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1 **Widespread microbial mercury methylation genes in the global ocean**

2 Emilie Villar^{1,2}, Lea Cabrol^{1*}, Lars-Eric Heimbürger-Boavida^{1*}

3

4 ¹Aix Marseille Université, Univ Toulon, CNRS, IRD, Mediterranean Institute of Oceanography

5 (MIO) UM 110, 13288, Marseille, France

6 ²Sorbonne Université, Université Pierre et Marie Curie - Paris 6, CNRS, UMR 7144 (AD2M),

7 Station Biologique de Roscoff, Place Georges Teissier, CS90074, Roscoff, 29688 France

8 *Both authors contributed equally to this work.

9 Corresponding Author: Léa Cabrol, lea.cabrol@mio.osupytheas.fr

10

11 **Abstract**

12 Methylmercury is a neurotoxin that bioaccumulates from seawater to high concentrations in

13 marine fish, putting human and ecosystem health at risk. High methylmercury levels have

14 been found in the oxic subsurface waters of all oceans, yet only anaerobic microorganisms

15 have been identified so far as efficient methylmercury producers in anoxic environments.

16 The microaerophilic nitrite oxidizing bacteria *Nitrospina* has been previously suggested as a

17 possible mercury methylator in Antarctic sea ice. However, the microorganisms processing

18 inorganic mercury into methylmercury in oxic seawater remain unknown. Here we show

19 metagenomic evidence from open ocean for widespread microbial methylmercury

20 production in oxic subsurface waters. We find high abundances of the key mercury

21 methylating genes *hgcAB* across all oceans corresponding to taxonomic relatives of known

22 mercury methylators from Deltaproteobacteria, Firmicutes and Chloroflexi. Our results

23 identify *Nitrospina* as the predominant and widespread key player for methylmercury
24 production in the oxic subsurface waters of the global ocean.

25

26 **Introduction**

27 Human activities release 2500 tons of inorganic mercury (Hg) every year and have added 55
28 000 tons of Hg to the global ocean since the industrial revolution ¹. Humans are exposed to
29 Hg in the form of methylmercury (MeHg), mainly through the consumption of marine fish.
30 The Minamata Convention (www.mercuryconvention.org) aims to protect human health
31 from the adverse effects of Hg *via* the reduction of anthropogenic, inorganic Hg emissions.
32 To understand the efficacy and time-scales of lowered Hg emissions to reduce fish MeHg
33 levels, we must fully understand the origin of marine MeHg. Microorganisms play a central
34 role in Hg transformations. We must identify the Hg methylating microbes and the factors
35 controlling their distribution in order to better constrain MeHg production in the global
36 ocean.

37 As the only cultured microbes known to produce MeHg to date are anaerobic, research
38 focused for many years on a MeHg source in anoxic marine sediments ²⁻⁵. However, several
39 lines of independent evidence speak in favour of *in situ* MeHg production in oxic seawater as
40 the main source of fish MeHg. Recent large scale oceanographic expeditions found
41 subsurface MeHg maxima in every ocean basin ^{4,6}. The proportion of MeHg to inorganic Hg
42 throughout the oxic seawater column is higher than those found in anoxic sediments.

43 Laboratory experiments show that Hg methylation can occur in anaerobic microniches that
44 occur within sinking particles in oxic waters ⁷. Bianchi et al. ⁸ provide compelling evidence
45 that anaerobic microbes thrive in anoxic microenvironments of sinking particulate organic
46 matter. Independently, incubation experiments with isotopically labelled Hg spikes show

47 significant *in situ* Hg methylation in oxic seawater⁹. Additional evidence stems from Hg
48 stable isotope signatures of marine fish, that can only be explained if 60-80% of the MeHg is
49 produced in open ocean subsurface waters¹⁰. Lastly, a pioneering study found a compound
50 specific $\delta^{13}\text{C}$ signature of fish tissue MeHg similar to algal $\delta^{13}\text{C}$, suggesting that MeHg is
51 produced in the open ocean water column¹¹.

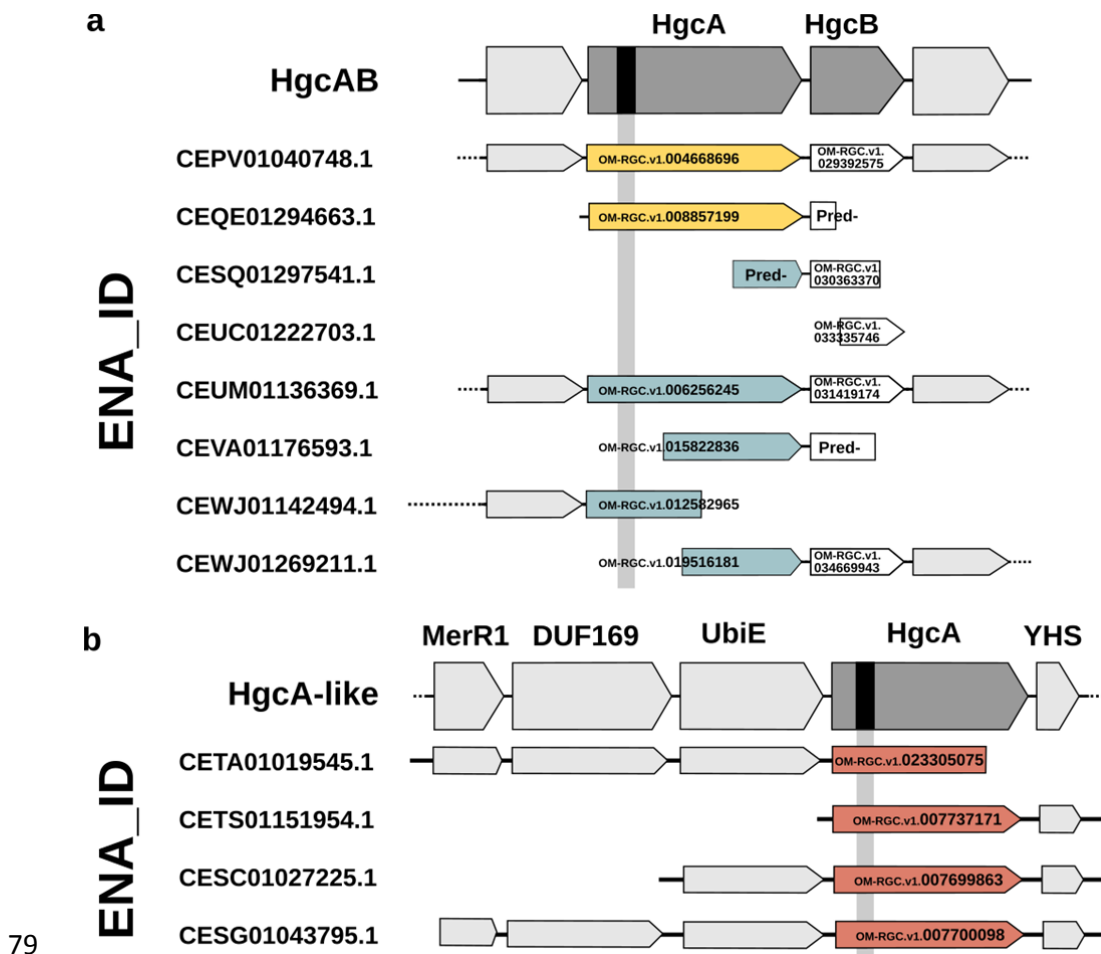
52 A major breakthrough has been made with the discovery of two key genes, *hgcA* and *hgcB*,
53 that control Hg methylation in model anaerobic Hg-methylators⁵. The presence of the *hgcAB*
54 operon predicts Hg methylation capacity in diverse microorganisms². A screening of publicly
55 available microbial metagenomes found the *hgcAB* genes in nearly all anaerobic
56 environments, but the study only rarely detected the genes in pelagic marine water column
57 metagenomes in the open ocean¹². In antarctic sea ice a marine microaerophilic nitrite
58 oxidizing bacterium belonging to the *Nitrospina* genus has been recently identified as a
59 potential Hg methylator with HgcA-like proteins¹³. We aim to resolve the paradox between
60 the wealth of geochemical evidence for *in situ* MeHg production and the absence of known
61 anaerobic Hg methylators in the open ocean. Metagenomic data from 243 *Tara* Oceans
62 samples from 68 open ocean locations covering most ocean basins was analysed to generate
63 an ocean microbial reference gene catalog¹⁴. We screened the *Tara* Oceans metagenomes
64 for the presence of the key *hgcA* methylating gene and provide compelling evidence on the
65 potential key players producing MeHg in the open ocean.

66 Results and Discussion

67 **Identification of HgcAB homologs in the ocean gene catalog**

68 Ten *hgcA* and 5 *hgcB* homolog genes were identified in the Ocean Microbial Reference Gene
69 Catalog¹⁴ (OM-RGC), 6 scaffolds presenting simultaneously *hgcA* and *hgcB* (Fig. 1,

70 Supplementary Table 1). Alignment of HgcA sequences revealed 7 sequences with the
 71 conserved NVWCAA motif⁵ and one sequence with the modified NIWCAA motif on the ‘cap
 72 helix’ region. Mutation experiments previously showed that the structure of the putative
 73 ‘cap helix’ region harbouring Cys93 is crucial for methylation function¹⁵. Two HgcA
 74 sequences were truncated (OM-RGC.v1.019516181, OM-RGC.v1.015822836), preventing
 75 inspection of their conserved motif, but they could be unequivocally assigned to HgcA
 76 sequences based on their phylogenetic placement and high similarity with known HgcA
 77 sequences (Fig. 2). The 5 HgcB sequences presented the conserved motif ECGAC⁵
 78 (Supplementary Table 1).



80 **Figure 1 | The genomic context of the HgcA orthologs. a, HgcAB operon. b, HgcA-like proteins. The**
 81 12 retrieved scaffolds are identified by their ENA_ID on the left of the figure. The solid lines represent

82 the extent of the scaffig sequence and the dashed lines indicate that the scaffig sequence is longer
83 than the represented part. The location of the conserved motif is indicated on the HgcA box by a
84 black bar. When present in *Tara* Oceans samples, the corresponding gene identifier is indicated on
85 the bar for HgcA and HgcB, or indicated as (Pred-) if the gene was incomplete and the protein
86 sequence was partially predicted. The colour of the HgcA boxes refers to the biogeographical
87 clustering as defined in Fig.3 (Cluster 1 in blue, Cluster 2 in yellow, Cluster 3 in red). For Cluster 3
88 sequences (assigned to *Nitrospina*), the genomic context was enlarged to show the closest sequences
89 (MerR1: mercuric resistance operon regulatory protein, UbiE: Ubiquinone/menaquinone
90 biosynthesis C-methyltransferase, DUF169: Hypothetical protein with DUF 169 motif, YHS:
91 Hypothetical protein with YHS domain).

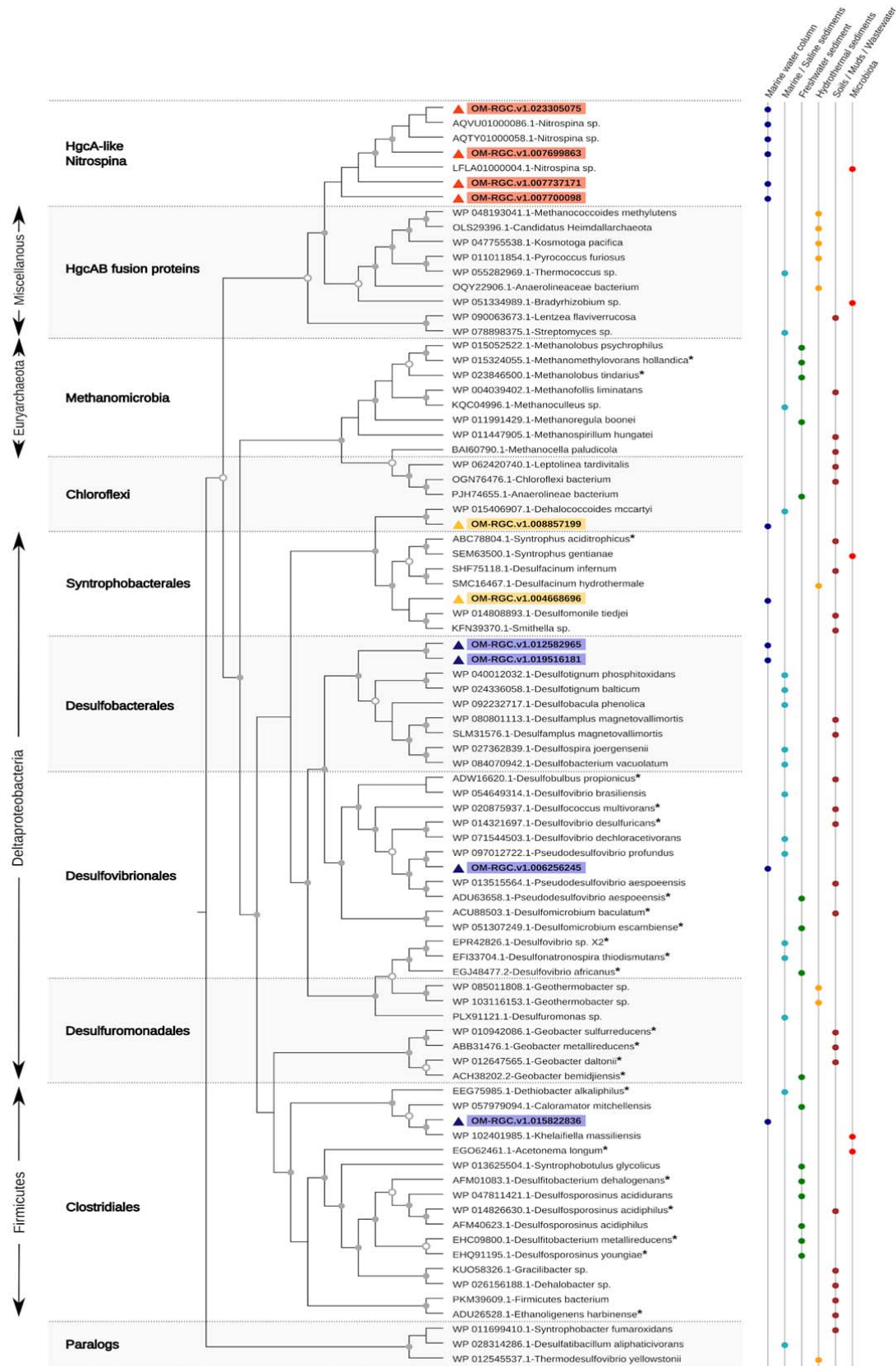
92

93 **HgcA sequences found in the Tara Oceans assemblies covered nearly all known Hg** 94 **methylators**

95 Phylogenetic placement of the 10 sequences found in the *Tara* Oceans assemblies covered
96 nearly all known Hg methylators (Fig. 2). Four sequences (OM-RGC.v1.007700098, OM-
97 RGC.v1.007737171, OM-RGC.v1.023305075, OM-RGC.v1.007699863) were closely related to
98 the HgcA-like proteins described by Gionfriddo et al.¹³ for *Nitrospina* sp. The *Nitrospinae*
99 phylum has been described as a distinct phylogenetic group of lithoautotrophic nitrite
100 oxidizing bacteria exclusively found in marine environments¹⁶, particularly abundant in
101 oxygen-deficient zones¹⁷.

102 The remaining 6 HgcA sequences were distributed between Deltaproteobacteria, Firmicutes
103 and Chloroflexi phyla. Within Deltaproteobacteria, three orders were represented, namely
104 *Desulfovibrionales*, *Desulfobacterales* and *Syntrophobacterales*. OM-RGC.v1.006256245 was
105 most closely related to HgcA from *Pseudodesulfovibrio profundus*, a strictly anaerobic
106 piezophilic sulfate-reducer bacteria (SRB) previously isolated from marine sediment¹⁸. It
107 belongs to the *Desulfovibrionales* order, which contains several members with confirmed
108 Hg-methylating capacity, such as the model species *Desulfovibrio desulfuricans* with

109 exceptionally high Hg-methylation rates, isolated from estuarine sediments¹⁹. OM-
110 RGC.v1.019516181 and OM-RGC.v1.012582965 belonged to *Desulfobacterales*, a well-known
111 order of SRB containing efficient Hg-methylators such as *Desulfobulbus propionicus* and
112 *Desulfococcus multivorans*. Finally, OM-RGC.v1.004668696 belonged to
113 Syntrophobacterales. The closest relative of OM-RGC.v1.004668696 with strong confirmed
114 methylation potential was the non-SRB obligate syntroph *Syntrophus aciditrophicus*².
115 Syntrophic bacteria are important Hg-methylators in low-sulfate ecosystems^{20,21}, where
116 they degrade OM in association with H₂-consuming microorganisms such as sulfate-
117 reducers, iron-reducers and methanogens.
118 Within Firmicutes, OM-RGC.v1.015822836 was tightly related to HgcA from recently isolated
119 human gut bacteria *Khelaifiella* in the Clostridiales order²². Their closest relative with
120 confirmed methylation potential is the non-SRB *Dethiobacter alkaliphilus*, with low to
121 moderate Hg-methylation capacity².
122 OM-RGC.v1.008857199 was related to Chloroflexi, a phylum for which several *hgcAB*-
123 carriers have been identified, but for which experimental confirmation of Hg methylation
124 capacity is still needed. This sequence clusters tightly with HgcA from *Dehalococcoides*
125 *mccartyi*, which has been reported as a potential methylator, albeit in minor abundance, in
126 freshwater marshes²⁰. These two sequences are separated from other Chloroflexi HgcA
127 sequences and more closely related to HgcA sequences from Syntrophobacterales, showing
128 that the taxonomy and the HgcA-phylogeny are not always congruent. The phylogenetically
129 irregular distribution of *hgcA* can be an indication of horizontal gene transfers (HGT) and/or
130 gene deletions in response to stress, suggesting the prevalent influence of environment on
131 Hg-methylation ability²³.



133 **Figure 2 | Phylogenetic tree of HgcA homolog sequences found in the *Tara* Oceans assemblies**

134 Maximum likelihood phylogenies were inferred using PhyML Best AIC Tree with the best model of
135 sequence evolution Blosum62+I+G+F. Branch support was calculated using the non-parametric
136 Shimodaira-Hasegawa-like approximate likelihood ratio test. The triangle colour refers to the
137 biogeographical clustering of the HgcA sequences retrieved from *Tara* Oceans assemblies, as defined
138 in Fig.3 (Cluster 1 in blue, Cluster 2 in yellow, Cluster 3 in red). The tree was rooted using 3
139 paralogues from confirmed non-Hg methylating bacteria. Sequences from experimentally confirmed
140 mercury methylators were indicated with an asterisk. Support values using 1,000 resamples are
141 shown when >50 and coloured squares indicate the isolation source.

142

143 Among the 10 HgcA sequences found in the gene ocean catalogue, none was affiliated to
144 methanogenic *Archaea*. Even if the co-existence of methanogens and sulfate-reducers has
145 been evidenced in marine sediments²⁴, sulfate reduction usually outcompetes
146 methanogenesis in seawater under non-limiting sulfate concentrations²⁵. Our results thus
147 show that Hg-methylators in the ocean span a large taxonomic diversity, not limited to
148 sulfate-reducing bacteria.

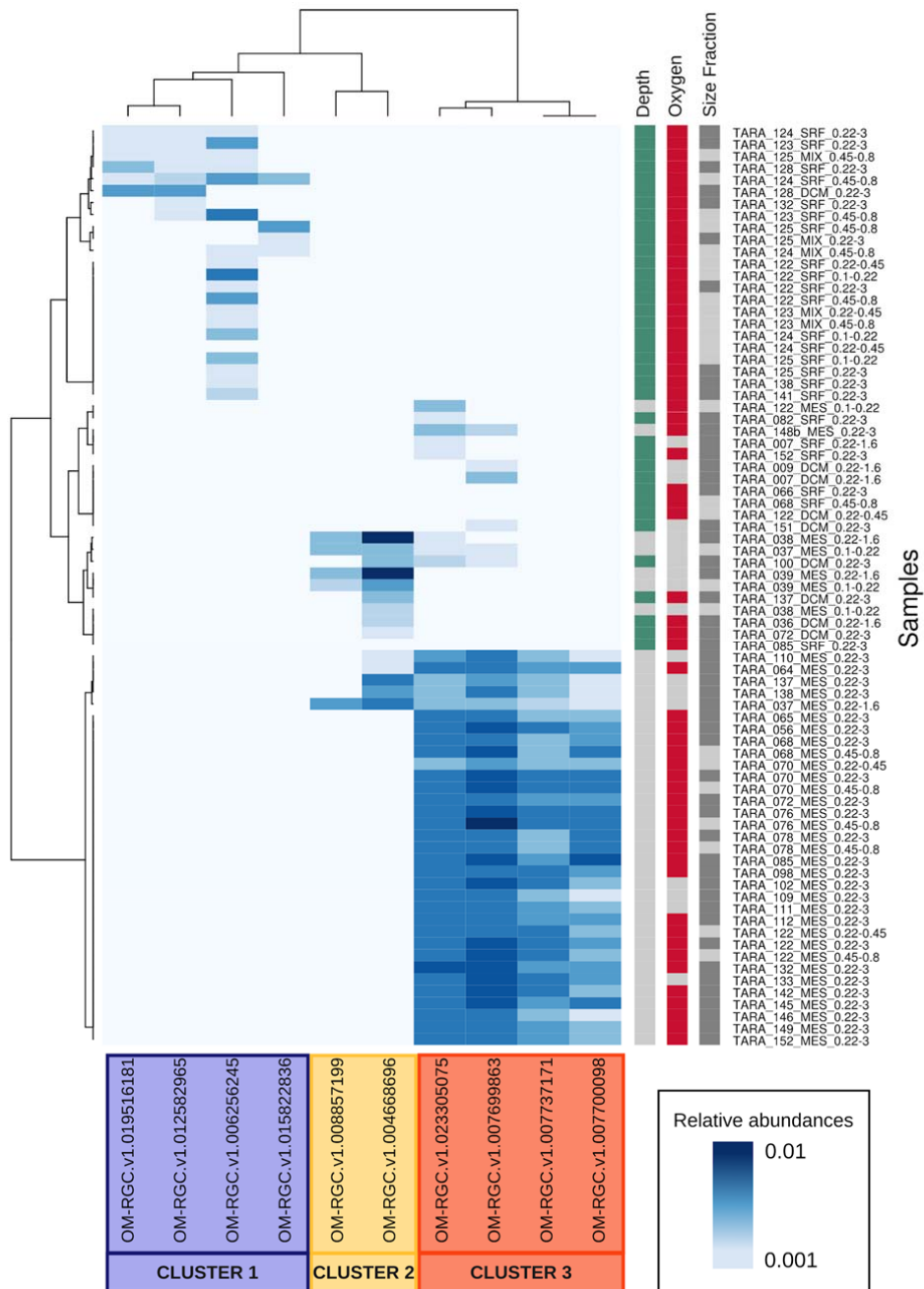
149

150 **Biogeography distinguishes three groups of putative marine Hg methylators**

151 Once clearly identified and phylogenetically assigned, the biogeographic distribution
152 patterns of *hgcA* was evaluated. The 10 HgcA sequences were identified in 77 samples out
153 of the 243 available *Tara* Oceans metagenomes and were clearly distributed in three clusters
154 according to their abundance patterns (Fig. 3). The biogeographic clustering was consistent
155 with the HgcA-phylogeny.

156 Cluster 1 gathered Desulfobacterales, Clostridiales and Desulfovibrionales HgcA sequences,
157 exclusively present in 23 oxic surface waters (< 120 m-depth, > 10 μM_{O_2}). Highest
158 abundances were found in the photic zone of the Pacific Ocean, especially in the area
159 surrounding the Marquesas Islands. This region is characterized by extensive plankton

160 blooms triggered by a physico-chemical phenomenon called Island Mass Effect related to
161 iron fertilization. In this Cluster, the HgcA sequence OM-RGC.v1.006256245 related to the
162 *Desulfovibrionales* order (containing most of the experimentally confirmed Hg-methylators)
163 was the most frequent and abundant in the 23 oxic samples.
164 The phylogenetic placement of the two sequences grouped in Cluster 2 is poorly supported.
165 The most abundant sequence was related to *Smithella* and *Desulfomonile tiedjei*
166 (Syntrophobacterales) while the other one was close to Chloroflexi (Fig. 2, Supplementary
167 Table 2). HgcA sequences from Cluster 2 were identified in 15 surface and subsurface
168 samples, mostly in suboxic waters : sequences found in samples with oxygen concentration
169 below 10 μM accounted for 98% of total Cluster 2 abundances (Supplementary Figure 2).
170 The highest abundances of Cluster 2 HgcA sequences were found in the subsurface waters of
171 the northern stations within the Arabian Sea Oxygen Minimum Zone (Stations TARA_036 to
172 TARA_039), under the influence of a previous major bloom event, where high particle
173 concentrations and strong anaerobic microbial respiration have been reported²⁶. Cluster 2
174 sequences were also found in lower abundance in the shallow anoxic zone of the Pacific
175 North Equatorial Counter Current (Stations TARA_137 and TARA_138, see methods).



176

177 **Figure 3 | Distribution of HgcA in Tara Oceans samples**

178 HgcA relative abundance (from 0.01 to 0.1) is indicated by the white-blue gradient. The hierarchical
 179 clustering highlighted three gene clusters with high abundances in specific samples with marked
 180 environmental features, as suggested by colored squares. Surface samples were collected in the
 181 upper layer (< 120 m-depth, in green) while subsurface were collected below 120 m-depth (in grey).
 182 Seawater was considered as oxic when O₂ > 10 μM (in red) and suboxic when O₂ < 10 μM (in grey).

183 Larger size fraction samples are in dark grey (0.22-3 μm) and smaller size fractions samples (<0.8 μm)
184 are in light grey.

185

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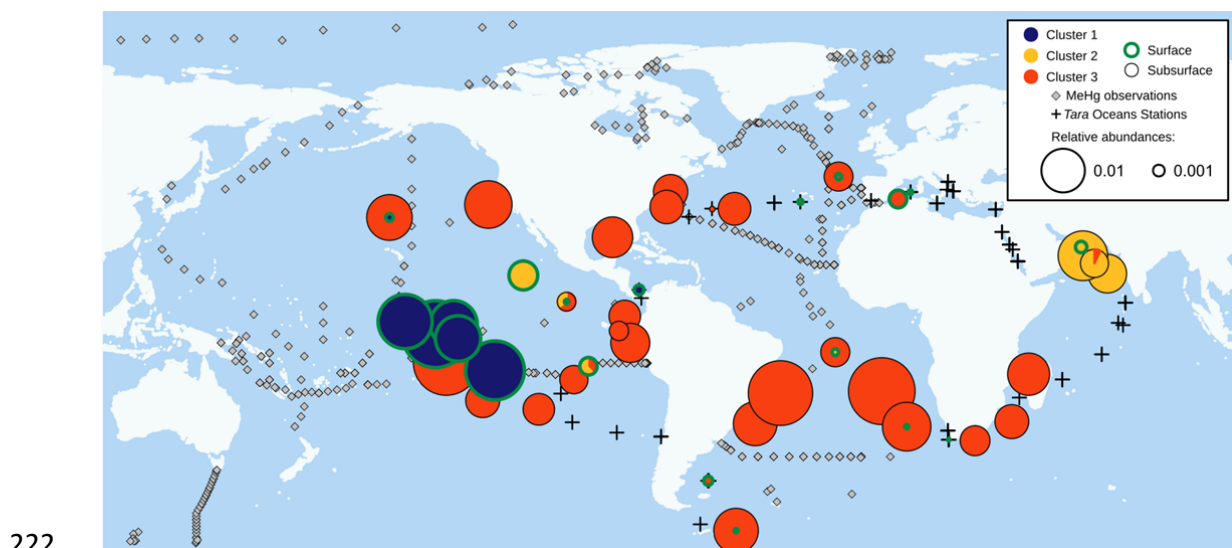
187 The most abundant HgcA-like proteins were grouped in Cluster 3 and were exclusively
188 assigned to *Nitrospina*. These *Nitrospina* HgcA-like proteins were found in 47 samples,
189 widespread across all sampled ocean basins. They were almost exclusively found in
190 subsurface water (> 120 m-depth) and were more frequent in the oxic waters (> 10 μM_{O_2}).
191 Subsurface oxic waters accounted for 84% of total *Nitrospina*-HgcA abundance
192 (Supplementary Figure 2). Their highest relative abundance was found in the South Atlantic
193 and the South Pacific Oceans (Fig. 4, Supplementary Table 2). *Nitrospina* HgcA abundance
194 was positively correlated to nitrate concentration (R 0.54, P < 0.005), which is consistent
195 with *Nitrospina*'s role as nitrate producer through nitrite oxidation, and with the well-known
196 nitrate enrichment with depth in the ocean.

197

198 ***Nitrospina* as the most predominant and widespread methylator in the open ocean**

199 The predominant and widespread HgcA-like homologs were phylogenetically extremely
200 close to the *Nitrospina*-related ones (Supplementary Figure 1) previously identified by
201 metagenomic analysis as potential Hg-methylators within Antarctic sea ice and brine, and
202 further detected by PCR in seawater samples below the ice¹³. The four *Nitrospina* HgcA-like
203 sequences from our study were distinct from HgcA in confirmed Hg-methylators, and also
204 from HgcAB fusion proteins reported in environmental metagenomes¹² (Figure 2). The few
205 cultured strains harboring a fused *hgcAB* gene (*Methanococcoides methylutens* and
206 *Pyrococcus furiosus*) were unable to produce MeHg in experimental conditions^{12,27}. Through
207 sequence alignment against Protein Data Bank templates, we confirmed that the four

208 *Nitrospina* HgcA-like homologs showed high conservation of six residue positions involved in
209 cobalamin binding, which is mandatory for methyl group transfer to Hg¹³ (Supplementary
210 Fig. 1). Protein structure modelling suggests that some *Nitrospina* species may be capable of
211 Hg-methylation. The observed mutations (N71 and C74) do not suppress Hg methylation
212 capacity, according to mutagenesis experiments in the model methylator *D. desulfuricans*
213 ND132¹⁵. The strictly conserved cysteine facilitates the transfer of methyl groups to
214 inorganic Hg²⁸. The two current *Nitrospina* isolates (*N. gracilis* and *N. watsonii*) have not
215 been experimentally tested to date for their Hg-methylation capacity. *N. gracilis* genome
216 lacks the *hgcA* gene. The complete genome of *N. watsonii* is not available. From the 12
217 *Nitrospina* genome assemblies available on NCBI at the time of writing, we found HgcA-like
218 proteins (harbouring the six mandatory residues for Hg-methylation) in three strains only:
219 SCGC AAA288-L16 (single cell whole genome from 770 m-deep ALOHA station, North Pacific
220 Ocean), AB-629-B06 (single cell whole genome from dark ocean water column) and LS_NOB
221 (from a deep-sea sponge holobiont; Supplementary Fig. 1).



222
223 **Figure 4 | HgcA biogeography**

224 Circle sizes are proportional to the cumulated HgcA homolog genes abundances at each station. The
225 pie charts indicate the cluster attribution (legend in the chart), and their border color indicates the

226 sampling depth: surface samples (<120 m-depth) in green and subsurface samples (> 120 m-depth) in
227 grey. *Tara* Oceans stations without detected *HgcAB* genes are represented by black crosses and
228 seawater MeHg profiles from the literature (Supplementary Text 1) by black diamonds.

229

230 Mercury methylation has long been described for anaerobic environments ¹² and *hgcA* genes
231 have been found exclusively in anaerobic Bacteria and Archea ⁵. Yet, we find the most
232 abundant HgcA homologs are strongly dominant in oxic subsurface samples, where they
233 coincide with the subsurface MeHg concentration peaks ⁶, and are carried by the nitrite
234 oxidizing bacteria *Nitrospina*, usually considered as aerobic.

235 Several clues may explain this apparent contradiction. First, it is increasingly recognized that
236 anaerobic processes can occur in anoxic niches such as organic matter aggregates in the
237 middle of oxic waters ⁸. *Nitrospina* sequences were predominantly present in the larger size
238 fractions (accounting for 78% of total HgcA abundances), suggesting that Hg-methylation -as
239 other anaerobic processes- might be associated with particles, where anoxic niches are likely
240 to be favourable to *Nitrospina* methylating activity. Several features suggest the adaptation
241 of *Nitrospina* to low-oxygen environments. *Nitrospina* have been detected as particularly
242 abundant (up to 10% of the bacterial community) in several upwelling and oxygen-deficient
243 zones ²⁹.

244 Comparative genomics revealed a close evolutionary relationship between *Nitrospina* and
245 Anammox bacteria, including horizontal gene transfer events, suggesting the coexistence of
246 these organisms in hypoxic or anoxic environments ¹⁶, as confirmed in incubation
247 experiments ^{30,31}. Genome analysis of several *Nitrospina* strains revealed unexpected
248 adaptation features to low-oxygen environments: no ROS defence mechanism, dependence
249 on highly oxygen-sensitive enzymes for carbon fixation, and high O₂-affinity cytochromes
250 ^{16,32}.

251 *Nitrospina* can play diverse ecological roles beyond the nitrogen cycle³³. *Nitrospina* can use
252 alternative anaerobic pathways to gain energy, using other terminal electron acceptors than
253 O₂ during fermentation under hypoxic or anaerobic conditions, such as sulfur compounds or
254 metal oxides. Their capacity to cope with environmental Hg through methylation is worth
255 considering, since their genome is well equipped against other toxic compounds (arsenate-
256 and mercuric-reductase, metallic cation transporters, multidrug export system)^{16,32}.
257 Mercury methylation potential might have been acquired by horizontal gene transfer.
258 Within the four *Nitrospina* scaffolds harbouring *hgcA*, other neighbour genes related to
259 methyl group transfer and Hg metabolism are found, such as the *merR1* regulator of the *mer*
260 operon involved in Hg resistance, the *ubiE* methyltransferase and the putative metal-binding
261 YHS domain (Fig. 1). This genomic context can lead to hypothesize that the expression of
262 these genes, including *hgcA*, is under the same Hg-induced regulation as the *mer* operon,
263 triggered by *merR*.
264 The choice of *hgcAB* as an indicator of Hg-methylation has to be discussed. First, the
265 presence of *hgcAB* appears necessary but not sufficient for Hg methylation. Indeed,
266 unsuccessful attempts to transfer Hg-methylation capacity to a non-Hg-methylating strain
267 suggest that unidentified additional genes might be needed for effective MeHg production
268¹⁵. Several critical steps are involved in the Hg-methylation process, such as Hg(II) sensing,
269 cellular uptake of Hg(II) by active transport, methyl-group providing and transfer, and MeHg
270 export from the cell. All these steps could be targeted as functional markers of Hg-
271 methylation in the environment in order to provide a more complete picture of the process.
272 Second, the exact contribution of *HgcAB* to Hg-methylation is not well understood. Since Hg
273 methylation does not confer Hg resistance, it cannot be considered as a protection
274 mechanism against Hg toxicity¹⁹. In model strains *D. dechloroacetivorans*, net Hg

275 methylation was not clearly induced by inorganic Hg and not significantly correlated to
276 *hgcAB* gene expression levels, but rather influenced by environmental factors, growth
277 conditions and energetic metabolism^{19,34}. The variability of the methylation potential has
278 been evidenced in different strains, and the implication of *hgcAB* might also vary between
279 strains. Such functional gene approaches are powerful to track biogeochemical potentials in
280 extended environments but remain limited to well described metabolic pathways, ignoring
281 genes with unknown function³⁵.

282 Here, we bring metagenomic evidence for widespread presence of microbial Hg-methylators
283 in the global ocean, thus reconciling with previous geochemical hints pointing to *in situ*
284 MeHg production in the water column. The key Hg-methylating genes found across all
285 oceans corresponded to taxonomic relatives of known Hg-methylators from
286 Deltaproteobacteria, Firmicutes and Chloroflexi phyla. We further identified the
287 microaerophilic NOB *Nitrospina* as the potential dominant Hg-methylator in the global
288 ocean, ubiquitous at the DNA-level, and favoured by oxic subsurface waters (Supplementary
289 Figure 2). A critical next step would be to examine their *hgcA* expression levels and to
290 evaluate Hg-methylation capacity in *Nitrospina* cultures. Further studies should also
291 determine the physicochemical parameters controlling *Nitrospina* Hg-methylation activity
292 level, in order to better understand how they will respond to expected global changes. Our
293 results open new avenues for disentangling the functional role of microorganisms in marine
294 Hg cycling. Our analysis of the *Tara* Oceans metagenomes reveals global distribution of the
295 key Hg methylating genes (*hgcA* and *hgcB*) and pinpoints *Nitrospina* as responsible for
296 widespread open ocean MeHg production in subsurface oxic seawater. Our study implicates
297 the subsurface oxic waters of all oceans as potential source of MeHg that should be
298 considered in the global Hg-cycle budgets, and identifies microbial target for further

299 research on marine MeHg production. We hypothesize that besides anthropogenic Hg
300 emissions, ongoing global climate change might have a previously underestimated effect on
301 *in situ* marine MeHg production by water-column microorganisms, by disturbing microbial
302 assemblages, activity, and environmental drivers governing Hg-methylation.

303 Methods

304 **Identification of HgcAB environmental sequences in oceanic metagenomes.**

305 *hgcA* and *hgcB* genes encode for a putative corrinoid protein, HgcA, and a [2Fe-4S]
306 ferredoxin, HgcB, serving respectively as methyl group carrier and electron donor for
307 corrinoid cofactor reduction. HgcA and HgcB homologs were retrieved by searching Hidden
308 Markov Model profiles (HMM)³⁶ provided by Podar et al.¹² in the Ocean Microbial
309 Reference Gene Catalog¹⁴ (OM-RGC) using the Ocean Gene Atlas³⁷ ([http://tara-
310 oceans.mio.osupytheas.fr/ocean-gene-atlas/](http://tara-oceans.mio.osupytheas.fr/ocean-gene-atlas/)). The OM-RGC is the most exhaustive catalogue
311 of marine genes to date including datasets from *Tara* Oceans metagenomic assemblies and
312 other publicly available marine genomic and metagenomic datasets. We applied an e-value
313 threshold of 1e-20. The corresponding scaffolds (i.e. the assembled sequences where the
314 homolog genes were predicted) were retrieved from the raw assemblies deposited at ENA
315 (Supp Data 1 & 4). Eight scaffolds without *Tara* Oceans mapped reads were discarded. The
316 remaining scaffolds were annotated using Prokka with default parameters³⁸. The resulting
317 translated sequences were aligned separately for HgcA and HgcB using Jalview 2.10 and
318 alignments were cleaned manually³⁹. For further analysis, we kept HgcA sequences if they
319 possess the conserved motif NVWCAA⁵, or if the neighbouring HgcB sequence was present
320 on the scaffold.

321 **HgcA phylogenetic analysis.**

322 A phylogenetic tree was built from the 10 HgcA sequences kept, 55 HgcA protein sequences
323 representative of known Hg-methylator clades belonging to Archaea, Firmicutes, Chloroflexi
324 and Deltaproteobacteria, including 18 experimentally-confirmed Hg-methylators (initially
325 published by Parks et al. ⁵, and updated at
326 <https://www.esd.ornl.gov/programs/rsfa/data.shtml>), as well as 9 HgcAB fusion proteins ¹³
327 and 3 HgcA-like proteins predicted from *Nitrospina* genome assemblies using Prokka ³⁸. The
328 tree was rooted with 3 paralogs from confirmed non-Hg-methylating strains ¹³. The closest
329 sequences (i.e. best e-value match) of each environmental HgcA sequence were retrieved
330 using BLASTp against non-redundant RefSeq protein database excluding sequences from
331 uncultured organisms ⁴⁰, and included in the tree.

332 The 80 sequences were aligned using MAFFT ⁴¹ and gap-containing sites were removed using
333 the mode gappyout of TrimAl ⁴². Maximum likelihood phylogenies were inferred using
334 PhyML Best AIC Tree (version 1.02b) implemented in Phylemon ⁴³ (version 2.0) with the best
335 model of sequence evolution Blosum62+I+G+F. Branch support was calculated using the
336 non-parametric Shimodaira-Hasegawa-like approximate likelihood ratio test. The final tree
337 was edited using Evolview ⁴⁴, especially by annotating the isolation sites retrieved from
338 Genomes OnLine Database ⁴⁵.

339 **Conserved sites in HgcA.**

340 Four sequences from OM-RGC related to *Nitrospina* were aligned with the same Protein
341 Data Bank (PDB) templates as Gionfriddo et al. ¹³, as well as the 3 HgcA-like proteins from
342 *Nitrospina* genome assemblies, and conserved residues were checked. The chosen PDB
343 structural templates (4djd_C, 2h9a_A, 4C1n_C, 2ycl_A) were the gamma subunit of the
344 corrinoid S-Fe acetyl-CoA decarbonylase/synthase complex, identified by Gionfriddo et al. ¹³
345 as the closest and most complete relative to currently unresolved HgcA structure.

346 **Biogeography of HgcA.**

347 Relative HgcA abundances in *Tara* Oceans samples were obtained from the Ocean Gene
348 Atlas³⁷. We screened 243 metagenomes from 68 sites covering the World Ocean except
349 Arctic, sampled at different depths from surface to 500 m-depth, covering six different size
350 fractions ranging from 0 to 3 μm . Environmental data were obtained from Pesant et al.⁴⁶
351 (Supplementary Table 2). For the following analysis, we considered two depths classes
352 (surface samples < 120 m-depth, subsurface samples > 120 m-depth), two particle size
353 fractions (< 5 μm , < 0.8 μm), two oxic states (oxic: $\text{O}_2 > 10 \mu\text{M}$, suboxic: $\text{O}_2 < 10 \mu\text{M}$).
354 HgcA relative abundance was calculated as follows: the length-normalized count of genes
355 read was divided by the median of the length-normalized counts of a set of ten universal
356 single copy marker genes^{47,48}. Thus, relative abundance represents the fraction of bacteria
357 harbouring the *hgcA* gene within the assembled genomes. A heatmap of the relative gene
358 abundances in *Tara* Oceans samples was generated in R⁴⁹ using the heatmap.2 function in
359 the ggplot CRAN library. Dendrograms were computed using hclust default parameters from
360 Ward distance index based on presence/absence of the genes ('binary' option). Genes were
361 clustered into three groups (Cluster 1, Cluster 2 and Cluster 3) according to their abundance
362 pattern on the heatmap. The geographic origin of the *hgcA* genes retrieved from the *Tara*
363 Oceans samples was plotted on a global map using the "mapplots" R package. At each
364 station, the cumulated abundance and phylogenetic affiliation of the retrieved *hgcA* genes
365 were represented on the map by the size and colour of the points. Cluster distribution was
366 also plotted against depth and oxygen concentration at each station to depict the
367 environmental conditions where each Cluster flourishes (Supplementary Figure 2). Tracks of
368 MeHg records from previous campaigns were searched in the literature (Supplementary Text
369 1) and georeferenced on the map.

370

371 References

- 372 1. Outridge, P. M., Mason, R. P., Wang, F., Guerrero, S. & Heimbürger-Boavida, L. E.
373 Updated Global and Oceanic Mercury Budgets for the United Nations Global Mercury
374 Assessment 2018. *Environ. Sci. Technol.* **52**, 11466–11477 (2018).
- 375 2. Gilmour, C. C. *et al.* Mercury methylation by novel microorganisms from new
376 environments. *Environ. Sci. Technol.* **47**, 11810–11820 (2013).
- 377 3. Gilmour, C. C. *et al.* Sulfate-Reducing Bacterium *Desulfovibrio desulfuricans* ND132 as
378 a Model for Understanding Bacterial Mercury Methylation. *Appl. Environ. Microbiol.*
379 **77**, 3938–3951 (2011).
- 380 4. Mason, R. P. *et al.* Mercury biogeochemical cycling in the ocean and policy
381 implications. *Environ. Res.* **119**, 101–117 (2012).
- 382 5. Parks, J. M. *et al.* The genetic basis for bacterial mercury methylation. *Science (80-.)*.
383 **339**, 1332–1335 (2013).
- 384 6. Schlitzer, R. *et al.* The GEOTRACES Intermediate Data Product 2017. *Chem. Geol.* **493**,
385 210–223 (2018).
- 386 7. Ortiz, V. L., Mason, R. P. & Evan Ward, J. An examination of the factors influencing
387 mercury and methylmercury particulate distributions, methylation and demethylation
388 rates in laboratory-generated marine snow. *Mar. Chem.* **177**, 753–762 (2015).

- 389 8. Bianchi, D., Weber, T. S., Kiko, R. & Deutsch, C. Global niche of marine anaerobic
390 metabolisms expanded by particle microenvironments. *Nat. Geosci.* 1–6 (2018).
391 doi:10.1038/s41561-018-0081-0
- 392 9. Lehnherr, I., St. Louis, V. L., Hintelmann, H. & Kirk, J. L. Methylation of inorganic
393 mercury in polar marine waters. *Nat. Geosci.* **4**, 298–302 (2011).
- 394 10. Blum, J. D. Marine mercury breakdown. *Nat. Geosci.* **4**, 139–140 (2011).
- 395 11. Masbou, J. *et al.* Carbon Stable Isotope Analysis of Methylmercury Toxin in Biological
396 Materials by Gas Chromatography Isotope Ratio Mass Spectrometry. *Anal. Chem.* **87**,
397 11732–11738 (2015).
- 398 12. Podar, M. *et al.* Global prevalence and distribution of genes and microorganisms
399 involved in mercury methylation. *Sci. Adv.* **1**, (2015).
- 400 13. Gionfriddo, C. M. *et al.* Microbial mercury methylation in Antarctic sea ice. *Nat.*
401 *Microbiol.* **1**, 16127 (2016).
- 402 14. Sunagawa, S. *et al.* Ocean plankton. Structure and function of the global ocean
403 microbiome. *Science* **348**, 1261359 (2015).
- 404 15. Smith, S. D. *et al.* Site-directed mutagenesis of HgcA and HgcB reveals amino acid
405 residues important for mercury methylation. *Appl. Environ. Microbiol.* **81**, 3205–17
406 (2015).
- 407 16. Lückner, S., Nowka, B., Rattei, T., Spieck, E. & Daims, H. The Genome of *Nitrospina*
408 *gracilis* Illuminates the Metabolism and Evolution of the Major Marine Nitrite

- 409 Oxidizer. *Front. Microbiol.* **4**, 27 (2013).
- 410 17. Spieck, E., Keuter, S., Wenzel, T., Bock, E. & Ludwig, W. Characterization of a new
411 marine nitrite oxidizing bacterium, *Nitrospina watsonii* sp. nov., a member of the
412 newly proposed phylum “Nitrospinae”. *Syst. Appl. Microbiol.* **37**, 170–176 (2014).
- 413 18. Cao, J. *et al.* *Pseudodesulfovibrio indicus* gen. nov., sp. nov., a piezophilic sulfate-
414 reducing bacterium from the Indian Ocean and reclassification of four species of the
415 genus *Desulfovibrio*. *Int. J. Syst. Evol. Microbiol.* **66**, 3904–3911 (2016).
- 416 19. Gilmour, C. C. *et al.* Sulfate-reducing bacterium *Desulfovibrio desulfuricans* ND132 as
417 a model for understanding bacterial mercury methylation. *Appl. Environ. Microbiol.*
418 **77**, 3938–51 (2011).
- 419 20. Bae, H.-S., Dierberg, F. E. & Ogram, A. Syntrophs dominate sequences associated with
420 the mercury methylation-related gene *hgcA* in the water conservation areas of the
421 Florida Everglades. *Appl. Environ. Microbiol.* **80**, 6517–26 (2014).
- 422 21. Sorokin, D. Y., Tourova, T. P., Mußmann, M. & Muyzer, G. *Dethiobacter alkaliphilus*
423 gen. nov. sp. nov., and *Desulfurivibrio alkaliphilus* gen. nov. sp. nov.: two novel
424 representatives of reductive sulfur cycle from soda lakes. *Extremophiles* **12**, 431–439
425 (2008).
- 426 22. Tidjani Alou, M. *et al.* Gut Bacteria Missing in Severe Acute Malnutrition, Can We
427 Identify Potential Probiotics by Culturomics? *Front. Microbiol.* **8**, 899 (2017).
- 428 23. Regnell, O. & Watras, C. J. Microbial Mercury Methylation in Aquatic Environments: A

- 429 Critical Review of Published Field and Laboratory Studies. *Environ. Sci. Technol.* **53**, 4–
430 19 (2019).
- 431 24. Sela-Adler, M. *et al.* Co-existence of Methanogenesis and Sulfate Reduction with
432 Common Substrates in Sulfate-Rich Estuarine Sediments. *Front. Microbiol.* **8**, 766
433 (2017).
- 434 25. Pak, K. R. & Bartha, R. Mercury methylation by interspecies hydrogen and acetate
435 transfer between sulfidogens and methanogens. *Appl. Environ. Microbiol.* **64**, 1987–
436 1990 (1998).
- 437 26. Roullier, F. *et al.* Particle size distribution and estimated carbon flux across the
438 Arabian Sea oxygen minimum zone. *Biogeosciences* **11**, 4541–4557 (2014).
- 439 27. Gilmour, C. C., Bullock, A. L., McBurney, A., Podar, M. & Elias, D. A. Robust mercury
440 methylation across diverse methanogenic Archaea. *MBio* **9**, (2018).
- 441 28. Zhou, J., Riccardi, D., Beste, A., Smith, J. C. & Parks, J. M. Mercury Methylation by
442 HgcA: Theory Supports Carbanion Transfer to Hg(II). *Inorg. Chem.* **53**, 772–777 (2014).
- 443 29. Levipan, H. A., Molina, V. & Fernandez, C. Nitrospina-like bacteria are the main drivers
444 of nitrite oxidation in the seasonal upwelling area of the Eastern South Pacific (Central
445 Chile ~36°S). *Environ. Microbiol. Rep.* **6**, 565–573 (2014).
- 446 30. Füssel, J. *et al.* Nitrite oxidation in the Namibian oxygen minimum zone. *ISME J.* **6**,
447 1200–1209 (2012).
- 448 31. Beman, J. M., Leilei Shih, J. & Popp, B. N. Nitrite oxidation in the upper water column

- 449 and oxygen minimum zone of the eastern tropical North Pacific Ocean. *ISME J.* **7**,
450 2192–2205 (2013).
- 451 32. Ngugi, D. K., Blom, J., Stepanauskas, R. & Stingl, U. Diversification and niche
452 adaptations of Nitrospina-like bacteria in the polyextreme interfaces of Red Sea
453 brines. *ISME J.* **10**, 1383–1399 (2016).
- 454 33. Daims, H., Lückner, S. & Wagner, M. A New Perspective on Microbes Formerly Known
455 as Nitrite-Oxidizing Bacteria. *Trends Microbiol.* **24**, 699–712 (2016).
- 456 34. Goñi-Urriza, M. *et al.* Relationships between bacterial energetic metabolism, mercury
457 methylation potential, and *hgcA/hgcB* gene expression in *Desulfovibrio*
458 *dechloroacetivorans* BerOc1. *Environ. Sci. Pollut. Res.* **22**, 13764–13771 (2015).
- 459 35. Reed, D. C., Algar, C. K., Huber, J. A. & Dick, G. J. Gene-centric approach to integrating
460 environmental genomics and biogeochemical models. *Proc. Natl. Acad. Sci. U. S. A.*
461 **111**, 1879–84 (2014).
- 462 36. Eddy, S. R. Accelerated profile HMM searches. *PLoS Comput. Biol.* **7**, e1002195 (2011).
- 463 37. Villar, E. *et al.* The Ocean Gene Atlas: exploring the biogeography of plankton genes
464 online. *Nucleic Acids Res.* **46**, W289–W295 (2018).
- 465 38. Seemann, T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**, 2068–
466 2069 (2014).
- 467 39. Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M. & Barton, G. J. Jalview
468 Version 2—a multiple sequence alignment editor and analysis workbench.

- 469 *Bioinformatics* **25**, 1189–1191 (2009).
- 470 40. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment
471 search tool. *J. Mol. Biol.* **215**, 403–410 (1990).
- 472 41. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7:
473 improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
- 474 42. Capella-Gutierrez, S., Silla-Martinez, J. M. & Gabaldon, T. trimAl: a tool for automated
475 alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–
476 1973 (2009).
- 477 43. Sanchez, R. *et al.* Phylemon 2.0: a suite of web-tools for molecular evolution,
478 phylogenetics, phylogenomics and hypotheses testing. *Nucleic Acids Res.* **39**, W470–
479 W474 (2011).
- 480 44. Gao, S. *et al.* Evolveview v2: an online visualization and management tool for
481 customized and annotated phylogenetic trees. *Nucleic Acids Res.* **44**, W236–W241
482 (2016).
- 483 45. Mukherjee, S. *et al.* Genomes OnLine Database (GOLD) v.6: data updates and feature
484 enhancements. *Nucleic Acids Res.* **45**, D446–D456 (2017).
- 485 46. Pesant, S. *et al.* Open science resources for the discovery and analysis of Tara Oceans
486 data. *Sci. Data* **2**, 150023 (2015).
- 487 47. Mende, D. R., Sunagawa, S., Zeller, G. & Bork, P. Accurate and universal delineation of
488 prokaryotic species. *Nat. Methods* **10**, 881–884 (2013).

489 48. Sunagawa, S. *et al.* Metagenomic species profiling using universal phylogenetic
490 marker genes. *Nat. Methods* **10**, 1196–1199 (2013).

491 49. R Development Core Team. R: A Language and Environment for Statistical Computing.
492 (2008).

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