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Brief Report

Widespread microbial mercury methylation genes in the global ocean

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Summary

Methylmercury is a neurotoxin that bioaccumulates from seawater to high concentrations in marine fish, putting human and ecosystem health at risk. High methylmercury levels have been found in the oxic subsurface waters of all oceans, but only anaerobic microorganisms have been shown to efficiently produce methylmercury in anoxic environments. The microaerophilic nitrite-oxidizing bacteria *Nitrospina* have previously been suggested as possible mercury methylating bacteria in Antarctic sea ice. However, the microorganisms responsible for processing inorganic mercury into methylmercury in oxic seawater remain unknown. Here, we show metagenomic and metatranscriptomic evidence that the genetic potential for microbial methylmercury production is widespread in oxic seawater. We find high abundance and expression of the key mercury methylating genes *hgcAB* across all ocean basins, corresponding to the taxonomic relatives of known mercury methylating bacteria from *Deltaproteobacteria*, *Firmicutes* and *Chloroflexi*. Our results identify *Nitrospina* as the predominant and widespread microorganism carrying and actively expressing *hgcAB*. The highest *hgcAB* abundance and expression occurs in the oxic subsurface waters of the global ocean where the highest MeHg concentrations are typically observed.

Introduction

Human activities release 2500 tons of inorganic mercury (Hg) every year and have added 55 000 tons of Hg to the global ocean since the industrial revolution (Outridge *et al*., 2018). Humans are exposed to Hg in the form of methylmercury (MeHg), mainly via marine fish consumption. The Minamata Convention (www.mercuryconvention.org) aims to protect human health from the adverse effects of Hg by decreasing anthropogenic inorganic Hg emissions. We must fully understand the origin of marine MeHg to evaluate the efficacy and time-scales of lowered Hg emissions aimed at decreasing fish MeHg levels. Microorganisms play a central role in Hg transformations. We must identify Hg methylating microbes and the factors controlling their distribution to better constrain MeHg production in the global ocean.

Since the only cultured microbes known to produce MeHg are anaerobic, research has focused for many years on a MeHg source in anoxic marine sediments (Gilmour *et al*., 2011; Mason *et al*., 2012; Gilmour *et al*., 2013; Parks *et al*., 2013). However, several lines of independent evidence support *in situ* MeHg production in oxic seawater as the main source of fish MeHg. Recent large-scale oceanographic expeditions found subsurface MeHg maxima in every ocean basin (Mason *et al*., 2012; Schlitzer *et al*., 2018). The proportion of MeHg to inorganic Hg throughout the oxic seawater column is higher (about 40%) than in anoxic sediments (about 5%). Laboratory experiments show that Hg methylation can occur in anoxic microniches that occur within sinking particles in oxic waters (Ortiz *et al*., 2015). Bianchi and colleagues (2018) provide compelling evidence that anaerobic microbes thrive in the anoxic microenvironments of sinking particulate organic matter. Independently, incubation experiments with isotopically labelled Hg spikes show significant *in situ* Hg methylation in oxic seawater (Lehnherr *et al*., 2011). Additional evidence stems from Hg stable isotope signatures of marine fish, which can

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only be explained if 60%–80% of the MeHg is produced in open ocean subsurface waters (Blum, 2011). Finally, a pioneering study found a compound-specific $\delta^{13}C$ signature of fish tissue MeHg similar to algal $\delta^{13}C$, suggesting that MeHg is produced in the open ocean water column (Masbou *et al*., 2015).

A major breakthrough was made with the discovery of two key genes, *hgcA* and *hgcB*, which control Hg methylation in model anaerobic Hg-methylating bacteria (Parks *et al*., 2013). The presence of the *hgcAB* operon predicts Hg methylation capacity in diverse microorganisms (Gilmour *et al*., 2013). A recent methodological evaluation study established that *hgcAB* gene identification in metagenomic datasets using Pfam/HMM models is a reliable method to determine Hg-methylating organism abundance and diversity (Christensen *et al*., 2019). A screening of publicly available microbial metagenomes found *hgcAB* genes in nearly all anoxic environments, but the study only rarely detected the genes in pelagic marine water column metagenomes in the open ocean (Podar *et al*., 2015). In Antarctic sea ice marine microaerophilic nitrite-oxidizing bacteria belonging to the *Nitrospina* genus haves been recently identified as potential Hg methylating bacteria with HgcA-like proteins (Gionfriddo *et al*., 2016). We aim to resolve the paradox between several biogeochemical evidences for *in situ* MeHg production and the absence of known anaerobic Hg methylating prokaryotes in the open ocean. Metagenomic and metatranscriptomic data from 243 and 187 *Tara* Oceans samples, collected from 68 and 108 open ocean locations covering most ocean basins respectively, were analysed to generate an ocean microbial reference gene catalogue (Sunagawa *et al*., 2015; Salazar *et al*., 2019). We screened the *Tara* Oceans metagenomes and metatranscriptomes for the presence of the key *hgcA* methylating gene. We provide compelling evidence of the potential bacterial key players involved in MeHg production in the open ocean. The experimental demonstration of MeHg production by seawater microorganisms was beyond the scope of this study.

Results and discussion

Identification of HgcAB homologues in the ocean gene catalogue

Twelve scaftigs were identified by Hidden Markov Model search of *hgcA* and *hgcB* in the Ocean Microbial Reference Gene Catalogue (Sunagawa *et al*., 2015) (OM-RGC), performed at the Ocean Gene Atlas website (Villar *et al*., 2018). Six scaftigs contiguously contained *hgcA* and *hgcB* (Fig. 1, Table S1, Text S1). The other six scaftigs contained either *hgcA* or *hgcB* sequence because of an incomplete assembly. Alignment of HgcA sequences revealed seven sequences with the conserved NVWCAA motif (Parks *et al*., 2013) and one sequence with the modified NIWCAA motif in the 'cap helix' region. Mutation experiments previously showed that the structure of the putative 'cap helix' region harbouring Cys93 is crucial for methylation capacity (Smith *et al*., 2015). Two HgcA sequences were truncated (OM-RGC.v1.019516181, OM-RGC.v1.015822836), preventing inspection of their conserved motif. However, they could be unequivocally assigned to HgcA sequences based on their phylogenetic placement and high similarity with known HgcA sequences (Fig. 2). The five HgcB sequences contained the conserved motif ECGAC (Parks *et al*., 2013) (Table S1). Eventually, 10 HgcA sequences were considered for the following analysis, based on the presence of the conserved motif NVWCAA, and/or the presence of the neighbouring HgcB sequence on the scaftig, and the corresponding complete protein sequence prediction in *Tara* Oceans samples (Fig. 1).

Fifty-four percent of the 77 metagenomic samples containing the *hgcA* gene with paired metatranscriptome data available contained the *hgcA* transcript with the identical sequence in the corresponding metatranscriptome, indicating active expression of the gene (Table S2, Fig. S3).

HgcA sequences found in the Tara Oceans assemblies covered nearly all known Hg methylating bacteria

The 10 HgcA sequences found in the *Tara* Oceans assemblies were phylogenetically diverse (Fig. 2, Text S1). Nine of the 10 *hgcA* genes have a corresponding transcript in the metatranscriptomic dataset, indicating effective expression and probable activity in all phylogenetically distinct taxa (Table S2). Four sequences (OM-RGC.v1.007700098, OM-RGC.v1.007737171, OM-RGC.v1.023305075 and OM-RGC.v1.007699863) were closely related to the HgcA-like proteins described by Gionfriddo and colleagues (2016) for *Nitrospina* sp. The *Nitrospinae* phylum is a phylogenetically distinct group of lithoautotrophic nitrite-oxidizing bacteria exclusively found in marine environments (Lücker *et al*., 2013), and they are particularly abundant in oxygen-deficient zones (Spieck *et al*., 2014).

The remaining six HgcA sequences were distributed between *Deltaproteobacteria*, *Firmicutes* and *Chloroflexi* phyla. Three orders were represented within *Deltaproteobacteria*: *Desulfovibrionales*, *Desulfobacterales* and *Syntrophobacterales*. OM-RGC.v1.006256245 was most closely related to HgcA from *Pseudodesulfovibrio profundus*, a strictly anaerobic piezophilic sulphate-reducing bacteria (SRB) previously isolated from marine sediment (Cao *et al*., 2016). OM-RGC.v1.006256245 belongs to the *Desulfovibrionales* order, which contains several members

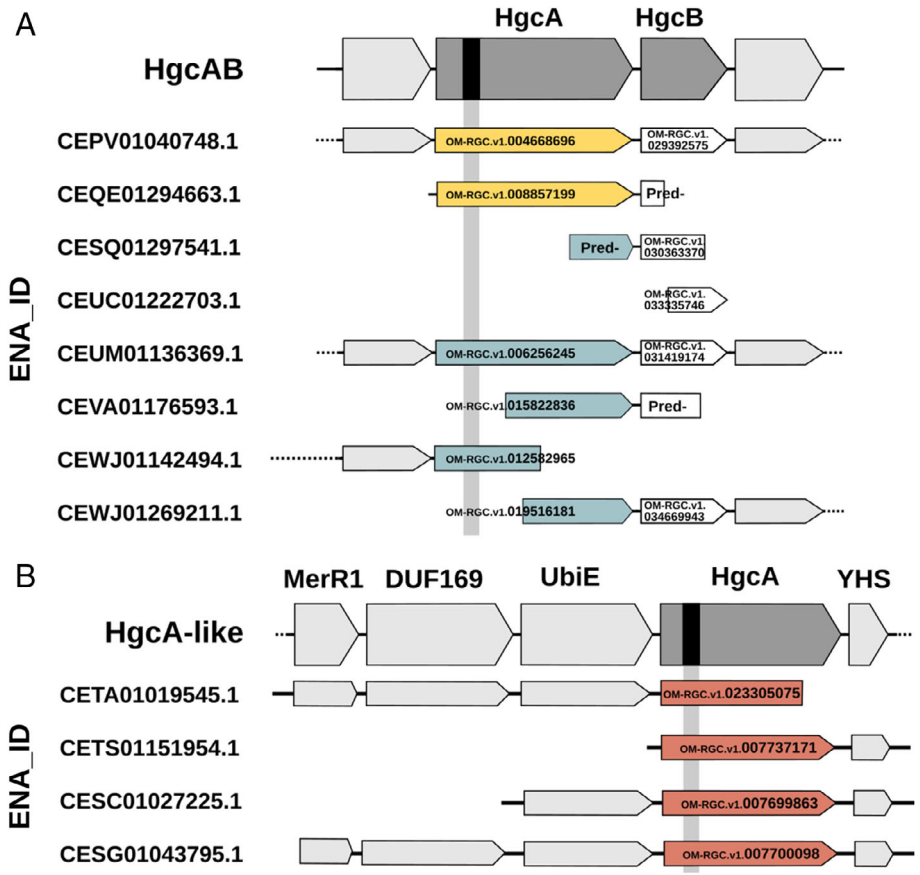

Fig. 1. The genomic context of HgcA orthologues. A, HgcAB operon. B, HgcA-like proteins. HgcA and HgcB homologues were retrieved by searching Hidden Markov Model profiles (HMM) in the Ocean Microbial Reference Gene Catalogue (OM-RGC). The 12 corresponding scaftigs (i.e. the assembled sequences where the homologue genes were predicted) retrieved from the raw assemblies deposited at ENA are represented on the figure. Coding DNA sequences (CDS) along the scaftig were predicted and annotated using Prokka with default parameters. Grey boxes show the CDS, solid lines represent the extent of the scaftig sequence and dashed lines indicate that the scaftig sequence is longer than the represented section. The resulting translated sequences were aligned separately for HgcA and HgcB using Jalview 2.10, and alignments were cleaned manually. For further analysis, we kept 10 HgcA sequences based on the presence of the conserved motif NVWCAA or the presence of the neighbouring HgcB sequence on the scaftig. The location of the conserved motif is indicated on the HgcA box by a black bar. When present in *Tara* Oceans samples, the corresponding gene identifier is indicated in the HgcA and HgcB boxes or indicated as (Pred-) if the gene was incomplete and the protein sequence was only partially predicted. The colour of the HgcA boxes refers to the biogeographical clustering as defined in Fig. 3 (Cluster 1 in blue, Cluster 2 in yellow and Cluster 3 in red). For Cluster 3 sequences (assigned to *Nitrospina*), the genomic context was enlarged to show the closest sequences (MerR1: mercuric resistance operon regulatory protein, UbiE: Ubiquinone/menaquinone biosynthesis C-methyltransferase, DUF169: Hypothetical protein with DUF 169 motif, YHS: Hypothetical protein with YHS domain).

with confirmed Hg-methylating capacity, like the model species *Desulfovibrio desulfuricans*, which has exceptionally high Hg-methylation rates (Gilmour *et al*., 2011). OM-RGC.v1.019516181 and OM-RGC.v1.012582965 belonged to *Desulfobacterales*, a well-known order of SRB containing efficient Hg-methylating bacteria like *Desulfobulbus propionicus* and *Desulfococcus multivorans*. Finally, OM-RGC.v1.004668696 belonged to *Syntrophobacterales*. The closest relative of OM-RGC.v1.004668696 with high methylation potential was the non-SRB obligate syntroph *Syntrophus aciditrophicus* (Gilmour *et al*., 2013). Syntrophic bacteria are important Hg-methylating bacteria in low-sulphate ecosystems (Sorokin *et al*., 2008; Bae *et al*., 2014). They degrade OM in association with H_2-consuming microorganisms such as SRB, iron-reducing bacteria and methanogens.

Within *Firmicutes*, OM-RGC.v1.015822836 was closely related to HgcA from recently isolated human gut bacteria *Khelaifiella* in the Clostridiales order (Tidjani Alou *et al*., 2017). Their closest relative with confirmed methylation potential is the non-SRB *Dethiobacter alkaliphilus*, which has low to moderate Hg-methylation capacity (Gilmour *et al*., 2013).

OM-RGC.v1.008857199 was related to *Chloroflexi*, a phylum for which several *hgcAB*-carriers have been identified, but for which experimental confirmation of Hg-methylation capacity is still needed. The closest HgcA relative from OM-RGC.v1.008857199 belongs to

Dehalococcoides mccartyi, previously reported as a potential Hg-methylating bacterium poorly abundant in freshwater marshes (Bae *et al*., 2014). These two sequences are separated from other *Chloroflexi* HgcA sequences, and they are more closely related to HgcA sequences from *Syntrophobacterales*. These results

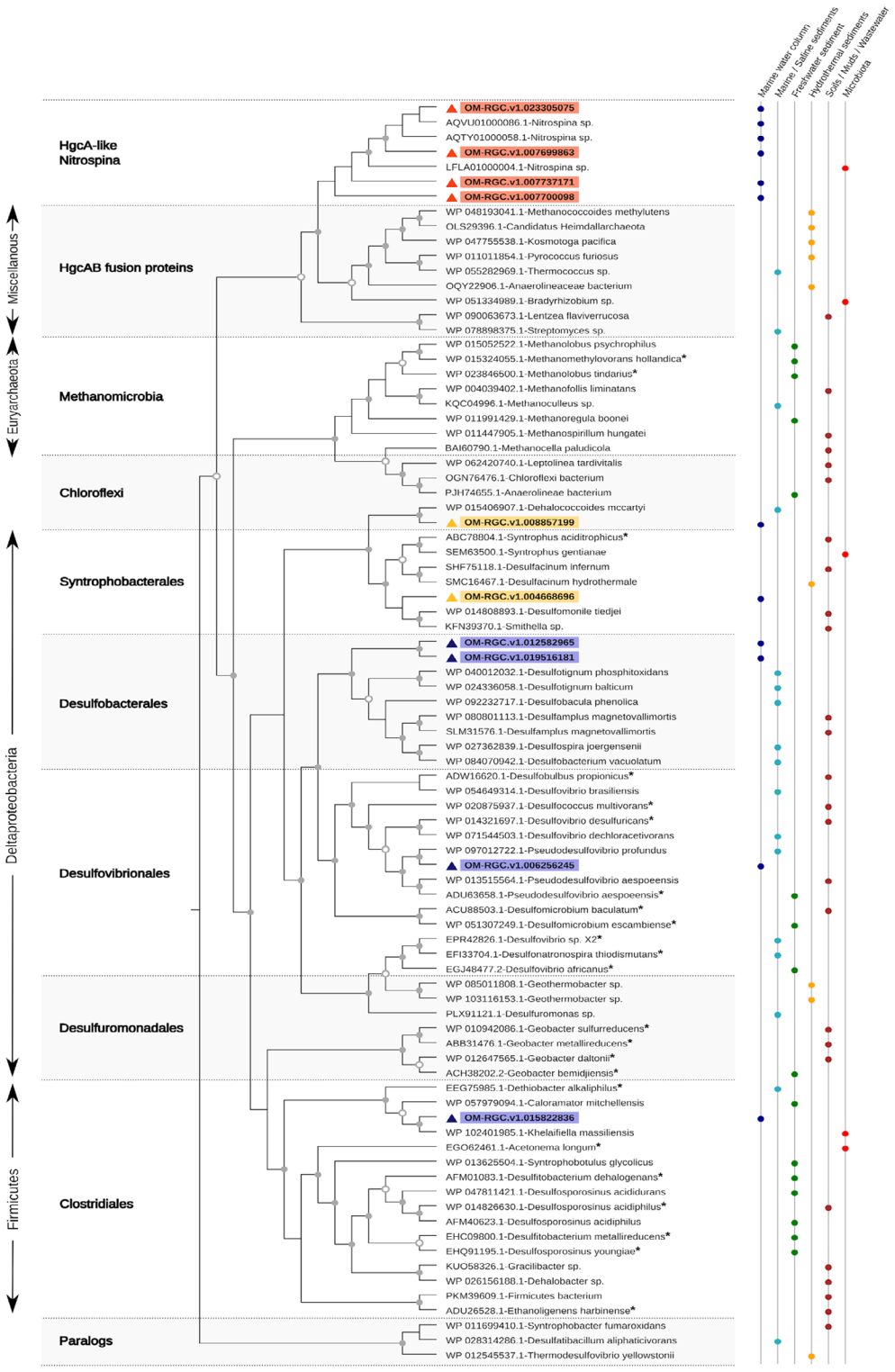

Fig. 2. Legend on next page.

show that the taxonomy and the HgcA-phylogeny are not always congruent. The phylogenetically irregular distribution of *hgcA* can be an indication of horizontal gene transfers (HGT) or gene deletions in response to stress, suggesting the environment's prevalent influence on Hg-methylation ability (Regnell and Watras, 2019).

None of the 10 HgcA sequences found in the gene ocean catalogue was affiliated to methanogenic *Archaea*. Even if the coexistence of methanogens and SRB was shown in marine sediments (Sela-Adler *et al*., 2017), sulphate reduction usually outcompetes methanogenesis in seawater under non-limiting sulphate concentrations (Pak and Bartha, 1998). Thus, our results show that Hg-methylating prokaryotes in the ocean span a large taxonomic diversity not limited to SRB.

Biogeography distinguishes three groups of phylogenetically separated putative marine Hg-methylating bacteria

The screening of the 243 available *Tara* Ocean metagenomes revealed that 77 samples contained reads mapping to at least one of the 10 previously identified *hgcA* sequences. The 10 HgcA sequences were clearly distributed in three clusters according to their abundance patterns (Fig. 3, Text S1). The biogeographic clustering was consistent with the HgcA phylogeny. For all three clusters, the stations with the highest *hgcA* gene abundance also had the highest *hgcA* transcript abundance (Table S2).

Cluster 1 contained *Desulfobacterales*, *Clostridiales* and *Desulfovibrionales* HgcA sequences. They were present in 23 metagenomic samples and eight metatranscriptomic samples, and they all exclusively came from oxic surface waters (<120 m-depth, >10 μM_{O2}). The highest frequency and abundance of Cluster 1-*hgcA* genes and transcripts were found in the photic zone of the South Pacific Ocean, especially in the area surrounding the Marquesas Islands. This region is characterized by extensive plankton blooms triggered by a physico-chemical phenomenon called the Island Mass Effect, which is related to iron fertilization (Caputi *et al*., 2019). The HgcA sequence OM-RGC.v1.006256245 related to the *Desulfovibrionales* order (containing most of the experimentally confirmed Hg-methylating bacteria) was the most frequent and abundant in this cluster among the 23 oxic samples.

The phylogenetic placement of the two sequences grouped in Cluster 2 is poorly supported. The most abundant gene and transcript sequence in Cluster 2 was related to *Syntrophobacterales* (with *Smithella* and *Desulfomonile tiedjei* as closest relatives), and the other one was close to *Chloroflexi* (Fig. 2, Table S2). HgcA sequences from Cluster 2 were identified in 15 surface and subsurface samples, mostly in suboxic waters. Sequences found in samples with oxygen concentrations below 10 μM accounted for 98% of all Cluster 2 abundances (Fig. S2). Cluster 2 sequences were also found in nine metatranscriptomic samples corresponding to the same stations. The highest abundances of Cluster 2 *hgcA* gene and transcript sequences were found in the subsurface waters of the northern stations within the Arabian Sea Oxygen Minimum Zone (Stations TARA_036 to TARA_039) under the influence of a previous major bloom event, where high particle concentrations and strong anaerobic microbial respiration were reported (Roullier *et al*., 2014). Cluster 2 sequences were also less abundant in the shallow anoxic zone of the Pacific North Equatorial Counter Current (Stations TARA_137 and TARA_138, see methods).

The most abundant *hgcA* genes and transcripts were grouped in Cluster 3 and exclusively assigned to *Nitrospina*. These *Nitrospina* HgcA-like proteins were found in 47 metagenomic samples and 22 metatranscriptomic samples, showing that they are widespread across all sampled ocean basins, including in seven Arctic stations from the *Tara* Oceans Polar Circle campaign. They were almost exclusively found in subsurface water (>120 m-depth), and they were more frequent in oxic waters (>10 μM_{O2}). Subsurface oxic waters accounted for 84% of total *Nitrospina*-HgcA abundance (Fig. 2). The highest relative abundance of *Nitrospina hgcA* genes was found in the South Atlantic and the South Pacific Oceans (Fig. 4, Table S2). *Nitrospina hgcA* abundance was positively correlated to nitrate concentration (R 0.54, $P < 0.005$), which is consistent with

Fig. 2. Phylogenetic tree of HgcA homologue sequences found in the *Tara* Oceans assemblies. The phylogenetic tree was built with the 10 HgcA sequences from this study and 60 HgcA protein sequences belonging to *Archaea*, *Firmicutes*, *Chloroflexi* and *Deltaproteobacteria*, including 23 sequences from experimentally confirmed Hg-methylating prokaryotes (indicated with an asterisk), and nine HgcAB fusion proteins and three HgcA-like proteins predicted from *Nitrospina* genome assemblies using Prokka. The tree was rooted using three paralogues from confirmed non-Hg methylating bacteria. The closest relative sequences (i.e. best e-value match) of each HgcA sequence here were retrieved using BLASTp against the non-redundant RefSeq protein database excluding sequences from uncultured organisms. The 85 sequences were aligned using MAFFT, and gap-containing sites were removed using the mode gappyout of TrimAl. Maximum likelihood phylogenies were inferred using PhyML Best AIC Tree with the best model of sequence evolution Blosum62 + I + G + F implemented in Phylemon (version 2.0). Branch support was calculated using the non-parametric Shimodaira–Hasegawa-like approximate likelihood ratio test. The triangle colour refers to the biogeographical clustering of the HgcA sequences retrieved from *Tara* Oceans assemblies, as defined in Fig. 3 (Cluster 1 in blue, Cluster 2 in yellow and Cluster 3 in red). Support values using 1000 resamples are shown when >50, and coloured squares indicate the isolation source.

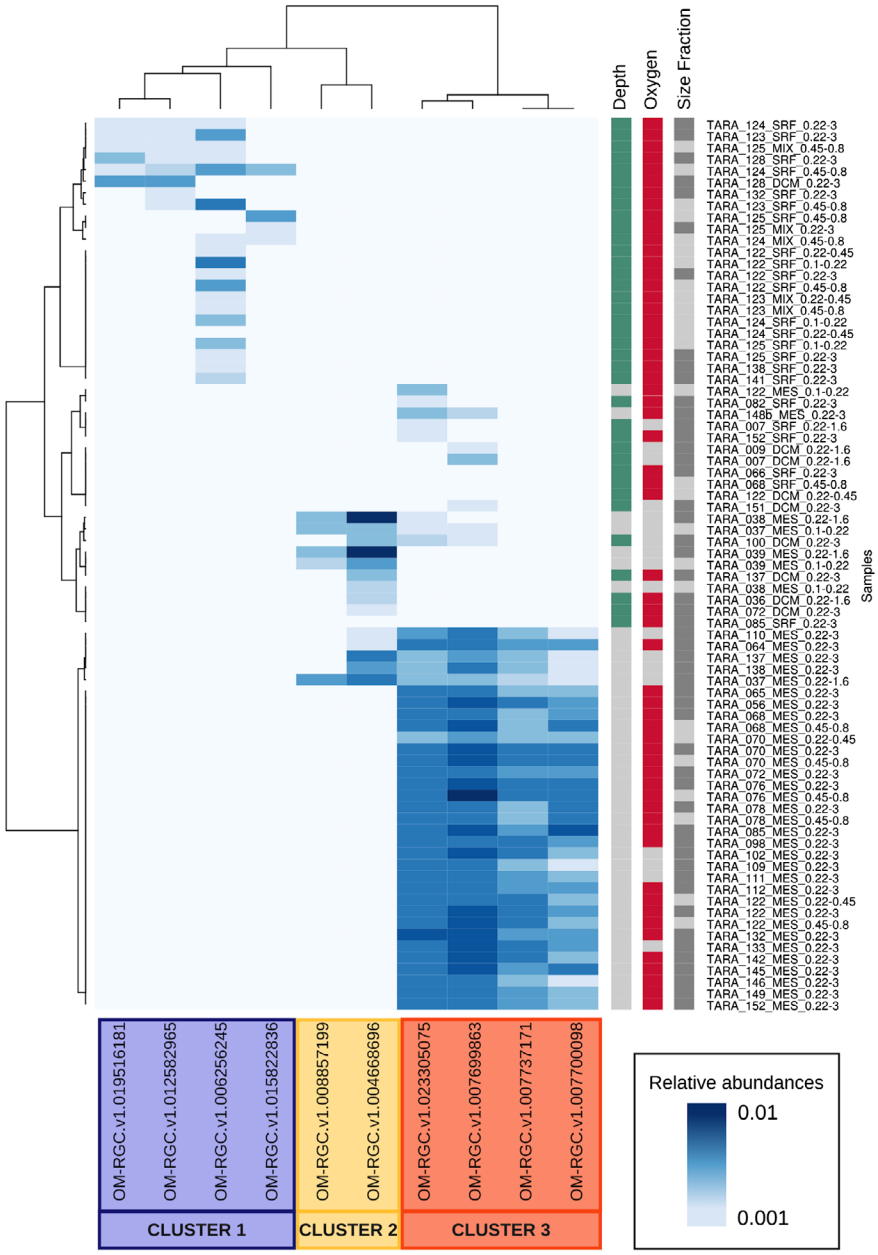

Fig. 3. Distribution of HgcA in *Tara* Oceans samples. We screened 243 metagenomes from 68 sites covering the Global Ocean (except Arctic), sampled at different depths from the surface to 500 m deep, and extracted from six different size fractions ranging from 0 to 3 μm. Relative *hgcA* abundances in Tara Oceans samples were obtained from the Ocean Gene Atlas and represented by the white-blue gradient. The relative abundances represent the fraction of bacteria harbouring the *hgcA* gene within the assembled genomes. The hierarchical clustering highlighted three gene clusters with contrasting high abundances in specific samples with marked environmental features, as indicated by the coloured squares on the right. Surface samples were collected in the upper layer (<120 m-depth, in green), and subsurface samples were collected below 120 m deep (in grey). Seawater was considered oxic when O_2 > 10 μM (in red) and suboxic when O_2 < 10 μM (in grey). Larger size fraction samples are in dark grey (0.22–3 μm) and smaller size fractions samples (<0.8 μm) are in light grey. The heatmap was generated in R using the heatmap.2 function in the ggplot CRAN library. Dendrograms were computed using hclust default parameters from Ward distance index based on the presence or absence of the genes ('binary' option).

Nitrospina's role as a nitrate producer via nitrite oxidation. Nitrate is known to be enriched with increasing depth in the ocean. *Nitrospina-hgcA* transcripts are more widely distributed than Cluster 1 and Cluster 2 transcripts (Fig. S3). The higher abundance of Cluster 1 and Cluster 2 transcripts in few (three) stations must be interpreted carefully because the abundance of metatranscriptomic reads does not solely and directly reflect the differential expression of a gene. Transcript abundance is also a function of the bacterial abundance and maybe biased by

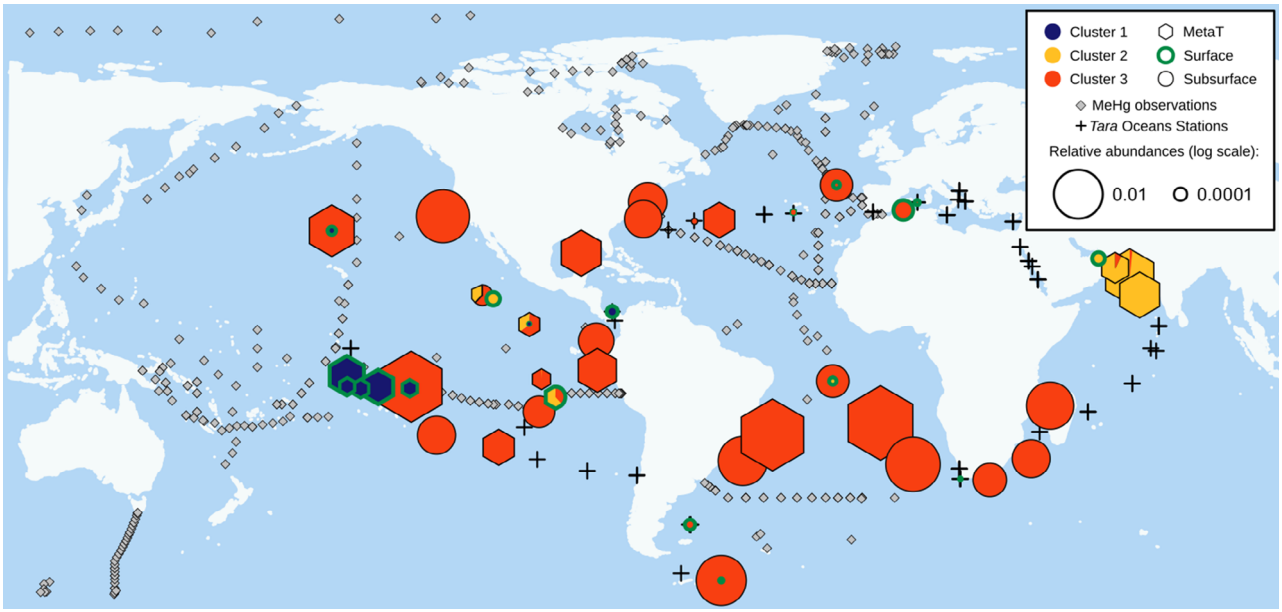

Fig. 4. HgcA biogeography. The distribution of HgcA in the Global Ocean is represented by coloured symbols. Circles indicate stations where *hgcA* genes were found in metagenomes only, and hexagons represent stations where *hgcA* transcripts were also found in metatranscriptomes. Symbol size is proportional to the cumulated HgcA homologue genes abundances at each station. The pie charts indicate the cluster attribution: Cluster 1 in blue (*Desulfobacterales*, *Clostridiales* and *Desulfovibrionales*), Cluster 2 in yellow (*Syntrophobacterales* and *Chloroflexi*) and Cluster 3 in red (*Nitrospina*). The circle border colour indicates the sampling depth: surface samples (<120 m deep) in green and subsurface samples (>120 m deep) in grey. Tara Oceans stations without detected HgcAB genes are represented by black crosses and seawater Hg species observations by black diamonds (Text S1). The global map was drawn using the 'mapplots' R package.

several technical issues such as difference of coverage between samples (Salazar *et al*., 2019). As an example, the apparent overexpression of Cluster 1-*hgcA* in the Marquesas Islands could result from oversequencing at these stations.

Microaerobic Nitrospina *as the most predominant and widespread* hgcA*-carrying and expressing bacteria in the oxic open ocean*

The predominant and widespread HgcA-like genes and transcripts in the global ocean were phylogenetically extremely close to the *Nitrospina*-related genes (Fig. S1) previously identified by metagenomic analysis within Antarctic sea ice and brine, and further detected by PCR in seawater samples below the ice (Gionfriddo *et al*., 2016). The four *Nitrospina* HgcA-like sequences from our study were distinct from HgcA in confirmed Hg-methylating bacteria and from HgcAB fusion proteins reported in environmental metagenomes (Podar *et al*., 2015) (Fig. 2). The few cultured strains harbouring a fused *hgcAB* gene (*Methanococcoides methylutens* and *Pyrococcus furiosus*) could not produce MeHg in experimental conditions (Podar *et al*., 2015; Gilmour *et al*., 2018). We used sequence alignment against Protein Data Bank (PDB) templates to confirm that the four *Nitrospina* HgcA-like homologues showed high conservation of six residue positions involved in cobalamin binding, which is mandatory for methyl group transfer to Hg (Gionfriddo *et al*., 2016) (Fig. S1). Among these six residues, the two substitutions (N71 and C74) observed in *Nitrospina* HgcA sequences (compared with the cobalamin interaction domain in PDB templates) do not suppress Hg methylation capacity based on mutagenesis experiments in the model methylating bacterium *D. desulfuricans* ND132 (Smith *et al*., 2015). The strictly conserved cysteine facilitates the transfer of methyl groups to inorganic Hg (Zhou *et al*., 2014). Therefore, protein structure modelling suggests that some *Nitrospina* species may be capable of Hg-methylation. The two current *Nitrospina* isolates (*N. gracilis* and *N. watsonii*) have not been experimentally tested for their Hg-methylation capacity. The *N. gracilis* genome lacks the *hgcA* gene. The complete genome of *N. watsonii* is not available. From the 12 *Nitrospina* genome assemblies available on NCBI at the time of writing, we found HgcA-like proteins (harbouring the six mandatory residues for Hg-methylation) in three strains only: SCGC AAA288-L16 (single-cell whole-genome from 770 m-deep ALOHA station, North Pacific Ocean), AB-629-B06 (single-cell whole-genome from dark ocean water column) and LS_NOB (from a deep-sea sponge holobiont; Fig. S1).

Mercury methylation has long been described in anoxic environments (Podar *et al*., 2015), and *hgcA* genes have

been found exclusively in anaerobic *Bacteria* and *Archaea* (Parks *et al*., 2013). However, we find that the most abundant HgcA homologues are strongly dominant in oxic subsurface samples, where they coincide with the MeHg peaks typically observed in the subsurface waters (Mason *et al*., 2012; Schlitzer *et al*., 2018). They are carried by the nitrite-oxidizing bacteria *Nitrospina*, usually considered to be aerobic organisms.

Several clues may explain this apparent contradiction between anoxic process and oxic environment. First, it is increasingly recognized that anaerobic processes can occur in anoxic niches such as organic matter aggregates in the middle of oxic waters (Bianchi *et al*., 2018). Here, *Nitrospina* sequences were predominantly present in the larger size fractions (accounting for 78% of total HgcA abundances), suggesting that Hg-methylation and other anaerobic processes might be associated with particles, where anoxic niches are likely to be favourable to *Nitrospina* methylating activity. Second, several features suggest that *Nitrospina* is adapted to suboxic environments despite being considered to be an aerobic organism. *Nitrospina* have been detected as particularly abundant (up to 10% of the bacterial community) in several upwelling and oxygen-deficient zones (Levipan *et al*., 2014). Genome analysis of several *Nitrospina* strains revealed unexpected adaptation features to low-oxygen environments. For instance, they have no ROS defence mechanism, they depend on highly oxygen-sensitive enzymes for carbon fixation and they have high O_2-affinity cytochromes (Lücker *et al*., 2013; Ngugi *et al*., 2016). Moreover, since *Nitrospina* and Anammox bacteria have a close evolutionary relationship, share genetic material through HGT, and coexist in anoxic environments (as confirmed by incubations) (Füssel *et al*., 2012; Beman *et al*., 2013; Lücker *et al*., 2013), we can thus assume that *Nitrospina* can live in anoxic environments. *Nitrospina* can use alternative anaerobic pathways to gain energy using other terminal electron acceptors besides O_2 during fermentation under hypoxic or anoxic conditions. The alternative electron acceptors can include sulphur compounds or metal oxides (Daims *et al*., 2016).

Nitrospina is first known as NOB, but it can play diverse ecological roles beyond the nitrogen cycle (Daims *et al*., 2016). The capacity of *Nitrospina* to cope with environmental Hg through methylation is worth considering since its genome is well equipped against other toxic compounds (arsenate- and mercuric-reductase, metallic cation transporters and multidrug export system) (Lücker *et al*., 2013; Ngugi *et al*., 2016). Mercury methylation potential might have been acquired by HGT. Other neighbouring genes related to methyl group transfer and Hg metabolism are found within the four *Nitrospina* scaftigs harbouring *hgcA*, including the *merR1* regulator of the *mer* operon involved in Hg resistance, the *ubiE* methyltransferase and the putative metal-binding YHS domain (Fig. 1). This genomic context suggests the hypothesis that here, the expression of these genes, including *hgcA*, might be under the same Hg-induced regulation as the *mer* operon, which is triggered by *merR*.

The choice of *hgcAB* as an indicator of Hg-methylation is debatable. First, the presence of *hgcAB* appears to be necessary but not sufficient for Hg-methylation. Indeed, unsuccessful attempts to transfer Hg-methylation capacity to a non-Hg-methylating strain suggest that unidentified additional genes might be needed for effective MeHg production (Smith *et al*., 2015). Several critical steps are involved in the Hg-methylation process, including Hg(II) sensing, cellular uptake of Hg(II) by active transport, methyl group providing and transfer and MeHg export from the cell. All these steps could be targeted as functional markers of Hg-methylation in the environment to provide a more complete picture of the process. Second, the exact contribution of HgcAB to Hg-methylation is not well understood. Since Hg methylation does not confer Hg resistance, it cannot be considered as a protection mechanism against Hg toxicity (Gilmour *et al*., 2011). In the model strain *D*. *dechloroacetivorans*, net Hg-methylation was not clearly induced by inorganic Hg additions and not significantly correlated to *hgcAB* gene expression levels, but it was instead influenced by environmental factors, growth conditions and energetic metabolism (Gilmour *et al*., 2011; Goñi-Urriza *et al*., 2015). A recent metaproteomic study confirmed that HgcA protein abundance was independent of total Hg and MeHg concentrations across large gradients (Christensen *et al*., 2019). HgcA protein expression was not likely to be induced by Hg exposure (in the range tested by the authors), and it may be connected to an alternative function encoded by *hgcAB* like a role in C1 metabolism. The presence of the *hgcA* gene conveys the genomic potential for Hg-methylation, but net MeHg production also depends on various biogeochemical controls like Hg-speciation, Hg-particle interactions, Hg bioavailability and photolytic or cellular activity (Bravo and Cosio, 2019). This explains the difficulty in finding correlations between *hgcA* abundance and MeHg concentration. The influence and interdependence of abiotic processes must also be considered. Environmental MeHg concentrations are the results of *in situ* and past Hg-methylation and demethylation. Therefore, it is complicated to try to directly use ambient MeHg concentration as an indicator of Hg-methylation activity. Methylation potential variability has been shown in different strains, and the contribution of *hgcAB* might also vary between strains. Such functional gene approaches are powerful for tracking biogeochemical potentials in extended environments, but they remain limited to well-described metabolic pathways and ignore genes with unknown functions (Reed *et al*., 2014).

Conclusion

Our analysis of the *Tara* Oceans metagenomes and metatranscriptomes reveals the global distribution of the key Hg methylating genes (*hgcA* and *hgcB*) in the open ocean and pinpoints *Nitrospina* as one of the most predominant and widespread bacteria potentially involved in MeHg production in subsurface oxic seawater. We show metagenomic and metatranscriptomic evidence for widespread presence and expression of the key Hg-methylating genes in oxic subsurface, thus reconciling with previous geochemical hints pointing to *in situ* MeHg production in the water column. The *hgcA* genes and transcripts found across all oceans corresponded to taxonomic relatives of known Hg-methylating bacteria from *Deltaproteobacteria*, *Firmicutes* and *Chloroflexi* phyla. We further identified the microaerophilic NOB *Nitrospina* as the potential predominant and widespread Hg-methylating bacteria in the global ocean, both carrying and actively expressing the *hgcA* gene. *Nitrospina*-related *hgcA* was ubiquitous at the DNA and RNA levels, and it was favoured in oxic subsurface waters (Fig. S2). *hgcA* expression was demonstrated in all the phylogenetic taxa found in this study, and it is widespread across all the oceanic basins. This provides an additional clue regarding *in situ* Hg-methylation. Multiple lines of evidence are needed to support *Nitrospina* as a dominant mercury-methylating microorganism. A critical next step is to evaluate Hg-methylation capacity in pure *Nitrospina* cultures or enrichment from seawater to test the contribution of these organisms to Hg-methylation. Further studies should also determine the physicochemical parameters controlling the *Nitrospina* Hg-methylation activity level to better understand how they will respond to expected global changes. Our results open new avenues for disentangling the functional role of microorganisms in marine Hg cycling. Our study implicates the subsurface oxic waters of all oceans as potential sources of MeHg that should be considered in the global Hg-cycle budgets, and it identifies microbial targets for further research on marine MeHg production.

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AUTHOR CONTRIBUTIONS

E.V., L.C., and L.E.H.B. wrote the manuscript. E.V. performed the bioinformatic analyses with the scientific support of L.C.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting Information
Table S1 Summary of the hgcAB homologues found in oceanic metagenomes
Table S2 HgcA homolog abundances in metagenomes and metatranscriptomes, and environmental parameters for each sample
Data S1 Scaftig sequences
Data S2 HgcA protein sequences
Data S3 HgcB protein sequences