspired by our question about possible endemic gene
443 content in the Red Sea: based on the geographic isolation of the Red Sea and its unique
444 combination of physicochemical conditions (simultaneously high irradiance, high salinity, high
445 temperature, and low nutrients), do genomes isolated from the Red Sea exhibit endemic OG
446 content encoding adaptive functions for this environment? The answer that emerged to this
447 question is that there were some indications of possible endemic adaptations to the Red Sea;
448 however, there were no new pathways identifiable, most of the OGs with annotated functions
449 were found in only one or two SAGs, and the majority of OGs encoded hypothetical proteins
450 with no assigned function.

451 The majority of RS-OGs were hypothetical proteins: 82% (723 of 878) for SAR11 and 91% (87
452 of 96) for *Prochlorococcus*. It was difficult to infer possible adaptive functions for OGs with no
453 predicted functions; however, these OGs may be referenced later when new approaches for
454 annotating conserved hypotheticals are developed. The remaining non-hypothetical OGs (155
455 SAR11, 9 *Prochlorococcus*), i.e., those with predicted functions, are listed in Table S2. While
456 we could not detect a widespread signature of adaptation to the Red Sea environment—i.e., RS-
457 OGs with annotated functions represented across multiple SAGs—below we highlight a few

458 sparsely represented RS-OGs that may have adaptive functionality in the Red Sea environment,
459 some with possible biotechnological potential.

460 Among *Prochlorococcus* SAGs, none of the 9 non-hypothetical RS-OGs (Table S2) were found
461 in more than one SAG. One OG (proch20425) found in SCGC AAA795-M23 encodes UvrABC
462 system protein B, responsible for repair of DNA damage. We could posit that this enzyme is
463 found preferentially in the Red Sea because of the year-round high irradiance, which increases
464 the rate of DNA damage in cells.

465 Among SAR11 SAGs, there were 21 non-hypothetical RS-OGs found in two or more SAGs and
466 another 134 found in only one SAG (Table S2). These OGs show links to high light adaptation,
467 motility, and nitrogen and phosphorus assimilation. One OG (pelag14710, found in one SAG)
468 encodes a photolyase enzyme that repairs damaged DNA caused by exposure to ultraviolet light.
469 Pyrophosphatase (pelag15064, found in one SAG) is involved in the hydrolysis of inorganic
470 pyrophosphate into two orthophosphates and may have a role in phosphorus utilization.
471 Allantoinase (pelag15247) and urease accessory protein UreF (pelag14490) are each found in
472 one SAR11 SAG. These enzymes involved in phosphorus and nitrogen metabolism may provide
473 an adaptive advantage in the Red Sea, which exhibits co-limitation to both elements and may be
474 relatively more nitrogen-limited (12, 15). Several of the SAR11 RS-OGs encode enzymes with
475 biotechnological relevance. DNA polymerase I (pelag12679, pelag14776, pelag14807) from this
476 higher temperature environment could have heat-resistant properties, for example, marginal
477 thermostability conferred by amino acid substitutions (56).

478 After the major analyses had been completed for this study, two SAR11 genomes (46) and one
479 *Prochlorococcus* genome (27) derived from cultivated strains were sequenced, and four
480 *Prochlorococcus* genomes were assembled from metagenomes (57). Of the SAR11 genomes,
481 one was assigned to subclade Ia and the other to subclade Ib (46). Of note, the subclade Ia
482 genome (RS39) contained several OGs also found among the Red-Sea-associated SAR11 OGs:
483 3-oxoacyl-acyl-carrier-protein synthase, ABC branched amino acid transporter,
484 arylsulfotransferase, formate dehydrogenases, glycosyl transferases, methyltransferases, sialic
485 acid synthase, sucrose synthase, sulfotransferases, and a type II restriction–modification system.
486 Several of these functions may play roles in one-carbon and sugar metabolism by SAR11 in the
487 Red Sea (46). The *Prochlorococcus* genome was assigned to the HLII ecotype and notably

488 contained a pathway for biosynthesis of the osmolyte (compatible solute) glucosylglycerol (27).
489 This pathway represents a possible adaptation to the higher salinity of the Red Sea. However, the
490 three genes in this pathway were not found among the Red-Sea-associated *Prochlorococcus*
491 OGs, nor were they found elsewhere among the retained or removed contigs from the Red Sea
492 SAGs (BLASTN).

493 **Expression of ortholog groups in the Red Sea water column**

494 To further test the idea that there could be OGs of ecological importance endemic to the Red Sea,
495 we analyzed metatranscriptomes from the Red Sea. Any OGs with functional roles would be
496 expected to be expressed in the Red Sea water column. We collected seawater and filtered the
497 prokaryotic fraction from a station in the central Red Sea over a broad temporal and depth range:
498 samples were collected at four depths and 13 timepoints over a 48-hour period. We extracted and
499 sequenced RNA from these samples and mapped the reads to the Red Sea SAGs.

500 We found that around two-thirds of RS-OGs were expressed in one or more sample: 64% for
501 SAR11 (Fig. 3b), 66% for *Prochlorococcus* (Fig. 3d). This was more than the fraction of non-
502 RS-OGs expressed: 32% for SAR11 (Fig. 3a), 20% for *Prochlorococcus* (Fig. 3c). We were
503 curious if the high fraction of non-RS-OGs that were unexpressed was due to many of these OG
504 being singletons (OGs having only one member). To the contrary, heatmaps of OG size
505 vs. number of metatranscriptomes in which the OG was found (Fig. 3, inset) do not show a high
506 density of singleton OGs having no expression in non-RS-OGs, and rather the trend toward
507 singletons is more common in RS-OGs.

508 Of OGs expressed in at least one sample, non-RS-OGs (Fig. 3a,c) tended to be expressed in more
509 samples than RS-OGs (Fig. 3b,d). This is consistent with many of the non-RS-OGs being core
510 genes, many of which are housekeeping genes that are often constitutively expressed. Overall,
511 the expression patterns indicate that the majority of RS-OGs are transcribed to messenger RNA,
512 consistent with the synthesis of functional gene products.

513 **Distribution of ortholog groups across the global ocean**

514 The analysis to this point has focused on the distribution of OGs among cultured and single-cell
515 genomes and their expression in the Red Sea water column. A set of OGs has been found that is

516 exclusive to Red Sea genomes (to date), and a majority of them are expressed in the water
517 column. However, we cannot rule out the possibility that these OGs appear endemic only
518 because more genomes are not available from around the World Ocean. If we extended our
519 search to global marine metagenomes, instead of just genomes, would we in fact find these
520 putative endemic OGs in other seas?

521 To investigate the possibility that, contrary to our original hypothesis, there may be few truly
522 endemic OGs in the Red Sea microbial community, we analyzed metagenomes collected from
523 across the global ocean by the *Tara* Oceans expedition. We searched for SAR11 and
524 *Prochlorococcus* OGs in 139 prokaryote-fraction metagenomes from the *Tara* Oceans expedition
525 (44), which come from several depths in the water column: surface, mixed layer, deep
526 chlorophyll maximum, and mesopelagic zone. We queried the dataset to determine what fraction
527 of all OGs and what fraction of RS-OGs could be found outside the Red Sea. If RS-OGs
528 represent endemic gene content of the Red Sea, we would expect to find them absent from
529 metagenomes from other regions. Our approach was complementary to a recent study that
530 analyzed the global metapangenome of *Prochlorococcus* in the *Tara* metagenomes, showing the
531 distributions of gene clusters (OGs) with strain-level resolution across the *Tara* samples (58). In
532 the work here, we employed rarefaction and ordination techniques, with a particular focus on
533 RS-OGs.

534 The presence or absence of SAR11 and *Prochlorococcus* orthologs in *Tara* Oceans prokaryote-
535 fraction metagenomes (supplemental files 7 and 8) was plotted as rarefaction curves (Fig. 4).
536 *Tara* Oceans metagenomes were added randomly one by one, and the fraction of SAR11 and
537 *Prochlorococcus* OGs found was tallied and plotted. The rarefaction curves show the average \pm
538 standard deviation of 1000 permutations. They also show the best-case (and worst-case)
539 scenarios, that is, the fraction of OGs found if each new metagenome adds the most (or fewest)
540 new OGs. Between 70–85% of OGs could be found in one or more *Tara* Oceans metagenome
541 (Fig. 4), and in the best-case scenarios it took at most ten metagenomes to find 90% of these OGs
542 (Table S3). The percentage of OGs not found (15–30%) was independent of whether they were
543 ‘Red-Sea-associated’ or not. This result combined with the rarefaction analysis suggests these
544 OGs would be unlikely to be found in the *Tara* samples with deeper sequencing. It is possible

545 that some OGs may be rare and/or divergent enough to be undetectable with the current
546 methodological approach.

547 Across the 139 *Tara* Oceans prokaryote-fraction metagenomes, we found 84.9% (4475/5272) of
548 all SAR11 OGs in one or more metagenomes (leaving 15.1% not found; Fig. 4a) and 72.2%
549 (7537/10439) of all *Prochlorococcus* OGs in one or more metagenome (leaving 27.8% not
550 found; Fig. 4c). In the best-case scenarios, it took only 5 metagenomes to find 90% of the
551 ‘found’ SAR11 OGs and 50 metagenomes to find 99%; it took only 10 metagenomes to find
552 90% of the ‘found’ *Prochlorococcus* OGs and 60 metagenomes to find 99% (Table S3). The
553 fractions of OGs found were similar for RS-OGs, where 81.2% (713/878) of SAR11 OGs were
554 found (leaving 18.8% not found; Fig. 4b) and 69.8% (67/96) of *Prochlorococcus* OGs were
555 found (leaving 30.2% not found; Fig. 4d). That is, RS-OGs were about as likely to be found
556 across the World Ocean as non-RS-OGs. For both SAR11 (Fig. S5a) and *Prochlorococcus* (Fig.
557 S5b), considering the number of *Tara* metagenomes in which each OG was found, RS-OGs were
558 less likely to be found in a large fraction of metagenomes, relative to all OGs. This is not
559 surprising: the set of non-RS-OGs contains all of the core OGs, which would be expected to be
560 found in most if not all samples.

561 To evaluate whether *Tara* Red Sea metagenomes contained any RS-OGs not already found in the
562 non-Red Sea metagenomes, we tested scenarios where the Red Sea metagenomes were added
563 last in the rarefaction analysis. There was no change in the mean curve of cumulative SAR11
564 OGs found when the six *Tara* Red Sea metagenomes were added last (Fig. 4b): all of the SAR11
565 RS-OGs could be found without examining the Red Sea metagenomes. In contrast, there were
566 five *Prochlorococcus* RS-OGs that were added to the cumulative total when the *Tara* Red Sea
567 metagenomes were added last (Fig. 4d). These five OGs, all with unknown function, represent a
568 small fraction of the total *Prochlorococcus* pangenome (10439 OGs total). Given the available
569 genomes, this study may have uncovered a small set of OGs (Table S2) that possibly reflect gene
570 content endemic to or generally associated with Red Sea environmental conditions, and this
571 marks an area for further research. In light of this metagenomic analysis, however, it appears that
572 the putative RS-OGs provide a relatively minor contribution to the whole and that these new
573 SAR11 and *Prochlorococcus* genomes from the Red Sea generally reflect global pangenomes.

574 Finally, we were curious if OG composition as a whole could show the Red Sea metagenomes to
575 be different from the other metagenomes, despite the lack of evidence of endemic OGs. More
576 generally, could the relative abundance of OGs across *Tara* be used to distinguish populations of
577 *Prochlorococcus* and SAR11?

578 We used the tables of OG counts in the 63 *Tara* surface (SRF) prokaryote-fraction metagenomes
579 to do PCA ordination on the *Tara* metagenomes (Fig. 5; top OGs driving separation among the
580 metagenomes provided in Table S4). SAR11 OG composition (Fig. 5a) was not obviously
581 structured by temperature differences in the temperate and tropical ranges, though Red Sea
582 samples clustered together, and polar samples were separate from the others. *Prochlorococcus*
583 OG composition (Fig. 5b), however, was structured by temperature differences in the temperate
584 and tropical ranges. The four Red Sea samples were split, with two samples clustering with the
585 warm samples and two samples with the cooler samples. These Red Sea samples are positioned
586 where they would be expected based on temperature: the two southern samples (latitude: 18.4
587 °N, 22.0 °N) were warmer (temperature: 27.6 °C, 27.3 °C) and clustered with other
588 warm/tropical samples (left side of PC1 in Fig. 5b); the two northern samples (latitude: 23.36 °N,
589 27.16 °N) were cooler (temperature: 25.8 °C, 25.1 °C) and clustered closer to the cool/temperate
590 samples (right side of PC1 in Fig. 5b). Note these temperatures are lower than average Red Sea
591 surface waters because the *Tara* Red Sea samples were collected in winter (January); by contrast,
592 the Red Sea samples in the World Ocean Database (see above) were collected in spring (April).
593 Given that temperature tolerances generally lack known genetic markers (59), these data suggest
594 an area for future investigation.

595 In summary, the analysis of *Prochlorococcus* and SAR11 OGs in *Tara* Oceans metagenomes
596 shows that (i) most “Red-Sea-associated” OGs are actually widely distributed across the World
597 Ocean, not endemic to the Red Sea; and (ii) OG distribution patterns as a whole, taking relative
598 abundance into account, place the Red Sea on a continuum with other seas, with patterns
599 explained by environmental factors including temperature. Supporting this idea, differences in
600 the relative abundance of OGs—with physicochemical properties covarying with OG
601 functions—have been observed among the North Pacific, Sargasso Sea, Mediterranean Sea, and
602 Red Sea in previous comparative metagenomics studies (11, 12). Despite the Red Sea existing at
603 the periphery of multiple physicochemical parameters in the World Ocean, its distinctiveness

604 may best be revealed by the relative abundance of OGs rather than in the wholesale presence or
605 absence of OGs. In addition to this general pattern, this effort also identified a small set of
606 putative and non-hypothetical proteins that warrant further ecological and biotechnological
607 study.

608 **Conclusions and future directions**

609 Here we analyzed SAR11 and *Prochlorococcus* SAGs from an undersampled ocean region, the
610 Red Sea. This single-cell sequencing effort included SAR11 SAGs from undersampled clades
611 and provided the first genomes from SAR11 subclades 1b and 1d. Our analysis of these genomes
612 provided significant contributions to the reference databases of these organisms, adding 878 new
613 ortholog groups to the SAR11 pangenome and 96 new ortholog groups to the *Prochlorococcus*
614 pangenome. We described a new method called “OG ordination” that uses PCA of ortholog
615 group composition to resolve phylogenetic differences in closely related genomes and used it to
616 distinguish *Prochlorococcus* ecotypes LLII and LLIII in our samples.

617 How marine microbes are able to respond to a changing ocean will be critical to understanding
618 the future biosphere of planet Earth. At the population and community levels, the cosmopolitan
619 distribution of genetic functions may confer an advantage, enabling marine microbial
620 populations and communities, as a whole, to rapidly respond and adapt to changing ocean
621 conditions. Here we generally considered the Baas Becking hypothesis (“Everything is
622 everywhere, but the environment selects”) from the perspective of gene ortholog groups (“Every
623 OG is everywhere, but the environment selects”). The overall data analysis lends support to the
624 Baas Becking hypothesis as applied to OGs. We described a small set of OGs that may be related
625 to Red Sea environmental conditions and that mark areas for further investigation. However, the
626 overall analysis was not consistent with endemism as a primary feature. Instead, we found Red
627 Sea OGs to be nearly as prevalent across global ocean metagenomes as in Red Sea
628 metagenomes. This view was supported by analysis of OG relative abundance rather than
629 absolute presence–absence of OGs. Perhaps OGs may be present but undetectable in a region,
630 and they become detectable after OG frequencies increase in response to environmental
631 conditions (via the growth of cells containing those OGs). Therefore, genomic adaptations in a
632 given ocean region may not simply reflect the presence of OGs unique to a region, but rather the
633 relative abundance of generally cosmopolitan OGs.

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638 Figure Legends

639 **Figure 1.** Maximum-likelihood proteomic trees for single-cell genomes from this study (bold),
640 plus a representative set of cultured genomes. Trees were built from concatenated alignments of
641 (a) 89 SAR11 and (b) 96 *Prochlorococcus* single-copy orthologous genes. Bootstrap values are
642 indicated at the nodes (solid circles $\geq 80\%$ and open circles $\geq 50\%$). Scale bar equals 0.1 change
643 per site. The Red Sea SAR11 SAGs cluster with subclades Ia, Ib, Id, and II. The Red Sea
644 *Prochlorococcus* SAGs all cluster with ecotype HLII.

645 **Figure 2.** PCA ordination of SAGs and genomes based on (a, b) hexanucleotide (6-mer)
646 composition and (c, d) ortholog group (OG) composition. Genomes are colored by clade; single-
647 cell genomes from the Red Sea (this study) are circled in black. OG counts, prior to PCA
648 ordination, were subsampled to 800 (SAR11) or 1400 (*Prochlorococcus*). While both nucleotide
649 composition and OG composition cluster genomes into discrete groups by clade, OG
650 composition differentiate clades more clearly, as exemplified by the separation of
651 *Prochlorococcus* clades LLII and LLIII (panel d).

652 **Figure 3.** Expression of SAG ortholog groups (OGs) in Red Sea metatranscriptomes. The 52
653 metatranscriptomes span a broad range of the water column at a station in the central Red Sea:
654 four depths and 13 timepoints over a 48-hour period (every 4 hours). Histograms show the
655 number of metatranscriptomes found in (a) SAR11 non-RS-OGs, (b) SAR11 RS-OGs, (c)
656 *Prochlorococcus* non-RS-OGs, and (d) *Prochlorococcus* RS-OGs. Heatmaps (inset) show the
657 density of OGs based on OG size (number of total copies across the SAGs) and the number of
658 metatranscriptomes an OG is found in. RS-OGs were more likely than other OGs to be expressed
659 in one or more samples, and non-RS-OGs that were expressed were more likely to be expressed
660 in a high number of samples.

661 **Figure 4.** Rarefaction analysis showing the proportion of (a, c) all OGs and (b, d) RS-OGs of
662 SAR11 and *Prochlorococcus* observed in *Tara* Oceans metagenome samples. Curves show the
663 cumulative number of OGs observed in *Tara* Oceans samples (e -value $< 1e-5$) as more samples
664 are added. Yellow lines show the average \pm standard deviation of 1000 permutations of
665 randomly added samples. Blue lines show the “best-case scenario” (each sample added is that
666 with the most number of new OGs observed) and “worst-case scenario” (each sample added is
667 that with the fewest number of new OGs observed). Red lines show the mean of 1000
668 permutations of randomly added samples but with Red Sea samples (031_SRF_0.22-1.6,
669 032_DCM_0.22-1.6, 032_SRF_0.22-1.6, 033_SRF_0.22-1.6, 034_DCM_0.22-1.6,
670 034_SRF_0.22-1.6) added last. As more *Tara* metagenome samples are added to the analysis, the
671 number of new OGs identified approaches a plateau where new samples do not reveal many new
672 OGs. The same is true with RS-OGs, even when samples from the Red Sea are added last, with
673 the exception of 5 *Prochlorococcus* OGs (proch20367, proch20368, proch20390, proch20423,
674 and proch20438).

675 **Figure 5.** Principal components analysis of *Tara* Oceans surface samples by the abundance of
676 (a) SAR11 and (b) *Prochlorococcus* OGs. The ordination shows the similarity of *Tara* Oceans
677 samples to each other along the first two principal components. Samples are colored by *Tara*
678 temperature categories: ‘polar’ samples (<10 °C) are dark blue, ‘temperate’ samples (10–20 °C)
679 are light blue, ‘tropical’ samples (>20 °C) are orange, and Red Sea ‘tropical’ samples are orange
680 with black edges. Red Sea samples and *Tara* samples generally show more separation based on
681 temperature when ordinated by *Prochlorococcus* OG composition than by SAR11 OG
682 composition.

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Table 1. Genomic features of *Prochlorococcus* and SAR11 single-cell genomes. Single cells were isolated from a surface sample from the Eastern Red Sea (19.75 °N, 40.05 °E). *Prochlorococcus* clades are ecotypes; SAR11 clades are subclades. Completeness is reported as the fraction of 1144 *Prochlorococcus* or 649 SAR11 single-copy core OGs found in each SAG; completeness is also reported as the percent of bacterial single-copy core OGs present as determined by CheckM. Redundancy of bacterial single-copy core OGs is defined as the “contamination” parameter from the CheckM software.

Genus	SAG ref. no.	Clade	Contigs	Assembled size (bp)	Genes	Single-copy core genes	Completeness (core, %)	Completeness (CheckM, %)	Redundancy (CheckM, %)	G+C (%)
<i>Prochlorococcus</i>	SCGC AAA795-F05	HLII	136	1,418,374	1632	1033	90.2	78.6	0.27	31.4
<i>Prochlorococcus</i>	SCGC AAA795-I06	HLII	120	1,388,767	1604	981	85.9	77.5	0.10	31.1
<i>Prochlorococcus</i>	SCGC AAA795-I15	HLII	221	1,282,941	1428	989	86.6	70.7	0.97	31.3
<i>Prochlorococcus</i>	SCGC AAA795-J16	HLII	85	1,463,721	1691	1033	90.3	78.7	0.52	31.0
<i>Prochlorococcus</i>	SCGC AAA795-M23	HLII	93	1,443,989	1710	1012	88.7	74.6	0.34	31.2
SAR11	SCGC AAA795-A08	Ia	61	374,567	384	158	24.3	24.5	0.00	28.3
SAR11	SCGC AAA795-A20	Ia	63	1,140,609	1199	584	90.0	76.7	0.00	29.1
SAR11	SCGC AAA795-B16	Ib	95	551,717	600	331	51.0	34.7	0.06	29.4
SAR11	SCGC AAA795-C09	Ia	82	667,038	734	390	60.1	44.6	0.88	28.4
SAR11	SCGC AAA795-C10	Ia	55	477,445	503	213	32.8	34.9	0.23	29.3
SAR11	SCGC AAA795-D22	Ia	68	1,010,421	1082	555	85.5	69.9	0.60	28.8
SAR11	SCGC AAA795-E07	II	101	681,366	737	418	64.4	56.9	1.37	29.7
SAR11	SCGC AAA795-E22	Ib	63	801,227	820	417	64.3	47.6	0.34	29.0
SAR11	SCGC AAA795-F16	Ib	74	945,491	1017	509	78.4	65.9	0.00	29.1
SAR11	SCGC AAA795-G15	II	62	294,337	342	132	20.3	19.1	0.46	30.5
SAR11	SCGC AAA795-J21	Ia	77	872,902	954	404	62.2	51.5	0.70	29.1
SAR11	SCGC AAA795-K18	Ia	114	731,292	782	314	48.4	48.7	0.70	29.9
SAR11	SCGC AAA795-L23	Ia	150	834,822	910	489	75.3	54.4	0.60	27.8
SAR11	SCGC AAA795-M18	Ib	61	1,050,527	1072	456	70.3	58.9	1.41	29.2
SAR11	SCGC AAA795-M22	Ib	80	860,157	921	515	79.4	64.2	0.13	29.4
SAR11	SCGC AAA795-N08	Ia	157	575,315	622	272	41.9	33.3	0.55	29.1
SAR11	SCGC AAA795-N17	II	94	611,592	620	361	55.6	38.0	0.42	29.5
SAR11	SCGC AAA795-O19	Ia	62	804,609	862	379	58.4	54.2	0.04	29.1
SAR11	SCGC AAA795-O20	Ia	62	1,009,143	1074	526	81.0	69.0	0.04	29.0
SAR11	SCGC AAA795-P11	Ia	127	977,727	1021	485	74.7	52.4	1.32	29.2
SAR11	SCGC AAA797-I19	Ia	77	1,016,895	1071	468	72.1	66.4	0.59	29.2









