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Method

Transcriptome reconstruction and functional analysis of eukaryotic marine plankton communities via high-throughput metagenomics and metatranscriptomics

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Large-scale metagenomic and metatranscriptomic data analyses are often restricted by their gene-centric approach, limiting the ability to understand organismal and community biology. De novo assembly of large and mosaic eukaryotic genomes from complex met-omics data remains a challenging task, especially in comparison with more straightforward bacterial and archaeal systems. Here, we use a transcriptome reconstruction method based on clustering co-abundant genes across a series of metagenomic samples. We investigated the co-abundance patterns of ~37 million eukaryotic unigenes across 365 metagenomic samples collected during the Tara Oceans expeditions to assess the diversity and functional profiles of marine plankton. We identified ~12,000 co-abundant gene groups (CAGs), encompassing ~7 million unigenes, including 924 metagenomics-based transcriptomes (MGTs, CAGs larger than 500 unigenes). We demonstrated the biological validity of the MGT collection by comparing individual MGTs with available references. We identified several key eukaryotic organisms involved in dimethylsulfoniopropionate (DMSP) biosynthesis and catabolism in different oceanic provinces, thus demonstrating the potential of the MGT collection to provide functional insights on eukaryotic plankton. We established the ability of the MGT approach to capture interspecies associations through the analysis of a nitrogen-fixing haptophyte-cyanobacterial symbiotic association. This MGT collection provides a valuable resource for analyses of eukaryotic plankton in the open ocean by giving access to the genomic content and functional potential of many ecologically relevant eukaryotic species.

[Supplemental material is available for this article.]

As an alternative to individual genome or transcriptome sequencing, environmental genomics has been used for many years to access the global genomic content of organisms from a given environment (Joly and Faure 2015). However, large-scale metagenomic and metatranscriptomic data analyses are often restricted by their gene-centric approach, limiting the ability to draw an integrative functional view of sampled organisms. Nevertheless, constructing gene catalogs from environmental samples provides a useful framework for a general description of the structure and functional capabilities of microbe-dominated communities (Venter et al. 2004; Qin et al. 2010; Brum et al. 2015; Sunagawa et al. 2015; Carradec et al. 2018). Gene-centric approaches allow deep and detailed exploration of communities of organisms, but they are usually undermined by limited contextual information for different genes, apart from taxonomic affiliation based on sequence similarity.

Several methods have been developed to shift the scientific paradigm from a gene-centric to an organism-centric view of environmental genomic and transcriptomic data. These methods use direct assemblies of metagenomic reads to generate contigs that encompass several genes. High recovery of bacterial and archaeal genomes has been achieved through traditional assembly strategies from both high- and low-diversity environmental samples (Dick et al. 2009; Albertsen et al. 2013; Tully et al. 2018). However, when dealing with complex communities of organisms, traditional genome assembly approaches are often impaired by the large amount of sequence data and the genome heterogeneity.

To circumvent these limitations, approaches based on reference genomes have been proposed (Caron et al. 2009; Pawlowski et al. 2012). Other approaches are based on binning of assembly contigs across a series of samples to extract information (Sharon et al. 2012; Albertsen et al. 2013). Significant results for bacteria and archaea have been achieved so far (Parks et al. 2017; Delmont et al. 2018; Nayfach et al. 2019; Pasolli et al. 2019). However, eukaryotic organisms have larger, more complex genomes that require significantly higher sequence coverage than bacteria and archaea. Even in cases where significantly long contigs can be obtained, the mosaic structure of eukaryotic genes and the difficulty of predicting them de novo from a genome
sequence leads to poor gene recovery (Boeuf et al. 2019). Only eu-
karyotes possessing small genomes and a high proportion of
mono-exonic genes are expected to provide results similar to those
achieved for bacteria or archaea. Recently, a semisupervised meth-
method based on a model trained with a set of diverse references allowed
eukaryotic genome reconstruction from complex natural environ-
ments (West et al. 2018; Olm et al. 2019).

Novel reference-independent clustering approaches that
produce genomes from metagenomic data have recently been de-
2014; Delmont et al. 2018; Kang et al. 2019) and successfully ap-
plied to prokaryote-dominated communities. One of these ap-
proaches efficiently delineated co-abundant gene groups (CAGs),
the largest of which were termed metagenomic species (MGS),
across a series of human gut microbiome samples (Nielsen et al.
2014). This method uses the metagenomic abundance profiles of
a reference gene catalog, determined by stringent mapping of
raw metagenomic reads onto sequences in this catalog, and defines
clusters of genes showing similar variations of abundance profiles
across a collection of samples.

The vast majority of the planktonic biomass in the global
ocean consists of single-cell eukaryotes and multicellular zoo-
plankton (Dorich and Packard 1989; Gasol et al. 1997). Globally,
these organisms play an important role in shaping the biogeo-
chemical cycles of the ocean and significantly impact food webs
and climate. Despite recent advances in understanding their taxo-
nomic and gene functional compositions (Venter et al. 2004; Joly
and Faure 2015; Carradec et al. 2018), little is known about the bio-
geographical preferences and metabolic potential of many eukary-
otic plankton species from an organism-centric perspective.
Several collections of reference marine eukaryote organisms’
sequences have been created, the largest one being the Marine
Microbial Eukaryotic Transcriptome Sequencing Project
(MMETSP) collection (Keeling et al. 2014). However, the majority
of fully sequenced marine eukaryotic genomes or transcriptomes
are derived from cultured organisms. Due to the limited availabil-
ity of cultured representatives of many dominant in the open
ocean plankton, including zooplankton representatives, reference
sequences represent only a small fraction of the natural biological
diversity (De Vargas et al. 2015; Sibbald and Archibald 2017).

Here, we used the rationale of this reference-independent,
gene co-abundance method (Nielsen et al. 2014) to delineate
transcriptomes by mapping metagenomic sequencing data ob-
tained from 365 metagenomic read sets generated from marine
water samples collected from the global ocean during the Tara
Oceans expedition onto the metatranscriptome-derived Marine
Atlas of Tara Oceans Unigenes (MATOU-v1 catalog [Carradec
et al. 2018]) obtained from the same set of Tara Oceans stations
(Supplemental Fig. S1). The samples were collected from all the
major oceanic provinces except the Arctic, typically from two pho-
tic zone depths (subsurface [SRF] and deep-chlorophyll maximum
[DCM]) and across four size fractions (0.8–5 μm, 5–20 μm, 20–180
μm, and 180–2000 μm).

Results

Construction of the MGT collection

Of the 116,849,350 metatranscriptomic-based unigenes of the
MATOU-v1 catalog, 37,381,609 (32%) were detected by metage-
nomic reads mapping in at least three different Tara Oceans sam-
dles and displayed no more than 90% of their total genomic
occurrence signal in a single sample. The metagenomic RPKM-
based abundance matrix of these unigenes was submitted to a can-
opy clustering process (see Methods) that regrouped unigenes
based on the covariation of their genomic abundance across the
samples. Of these unigenes, 7,254,163 (19.5%) were clustered
into 11,846 co-abundance gene groups with sizes varying from 2
to 226,807 unigenes. Nine hundred twenty-four CAGs consisting
of at least 500 unigenes were termed metagenomics-based tran-
scriptomes (MGTs) as they may constitute a significant part of an
organism’s transcriptome and which encompass 6,946,068 uni-
genies (Supplemental Table S1). For subsequent analyses, we focused
on these more complete 924 MGTs since they more accurately rep-
resent organisms’ transcriptomes (Supplemental Fig. S1). This MGT
collection recruited a significant number of metagenomic reads
across Tara Oceans stations with an average of 58.5% (up to
94.5% for some samples) of the reads (Supplemental Fig. S2). The
average number of taxonomically assigned unigenes across the MGTs
was 44.6% (up to 99.5%). We have detected 22 MGTs with com-
pleteness higher than 50% (average contamination 16%) and 58
MGTs with completeness higher than 20% (average contamination
10.5%). Contamination was computed using a set of 83 pro-
tistan-specific single-copy core genes (Simão et al. 2015) or a set
of 139 bacterial-single-copy core genes (Campbell et al.
2013) within the Anvi’o package (ver 5.2) (Eren et al. 2015).
Since unigenes often represent not full genes but their fragments,
single-copy core genes may map to multiple unigenes representing
the same gene, which may lead to artificially high levels of con-
tamination. MGT completeness significantly improved after the
CAP3 assembly step (see Supplemental Material), which resulted
in 74 MGTs with completeness higher than 50% and 131 MGTs
with completeness higher than 20% (Supplemental Table S1).

Taxonomic diversity of the MGT collection

We studied the distribution of taxonomically assigned unigenes
for each MGT across major planktonic taxa. In several cases, we
observed a homogeneous distribution of taxonomic affiliations,
suggesting that the MGTs represented transcriptomes of individu-
al organisms (Supplemental Table S1; Supplemental Fig. S11). The
accuracy of the taxonomic affiliations of the unigenes varied
throughout the samples and depended on (1) the conservation lev-
el of a given sequence across species and (2) the adequacy and ro-
bustness of a reference database in regard to a given taxonomic
unit (Carradec et al. 2018).

For each MGT, global taxonomic affiliation was determined
by the taxonomic node that covered at least 75% of the taxonom-
ically assigned unigenes of that MGT (see Methods for more
detail). The MGT collection mostly comprised eukaryotic repre-
sentatives (728 MGTs: 78%, 6,380,849 unigenes), followed by bac-
teria (148 MGTs: 16%, 454,253 unigenes), archaea (2 MGTs: 0.2%,
2844 unigenes), and viruses (1 MGT: 0.1%, 877 unigenes). Presence of bacteria and archaea in the MATOU-v1 catalog, despite
using polyadenylated RNA for the sequencing step, can be ex-
plained by (1) the true nonpolyadenylated nature of these tran-
scripts or (2) the low level of eukaryotic annotations in regard to
prokaryotes in reference databases (Carradec et al. 2018).

In this study, we focused only on the MGTs from the domain Eukarya.

The overall taxonomic analysis of the MGT collection re-
vealed that most of the major eukaryotic marine planktonic king-
doms (Worden et al. 2012) were covered, with the exception of
Amoebozoa, Cryptophyta, and Rhodophyta (Fig. 1). Most of the
MGTs with a low-resolution global taxonomic assignment (i.e.,
those for which the taxonomic affiliation could be assigned only at the kingdom level or higher) were related to the Opisthokonta group (447 out of 728 MGTs) or unclassified Eukaryota (105), whereas the well-defined eukaryotic MGTs (i.e., those for which the taxonomic affiliation could be assigned at the class level or deeper) belonged to unicellular algae: Stramenopiles (62), Alveolata (48), Viridiplantae (30), and Haptophyceae (29) lineages. This low taxonomic resolution of the MGTs could be due to (1) a low number of zooplankton organisms, including representatives from the Opisthokonta group, in the reference databases or (2) the presence of associations of several organisms in a given MGT. Overall, these observations suggest that the MGTs correspond to either organisms with available transcriptomes or those without sequenced representatives. Taxonomic diversity of the MGT collection differs significantly compared to the collections of reference transcriptomes derived from cultured organisms (Supplemental Fig. S3), including the MMETSP project (Keeling et al. 2014). Possible explanations for this include (1) the essentially coastal origin of cultured strains and (2) the absence of zooplankton in the MMETSP selected organisms.

Comparison with available transcriptomes

To assess the biological validity of the obtained MGTs, we investigated the distribution of unigenes from two marine planktonic reference organisms in the MGT collection. We analyzed reference transcriptomes from a single-celled microeukaryote *Bathycoccus prasinos* and a small multicellular zooplankton *Oithona nana*. The rationale for choosing these organisms as references was as follows: (1) They play an important role in the functioning of marine ecosystems; (2) their transcriptomes were publicly available (Keeling et al. 2014; Madoui et al. 2017); (3) they were hypothesized to be present in the data sets because of their high abundance in marine waters as demonstrated by an 18S rDNA survey from the same samples (De Vargas et al. 2015); and (4) they cover a range of organisms with substantially different transcriptome sizes (5.6–24 Mb), from phytoplankton and zooplankton groups, and from small and large size fractions of planktonic communities. We were able to recover an average of 68% (up to 77%) of the reference transcriptomes utilizing the MGT unigenes with at least 95% sequence identity over at least 50 amino acids (Fig. 2).

Segregation of the *Bathycoccus* ecotypes

To assess the potential of the MGT approach to segregate closely related biological entities, we focused on the MGTs highly similar to the reference transcriptomes of *Bathycoccus prasinos*. *Bathycoccus* is a genus of green algae from the order Mamiellales which is ecologically relevant because it is widely distributed in the global ocean and contributes significantly to primary production (Vannier et al. 2016; Limardo et al. 2017). Recent omics-based studies demonstrated the existence of at least two ecotypes of *Bathycoccus* (B1 and B2) which have identical 18S rRNA sequences but whose orthologous proteins share only 82 ± 6% nucleotide identity (Vaulot et al. 2012; Vannier et al. 2016). Both of these *Bathycoccus* ecotypes were detected in the MGT collection, and 99% of the total number of unigenes similar to *B. prasinos* were divided into three MGTs (MGT-41, MGT-65, MGT-277) (Supplemental Table S2). We focused on MGT-41 and MGT-65 because they comprised 95.2% of the signal in this group. Pangenomic analysis demonstrated a clear segregation between the two MGTs (Fig. 3A). The average nucleotide identity (ANI) analysis indicated <90% sequence similarity between them,
Functional insights from MGTs

After demonstrating the biological validity of the MGTs, we studied their potential to assess the functional state of the ecosystem through the analysis of ecologically relevant metabolic pathways and individual marine organisms. We analyzed the expression patterns, taxonomic affiliation, and geographical distribution of the genes coding for the key enzymes involved in the cycling of dimethylsulfiniopropionate (DMSP). We also investigated the interspecies relationship between an uncultivated unicellular cyanobacterium Candidatus Atelocyanobacterium thalassa (UCYN-A) and a haptophyte picoplankton alga of the class Prymnesiophyceae.

**DMSP synthesis and degradation**

Eukaryotic plankton, along with bacteria, are actively involved in the cycling of DMSP, an ecologically relevant organosulfur compound that can reach high concentrations in marine waters. DMSP is the precursor of the climate-active gas dimethyl sulfide.
Reconstruction of marine eukaryotic transcriptomes

which allowed us to study their expression and biogeography from a genome-centric point of view (Supplemental Table S3).

Out of 1220 DSYB-related unigenes detected in the MATOU-v1 catalog, 1214 were taxonomically assigned to eukaryotes (Supplemental Fig. S6A). They were detected at all of the 66 sampling stations of the Tara Oceans cruise analyzed in this study and were mainly attributed to the pico-eukaryote size fraction (0.8–5 µm) (Supplemental Fig. S7). Forty-six DSYB-related unigenes were detected in 20 different MGTs, and their expression in 10 out of 20 MGTs represented more than 10% of the total DSYB expression in at least one sample. This further confirms the importance of the organisms related to these MGTs in the DMSP production (Fig. 4A). Two MGTs affiliated to the genus *Phaeocystis* (MGT-4 and MGT-13) were prevalent (up to 45% of the total DSYB expression signal) at the Southern Ocean stations (82–85), whereas a Haptophyte-related organism (MGT-29) was prevalent (from 10% to 35%, depending on the size fraction) at station 80 in the southern Atlantic. *Phaeocystis*-affiliated MGTs demonstrated high levels of DSYB expression both in the small size fraction (0.8–5 µm, corresponding to individual cells) and in large size fractions (20–180 and 180–2000 µm, corresponding to multicellular colonies which may form during *P. antarctica* blooms [Carlson et al. 1998; Smith et al. 1998; Wang et al. 2018]). A large portion of the total DSYB expression (from 10% to 70%) was also attributed to the Chloropicrophyceae lineage (previously referred to as Prasinophytes clade VII) (Lopes Dos Santos et al. 2017a,b; Turmel et al. 2019): MGT-44 at the

(DMS), the largest natural source of sulfur to the atmosphere. This compound is also important from an organismal point of view because of its ability to act as an osmolyte, antioxidant, predator deterrent, and cryoprotectant in phytoplankton (Stefels et al. 2007; Bullock et al. 2017).

We investigated the expression patterns and geographical distribution of the genes involved in the production and degradation of DMSP across *Tara* Oceans stations. While the role of bacteria in DMSP cycling is well described (Reisch et al. 2011; Johnston et al. 2016; Bullock et al. 2017; Carson et al. 2017), recent identification of eukaryote counterpart genes (1) *DSYB*, coding for a methyl-thiohydroxybutyrate methyltransferase, a key enzyme involved in the DMSP synthesis in eukaryotes (Carson et al. 2018), and (2) *Alma1* coding for dimethylsulfiniopropionate lyase 1, an enzyme responsible for the cleavage of DMSP into dimethyl sulfide and acrylate (Alcolombri et al. 2015; Johnson et al. 2016), allows investigation of the role of marine phytoplankton in the global DMSP cycle. Both of these genes were detected in the MGT collection, Pacific Ocean stations (93, 102, 109, 110, 122–125, 128, 136, 137, 139, and 144) and MGT-166 and MGT-179 at the Indian Ocean stations (36, 38, 39, and 41). In addition to these differences in their geographical distribution, MGT-166 and MGT-179 shared more genomic similarity between them (ANI = 95.23%), whereas MGT-44 was more distantly related (ANI = 86.86 and ANI = 85.97% with MGT-166 and MGT-179, respectively), suggesting that the former two may represent closely related organisms and the third one corresponds to a different transcriptome. However, given the small number of available Chloropicrophyceae-related references in DNA databases, this hypothesis requires further investigation.

It is well established that organisms from the classes Dinophyceae (dinoflagellates) and Prymnesiophyceae (coccolithophores) are major producers of DMSP, but only few studies are available that investigate the involvement of Chloropicrophyceae in DMSP production (Keller 1989; Keller et al. 1989; Kiene et al. 1997). Our data suggest that there are at least two distinct representatives of Chloropicrophyceae involved in DMSP biosynthesis and

![Figure 3](image-url)

**Figure 3.** The pangenomes of (A) *Bathycoccus prasinos* and (B) *Oithona nana* compared to available sequenced references. Each layer represents an MGT, a reference genome, or transcriptome. Gene clusters are organized based on their distribution across samples. The dendrogram in the center organizes gene clusters based on their presence or absence in the samples. The top right dendrogram represents the hierarchical clustering of the samples based on the abundance of gene clusters. (ANI) Average nucleotide identity, (SCG) single-copy core genes, (GC) gene cluster.
provide preliminary results on a possible pathway for DMSP production in this group.

We also investigated the expression of the Alma1 gene coding for a key enzyme of the DMSP degradation pathway (Alkolombri et al. 2015). We detected 1059 Alma1-related unigenes in the MATOU-v1 catalog (Supplemental Fig. S6B). The expression of these unigenes was detected mostly in the smallest size fraction (0.8–5 µm) at both depths (surface and DCM) at 66 sampled stations (Supplemental Fig. S8). No taxonomic affiliation was found for 153 Alma1 unigenes. The highest levels of Alma1 abundance and expression were detected at the Southern Ocean stations (Supplemental Fig. S8). Thirty-six Alma1-related unigenes were detected in 13 MGTs. Most of these Alma1 unigenes were taxonomically affiliated to the clade Alveolates (78%), followed by the family Haptophyceae (5%). However, 30 out of the 36 Alma1-related unigenes present in the MGT collection were concentrated in six MGTs taxonomically assigned to Haptophytes. Even though 48 MGTs possessed unigenes assigned to Alveolates, we did not detect any Alma1-containing MGTs affiliated to this group. In nine out of 13 MGTs containing Alma1 unigenes, Alma1 expression contributed more than 10% (in some cases, up to 40%) to the total Alma1 expression detected across 43 samples (Fig. 4B). MGTs demonstrating the highest levels of Alma1 expression were taxonomically assigned to Phaeocystis (MGT-4, MGT-13, and MGT-67) and Pelagomonas spp. (MGT-178).

Identification of interspecies associations

We investigated the interspecies relationship between an uncultivated diazotrophic unicellular cyanobacterium Candidatus Atelocyanobacterium thalassa (Zehr et al. 2008; Thompson et al. 2012) and a haptophyte picoplankton alga Braarudosphaera bigelowii (B. bigelowii) from the class Prymnesiophyceae. Both members of this association are abundant and widely distributed in the ocean and are ecologically relevant because of their ability to fix N2 (Zehr and Kudela 2011; Farnelid et al. 2016). Several UCYN-A genomes have been previously sequenced (Tripp et al. 2010; Bombar et al. 2014), whereas no genomic information is currently available for the algal host. Detected in the MATOU-v1 catalog were 2616 UCYN-A-affiliated unigenes (see Methods). They were distributed among 41 Tara Oceans stations and were mostly present in the small size fraction (0.8–5 µm). The majority of the UCYN-A-affiliated unigenes (96%) were detected in two MGTs: 1742 unigenes in MGT-29 and 771 unigenes in MGT-176 (Supplemental Fig. S9). In addition to the unigenes affiliated with the diazotrophic cyanobacterium,
MGT-29 also possessed ~20,000 unigenes taxonomically assigned to the Haptophyte clade and possibly representing the eukaryotic host of this symbiosis, a Prymnesiophyte closely related to B. bigelowii. Together with the observation that the host’s 18S rDNA V4 region was identified in the same samples as MGT-29, this suggests that the non-UCYN-A-affiliated genes of MGT-29 may be a part of the transcriptome of the host. Comparison of the MGTs comprising UCYN-A-related unigenes with reference genomes (Tripp et al. 2010; Bombar et al. 2014) and metagenome-assembled genomes (MAGs) (Parks et al. 2017; Delmont et al. 2018) demonstrated that MGT-29 unigenes covered 90.3% of the UCYN-A1 genome (Fig. 5). The estimated completeness of the UCYN-A genome computed based on a set of 139 bacterial-specific single-copy core genes (Campbell et al. 2013) was 82.7% for MGT-29 and 42.4% for MGT-176. The ANI value between MGT-29 and UCYN-A1 isolate ALOHA was 99.7%, which indicates a high genomic similarity. We hypothesize that MGT-176 may represent UCYN-A2 or another UCYN-A sublineage, because the ANI analysis demonstrated its higher genomic similarity with isolate SIO64986 (UCYN-A2) than isolate ALOHA (UCYN-A1) (97.1% and 94.3%, respectively).

In addition to the UCYN-A-related genes, MGT-29 also contained multiple core metabolism genes taxonomically assigned to the Haptophyte clade which may belong to the eukaryotic host of this symbiotic association, B. bigelowii (Supplemental Data Set S2). More specifically, we detected genes coding for enzymes driving major metabolic pathways in the haptophyte algae including glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway, the GS-GOGAT cycle (ammonium assimilation through sequential actions of glutamine synthetase [GS] and glutamate synthase [GOGAT]), as well as multiple genes affiliated with the metabolism of fatty acids and amino acids. We also observed the presence of the gene bacA, coding for the ABC transporter involved in the transport of vitamin B12.

In addition to the UCYN-A symbiosis with a single-celled haptophyte, we detected other known microbial associations in the MGT collection (Supplemental Table S4). For example, MGT-738 partially captured an association between a diatom from the class Coscinodiscophyceae (47 unigenes) and the nitrogen-fixing heterocystous cyanobacteria, Richelia intracellularis (132 unigenes), an abundant organism in tropical and subtropical waters (Janson et al. 1999; Lyimo 2011). This MGT collection also detected an association between a pennate diatom from the family Bacillariaceae and a tintinnid ciliate, genes from both of which were detected in MGT-136 (243 and 192 unigenes affiliated to the diatom and tintinnid, respectively) (Vincent et al. 2018).

Discussion

Many eukaryotic lineages of ocean plankton remain largely undersampled, and as a result, there are few sequenced representatives for many ecologically important marine eukaryotic organisms. The MGT collection reported here represents a valuable resource for studying a range of eukaryotic planktonic organisms, including those that are largely unexplored using traditional omics techniques.

The gene clustering approach applied here provides an organism-centric view of the most abundant plankton populations. This approach allowed us to focus on the diversity and functional potential of marine eukaryotic organisms across major taxonomic lineages. The 924 MGTs generated from the Tara Oceans data sets contain an impressive diversity of taxa, allowing for comparative genomic studies in major eukaryotic groups, including Ophistokonta, Haptophytes, Stramenopiles, Alveolates, Archaeplastida, and Rhizaria (Fig. 1). Additionally, this collection of MGTs provides the first glimpse of the genomic content of a variety of organisms currently not available in culture, including copepods, one of the most prevalent zooplankton of the photic open ocean. Only a small number of MGTs have closely related cultured references, suggesting that many MGTs represent organisms which have only distant relatives within the publicly available collections of sequenced genomes or transcriptomes. These MGTs provide access to valuable genomic information currently not accessible through other DNA-based resources. For example, limited availability of full genomes or transcriptomes representing heterotrophic organisms prevents advances in studying their distribution, population structure, and functional potential. Access to the zooplankton transcriptomes through the
MGT collection will increase our knowledge on their biogeography and complement the general lack of references for the copepods group. Alternatively, the analysis of the MGTs closely related to organisms which have sequenced representatives may lead to a re-evaluation of their genomic potential and provide additional information on their ecology.

DMSP biosynthesis and degradation by eukaryotes

We demonstrated the ability of the MGT approach to assess the contribution of eukaryotic plankton to ecologically important processes by focusing on the MGTs expressing genes coding for key enzymes involved in DMSP cycling. These genes included DSYB, coding for a methyl-thiohydroxybutyrate methyltransferase, a key enzyme of the eukaryotic DMSP synthesis pathway (Curson et al. 2018) and Alma1 coding for dimethylsulfiniopropionate lyase 1, an algal enzyme that cleaves DMSP into DMS and acrylate (Alcolombri et al. 2015). We revealed the importance of Phaeocystis spp. in the Southern Ocean as potential DMSP producers and degraders. Our data also indicated the involvement of at least two representatives from Chloropiconophyceae in the biosynthesis of DMSP. One of them was primarily active at the oligotrophic equatorial Pacific stations, while the other one appeared to be restricted to the low oxygen stations (the Arabian basin and upwelling stations in the East Pacific). However, involvement of Chloropiconophyceae in DMSP production is currently supported only by circumstantial evidence, mostly in early studies (Keller 1989; Keller et al. 1989; Kiene et al. 1997), which demonstrates a clear need for more sequenced references of this group of organisms. Our results also support recent findings stating that picoeukaryotes should be considered as important contributors to DMSP production through the DSYB pathway (Curson et al. 2018). These organisms may represent interesting targets for the experimental validation of their role in the global biogeochemical sulfur cycle and their impact on climate change as proposed in the CLAW hypothesis (Charlson et al. 1987; Ayers and Cainey 2007). Thus, the MGT approach allowed us to identify candidate organisms responsible for a large part of the eukaryotic DMSP biosynthesis and catabolism in the different regions of the open ocean.

Presence of different dominating groups of organisms involved in the DMSP cycling across oceanic regions suggests the importance of environmental conditions in shaping microbial community composition that defines the DMSP fate in the ocean. If environmental conditions change in a given ecological niche, we may expect that DMSP production and degradation rates would also change because of the transformations in the microbial community structure, which may lead to significant effects on climate change.

Interspecies associations

The importance of the MGT collection as a resource for studying marine interspecies interactions was demonstrated through detection of the ecologically relevant symbiosis between the metabolically streamlined nitrogen-fixing cyanobacterium UCYN-A and a single-celled haptophyte picoplankton alga. Initially, this symbiosis was discovered using a targeted approach that involved several culture-dependent and molecular techniques, proving it to be a challenging task (Zehr et al. 2017). Several UCYN-A sublineages have been defined, but limited information is currently available regarding their global distribution and, for some, the identity of the host (Thompson et al. 2014; Farnelid et al. 2016; Turk-Kubo et al. 2017). MGT-29 from our collection encompasses genes similar to those from UCYN-A1 strain ALOHA and genes taxonomically affiliated with the Haptophyte clade potentially representing the eukaryotic host. This suggests that MGT-29 may represent UCYN-A1 specifically associated with a closely related to B. bigelowii prymnesiophyte. More information is needed about the genomic content of the host cells for different UCYN-A sublineages to confidently state which symbiosis was detected.

No genomic information is available about these symbiotic hosts beyond 18S rRNA sequences (Hagino et al. 2013). As a result, many questions remain unanswered regarding the evolution of this symbiosis and the exact nature of the relationship between the two organisms. Through partial reconstruction of the host transcriptome, we provide the first glimpse of its genomic content, which will change the way in which this interspecies association can be studied. Better methods are needed for the accurate targeting of distinct UCYN-A/host associations, which will improve the understanding of the evolution and ecological characteristics of this symbiosis. Access to the genomic content of the host through the MGT collection, used in conjunction with the two closed UCYN-A genomes currently available in the databases (Tripp et al. 2010; Bombar et al. 2014), will provide a much-needed push in this direction.

Other ecologically important microbial associations were detected in the MGT collection. The diatom-cyanobacteria symbiotic populations captured in the MGT-738 which were previously observed in all major ocean basins (Foster and O’Mullan 2008) may encompass diatoms from several genera, including Hemiaulus, Rhizosolenia, and Chaetoceros. These associations may contribute as much new nitrogen (N) as the free-living diazotroph Trichodesmium, which is widely regarded as the most important player responsible for N2 fixation in the open ocean (Capone et al. 2008). It was reported that the contribution of the diatom symbioses to the global pool of N had been underestimated and that they should be included in global N models (Foster et al. 2011). In order to accurately do so, additional information on their genomic potential is required and can be accessed through the MGT collection.

Little is known about the nature of a diatom-tintinnid association detected in MGT-136. One hypothesis suggests a mutualistic symbiosis, where diatoms acquire increased motility and tintinnids benefit from silicification through increased protection (Vincent et al. 2018). Other data indicate that the tintinnid can be the only beneficiary of the association, whereas the diatom would play the role of the “victim” (Ambrecht et al. 2017). Recent studies suggest that diatom-tintinnid associations may be more common in the ocean than previously thought. However, their global ecological and biogeographical patterns remain poorly characterized (references within Vincent et al. 2018).

The MGT collection provides a valuable resource for the evaluation of these ecologically relevant associations by studying their distribution in major oceanic provinces and by exploring the expression patterns of key genes. These findings illustrate the ability of the MGT collection to depict more interspecies relationships in the ocean, thus potentially discovering previously unknown microbial associations (Supplemental Table S4), as well as to study their gene expression patterns.

Fragmentation of the MGTs

In addition to the MGTs, comprised of tens of thousands of unigenes, some of which cover eukaryotic reference transcriptomes
with a high level of completeness, we also detected a number of smaller gene clusters which cannot reliably cover a full eukaryotic transcriptome. Several reasons may lead to the presence of these smaller MGTs representing partial eukaryotic transcriptomes. In some cases, not all of the genes from a specific organism can be detected in all of the samples where this organism is present—some genes may be missing or present at levels below the achieved sequence coverage. This may lead to the fragmentation of the MGTs, i.e., to the fact that genes from the same organism may be allocated to multiple CAGs and MGTs of various sizes (comprised of a different number of unigenes). Several possible scenarios exist: (1) Some accessory genes may be present and expressed in some subpopulations and missing in others; or (2) a sufficient sequencing depth was not achieved for some of the samples, resulting in only a partial genomic coverage. Thus, for organisms with sequencing depths below or near the limit of detection, some genes may lack corresponding reads, which would lead to incomplete coverage of the transcriptome by metagenomic reads. The situation when several CAGs of various sizes represent the same organism has been observed for the prokaryotic compartment of a human gut microbiome (Nielsen et al. 2014).

Limitations and advantages of the MGT method

General limitations relevant to interpreting the gene co-abundance data obtained using the MGT approach described here include its inability to incorporate the accessory genes in the analysis due to their inherent nature of not being present in all strains and its intrinsic inability to segregate organisms that form obligate symbioses because of their identical gene co-abundance profiles.

A recently developed computational tool may solve the former problem (Plaza Oñate et al. 2019), although further analyses on environmental data sets are needed to confirm its accuracy and efficiency. Alternatively, postprocessing of the MGT collection using methods based on differential sequence coverage of genes may be effective in cases where a significant bias in genome copy number of the associated organisms exists. Another caveat specific to our data sets is that genes expressed below the level of detection may be overlooked because the gene reconstruction has been performed using the metatranscriptomics data. However, the MGT approach has a number of advantages compared to other metagenome and metatranscriptome assembly methods. These include: (1) access to genomic content of organisms not available in culture (including zooplankton species) because of the culture-independent assembly and clustering of sequence data; and (2) de novo definition of gene clusters which allows for the reconstruction of transcriptomes with no need for references.

In this study, we applied a gene co-abundance clustering approach on a series of samples provided by the Tara Oceans expedition and demonstrated its efficiency for reconstructing high-quality eukaryotic transcriptomes. The resulting MGT collection provides a valuable resource for a comprehensive analysis of the eukaryotic plankton in the open sunlit ocean by providing access to biogeography, genomic content, and functional potential of many ecologically relevant eukaryotic species. This universal methodological framework can be implemented for transcriptome reconstruction of microscopic eukaryotic organisms in any environment provided that both metagenomic and metatranscriptomic data are available.

Methods

Sampling of eukaryotic plankton communities

The samples were collected during the 2009–2013 Tara Oceans expeditions from all the major oceanic provinces except the Arctic. For the majority of stations, samples were collected from two depths in the photic zone: subsurface and deep-chlorophyll maximum. Planktonic eukaryotic communities were collected in the 0.8– to 2000-μm range and divided into four size fractions (0.8–5 μm, 5–20 μm, 20–180 μm, and 180–2000 μm). A detailed description of the sampling strategies and protocols is available in the Supplemental Material and in Pesant et al. (2015). Biogeochemical data measured during the expedition are available in the Supplemental Material and in the Pangaea database (https://www.pangaea.de/).

DNA and RNA libraries were constructed and sequenced as detailed in Alberti et al. (2017) and processed as described in Carradec et al. (2018). Briefly, the raw data were filtered and cleaned to remove low-quality reads, adapters, primers, and ribosomal RNA-like reads. Resulting metatranscriptomic reads were assembled using Velvet v.1.2.07 (Zerbino et al. 2009) with a k-mer size of 63. Isoform detection was performed using Oases 0.2.08 (Schulz et al. 2012). Contigs smaller than 150 bp were removed from further analysis. The longest sequence from each cluster of contigs was kept as a reference for the gene catalog. The MATOU-v1 unigene catalog is accessible at https://www.genoscope.cns.fr/tara/.

Abundance computing and canopy clustering

The raw metagenomic (metaG) reads from 365 samples were mapped against the MATOU-v1 catalog as described in Carradec et al. (2018). Briefly, raw metagenomic reads from each sample were compared with the MATOU-v1 unigenes using the BWA tool (version 0.7.4) (Li and Durbin 2009), and those covering at least 80% of the read length with at least 95% of identity were retained for further analysis. In the case of several possible best matches, a random one was picked. For each unigene in each sample, the metagenomic abundance was determined in RPKM (reads per kilo base per million of mapped reads). To improve the clustering efficiency, we selected unigenes detected with metagenomic reads in at least three different samples and which had no more than 90% of their total genomic occurrence signal in a single sample. These two criteria are the default parameters of the canopy clustering tool (–filter_min_obs 3 and –filter_max_top3_sample_contribution=0.9). The metagenomic RPKM-based abundance matrix of these unigenes was submitted to the canopy clustering algorithm described in Nielsen et al. (2014) (the original code is available in Supplemental Code and at https://www.genoscope.cns.fr/tara/), which is a density-based clustering that does not take into account the sequence composition, as opposed to most binning tools. We used a max Pearson's correlation difference of 0.1 to define clusters, and then clusters were merged if canopy centroids’ distances were smaller than 0.05 (250 k iterations, default parameters).

A total of 7,254,163 unigenes were clustered into 11,846 co-abundant gene groups of at least two unigenes. CAGs with more than 500 unigenes are hereafter termed metagenomics-based transcriptomes. Nine hundred twenty-four MGTs were generated which encompassed 6,946,068 unigenes (95.8%). Since this method has never been applied to eukaryotic data, a smaller cutoff of 500 unigenes was used (compared to the original method applied to prokaryote-dominated communities [Nielsen et al. 2014]) to increase the number of resulting MGTs potentially representing individual organisms. For each sampling filter, we determined the
fraction of metagenomics reads captured by the unigenes that compose the MGTs (Supplemental Fig. S2).

**Taxonomic assignment**

Taxonomic assignment of the unigenes is described in Carradec et al. (2018). Briefly, to determine a taxonomic affiliation for each of the unigenes, a reference database was built from UniRef90 (release of 2014-09-04 (Suzek et al. 2015)), the MMETSP project (release of 2014-07-30 (Keeling et al. 2014)), and Tara Oceans Single-cell Amplified Genomes (PRJEB6603). The database was supplemented with three Rhizaria transcriptomes (Callozoan, Phaeodarea, and Eucarytidia), available through the European Nucleotide Archive under the reference PRJEB18281 (https://www.ebi.ac.uk/ena/data/view/PRJEB18281) and transcriptomes of Oithona nana (Madouli et al. 2017), available through the European Nucleotide Archive under the reference PRJEB18938 (https://www.ebi.ac.uk/ena/data/view/PRJEB18938).

Sequence similarities between the gene catalog and the reference database were computed in protein space using DIAMOND (version 0.7.9) (Buchfink et al. 2015) with the following parameters: -e 1E-5 -k 500 -a 8 --more-sensitive. Taxonomic affiliation was performed using a weighted Lowest Common Ancestor approach. Subsequently, for each MGT, representative taxonomic level was determined by computing the deepest taxonomic node covering at least 75% of the taxonomically assigned unigenes of that MGT.

**Completeness and contamination assessment**

For each MGT, unigenes were further assembled using CAP3 (version date: 02/10/15) (Huang and Madan 1999). Assembled contigs and singletons were pooled, and completeness and contamination were computed using the Anvi’o package (version 5.2) (Eren et al. 2015) with default parameters and a set of 83 protistan-specific single-copy core genes (Simão et al. 2015) for eukaryotes or a set of 139 bacterial-specific single-copy core genes (Campbell et al. 2013) for bacteria (Supplemental Table S1). Average nucleotide identity was computed using the dnadiff tool from the MUMmer package (version 3.23) (Kurtz et al. 2004).

**Functional characterization**

DSYB-related unigenes were identified using Hidden Markov Models (HMMs) generated from 135 sequences extracted from Curson et al. (2018). These sequences were clustered using MMSegq2 (Steiniger and Söding 2017), and for each of the resulting 24 clusters, sequences were aligned using MUSCLE (Edgar 2004). HMM construction and unigenes catalog scanning were performed using HMMer (Wheeler and Eddy 2013). The DSYB HMM profile had significant matches (e-value ≤ 10^-50) with 1220 unigenes in the MATOU-v1 catalog, 46 of which were found in the MGT collection (Supplemental Table S3).

Almal1-related unigenes were identified using HMMs generated from five sequences with demonstrated DMSP lyase activity, extracted from Alcolombri et al. (2015). These sequences were clustered using MMSegq2 (Steiniger and Söding 2017), and for each of the two resulting clusters, sequences were aligned using MAFFT v7.407 (Katoh and Standley 2013). HMM construction and unigenes catalog scanning were performed using HMMer (Wheeler and Eddy 2013). We identified 1069 positive unigenes (e ≤ 10^-50) from the MATOU-v1 catalog; 36 of them were found in the MGT collection (Supplemental Table S3).

Unigene expression values were computed in RPKM. The expression of DSYB- and Almal1-related unigenes was normalized to the total number of reads mapped to a given MGT and to the total number of reads in a given sample (Fig. 4).

**Comparison with reference transcriptomes**

To assess the biological validity of the resulting MGT, the reference transcriptomes of Bathycoccus prasinos from the MMETSP database (release of 2014-07-30 (Keeling et al. 2014)) and the reference transcriptome of Oithona nana from Genoscope (Madouli et al. 2017) were used. Sequence similarities between the unigenes and the reference transcriptomes were computed in protein space using DIAMOND (version 0.7.9) (Buchfink et al. 2015) with the following parameters: -e 1E-5 -k 500 -a 8, and positive matches were defined as ≥95% identity over at least 50 amino acids.

**Identification of potential interspecies interactions**

We screened the MGT collection for potential interspecies associations by focusing on the MGTs that meet two criteria: (1) These MGTs must contain at least 10 unigenes from two different subkingdom taxonomic units; and (2) the number of unigenes associated with one of these taxonomic units must account for at least 5% of the number of unigenes associated with the other taxonomic unit. For example, MGT-29 contains 19,652 unigenes assigned to Haptophyceae and 1940 unigenes (9.9%) assigned to cyanobacteria. All the MGTs that met these criteria are listed in Supplemental Table S4. See Supplemental Material for more detail.

**Statistical analysis**

All statistical analyses and graphical representations were conducted in R (v 3.3.2) (R Core Team 2019) with the R package ggplot2 (v 2.2.1). The taxonomic dendrogram shown in Figure 1 was built using the phyloT and NCBI Taxonomy (https://www.ncbi.nlm.nih.gov/taxonomy) toolkits of the Python ETE3 package and visualized using iTOL (Letunic and Bork 2016). The world maps were obtained using the R packages grid (v 3.3.2) and maps (v 3.2.0). Inkscape 0.92.3 was used to finalize the figures.

**Data access**

Sequencing data generated in this study have been submitted to the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) under accession number PRJEB4352 for the metagenomics data and PRJEB6609 for the metatranscriptomics data. The unigene catalog generated in this study has been submitted to the ENA under accession number ERZ480625. The MGT collection data and environmental data are available in Supplemental Material in Supplemental Data Set S1, at https://www.genoscope.fr/tara/, and in the Pangaea database (https://www.pangaea.de/). MGT nucleic sequences in FASTA format and MGT post-assemblies generated through CAP3 are available at https://www.genoscope.fr/tara/. See Supplemental Material for more detail.

**Competing interest statement**

The authors declare no competing interests.

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