Survey of the green picoalga *Bathycoccus* genomes in the global ocean

Thomas Vannier[1,2,3], Jade Leconte[1,2,3], Yoann Seeleuthner[1,2,3], Samuel Mondy[1,2,3], Eric Pelletier[1,2,3], Jean-Marc Aury[1], Colomban de Vargas[4], Michael Sieracki[5], Daniele Iudicone[6], Daniel Vaulot[4], Patrick Wincker*[1,2,3] & Olivier Jaillon*[1,2,3]

[1]CEA - Institut de Génomique, GENOSCOPE, 2 rue Gaston Crémieux, 91057 Evry France.
[2]CNRS, UMR 8030, CP5706, Evry France.
[3]Université d'Evry, UMR 8030, CP5706, Evry France.
[4]Sorbonne Universités, UPMC Université Paris 06, CNRS, UMR7144, Station Biologique de Roscoff, 29680 Roscoff, France.
[5]National Science Foundation, 4201 Wilson Blvd., Arlington, VA 22230, USA.
[6]Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy
*Correspondence: Olivier Jaillon (ojaillon@genoscope.cns.fr) and Patrick Wincker (pwincker@genoscope.cns.fr).

ABSTRACT

Bathycoccus is a cosmopolitan green micro-alga belonging to the Mamiellophyceae, a class of picophytoplankton that contains important contributors to oceanic primary production. A single species of *Bathycoccus* has been described while the existence of two ecotypes has been proposed based on metagenomic data. A genome is available for one strain corresponding to the described phenotype. We report a second genome assembly obtained by a single cell genomics approach corresponding to the second ecotype. The two *Bathycoccus* genomes are divergent enough to be unambiguously distinguishable in whole DNA metagenomic data although they possess identical sequence of the 18S rRNA gene including in the V9 region. Analysis of 122 global ocean whole DNA metagenome samples from the *Tara*-Oceans expedition reveals that populations of *Bathycoccus* that were previously identified by 18S rRNA V9 metabarcodes are only composed of these two genomes. *Bathycoccus* is relatively abundant and widely distributed in nutrient rich waters. The two genomes rarely co-occur and occupy distinct oceanic niches in particular with respect to depth. Metatranscriptomic data provide evidence for gain or loss of

29 highly expressed genes in some samples, suggesting that the gene repertoire is modulated by
30 environmental conditions.

Introduction

Phytoplankton, comprising prokaryotes and eukaryotes, contribute to nearly half of the annual global primary production[1]. Picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* dominate the prokaryotic component[2]. However, small eukaryotes (picoeukaryotes; < 2µm) can be major contributors to primary production[3,4]. In contrast to cyanobacteria, the phylogenetic diversity of eukaryotic phytoplankton is wide, with species belonging to virtually all photosynthetic protist groups[5]. Among them, three genera of green algae belonging to the order Mamiellales (class Mamiellophyceae[6]), *Micromonas, Ostreococcus* and *Bathycoccus* are particularly important ecologically because they are found in a wide variety of oceanic ecosystems, from the poles to the tropics[7–12]. The cosmopolitan distribution of these genera raises the questions of their diversity and their adaptation to local environmental conditions. These genera exhibit genetic diversity: for example, there are at least three genetically different clades of *Micromonas* with different habitat preferences[12,13]. One ecotype of *Micromonas* seems to be restricted to polar waters[8,14]. *Ostreococcus* which is the smallest free-living eukaryotic cell known to date with a cell size of 0.8 µm[15] can be differentiated into at least four clades. Two *Ostreococcus* species have been formerly described: *O. tauri* and *O. mediterreanus*[15,16]. Among these *Ostreococcus* clades, different strains seem to be adapted to different light ranges[17]. However, the ecological preferences of *Ostreococcus* strains are probably more complex, implying other environmental parameters such as nutrients and temperature[9].

The genus *Bathycoccus* was initially isolated at 100 m from the deep chlorophyll maximum (DCM) in the Mediterranean Sea[18] and cells with the same morphology (body scales) had been reported previously from the Atlantic Ocean[19]. *Bathycoccus* has been since found to be widespread in the oceanic environment, in particular in coastal waters[20,21], and one genome sequence from a coastal strain is available[22]. Metagenomic data have suggested the existence of two *Bathycoccus* ecotypes[10,11,23], recently named BI and BII[11]. These two ecotypes have identical 18S rRNA sequences and therefore cannot be discriminated when using metabarcodes such as the V4 or V9 regions of the 18S rRNA genes[10]. However information on the ocean-wide distribution and the ecological preferences of these two ecotypes are lacking.

Mapping of metagenomic reads onto whole genomes (fragment recruitment) has been shown to be an

efficient way to assess the distribution of oceanic bacterial populations[24,25]. The paucity of eukaryotic genomes and metagenomes has prevented this approach to be applied on a large scale to eukaryotes. Therefore the determination of the geographical distribution and ecological preferences of marine eukaryotic species has relied on the use of marker genes such as 18S rRNA or ITS (internal transcribed spacer)[26] and more recently on metabarcodes[27]. One major problem is the absence of reference genomes for many marine eukaryotes as a consequence of the difficulty to cultivate them. To overcome this limitation, Single Cells Genomics is a very promising approach[28,29]. However, this approach has been largely used for bacteria[30] and numerous technical challenges have limited the recovery of eukaryotic genomes with this approach[28,31–33]. The most complete assembly obtained so far is for an uncultured stramenopile belonging to the MAST-4 clade and contains about one third of the core eukaryotic gene set[33]. Recently, the *Tara* Oceans expedition collected water samples from the photic zone of hundreds of marine sites from all oceans and obtained physicochemical parameters, such as silicate, nitrate, phosphate, temperature and chlorophyll[34–36]. This expedition also led to the massive sequencing of the V9 region from 18S ribosomal gene providing a description of the eukaryotic plankton community over wide oceanic regions[27]. During this expedition a large number of metagenomic data and single-cell amplified genomes (SAGs[37]) have also been acquired. Here, we introduce a novel genome assembly for *Bathycoccus* based on the sequence assembly of four SAGs obtained from a *Tara* Oceans sample collected in the Arabian Sea. Comparison of this assembly with the reference sequence of *Bathycoccus* strain RCC1105[22] unravels substantial genomic divergence. We investigated the geographical distributions of these two genomes by mapping onto them the short reads of a large set of metagenomes obtained in multiple marine basins from the *Tara* Oceans survey[35,38]. We also determined the genomic properties and habitat preferences of these two *Bathycoccus*.

Results.

Genome structure of *Bathycoccus* TOSAG39-1.

We obtained a new *Bathycoccus* SAG assembly (TOSAG39-1) by the single cell genomics approach from

four single cells collected from a single sample during the *Tara* Oceans expedition. We presumed these cells were from the same population and combined their genomic sequences to improve the assembly. The length of the final combined-SAGs assembly is 10.3 Mb comprising 2 345 scaffolds. Half of the assembled genome lies in 179 scaffolds longer than 13.6 kb (N50 size). This assembly covers an estimated 64% of the whole genome when considering the proportion of identified eukaryotic conserved genes[39]. We verified that this combined SAG assembly has longer cumulative size, and a larger representation of the genome than each assembly obtained from sequences of a single-SAG. We also merged the four assemblies from single-SAGs and, after removing redundancies, we obtained a substantially lower genomic representation than for the combined-SAGs strategy (Table 1). We mapped the reads of each SAG-sequencing onto the final assembly to examine whether genomic variability among the sampled population might have affected the quality of the assembly. We did not detect any major genomic variability; contigs can be formed by reads from different cells (Supplementary Figure S1). In total, half of the assembly (52.2%) was generated by reads from a single cell and one third (30.5%) by two cells.

The approximate estimated genome size is 16 Mb and GC content is 47.2%, similar to what has been reported for RCC1105 (15 Mb and 48%, respectively). We predicted 6 157 genes (Supplementary Table 1), representing a higher gene density compared to RCC1105 (622 *vs*. 520 genes per Mb), probably because of the higher fragmentation of the SAG assembly (the coding base density is conversely higher in TOSAG39-1, 742 *vs*. 821 kb/Mb for the two assemblies, respectively, Supplementary Table 1). The photosynthetic capacity of TOSAG39-1, presumed from the chlorophyll autofluorescence in the cell sorting step, was verified by the presence of plastid contigs (removed during quality control filtering) and by the presence of nuclear photosynthetic gene families (encoding RuBisCo synthase, starch synthase, alternative oxidase and chlorophyll a/b binding proteins) in the final assembly.

Previous comparisons of Mamiellales genomes demonstrated global conservation of chromosomal locations of genes between *Bathycoccus*, *Ostreococcus* and *Micromonas*[22]. These genera all possess outlier chromosomes (one part of chromosome 14 and the entire chromosome 19 for *Bathycoccus*) that display an atypical GC% and numerous small, unknown, non-conserved genes. We detected almost

perfect co-linearity between non-outlier chromosomes of RCC1105 and orthologous regions of TOSAG39-1 scaffolds (Supplementary Figure S2). However, there is a significant evolutionary divergence between the genomes: the orthologous proteins are only 78% identical on average (Supplementary Figure S3). Only 26 genes are highly conserved (> 99% identity), they are distributed on 14 chromosomes (including outlier chromosome 14) and did not display any clustering. As expected, chromosome 19 did not fit this pattern: we could not align most of its genes by direct BLAST comparison. Some traces of homology were observed for nine genes (62% protein identity). One of the twenty longest scaffolds of TOSAG39-1 had characteristics similar to chromosome 19. This scaffold could not be aligned to RCC1105 and has the lowest GC content (0.44 *vs*. 0.48% for the other scaffolds on average).

Manual curation of alignments to analyze synteny along the twenty longest TOSAG39-1 scaffolds showed that 90% of genes are collinear between the two genomes, 5% are shared outside syntenic blocks, and 5% are specific to TOSAG39-1. The three rRNA genes (18S or small subunit (SSU), 5S, 23S or large subunit (LSU)), used as phylogenetic markers in many studies, are identical between the two genomes. The SSU and LSU genes of TOSAG39-1 have introns. The SSU intron (440 bp) is at the same position as in RCC1105, but is only 91% similar. The LSU intron (435 bp) is only present in TOSAG39-1. The internal transcribed spacers (ITS) are different between the two TOSAG39-1 and the RCC1105 assemblies (82% and 86% for ITS1 and ITS2, respectively) but closer to those of two *Bathycoccus* oceanic strains from the Indian Ocean (RCC715 and RCC716) (Supplementary Figure S4) and of a metagenome from the Atlantic Ocean DCM[40]. We also looked at the plastid 16S marker gene[41] and to the PRP8 intein gene that has been proposed as markers for *Bathycoccus*[10]. The plastid 16S sequences of the two *Bathycoccus* genomes share 92% identical nucleotides, and PRP8 is lacking from the TOSAG39-1 assembly.

We were able to determine the affiliation of three metagenomes[23,40] containing *Bathycoccus* and two *Bathycoccus* transcriptomes of the MMETSP database[42] (Supplementary Figures S5). Metagenomes T142 and T149 from the South East Pacific[23] and transcriptome MMETSP1399 (strain CCMP1898, which is the type strain for *Bathycoccus prasinos*) correspond, or are closely related to RCC1105. The tropical Atlantic Ocean metagenome[40] and transcriptome MMETSP1460 (strain RCC716 from the Indian Ocean)

correspond, or are closely related to TOSAG39-1. Direct amino acid BLAST[43] comparison of TOSAG39-1 and RCC1105 versus metagenomes T142 and T149 demonstrates the presence of additional genomes in these samples that were obtained by flow cytometry sorting of natural picoplankton populations (Supplementary Figure S5).

Oceanic distribution of *Bathycoccus* genomes.

We analyzed the worldwide distribution of the two *Bathycoccus* genomes using metagenomic samples from the *Tara* Oceans expedition. Metagenomic short reads obtained from 122 samples taken at 76 sites and covering 24 oceanic provinces were mapped onto the two *Bathycoccus* genomes RCC1105 and TOSAG39-1. Among the four eukaryotic size fractions sampled in this expedition (0.8–5 µm, 5–20 µm, 20–180 µm, 180–2000 µm) statistically significant mapping was only obtained for the 0.8–5-µm fraction, which matches the cellular size of *Bathycoccus* (1.5–2.5 µm[18]). The percentage of filtered mapped metagenomic reads for every gene and station was used to estimate the relative genomic abundance of *Bathycoccus*. We compared final counts of genome abundances with counts based on amplicon sequences of the V9 region of the 18S rRNA gene[27] which does not distinguish RCC1105 from TOSAG39-1 because their 18S rRNA gene sequences are identical. The V9 data demonstrated the wide distribution of *Bathycoccus* in marine waters, with maximum relative abundance reaching 2.6% of all reads. The *Bathycoccus* metabarcode was represented by more than 1% of reads in 13% of the samples. *Bathycoccus* sequences were detected in whole metagenome reads from the same samples where Bathycoccus was detected with 18S rRNA metabarcodes (Figure 1). For each sample displaying a V9 signal, we detected the presence of the genomes of either RCC1105, TOSAG39-1, or both. In addition, the relative abundances estimated from V9 metabarcodes were correlated with the sum of the relative genomic abundances of TOSAG39-1 and RCC1105 (Supplementary Figure S6). Therefore, the *Bathycoccus* populations detected by the V9 metabarcode are likely to correspond to these two genomes only, and not to a third yet unknown genome.

160 Among the 58 samples where *Bathycoccus* metagenomics abundances represented more than 0.01% of the
161 total numbers of reads, in 91% of the cases a single genome was dominant, i.e. accounting for more than
162 70% of the reads. The two *Bathycoccus* showed similar proportions (i.e., between 40% and 60% of the
163 reads) in only two samples (stations TARA_006 and TARA_150 at DCM, Supplementary Figure S7).
164 The global distribution of the two *Bathycoccus* genomes revealed complex patterns. The RCC1105
165 genome was found mainly in temperate waters, both at the surface and at the DCM, whereas TOSAG39-1
166 appeared more prevalent in tropical zones and at the DCM (Figure 2). TOSAG39-1 was found in surface
167 water in only five winter samples from the Agulhas and Gulf Stream regions at stations undergoing strong
168 vertical mixing (Supplementary Table 2, Supplementary Figure S8). RCC1105 was detected more widely
169 in surface water and was restricted to two narrow latitudinal bands around 40°S and 40°N. Conversely,
170 TOSAG39-1 was found throughout a latitudinal range from 40°S to 39°N (Figure 2). In particular,
171 TOSAG39-1 was found in the tropical and subtropical regions in the Pacific, Atlantic and Indian Oceans.
172 In the equatorial and tropical Pacific Ocean, a region characterized by high nutrient and low chlorophyll
173 where phytoplankton is limited by iron[44], *Bathycoccus* was not detected (or only at very low abundance),
174 except close to the Galapagos Islands. We detected opposite trends in the presence of the two *Bathycoccus*
175 along the Gulf Stream: RCC1105 increased from west to east while TOSAG39-1 showed the reverse
176 trend. The two *Bathycoccus* also showed opposite trends at some stations that were relatively close but
177 located on both sides of important oceanographic boundaries. The first case was off South Africa, between
178 stations TARA_065 and TARA_066 (Supplementary Figure S8) located, respectively, in coastal,
179 temperate Atlantic and in Indian subtropical water from the Agulhas current[45].
180 The second case occurred in winter in the North Atlantic, downstream of Cape Hatteras (US East coast),
181 where station TARA_145 was in cold, nutrient-rich waters north of the northern boundary of the Gulf
182 Stream (also called the Northern Wall for its sharp temperature gradient) and TARA_146 was south of the
183 southern boundary, in the subtropical gyre (Figures 2 and Supplementary Figure S8).
184 Principal component analysis was used to assess the relationship between the genomic data and
185 environmental parameters determined *in situ*[36] complemented by satellite and climatology data

(Supplementary Information). Temperature, oxygen, sampling depth and PAR (photosynthetic active radiation), though with less significant p-values for the latter, were related to the segregation of the two genomes (Figures 3 and Supplementary Figure S9). The two *Bathycoccus* were found in temperature ranges from 0 to 32°C and from 7 to 28°C for RCC1105 and TOSAG39-1, respectively. On average, the TOSAG39-1 genome was found in waters 3°C warmer than was RCC1105 (21.5 *vs*. 18.4°C, p-value < 10^{-3}, Figures 3 and Supplementary Figure S10). Abundances were very low below 13°C for both genomes, and above 22°C for RCC1105. A similar discrimination was observed for oxygen: TOSAG39-1 was found in samples with lower oxygen content. For example, the TOSAG39-1 genome was abundant in the DCM of station 138 where O_2 was low (31.2 µM, figures 3, Supplementary Figure S9 and S10), though no stations were anoxic[46].

The two *Bathycoccus* were recovered from significantly different ranges of PAR, estimated from weekly averages of surface irradiance measurements extrapolated to depth using an attenuation coefficient derived from local surface chlorophyll concentrations[47] (Figures 3, Supplementary Figures S9 and S10, Supplementary Information). Both *Bathycoccus* could thrive in winter when the overall light availability is low (Supplementary Figure S8). Nutrient concentrations did not seem to explain the separation between the two *Bathycoccus*. We found RCC1105 in nutrient-rich surface waters and TOSAG39-1 mostly at the DCM in oligotrophic waters, close to the nutricline characterized by a significant upward flux of nutrients[48,49]. While RCC1105 was never abundant below 80 m, TOSAG39-1 extended down to almost 150 m (Figure 3 and Supplementary Figure S10).

Genomic plasticity.

For each genome, we searched for evidence of gene gain or loss by analyzing gene content variations at the different stations. Lost or gained genes could be considered as dispensable genes or as present only in some genomic variants, therefore, characterizing a "pan-genome" analogous to what is observed in bacterial populations[50]. We analyzed the coverage of metagenomic reads that were specifically mapped at high stringency onto one genome and looked for traces of gene loss. To avoid false positives caused by conserved genes, we restricted this analysis to samples where 98% of the genes from one of the two

212 *Bathycoccus* genome sequences were detected, and focused on genes that were detected in the
213 metagenomes of at least four samples, and not detected in at least five samples. Metatranscriptomic data
214 was used to select genes having an expression signal in at least six samples. Using these stringent criteria,
215 we detected about one hundred dispensable genes for each genome (Supplementary Tables 1, 4 and 5).
216 Half of the RCC1105 dispensable genes (50/108) are located on chromosome 19, representing 70% of the
217 genes on this chromosome. These genes have shorter coding and intronic regions than other genes
218 (Supplementary Table 1), which is a property of the genes predicted on outlier chromosome 19[22].
219 Dispensable genes on regular chromosomes also tend to be shorter. Additionally, the distribution of
220 dispensable genes on the genome is not random. Among the 72 genes of chromosome 19, 47 out the 50
221 dispensable genes are grouped in two long blocks at the chromosome end, leaving the first part of
222 chromosome 19 almost free of dispensable genes (Supplementary Figure S11). Dispensable genes also
223 appear clustered on regular chromosomes. Twenty-one out of 58 dispensable genes are in small blocks of
224 two to four gene-long cassettes, especially on chromosomes 2, 5 and 17 (Figures 4 and Supplementary
225 Figure S11). We verified the contiguity of the genomic regions around the dispensable genes by alignment
226 with assemblies of metagenomics reads (Supplementary Information). We analyzed the pattern of loss of
227 these dispensable cassettes in samples where they were not detected and obtained alignments that included
228 gaps in place of dispensable genes (Figure 4). Notably, cassette borders were at the same positions in the
229 various samples, showing a low diversity at these loci. This suggests that a common or single breakpoint
230 event occurred in the past. Fragment recruitments plots showed a homogenous decrease of read coverage
231 along the contiguous dispensable genes, confirming that genomic losses or gains occurred at the scale of
232 entire cassettes (Figures 4 and Supplementary Figure S11). We examined the synteny between RCC1105
233 and TOSAG39-1 for the regions corresponding to the two cassettes illustrated in Figure 4. We retrieved
234 the orthologous genes situated around the cassettes in two TOSAG39-1 scaffolds in a clear syntenic
235 relationship, but the cassettes genes were missing.
236 We observed an incomplete, but marked, depletion of read coverage for three contiguous genes on
237 chromosome 5. These genes immediately precede the longest dispensable gene cassette. This incomplete

read coverage depletion indicates that this genomic region only occurs in a sub-population, suggesting a sympatry or at least co-occurence of these two genomic forms. This pattern was observed in every oceanic basin (Figure 4B) with the longest dispensable gene cassette spanning seven genes.

The function of these dispensable genes is unclear. Only 15 dispensable genes located on RCC1105 non-outlier chromosomes possess a protein Pfam domain (Supplementary Information, Supplementary Table 3). However, several of these genes might be involved in genomic rearrangements because they contain reverse transcriptase and HNH endonuclease domains and this could be linked to their dispensability. Intriguingly, the average relative transcriptomic activity is higher in dispensable genes than in non-dispensable genes (0.73 *vs*. 0.56, Mann-Whitney-Wilcoxon test p-value=1.52E-4, Supplementary Table 1).

Beside these patterns suggesting gene gains or losses, we examined at a global level the genomic variation within populations of each *Bathycoccus*. This was done by fragment recruitment of the metagenomic reads of *Tara* Oceans samples onto the two reference assemblies. The distributions of nucleotide identities show a weak divergence between the reference assemblies and geographically distant samples, though higher for TOSAG39-1 than for RCC1105 (Supplementary Information, Supplementary Figure S12).

Discussion

We provide a novel *Bathycoccus* genome assembly using a single-cell genomics approach. This assembly is estimated to be 64% complete, which is, to our knowledge, the most complete eukaryotic genome obtained to date by this approach. This relatively high level of completion was reached through the combination of several independent cells originating from the same population. It has been described that the enzymatic amplification of DNA which is inherent to single-cell genomics induces strong biases in sequencing depth along the genome, leading to partial and fragmented assemblies[51]. Here, this caveat appears reduced as the combined-SAGs assembly is significantly less partial than the assembly obtained from each of the individuals SAGs.

This *Bathycoccus* SAG assembly is significantly different from the previously described genome assembly, originating from the coastal Mediterranean strain RCC1105. The former corresponds to the BI

clade and the latter to the BII clade as defined recently[11]. Orthologous proteins of these two genomes share only 78% identity, which is similar to the 74% of amino-acid identity shared by the two sequenced *Ostreococcus* isolates which belong to different clades[52].

A previous study[11] estimated a lower genetic distance (82% of identical nucleotides) between the two *Bathycoccus* using metagenomic data. This difference is probably as expected because of the reduced dataset of highly conserved and single copy genes (1 104 genes) considered in the latter analysis. The evolutionary distance that separates the protein coding genes of these two *Bathycoccus* is slightly smaller than the one between two vertebrate lineages separated by more than 400 million years (mammal and fish share 72% of identity[53]) and larger than the one reported between many model organisms (for example, human and mouse share 85% of identity[54,55]). This high divergence in protein coding genes and the frequent genes rearrangement in chromosomes is hardly compatible with chromatid pairing required for intercrossing[56] between the two *Bathycoccus*. Very few genes are highly conserved (> 99% identity) between the two *Bathycoccus* and conserved genes are not clustered, which makes active genetic exchange by homologous recombination unlikely. Therefore, although the two *Bathycoccus* share 100% similar rRNA gene sequences, these genomic differences reflect two different, probably cryptic, species. Identical rRNA sequences have been previously reported in the yeast *Saccharomyces cerevisiae sensu stricto* clade[57], or the haptophyte species *Emiliania huxleyi* and *Gephyrocapsa oceanica,* which also have identical 18S rRNA gene sequences, but quite different morphologies[58].

The combination of genomics and environmental data from a large set of oceanic samples revealed the distinct ecological preferences of the two *Bathycoccus* for depth, temperature, light and oxygen. TOSAG39-1 is usually found in warmer but deeper and darker water than RCC1105. TOSAG39-1 seems to be well adapted to the DCM conditions, which would explain its presence in oligotrophic marine zones where nutrients are found deeper.

Numerous marine bacteria show geographical variation of their gene repertoire[59–63] which affects genomic regions that generally represent only a few percent of the total genome[61] and has been proposed, in some cases, to result from horizontal transfer. In *Prochlorococcus*, genomic islands are thought to be related to

290 niche adaptation[63] because they host ecologically important genes[60]. A comparison of two
291 *Prochlorococcus* ecotypes revealed that differences in gene content were related to high-light *vs*. low-light
292 adaptation[64]. Such adaptations have been hypothesized in species closely related to *Bathycoccus*, like
293 *Ostreococcus*[17], but are still a matter of debate[9]Our data show that the depth and light ranges of the two
294 *Bathycoccus* are different but overlapping, with TOSAG39-1 extending deeper. Interestingly, the surface
295 samples where TOSAG39-1 was detected correspond to sites that undergo vertical mixing (Aghulas and
296 Gulf Stream). Temperature also seemed to influence the distribution of the two *Bathycoccus*, as for
297 example along the Gulf Stream where one type is more prevalent on the West side and is replaced by the
298 other type eastward as water cools down. Among eukaryotes, several examples of correspondence
299 between temperature and geographical distribution have been reported, such as for the heterotrophic
300 MAST-4[26,65] and the Arctic ecotype of *Micromonas*[8]. TOSAG39-1 was also observed at low O_2
301 concentrations at Costa Rica Dome station 138, an area of high biological production in the East
302 equatorial Pacific[66] where picoplankton can be very abundant[67]. This could reflect the fact that since
303 TOSAG39-1 is better adapted to low light conditions it could be found deeper in the water column where
304 suboxic conditions are developing, rather than having a specific capacity to withstand low O_2.
305 The wide geographical distribution and relatively high abundance of *Bathycoccus* observed here implies a
306 capability to thrive across a range of ecological niches. Dispensable genes could correspond to the
307 genomic traces of this adaptation. Intriguingly, dispensable *Bathycoccus* genes have genomic features
308 similar to those of chromosome 19 genes, such as a lower GC content. This suggests that these genes may
309 have been located on chromosome 19 ancestrally and have undergone subsequently inter-chromosomal
310 translocations. A recent experimental evolution experiment of *Ostreococcus tauri* inoculated with a large
311 quantity of virus, Otv5, provided evidence that genes on outlier chromosome 19 are up-regulated in viral-
312 resistant cell lines and that the size of this chromosome varies in resistant lines[68]. Our results on gene
313 content plasticity in Chromosome 19 is consistent with the immunity chromosome hypothesis, frequent
314 events of gene birth and gene loss may thus be the genomic traces of a microalgal – virus evolutionary
315 arm race.

316 Dispensable genes possess features of so-called *de novo* genes, genes emerging from previously
317 noncoding regions. These genes are an important class of unknown genes and challenge evolutionary
318 sciences[69,70]. It has been hypothesized that cosmopolitan bacteria would hold specific genes or gene
319 variants due to their ecological properties[71]. Cosmopolitan marine lineages are exposed to a range of
320 contrasted environmental constraints, raising the question of their genomic plasticity. The high turnover of
321 a certain class of genes restricted to some environmental conditions might be an evolutionary advantage
322 for rapid acclimation related to being cosmopolitan.
323
324 The amplification biases inherent to the Single Cell Genomics approach do not in general allow
325 recovering full genomes from environmental protists. However incomplete SAG assemblies are sufficient
326 to allow mapping of environmental metagenomes and to determine the distribution of genotypes that are
327 not resolved by traditional marker genes or metabarcodes. In the case of *Bathycoccus* we provide the
328 distribution of two clades, corresponding to the genomes of RCC1105 (clade B1) and to the genome of
329 TOSAG39-1 (clade B2) and identify environmental parameters underlying these distributions. Our
330 observations unfortunately do not cover all oceanic ecosystems, particularly the polar zones. Future
331 analysis of additional genomes and transcriptomes of wild and cultured *Bathycoccus* will improve the
332 accuracy of the environmental niches of the two types of *Bathycoccus*.
333
334 **Material and Methods**
335 During the *Tara* Oceans expedition[34,35], we collected and cryo-preserved samples at station TARA_039
336 situated in the Arabian Sea (Supplementary Figure S13, oceanographic conditions are available in
337 reference[36]). In the laboratory, single cells were sorted by flow cytometry based on their size and
338 chlorophyll autofluorescence. Four *Bathycoccus* cells were identified following DNA amplification and
339 18S rDNA sequencing[37]. The four amplified genomes were individually sequenced using Illumina HiSeq
340 technology, and a suite of tools was used to obtain single-cell final assembly (Supplementary
341 Information). Firstly, individual assemblies were generated using a colored de Bruijn graph-based

342 method[72] and then a final assembly, named here as TOSAG39-1, was generated comprising gap-reduced
343 scaffolded contigs, using SPAdes, SSPACE and GapCloser[73–75] (Supplementary Figure S14). The four
344 cells had identical 18S sequences and came from the same 4 mL sample, so it is reasonable to presume
345 they were of the same population.
346 Quality control filters detected and removed contigs or scaffolds that did not correspond to *Bathycoccus*
347 nuclear DNA (Supplementary Figure S14, Supplementary Information). Direct comparisons of sequence
348 assemblies detected putative DNA contamination from other SAGs that were sequenced in the same
349 laboratory and scaffolds corresponding to organelles.
350 We predicted exon-intron gene structures by integrating various coding regions data. We aligned the
351 reference protein set of the published *Bathycoccus* RCC1105 genome[22] to our assembly. We extracted and
352 sequenced polyA mRNA from *Tara* Oceans samples. We aligned this eukaryote metatranscriptome on
353 TOSAG39-1 assembly. We also used a public protein databank[76] and the Marine Microbial Eukaryote
354 Transcriptome Sequencing Project (MMETSP) collection of marine protist transcriptomes[42]. In addition,
355 we performed direct *ab initio* prediction by calibrating and running the Markov model implemented in
356 snap[77]. Integrating and combining all this evidence provided a final set of genes, using a process based on
357 Gmorse software rationale[78]. We evaluated the relative genomic abundance of each genome for two
358 sampled depths (surface and Deep Chlorophyll Maximum) at the 76 *Tara* Oceans stations (122 samples in
359 total, Supplementary Figure S13) by recruiting metagenomic reads[24]. We mapped metagenomic reads
360 directly from 0.8–5µm organism-size fraction samples onto genome assemblies, and estimated the relative
361 contribution of each *Bathycoccus* genome in the metagenomes. To obtain a proper genome abundance
362 estimate, we developed methods to select genome-specific signals only (Supplementary Information). We
363 discarded highly conserved genes that were detected by direct sequence comparisons.
364 A more detailed description of methods is available in the online supplementary information.
365
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Author Contributions

CdV, MS, PW and OJ designed the study. OJ wrote the paper, with significant inputs from DV, TV and PW. MS managed the single cell isolation; YS and JMA managed the SAG assembly and gene predictions. TV and OJ analyzed the genomic data, with significant input from JL, YS, SM, EP, JMA, DV and PW. TV, JL, DV, DI and OJ analyzed the oceanographic data. All authors discussed the results and commented on the manuscript.

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557 **Additional Information**

558 This article is contribution number XXX of *Tara* Oceans.

559 Physicochemical parameters from all *Tara* Oceans samples are available at Pangea
560 (http://doi.pangaea.de/10.1594/PANGAEA.840721); metagenomics reads can be downloaded at SRA
561 under identification study number PRJEB402 (https://www.ebi.ac.uk/ena/data/view/PRJEB402). The
562 sequences of TOSAG39-1 were deposited and are available at EMBL/DDBL/GenBank under accession
563 number XXX.

564 Supplementary information is available at Scientific Reports' website.

565 The authors declare no conflict of interest.

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571 **Figure Legends**

572 **Figure 1.** Comparisons of relative abundances of *Bathycoccus* in the 0.8-5µm size fraction samples from
573 *Tara* Oceans stations. Left: relative 18S rRNA V9 amplicons abundance (percent of reads). Right: relative
574 metagenomic abundances (percent of metagenomic reads) from direct mapping of metagenomic reads
575 onto two genome sequence assemblies (strain RCC1105 and TOSAG39-1, single cell assembly from an
576 Indian Ocean sample). Stations and depth (Surface or DCM) are indicated on the Y axis.

577 **Figure 2.** Geographical distribution of two *Bathycoccus* genomes, RCC1105 and TOSAG39-1, along
578 *Tara* Oceans expedition stations from recruitments of metagenomic reads. Top and bottom maps
579 correspond to the surface and deep chlorophyll maximum (DCM) samples respectively. Gray crosses
580 indicate *Tara* Oceans sampling stations and the sizes of the red or blue circles indicate the relative
581 genomic abundances of the two *Bathycoccus* types. We generated this map using R-package maps_2.1-6,
582 mapproj_1.1-8.3, gplots_2.8.0 and mapplots_1.4 (version R-2.13, https://cran.r-
583 project.org/web/packages/maps/index.html).

584 **Figure 3.** Relationships between environmental parameters and *Bathycoccus* genome abundance. Left:
585 Principal component analysis. We only considered stations where we detected 98% of the genes for one
586 *Bathycoccus* genome, and for which all environmental parameters were available (Oxygen, Nitrates,
587 Phosphates, Chlorophyll, Sampling Depth, Water Temperature and Salinity). Crosses indicate stations,
588 with a color scale corresponding to the water temperature. The distance to coast parameter corresponds to
589 the shortest geographical distance to the coast. The two *Bathycoccus* are distributed along temperature and
590 oxygen axes. Stars indicate parameters that statistically discriminate the two *Bathycoccus*. Right: Range of
591 values of temperature, oxygen and sampling depth for parameters where a significant difference was
592 detected between RCC1105 and TOSAG39-1.

593 **Figure 4.** Evidence for cassettes of dispensable genes in *B. prasinos* RCC1105. Left and right sides of the
594 figures represent fragment recruitment and genomic alignments of dispensable gene cassettes,
595 respectively. Fragment recruitments plots are displayed by marine zones (left legend). Each dot

corresponds to a given number of mapped reads at a given identity percent (indicated on the Y-axis). The density of mapped read is displayed as the black line plotted below each fragment recruitment plot. Gene positions are represented by black boxes on the top of the first fragment recruitment plot and dispensable genes are highlighted in red. Genomic alignments are represented as circos graphs[79] on which dispensable genes are colored in red, and other genes are represented by black boxes. Left side and right side of the genomic region are connected to metagenomics contigs (gray segments), leaving in-between the locus of the dispensable gene cassette that remains unconnected to any metagenomic contig. Connections correspond to blast alignments positions. A. 100- and 8.6-kb regions of chromosome 1 are represented on a fragment recruitment plot and on the circos graph, respectively. A two gene long cassette is represented. A massive decrease of read coverage appears on the fragment recruitment plot in all oceanic zones except in the Mediterranean Sea, which indicates that the two genes are present only in a sub-population in this basin. A similar pattern is observed in panel B for four consecutive genes for which fragment recruitment plots representing 100 kb of chromosome 5 suggest a presence in a Mediterranean sub-population and absence in other marine areas. The circos graph represents alignments along the 15.6-kb cassette locus with metagenomics contigs, which resulted in a gap that included three small genes (in blue) in addition to the four automatically detected dispensable genes. Fragment recruitment confirmed a significant, but not total, decrease of read coverage for these three genes in every oceanic zone, indicating that their presence or absence in the two sub-populations was widely distributed.

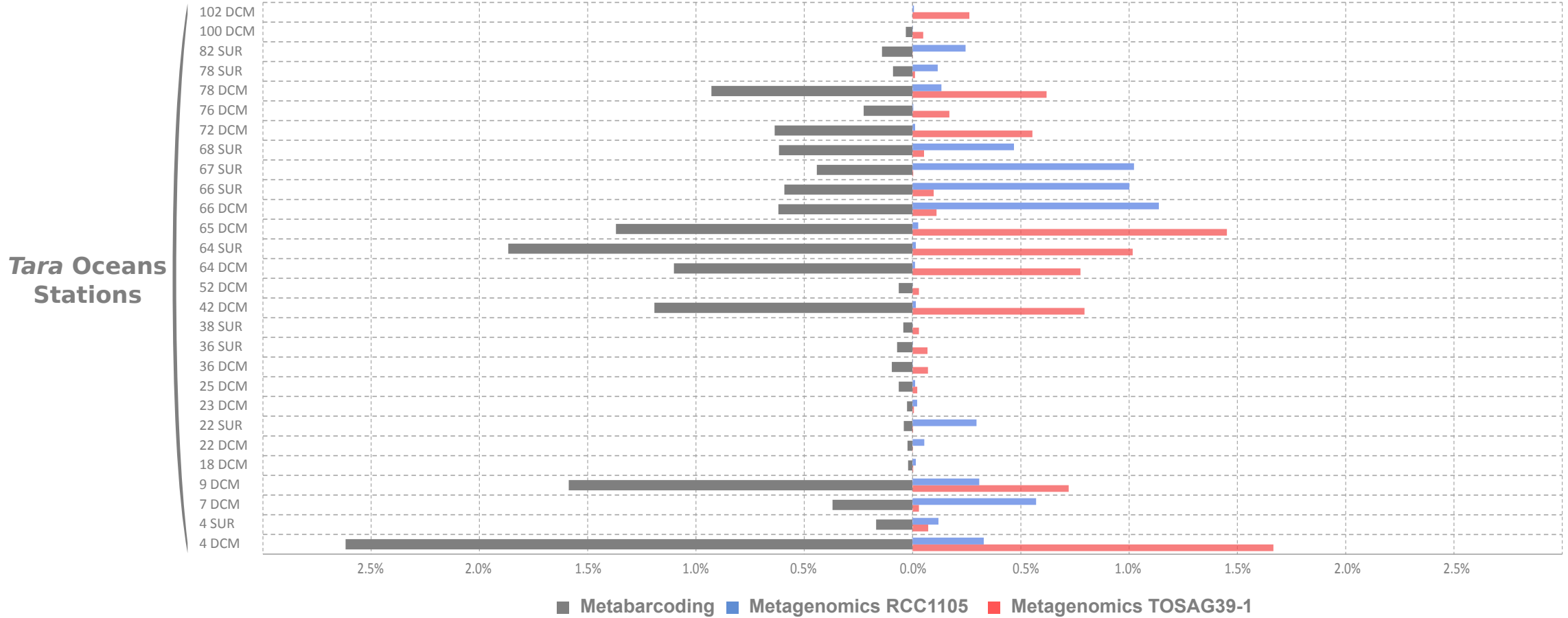
Table 1. Assembly summaries of TOSAG39-1.

SAG Assembly	Total Size (Mb)	N50 (kb)	NG50[(1)] (kb)	Genome Completion (%)
A	3.5	14.8	NA	30.8
B	4.7	14.5	NA	27.7
C	3.7	24.1	NA	21.5
D	4.1	18.1	NA	26.0
(A)+(B)+(C)+(D)[(2)]	8.0	16.6	0.9	44.6
Combined ABCD[(3)]	10.1	14.1	6.0	63.0

[1] The longest assembly contigs covering together half of the genome size (15 Mbp) are each longer than the NG50. This evaluation was not possible for the four individual cell assemblies for which the total assembly sizes are shorter than half of the genome size.

[2] A+B+C+D corresponds to a non-redundant merging of contigs from individual assemblies

[3] Combined ABCD corresponds to the co-assembly process.

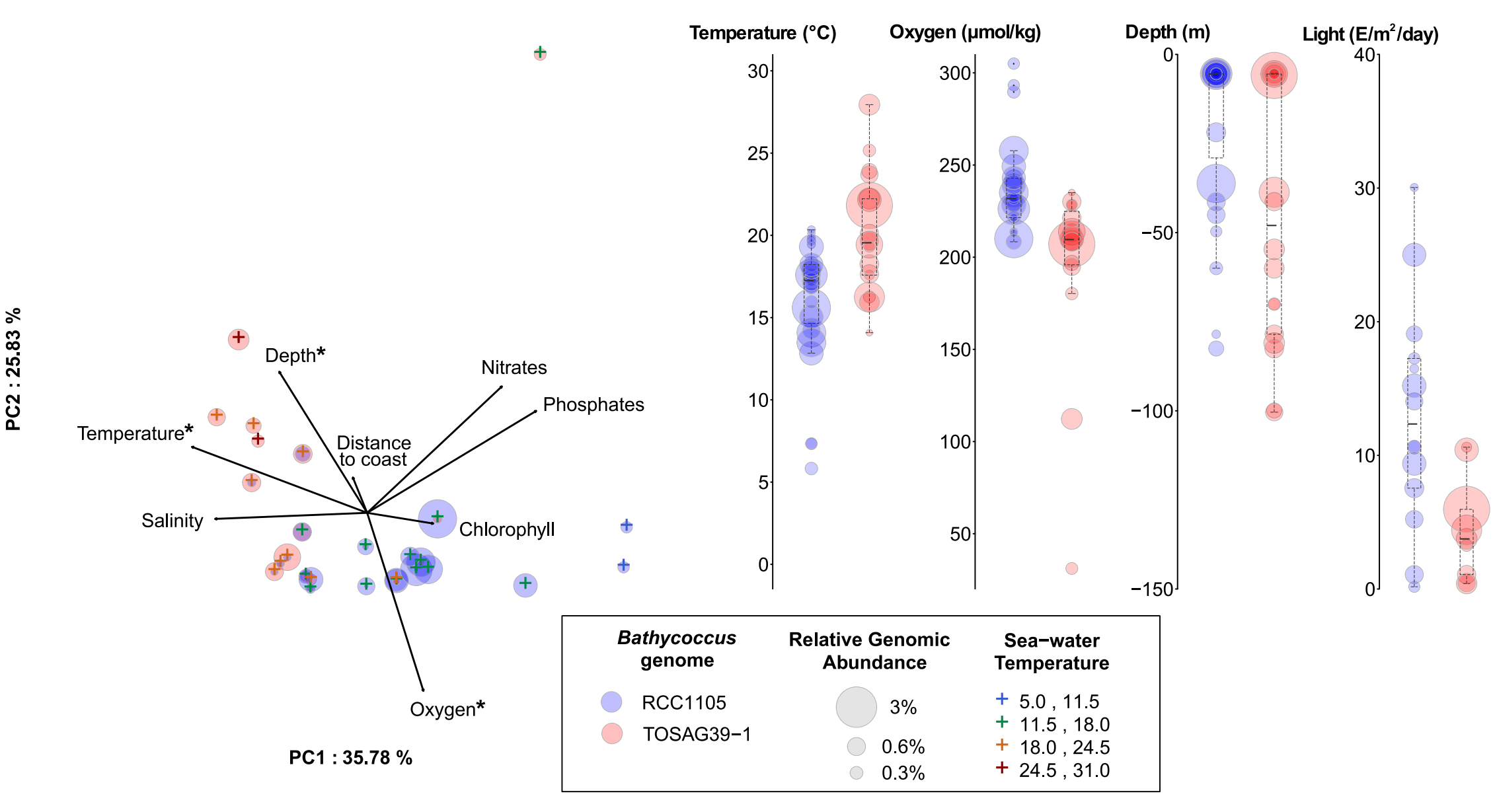

Tara Oceans Stations
102 DCM
100 DCM
82 SUR
78 SUR
78 DCM
76 DCM
72 DCM
68 SUR
67 SUR
66 SUR
66 DCM
65 DCM
64 SUR
64 DCM
52 DCM
42 DCM
38 SUR
36 SUR
36 DCM
25 DCM
23 DCM
22 SUR
22 DCM
18 DCM
9 DCM
7 DCM
4 SUR
4 DCM
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
0.5%
1.0%
1.5%
2.0%
2.5%
Metabarcoding
Metagenomics RCC1105
Metagenomics TOSAG39-1

Surface sea-water

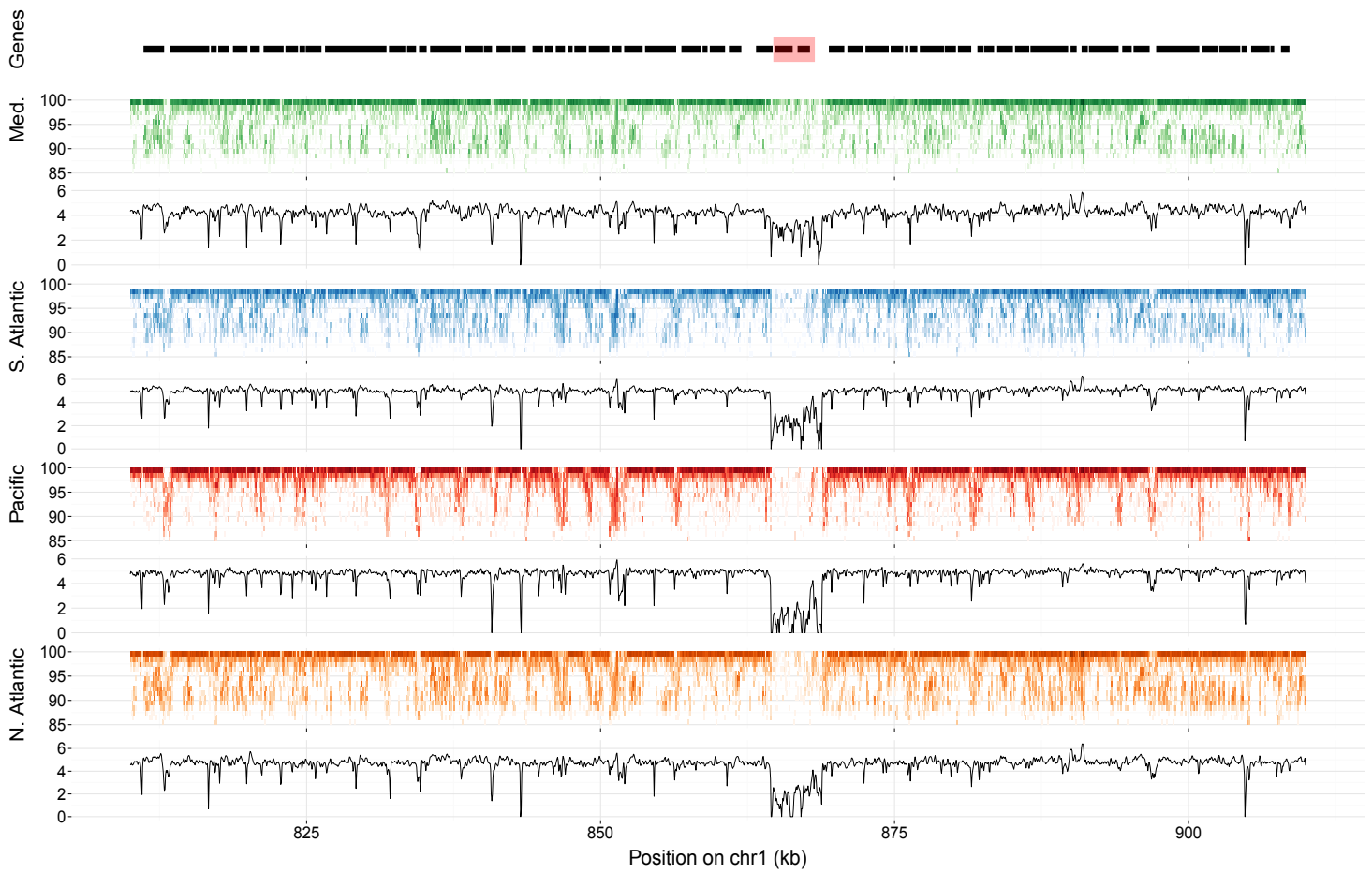
Deep Chlorophyll Maximum water layer

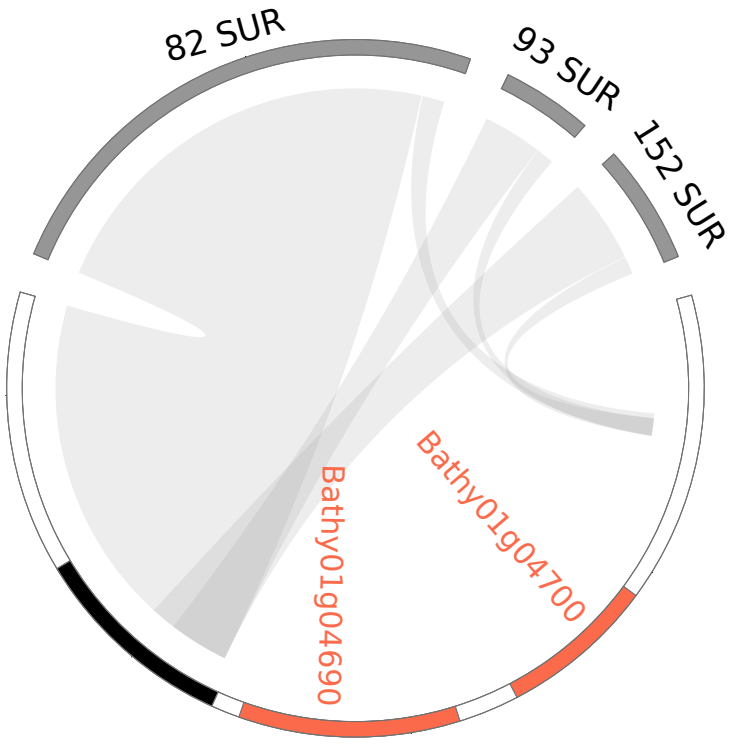
***Bathycoccus* genome :** RCC1105 TOSAG39−1

Relative Genomic Abundance : 3% 0.6% 0.3%


PC2 : 25.83 %
PC1 : 35.78 %
Depth*
Nitrates
Phosphates
Temperature*
Distance
to coast
Salinity
Chlorophyll
Oxygen*
Temperature (°C)
Oxygen (µmol/kg)
Depth (m)
Light (E/m²/day)
Bathycoccus genome
RCC1105
TOSAG39−1
Relative Genomic Abundance
3%
0.6%
0.3%
Sea−water Temperature
5.0 , 11.5
11.5 , 18.0
18.0 , 24.5
24.5 , 31.0

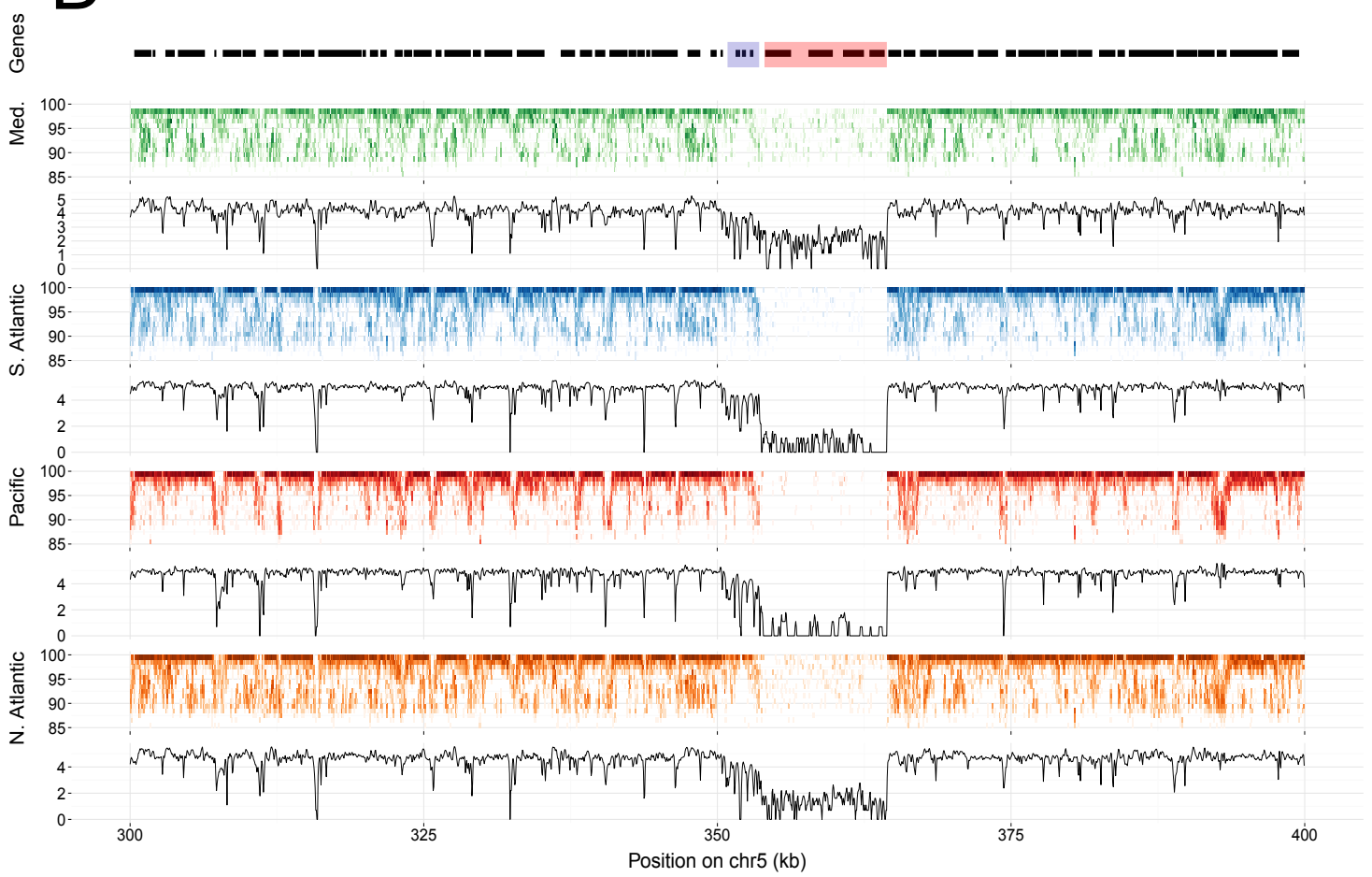
A

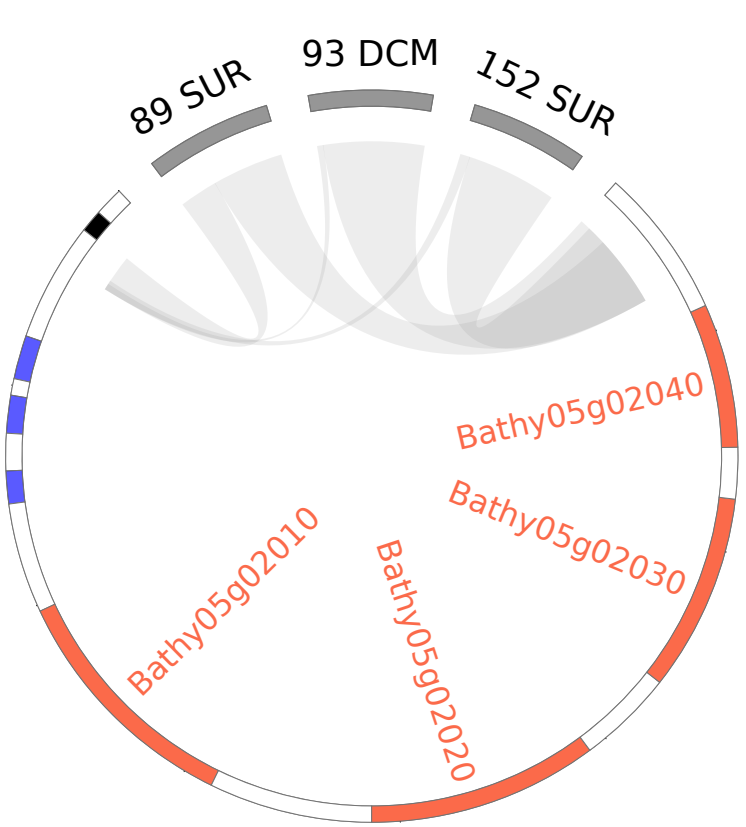




RCC1105 chr1
from 861298 to 869930

B





RCC1105 chr5
from 350000 to 365600