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Phylogeography and pigment type diversity of *Synechococcus cyanobacteria* in surface waters of the northwestern Pacific Ocean

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1 **Phylogeography and pigment type diversity of *Synechococcus***
2 **cyanobacteria in surface waters of the northwestern Pacific**
3 **Ocean**

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15 **Running Title:** *Synechococcus* community diversity in the northwestern Pacific Ocean

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27 **Summary**

28 The widespread unicellular cyanobacteria *Synechococcus* are major contributors to
29 global marine primary production. Here we report their abundance, phylogenetic diversity (as
30 assessed using the RNA polymerase gamma subunit gene *rpoC1*) and pigment diversity (as
31 indirectly assessed using the laterally transferred *cpeBA* genes, encoding phycoerythrin-I) in
32 surface waters of the northwestern Pacific Ocean, sampled over nine distinct cruises (2008-
33 2015). Abundance of *Synechococcus* was low in the subarctic ocean and South China Sea,
34 intermediate in the western subtropical Pacific Ocean, and the highest in the Japan and East
35 China seas. Clades I and II were by far the most abundant *Synechococcus* lineages, the former
36 dominating in temperate cold waters and the latter in (sub)tropical waters. Clades III and VI
37 were also fairly abundant in warm waters, but with a narrower distribution than clade II. One
38 type of chromatic acclimater (3dA) largely dominated the *Synechococcus* communities in the
39 subarctic ocean, while another (3dB) and/or cells with a fixed high phycourobilin to
40 phycoerythrobilin ratio (pigment type 3c) predominated at mid and low latitudes. Altogether,
41 our results suggest that the variety of pigment content found in most *Synechococcus* clades
42 considerably extends the niches that they can colonize and therefore the whole genus habitat.

43

44 **Key words:** cyanobacteria, *Synechococcus*, *rpoC1*, western Pacific Ocean, marginal sea,
45 pigment type, genetic diversity.

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48

49 **Abbreviations:** phycocyanobilin (**PCB**); phycoerythrobilin (**PEB**); phycourobilin (**PUB**);
50 type IV chromatic acclimaters (**CA4**); photosynthetically available radiation (**PAR**); the limit
51 of detection (**LOD**); 16S–23S internal transcribed spacer region (**ITS**); the operational
52 taxonomic unit (**OTU**); Non-metric multidimensional scaling analysis (**NMDS**); redundancy
53 analysis (**RDA**).

54

55 **Introduction**

56 Marine unicellular cyanobacteria belonging to the *Synechococcus* genus are ubiquitously
57 distributed in the euphotic layer of the ocean and play a key role in the marine ecosystems in
58 terms of global carbon biomass (Garcia-Pichel *et al.*, 2003; Buitenhuis *et al.*, 2012) and net
59 primary production (Liu *et al.*, 1998; Flombaum *et al.*, 2013). *Synechococcus* communities are
60 both phenotypically and phylogenetically diverse. *Synechococcus* cells harvest light using large
61 complexes called phycobilisomes, constituted by a core of allophycocyanin surrounded by six
62 radiating rods with highly variable phycobiliprotein and chromophore composition (Six *et al.*,
63 2007). Based on the different phycobiliprotein composition of phycobilisome rods,
64 *Synechococcus* strains can be divided into three main pigment types: type 1 containing only
65 phycocyanin (PC), type 2 containing both PC and phycoerythrin-I (PEI) and type 3 possessing
66 PC, PEI and phycoerythrin-II (PEII). PEI and PEII have different amino acid sequence and
67 bilin composition and content, with the latter always binding both phycoerythrobilin (PEB) and
68 phycourobilin (PUB), whereas the former either bind only PEB or both PEB and PUB (Ong
69 and Glazer, 1991; Six *et al.*, 2007). In terms of chromophorylation, phycobilisomes of pigment
70 type 1 bind only phycocyanobilin (PCB), type 2 bind both PCB and PEB, while type 3 bind
71 PCB, PEB and PUB. Pigment type 3 *Synechococcus* can be further divided into type 3a
72 (PUB/PEB<0.6), 3b ($0.6 \leq \text{PUB/PEB} < 1.6$), 3c ($\text{PUB/PEB} \geq 1.6$) and type 3d/e (variable
73 PUB/PEB), according to the *in vivo* PUB/PEB ratio of whole cells, as assessed using the ratio
74 of the fluorescence excitation peaks of these two chromophores, i.e. usually $\text{Ex}_{495 \text{ nm}}:\text{Ex}_{550 \text{ nm}}$
75 (Everroad *et al.*, 2006; Six *et al.*, 2007, Everroad and Wood, 2012; Humily *et al.*, 2013). Strains
76 displaying either high (type 3d) or low (type 3e) amplitude of variation of their PUB/PEB ratio

77 in response to change in light quality from blue to green light (and vice versa) are also called
78 type IV chromatic acclimators (CA4). Genes involved in the CA4 process are gathered into a
79 small genomic island with two possible configurations, CA4-A and CA4-B (Humily *et al.*,
80 2013). The chromatically acclimating strains that possess a CA4-A or a CA4-B island type are
81 called 3dA or 3dB, respectively, but it is noteworthy that a number of strains that possess such
82 an island do not actually chromatically acclimate and therefore display a fixed PUB/PEB ratio,
83 possibly due to a dysfunction of the CA4 regulatory machinery.

84 The distribution of pigment types has been studied in the marine environment using
85 various approaches, including epifluorescence microscopy (Wang *et al.*, 2011), flow cytometry
86 (Olson *et al.*, 1988), spectrofluorimetry (Lantoine and Neveux, 1997; Wood *et al.*, 1998) and
87 sequencing of clone libraries of *cpcBA* and *cpeBA* operons, encoding alpha and beta subunits
88 of PC and PEI, respectively (Liu *et al.*, 2014; Chung *et al.*, 2015). Indeed, in sharp contrast
89 with phylogenies obtained from housekeeping genes such as 16S rRNA or *rpoCI* genes,
90 phylogenies made using such phycobiliprotein-encoding genes reflect better pigment content
91 than vertical phylogeny (Six *et al.*, 2007; Everroad *et al.*, 2012; Humily *et al.*, 2014). While the
92 characterization of cultured *Synechococcus* isolates showed that different pigment types can
93 collect different wavelengths of light (Olson *et al.*, 1988; Olson *et al.*, 1990; Stomp *et al.*, 2004;
94 Six *et al.*, 2007), field studies suggest that these differences can allow them to colonize distinct
95 light quality niches of the marine environment (Wood, 1985; Wood *et al.*, 1999; Stomp *et al.*,
96 2007; Liu *et al.*, 2014). Pigment type 1 *Synechococcus* harvest optimally red-orange light and
97 dominate in turbid estuarine waters (Wang *et al.*, 2011; Liu *et al.*, 2014), whereas type 2 mainly
98 absorb yellow-green light and preferentially thrive in turbid coastal or continental shelf waters

99 (Wood *et al.*, 1998). The different pigment subtypes within type 3 would mainly occur in
100 mesotrophic or oligotrophic oceanic waters where green or blue light predominate, respectively
101 (Olson *et al.*, 1988; Wood *et al.*, 1998). Both cultivation-dependent and independent methods
102 used to study the pigment diversity of *Synechococcus* communities have shown that multiple
103 pigment types co-occur in marine surface waters (Choi and Noh, 2009; Haverkamp *et al.*, 2009;
104 Humily *et al.*, 2014; Larsson *et al.*, 2014).

105 The vertical phylogeny of the marine *Synechococcus* group has also been extensively
106 studied. Based on the 16S rRNA gene marker, marine *Synechococcus* have been classified into
107 three phylogenetic subclusters, 5.1, 5.2 and 5.3 (hereafter S5.1, S5.2 and S5.3; Dufresne *et al.*,
108 2008). S5.1, which is more diverse than the other two subclusters, contains at least sixteen
109 clades (Ahlgren and Rocop, 2012). S5.1 and S5.3 are mainly composed of pigment types 2 and
110 3, except the euryhaline clade VIII (within S5.1), which so far only comprises strains belonging
111 to pigment type 1. In contrast, S5.2 contains pigment types 1 and 2 (e.g., strains CB0101 and
112 CB0205; Chen *et al.*, 2006). Studies of the global distribution of *Synechococcus* community
113 composition have revealed a clear spatial partitioning for the main *Synechococcus* lineages
114 (Zwirgmaier *et al.*, 2008; Huang *et al.*, 2012; Mazard *et al.*, 2012; Sohm *et al.*, 2015; Farrant
115 *et al.*, 2016). Indeed, in agreement with the different thermal growth range and preference of
116 representative strains (Pittera *et al.*, 2014), clade II was found to be prevalent in warm
117 subtropical and tropical waters, while clades I and IV dominate in cold waters. The relative
118 abundance of *Synechococcus* lineages also undergo seasonal variations (Fuller *et al.*, 2003;
119 Post *et al.*, 2011; Chung *et al.*, 2015). For instance, clade III often occurs in summer in warm,
120 stratified surface waters, whereas clade XII are generally prevalent in fall and winter (Post *et*

121 *al.*, 2011). Although 16S rRNA gene was the first phylogenetic marker used to define
122 *Synechococcus* lineages (Urbach *et al.*, 1998; Fuller *et al.*, 2003), some of these lineages cannot
123 be discriminated by this marker, due to its too low taxonomic resolution. Therefore, several
124 other markers such as the 16S–23S internal transcribed spacer region (ITS; Ahlgren and Rocap,
125 2012) or the single copy genes *rpoCI* (Toledo and Palenik, 1997), *ntcA* (Penno *et al.*, 2006)
126 and *petB* (Mazard *et al.*, 2012) have been applied to study the genetic diversity of
127 *Synechococcus* at higher resolution.

128 The western Pacific Ocean, an area extending from 15 to 55 °N, exhibits a wide variety
129 of hydrographic conditions with surface water temperature ranging from > 29 °C throughout
130 the year in the tropical ocean to < 10 °C in the subarctic ocean, providing a comprehensive
131 framework to study the effects of physico-chemical parameters on *Synechococcus* community
132 structure. This area encompasses five marginal seas, including the South China Sea, the East
133 China Sea, the Japan Sea, the Sea of Okhotsk and the Bering Sea, located along the
134 northwestern boundary of the Pacific Ocean, and altogether covering the tropical, temperate
135 and frigid zones (Fig. 1A). The warm current Kuroshio (Sawada and Handa, 1998) and the cold
136 current Oyashio (Qiu, 2001) as well as their extension (the Northern Pacific current; Cummins
137 and Freeland, 2007) also create some unique environments in the western Pacific Ocean. For
138 example, the Tokara Strait where the Kuroshio Current flows out of the East China Sea to
139 Pacific Ocean, has water temperature higher than the adjacent regions and does not exhibit
140 strong seasonal variations (Nagata and Takeshita, 1985), whereas the western subtropical
141 Pacific (East of Taiwan) is part of the western Pacific warm pool, a typical oligotrophic
142 environment with low nutrient concentration. As some seasonal variations in the abundance of

143 *Synechococcus* cells have been reported in the South China Sea (Xia *et al.*, 2015a) and East
144 China Sea (Guo *et al.*, 2014), the present study mainly focused on late spring and summer (with
145 the exception of Tokara Strait samples that were sampled in fall), in order to lower potential
146 effects of seasonal variations on data interpretation. Besides covering a wide temperature and
147 nutrient gradients, the study region also encompasses a large variety of optically distinct water
148 bodies, from turbid coast to transparent open ocean waters. Therefore, this region likely
149 comprises most of the niche types of *Synechococcus* that are reported so far in global ocean.
150 Studying concomitantly the phylogenetic and pigment type composition of *Synechococcus*
151 communities across such a wide range of environments allowed us to reveal the latitudinal
152 distribution patterns of different clades and pigment types, to assess the relative importance of
153 temperature and other environmental factors (in particular light quality) in determining niche
154 partitioning of *Synechococcus* populations, and to unveil some of the strategies developed by
155 *Synechococcus* to adapt to these different niches.

156

157 **Results**

158 ***Synechococcus* is widespread in surface waters of the northwestern Pacific Ocean**

159 Flow cytometry analyses showed that *Synechococcus* ubiquitously occurred in the
160 surface waters (5-10 m depth) of northwestern Pacific Ocean (Fig. 1B). During the sampling
161 periods, which are mainly late spring to summer, *Synechococcus* abundance was low in the
162 subarctic waters (Sea of Okhotsk, Bering Sea and western subarctic Pacific Ocean; $0.7\text{-}6.7\times 10^3$
163 cells mL^{-1} , average 1.7×10^3 cells mL^{-1}) and South China Sea ($2.5\times 10^3\text{-}3.9\times 10^4$ cells mL^{-1} ,
164 average 7.1×10^3 cells mL^{-1}), slightly higher in western subtropical Pacific Ocean ($2.5\times 10^3\text{-}$

165 3.7×10^4 cells mL⁻¹, average 1.2×10^4 cells mL⁻¹), and the highest in the East China Sea (8.0×10^3 -
166 2.8×10^5 cells mL⁻¹, average 7.1×10^4 cells mL⁻¹) and Japan Sea (1.9×10^3 - 1.1×10^5 cells mL⁻¹,
167 average 6.1×10^4 cells mL⁻¹). In the Tokara Strait, a region located South of Japan that was
168 sampled in November 2012, the abundance of *Synechococcus* was fairly low (1.3 - 2.2×10^4 cells
169 mL⁻¹; average 1.7×10^4 cells mL⁻¹) but comparable with that in open ocean waters of the East
170 China Sea collected in August 2009, suggesting that this was not due to seasonal variations
171 (Fig. 1B). The abundances of *Synechococcus* were the most variable in the East China Sea,
172 with the highest ones occurring in the northern part and in the mid-shelf region and the lowest
173 ones in the estuary of Changjiang River. In the South China Sea, the abundance of
174 *Synechococcus* was slightly higher in coastal than in open ocean waters. In the subarctic ocean,
175 *Synechococcus* abundance was only around 5.0×10^2 cells mL⁻¹ in the Bussol Strait connecting
176 the Sea of Okhotsk and the Pacific Ocean, where a strong vertical mixing occurred. This was
177 the lowest *Synechococcus* abundance recorded in all regions investigated in this study (Fig.
178 1B).

179 Altogether, the abundance of *Synechococcus* in surface water showed a weak positive
180 correlation with temperature and a weak negative correlation with PO₄³⁻ (Table S1). However,
181 when looking at individual geographic regions, nutrients as well as temperature were often
182 found to play a strong role in influencing the local abundance of *Synechococcus*. For example,
183 cell density in the western subtropical Pacific Ocean was found strongly affected by PO₄³⁻,
184 whereas TIN, PO₄³⁻, as well as temperature had a positive influence in the subarctic ocean. In
185 the South China Sea, which displays a fairly low temperature with regard to the western
186 subtropical Pacific Ocean, potentially due to the occurrence of a local coastal upwelling, we

187 observed a weak negative relationship between temperature and *Synechococcus* abundance. In
188 contrast, no significant correlation was observed between *Synechococcus* abundance and
189 salinity, even in the East China Sea region that was influenced by local river input (Table S1).

190

191 **The different pigment types of *Synechococcus* colonize different niches in the** 192 **environment**

193 The diversity of *Synechococcus cpeBA* operon in each community was estimated using
194 the Shannon diversity index (Fig. S1). The Shannon diversity index of the 12 *Synechococcus*
195 communities ranged from 0 to 2.18. WSAP-C5, which was collected from the western subarctic
196 Pacific Ocean, had the lowest diversity, while TS-ST4, collected from the Tokara Strait, had
197 the highest diversity. Altogether, the diversity of *Synechococcus cpeBA* operon was higher in
198 tropical and subtropical waters than in temperate waters (Fig. S1).

199 Phylogenetic analysis of the *cpeBA* operon sequences allowed us to separate four groups
200 of pigment types (Fig. 2): 2, 3a, 3dA and the combination of 3c and 3dB, since the latter two
201 types cannot be discriminated using this marker gene (Humily *et al.*, 2014). It is worth noting
202 that the only two strains known so far to exhibit a pigment type 3b in culture (WH8103 and
203 WH8109) are both genetically similar to 3dB strains and possess a complete CA4-B island, but
204 they have lost their ability for chromatic acclimation (Humily *et al.*, 2013). Thus, pigment types
205 3b and 3dB also cannot be differentiated based on phylogenies using *cpeBA* (Humily *et al.*,
206 2014). With these caveats in mind, our phylogenetic analyses showed that all pigment types
207 detectable with this marker were present in the northwestern Pacific Ocean, but their relative
208 abundance exhibited large geographical variations (Fig. 3). *Synechococcus* pigment type 2 was

209 by far the least abundant, mainly occurring at ECS-KP01, a station influenced by a river plume.
210 Pigment type 3a was abundant in the Tokara strait and dominant in the whole East China Sea
211 region (Fig. 3). The group composed by type 3c and/or type 3dB was largely dominant at both
212 stations of the South China Sea as well as at the southernmost station of the Northern Pacific
213 Current. Most strikingly, chromatic acclimators of the 3dA type constituted almost the sole
214 pigment type detected in the western subarctic Pacific Ocean and Bering Sea, but occurred in
215 mixture with 3dB/3c and 3a types at the northernmost station of Northern Pacific Current.

216

217 ***Synechococcus* genetic diversity is much higher in the subtropics than in the subarctic**
218 **ocean**

219 In this study, the *rpoC1* gene of 33 samples from the northwestern Pacific Ocean (Fig.
220 1A) were sequenced. After cleaning, 1,739 high-quality sequences of each sample remained
221 for further analysis. The Shannon diversity index of *Synechococcus* communities ranged from
222 0.99 to 4.34 (Fig. S2). Generally, the diversity of *Synechococcus* in summer was low in the
223 subarctic ocean (Bering Sea, Sea of Okhotsk and western subarctic Pacific Ocean), slightly
224 higher in the South China Sea, and the highest in the East China Sea (Fig. S2). In particular,
225 ECS-DH13 and ECS-PN05 showed very high diversity index values. The Shannon diversity
226 based on the *rpoC1* sequences was systematically higher than that based on the *cpeBA*
227 sequences at all studied stations.

228 In the western Pacific Ocean, during summer, the *Synechococcus* community
229 composition at sites located above and below 40°N (Groups A and B on Fig. 4A, respectively)
230 were significantly different ($P=0.001$, One-way Analysis of Variance). Sites NPC-J6, JS-TE3,

231 JS-JY1, and JS-SH2, which lie between latitudes 34°N and 40°N did not fall in any of these
232 groups, indicating that the *Synechococcus* community compositions at those sites were unique
233 and distinct from all other stations. Since all Japan Sea samples were collected in spring, we
234 cannot exclude that their specificity results in part from seasonal variations. In the case of the
235 Northern Pacific Current cruise, the two southernmost samples from the (NPC-K1 and A1), a
236 region also sampled in spring, fell close to Tokara strait samples collected at the same latitude
237 but in winter. Yet the latter set of samples fell within group B, which mainly gathers low latitude
238 samples collected during summer, suggesting that latitude has a stronger effect on genetic
239 diversity than season. To better split *Synechococcus* communities falling within group B
240 (average similarity: 29.31%), we did a further NMDS analysis specifically on this group (Fig.
241 4B). In the East China Sea, *Synechococcus* communities formed two major sub-groups
242 composed of samples collected below and above 32°N, respectively (Fig. 4B). Similar result
243 was observed in the North Pacific Current (Fig. 4A). The coastal station ECS-KP01, which is
244 strongly influenced by the river discharge, did not group together with other East China Sea
245 samples.

246 More than 80% of reads in each sample were taxonomically assigned to known lineages
247 using the reference database, except stations SCS-SEATS, SCS-LE09 and WSTP-ST1, where
248 a higher number of unknown sequences were detected, potentially corresponding to novel
249 clades (Fig. S3). All three *Synechococcus* subclusters were detected but S5.1 was by far the
250 most abundant and widespread and constituted the only subcluster detected in the subarctic
251 ocean (Fig. S3). S5.2 was only found in 13 samples, and its relative abundance was lower than
252 1% in 8 of them. The highest abundance of this subcluster (15.5%) was detected at the coastal

253 ECS-KP01 station. S5.3 was widespread but generally occurred at low abundance in the
254 tropical and subtropical ocean and was virtually absent in cold waters of the Japan Sea, Sea of
255 Okhotsk, western subarctic Pacific Ocean and Bering Sea. Yet, its abundance was fairly high
256 in the Tokara Strait, especially at TS-ST5 (Figs. 5 and S3).

257 Altogether, 17 clades within S5.1 were detected in the western Pacific Ocean with clades
258 I, II, III and VI being the most abundant (Fig. 5). Clade I mainly occurred in the subarctic ocean,
259 while clade II widely dominated in tropical and subtropical areas. Compared to clade II, clade
260 III also occurred in warm waters, but with a lower abundance and a narrower distribution (Figs.
261 5 and S3). Clade VI was also widely distributed in warm East China Sea (DH04 and HH12)
262 and TS waters that are influenced by the Kuroshio Current. Clades VII and WPC2 exhibited a
263 similar niche as S5.3, all displaying a wider distribution than clade VI. Clades Miyav and IX
264 co-occurred with S5.2, mainly in waters influenced by a nutrient-rich and turbid river plume.
265 WPC1 was mainly found in the East China Sea, with a similar distribution to clade III. Clade
266 XV co-occurred with clade XVI and CRD1, especially in the Northern Pacific Current.

267 RDA analysis showed that temperature, which explained 56.0 % of the variance of
268 *Synechococcus* community, was by far the most important factor determining the distribution
269 of *Synechococcus* lineages in the northwestern Pacific Ocean (Fig. 6A). Clade I and IV relative
270 abundances were associated both with low temperature and high nutrients, while all other
271 clades showed reverse correlations with these parameters (Fig. 6A). This is consistent with the
272 occurrence of these two clades being almost restricted to high latitudes ($> 35^{\circ}\text{N}$), where the
273 surface water temperature was lower than 12°C , while clade II often accounted for more than
274 60 % of the sequences from 18°N to 34°N (Fig. 7A). Other important lineages, i.e. clades III,

275 VI and S5.3, were only abundant between 25°N and 35°N (Fig. 7B).

276 Focusing on stations with temperature higher than 25 °C then allowed us to better
277 discriminate the other important parameters driving the distribution of all lineages except
278 clades I and IV (Fig. 6B). Clade IX, Env-Miyav and S5.2 were positively correlated to TIN and
279 phosphate concentrations, consistent with their preference for river plume influenced waters
280 (Figs. 6 and S3). Clades II and III also appeared to be well separated on correlation biplots (Fig.
281 6B). However, the parameters explaining their respective distribution remain unclear even
282 though salinity could partially explain the observed differences, clade III being most abundant
283 in the East China Sea (Fig. S4), which displayed the lowest salinity of the studied area (Table
284 S2).

285

286 **Unveiling *Synechococcus* diversity within clade I in the western Pacific Ocean**

287 Although *Synechococcus* clade I is generally considered as typical of cold, mesotrophic
288 and eutrophic waters (Zwirgmaier *et al.*, 2008), members of this clade were also detected at
289 low abundance in warm waters of East China Sea, TS and Northern Pacific Current (Fig. 8).
290 Phylogenetic analysis of clade I *rpoC1* sequences showed that OTUs, which dominated in the
291 subarctic ocean (Bering Sea, Sea of Okhotsk and western subarctic Pacific Ocean) clustered
292 together. In contrast, OTUs occurring at lower latitude (East China Sea, Tokara Strait, Japan
293 Sea and Northern Pacific Current) could be split into five additional subclades, displaying
294 slightly different distribution patterns. Thus, one subclade is truly psychrophilic as it mainly
295 dominated in subarctic oceanic waters, where the surface water temperature was lower than
296 12 °C, while other subclades occur in warm waters with temperature ranging from 15 to 29 °C.

297 Fig. 8 also shows that the diversity of *Synechococcus* clade I was the highest between 34°N
298 and 40°N.

299

300 **Linking *Synechococcus* genetic diversity and pigment content.**

301 In order to examine the links between *Synechococcus* phylogeny, as assessed using the
302 *rpoC1* marker, and pigment content, as indirectly assessed using the *cpeBA* operon, a Spearman
303 rank correlation analysis was performed over the whole Northwestern Pacific Ocean dataset
304 (Fig. S5). It showed that pigment type 2 (no PUB) was strongly correlated with clades IX and
305 Env-Miyav, while type 3a (low PUB:PEB) was associated with clades III, V and WPC1.
306 Pigment type 3dA (one of the two types of chromatic acclimators) was strongly positively
307 correlated with clade I but anti-correlated with clade II. At last, the two indistinguishable
308 pigment types 3c (high PUB:PEB) and 3dB (the second chromatic acclimeter type) were
309 associated with clades II and UC-A. Thus, even if pigment types are not restricted to single
310 clades, they seem to be preferentially found in certain clades in the field.

311

312

313 **Discussion**

314 **Factors controlling the abundance of *Synechococcus* in the northwestern Pacific Ocean**

315 This study, which analyzed surface samples collected from nine distinct cruises in the
316 northwestern Pacific and its marginal seas, provides a comprehensive overview of the
317 variability of *Synechococcus* abundance in this vast oceanic region and novel insights on how
318 it relates with environmental factors. While the *Synechococcus* abundance was high in the

319 subtropical and temperate areas (25-40°N), especially near the coasts (East China Sea and
320 Japan Sea), it was fairly low in the tropical (South China Sea) and subarctic regions (Figs. 1B
321 and 7A). Using a parametric regression model, a recent study also predicted strong variations
322 of *Synechococcus* abundance with latitude, but when averaged over a year, the abundance peak
323 was located around 45°N (Flombaum *et al.*, 2013), i.e. at a significantly higher latitude than
324 observed here (around 33.5°N; Fig. 1B). This difference may arise in part from seasonal
325 variations in *Synechococcus* abundance (see e.g. Fig. S5 in Flombaum *et al.*, 2013) but also
326 from the fact that latitudinal variations of abundance could significantly differ between the
327 marginal seas studied here and the central part of the northwestern Pacific Ocean, and this local
328 distribution pattern may have been missed by the global model developed by Flombaum and
329 coworkers. Indeed, the highest cell densities reported here were all recorded in coastal seas
330 (Fig. 1B), with a maximal abundance of 2.8×10^5 *Synechococcus* cells mL⁻¹ in the East China
331 Sea in summer. This value is one order of magnitude higher than those reported during summer
332 1998 in the same area (Jiao *et al.*, 2005), but is comparable to those reported in other nutrient-
333 rich areas around the world, such as the Bedford Basin (Li, 1998) and the Martha Vineyard's
334 coastal observatory in summertime (Hunter-Cevera *et al.*, 2015), as well as the Arabian Sea
335 (Liu *et al.*, 1998) or areas influenced by local upwelling (Partensky *et al.*, 1996). It is however
336 much lower than those observed in the Costa Rica dome, where record abundances between
337 1.2×10^6 and 3.7×10^6 cells mL⁻¹ have been reported (Saito *et al.*, 2005).

338 A number of previous studies have suggested that the net abundance of *Synechococcus*
339 is mainly controlled by temperature and nutrients (Agawin *et al.*, 2000; Zwirgmaier *et al.*,
340 2008; Tai and Palenik, 2009). Here we found that when considering our whole dataset on

341 surface waters of the western North Pacific Ocean, *Synechococcus* abundance was only weakly
342 correlated with temperature and phosphate availability and not correlated with TIN availability
343 (Table S1). Yet, this overall trend clearly masks local disparities. As for the first parameter,
344 temperature seems to be more influential to *Synechococcus* abundance in low temperature
345 subarctic ocean than tropical/subtropical warm waters. This is in agreement with a previous
346 study showing that *Synechococcus* abundance is positively correlated to temperature only
347 below 14 °C (Li, 1998). Although Flombaum and coworkers predicted that the abundance of
348 *Synechococcus* cells in the world ocean will increase by 14% at the end of this century, due to
349 the global rise in sea surface temperature (Flombaum *et al.*, 2013), they also foresaw that this
350 increase will not equally affect all latitudes. Our results indeed suggest that in the northwestern
351 Pacific Ocean, effects of global change should be much more significant in the subarctic region,
352 where both surface temperature and *Synechococcus* abundance are low, than in temperate,
353 tropical or subtropical waters.

354 As concerns nutrient concentrations, our statistical analyses showed that *Synechococcus*
355 abundance was positively influenced by nutrient availability in the subarctic ocean but not in
356 the tropical/subtropical waters (Table S1). *Synechococcus* abundance in the latter regions was
357 higher in coastal and river plumes influenced waters than in oligotrophic oceanic waters and
358 low salinity estuarine waters (Fig. 1B). This is in agreement with the dome-shaped distribution
359 proposed by Liu *et al.*, (1998), which suggested that *Synechococcus* grow best in waters with
360 intermediate level of nitrate. Consistently, Chung and coworkers, who also studied the
361 distribution of *Synechococcus* in the East China Sea observed during non-flooding summer the
362 occurrence of a bloom of PE-rich *Synechococcus* on the outer boundaries of the Changjiang

363 River in water with a salinity comprised between 31 and 32 ppt (Chung *et al.*, 2014).

364

365 **Niche partitioning of marine *Synechococcus* pigment types**

366 The wide diversity of pigment types that we observed in the northwestern Pacific Ocean
367 translates the large variety of light environments encountered in this vast oceanic region, from
368 turbid estuaries to transparent open ocean waters (Fig. 3). Interestingly, while different
369 *Synechococcus* pigment types co-occurred within most samples, one phenotype generally
370 predominated. This observation supports the hypothesis that the spectral properties of seawater
371 exert a strong selective pressure on *Synechococcus* populations, favoring the pigment type
372 possessing phycobilisomes with light absorption characteristics matching at best the local
373 spectrum of photosynthetically available radiation (PAR). While low PUB:PEB cells (type 3a)
374 dominated the population all over the East China Sea, *Synechococcus* cells lacking PUB (type
375 2) were only abundant in a low transparency station (St. ECS-KP01, see Fig. 2 of Chung *et al.*,
376 2014). This indicates that the latter pigment type is well adapted to harvest light in these fairly
377 turbid waters (Olson *et al.*, 1988; Wood *et al.*, 1998), where the PAR spectrum is likely shifted
378 towards yellow/yellow-green light due to organic matter in suspension (Kirk, 1994), while
379 pigment type 3a can stand wider PAR spectra, extending from blue-green to yellow-green (see
380 e.g., Six *et al.*, 2007 for representative absorption spectra of pigment types 2 and 3). It is
381 noteworthy that although analysis of the *cpeBA* operon diversity does not allow to detect
382 *Synechococcus* type 1 cells since they lack phycoerythrin, this pigment type was probably also
383 present at station ECS-KP01, as these phycocyanin-rich *Synechococcus* were recently shown
384 to predominate in the highly turbid diluted waters of the Changjiang River (Chung *et al.*, 2014).

385 As mentioned above, type 3dB cells, (i.e. chromatic acclimators possessing a CA4-B genomic
386 island; Humily *et al.*, 2013), are not distinguishable from type 3c (i.e. cells with a fixed high
387 PUB:PEB ratio) based on analyses of the *cpeBA* operon. It is therefore not possible from our
388 data to precisely assess the respective habitat of these two pigment types, though it appears that
389 both are absent from turbid, coastal as well as high latitude waters. Previous studies using flow
390 cytometry or spectrofluorometry have concluded that blue, oligotrophic areas are populated
391 with type 3c cells (Olson *et al.*, 1988; Campbell and Iturriaga, 1988; Lantoiné and Neveux,
392 1997; Wood *et al.*, 1999; Haverkamp *et al.*, 2009; Everroad and Wood, 2012). Yet, it is worth
393 noting that these methods also cannot distinguish between type 3c and 3dB cells, since both
394 these pigment types exhibit high PUB:PEB ratios in blue light (Humily *et al.*, 2013). In contrast,
395 our study clearly indicated, for the first time, that type 3dA, i.e. chromatic acclimators
396 possessing a CA4-A genomic island, were by far the predominant pigment type in the subarctic
397 ocean area. Due to the low angle of the sun at these latitudes, light intensity decreases rapidly
398 with depth, resulting in an unstable light environment (Nosaka *et al.*, 2014). Thus, the ability
399 to perform chromatic acclimation seemingly constitutes an advantage in environments with
400 variable light conditions, consistent with a previous study showing the abundance of chromatic
401 acclimators in permanently mixed waters of the English Channel (Humily *et al.*, 2014).

402

403 **Niche partitioning of *Synechococcus* clades**

404 *Synechococcus* clades are known to partition more strongly along the horizontal scale
405 than with depth (Zwirgmaier *et al.*, 2008; Choi and Noh, 2009; Sohm *et al.*, 2015), indicating
406 that even though our study dealt only with populations from surface waters, the relative

407 abundance of clades that we observed at any given station is truly representative of the diversity
408 of local *Synechococcus* populations. Several studies have suggested that clades I through IV
409 are the most abundant clades at the global scale, with clades I and IV predominantly found in
410 cold and temperate waters, while clades II and III prefer warmer waters (Zwirgmaier *et al.*,
411 2008; Mella-Flores *et al.*, 2011; Post *et al.*, 2011; Huang *et al.*, 2012). Here we show that clade
412 I was by far the dominant clade in the subarctic ocean and temperate waters of Japan Sea and
413 Northern Pacific Current, whereas clade II appears to be its counterpart in subtropical and
414 tropical areas (Figs. 5 and S4), suggesting that these two dominant clades are mutually
415 exclusive. In contrast, clade IV was scarce over all the studied area, including cold waters, in
416 agreement with a previous report of low abundances of the latter clade in the Bering Sea (Huang
417 *et al.*, 2012).

418 Interestingly, a number of clade I sequences was also retrieved from East China Sea,
419 Tokara Strait and Northern Pacific Current (Fig. 8), consistent with previous studies reporting
420 the occurrence of this clade as a minor component of *Synechococcus* communities in tropical
421 and subtropical, warm waters (Fuller *et al.*, 2006; Ahlgren *et al.*, 2014). This wide distribution
422 of clade I might be due in part to the large microdiversity existing within this clade. A single
423 subclade encompassing eight OTUs (subclade I-F) dominated in the subarctic ocean cluster,
424 while OTUs present in warm and temperate waters (East China Sea, Tokara Strait and Northern
425 Pacific Current) appeared more diverse, clustering into 5 separate subclades (I-A through I-E;
426 Fig. 8). Interestingly, members of all six subclades co-occurred at intermediate latitudes (Japan
427 Sea and/or Northern Pacific Current), suggesting that the realized niches of warm- and cold-
428 adapted clade I populations overlap in this transition area in which genetic exchange could

429 occur, e.g., by lateral gene transfer. The wider abundance and geographic distribution of clade
430 I compared to clade IV could also possibly be explained by the fact that members of the former
431 clade are genetically more versatile than members of the latter clade, as suggested by
432 comparative genome analysis of strains representative of these two clades (Dufresne *et al.*,
433 2008; Scanlan *et al.*, 2009; Tai and Palenik, 2009)

434 Although clade III was locally abundant in warm waters (>25 °C), it had a more limited
435 distribution pattern than clade II and was only abundant in the nutrient-rich and/or low salinity
436 waters of East China Sea (Figs. 5 and S4). This observation is in sharp contrast with the realized
437 niche of this clade in the eastern basin of the Mediterranean Sea, a very oligotrophic, strongly
438 phosphate-depleted, high-salinity area, where clade III constitutes the locally dominant
439 *Synechococcus* taxon (Mella-Flores *et al.*, 2011; Farrant *et al.*, 2016). This suggests that, like
440 clade I, clade III encompasses several subpopulations with very distinct nutrient and/or salinity
441 preferenda, i.e. distinct ecotypes.

442 The fourth most abundant taxon in the northwestern Pacific was clade VI but its
443 geographical distribution was limited to the Tokara strait and the central part of East China Sea
444 (Figs. 5 and S4), suggesting that it has a narrow niche. Yet, correlation analyses did not allow
445 us to clearly identify factors delineating its niche, and its overall distribution is also not well
446 understood so far since counts of this clade have often been merged with those of the related
447 clades V, VII and/or CRD1 (Zwirgmaier *et al.*, 2008; Huang *et al.*, 2012). Thus, more field
448 and culture studies are clearly needed to characterize this group. A few other clades within S5.1
449 as well as members of S5.3 were also frequently encountered in northwestern Pacific Ocean
450 warm waters but generally at lower relative abundances (Fig. 5). Clade WPC1, which was first

451 reported in the East China Sea and Japan Sea (Choi and Noh 2009), may co-occur with clade
452 III, whereas *Synechococcus* clades VII and S5.3 occur in waters with variable nutrients and
453 light supply (Fuller *et al.*, 2006; Post *et al.*, 2011). Although previous studies suggested that
454 clades XV and XVI have a global distribution (Ahlgren and Rocap, 2006; Sudek *et al.*, 2015),
455 we rather suggest that these two clades mainly occur between 30° and 35° N/S (Ahlgren and
456 Rocap, 2006; Huang *et al.*, 2012; Sudek *et al.*, 2015) and in upwelling regions (Sohm *et al.*,
457 2015), i.e. in areas characterized by nutrient-rich and intermediate environmental conditions.
458 At last, members of clades IX and Miyav as well as S5.2 showed similar niches, with an
459 abundance peak in the river plume influenced waters (Fig. 5). These taxa were previously found
460 to be abundant in the Hong Kong estuarine waters (Xia *et al.*, 2015b), suggesting that they have
461 a high nutrient requirement and are possibly halotolerant, as it is the case for S5.2 (Chen *et al.*,
462 2006; Dufresne *et al.*, 2008).

463

464 **The combination of genetic and pigment diversity contributes to *Synechococcus* ubiquity**

465 Our phylogenetic tree based on *cpeBA* operon sequences clearly grouped together
466 members of several distinct phylogenetic clades (Fig. 2). This is consistent with several
467 comparative phylogenetic analyses based on the one hand on phycobilisome rod genes and on
468 the other hand on housekeeping genes (ITS, ribosomal proteins, etc.) or allophycocyanin genes
469 that have suggested that the variety of pigment types that occurs among lineages of S5.1 results
470 from multiple lateral transfers of PE-encoding genes between *Synechococcus* lineages during
471 the evolution of this genus (Six *et al.*, 2007; Haverkamp *et al.*, 2008; Haverkamp *et al.*, 2009).
472 Another study has also evoked a specific loss of *mpeBA* genes in pigment type 2 strains as an

473 alternative hypothesis (Everroad and Wood, 2012). Whatever the origin of this discrepancy
474 between *Synechococcus* clades and pigment types, it remains possible to assign with some
475 confidence a pigment type to a specific clade in the field, whenever there is a concomitant
476 dominance at one location of both one pigment type and one clade over all others. For instance,
477 clade I populations from the subarctic North Pacific Ocean are clearly almost exclusively of
478 type 3dA. This is consistent with the fact that all clade I strains able to chromatically acclimate
479 sequenced so far possess a CA4-A island (Humily *et al.*, 2013). Similarly, the comparison of
480 Figures 3 and 5 suggest that most cells at St. ECS-KP01 seemingly belong to clade Miyav and
481 have phycobilisomes of type 3a, while those at St. SCS-LE04 predominantly belong to clade
482 II and possess phycobilisomes of type 3c and/or 3dB (Figs. S5 and S6).

483 It also appears that members of *Synechococcus* communities belonging to a single clade
484 can possess phycobilisomes of different types, consistent with observations on isolates (Fig. 2,
485 but see also Six *et al.*, 2007; Haverkamp *et al.*, 2008; Haverkamp *et al.*, 2009; Everroad and
486 Wood 2012). For instance, although the *Synechococcus* community at St. ECS-KP13 was
487 largely dominated by clade II (Fig. 5), there were several co-occurring pigment types at this
488 station: 3a, 3c and/or 3dB (Fig. 3). It is noteworthy though that the low proportion of pigment
489 type 3dA sequences also found at this station is likely attributable to minor clades, possibly
490 WPC2 or UC-A (Fig. 5), since all clade II and III strains able to chromatically acclimate
491 sequenced thus far are of pigment type 3dB, that is possess a CA4-B island (Humily *et al.*,
492 2013).

493 In conclusion, although combining genetic and pigment diversity analyses has rarely been
494 applied to field populations prior to this study, this approach clearly brings interesting new

495 perspectives on the extent of the flexibility of the *Synechococcus* genus as a whole with regard
496 to environmental parameters. The highly variable phycobiliprotein composition and
497 chromophore content of *Synechococcus* antenna complexes, and hence particularly wide PAR
498 range in which cells of this genus can thrive, clearly confers this group a flexibility with regard
499 to light quality that is unique among marine phytoplankters. Yet, the adaptive capacity of
500 *Synechococcus* is not limited to the spectral properties of seawater, but also concerns other key
501 parameters, including temperature, salinity and nutrient availability, for which adaptive specific
502 traits have been selected by billions years of evolution (Dufresne *et al.*, 2008; Scanlan *et al.*,
503 2009; Pittera *et al.*, 2014). Altogether this adaptability explains the extraordinary ubiquity of
504 *Synechococcus* not only in the northwestern Pacific Ocean but more generally in the marine
505 environment. Our study provides some unprecedented general patterns of *Synechococcus*
506 abundance, pigment diversity and clade composition in a key oceanic region, the northwestern
507 Pacific Ocean. Despite the fact that most samples were collected during summer, we cannot
508 rule out that some of the observed variability actually arises from temporal changes, since
509 seasonal variations of *Synechococcus* abundance and community composition have been
510 reported in some regions of the western Pacific Ocean (Xia *et al.* 2015a) and elsewhere (Tai
511 and Palenik 2009). Thus, future studies are required to better decipher the effects of seasonality
512 on picocyanobacterial abundance and community composition over a wider part of the study
513 area but also to highlight potential effects of large multi-year oceanographic events, such as El
514 Niño.

515

516 **Experimental Procedures**

517 **Sample collection**

518 Samples were collected from surface waters (5-10 m depth) of the western Pacific Ocean
519 and its marginal seas in a total of nine cruises (Table S2, Fig. 1A). With the exception of the
520 cruise in the Tokara Strait, which was conducted in November, all other cruises were performed
521 in late spring to summer. Water was collected using Niskin bottles (12 L) attached to a
522 conductivity, temperature, and depth (CTD) rosette multi-sampler (Sea Bird Electronics, USA).
523 At each station, 0.5-3 L of seawater was pre-filtered through a 3.0 μm (47 mm) polycarbonate
524 membrane (PALL Corporation) and then filtered onto a 0.22 μm (47 mm) polycarbonate
525 membrane for DNA extraction. Membranes were frozen at $-80\text{ }^{\circ}\text{C}$ immediately after filtration.
526 Temperature and salinity of seawater were measured using a conductivity-temperature-depth
527 rosette system (CTD, Sea Bird Electronics). Seawater (1.8 mL) for flow cytometry analysis
528 was fixed with 0.5% (final concentration) seawater-buffered paraformaldehyde. All samples
529 were frozen in $-80\text{ }^{\circ}\text{C}$ until analysis. Inorganic nutrients including $\text{NO}_3^- + \text{NO}_2^-$ (limit of
530 detection (LOD): $0.1\ \mu\text{mol L}^{-1}$) and PO_4^{3-} (LOD: $0.08\ \mu\text{mol L}^{-1}$) were analyzed using the
531 Technicon AA3 Auto-Analyzer (Bran+Luebbe, Germany) onboard or a QuAAtro Auto-
532 Analyzer (Bran+Luebbe, Germany) on shore. Seawater samples for nutrient analysis were
533 filtered through $0.45\ \mu\text{m}$ acetate fiber membranes, except for the cruises in the Bering Sea,
534 Tokara Strait and Sea of Okhotsk, for which samples were not prefiltered before measurements.

535

536 **Analysis of *Synechococcus* abundance**

537 *Synechococcus* cells were enumerated using a Becton-Dickinson FACSCalibur flow
538 cytometer equipped with dual lasers of 488 nm and 635 nm with high flow rate (Liu *et al.*,

539 2014). Ten microliters of yellow-green fluorescent beads (1 μm , Polysciences, Warrington, PA,
540 USA) were added to each sample as an internal standard. Flow cytometric data were analyzed
541 using WinMDI software 2.9 (Joseph Trotter, Scripps Research Institute, La Jolla, CA, USA).
542 All *Synechococcus* abundance data were used to generate a contour plot (Fig. 1B) using the
543 contouring and shading of the Weighted-average gridding algorithm in Ocean Data View
544 (Schlitzer, 2009). X and Y scale-lengths were set as 10 per-km. The relationship between the
545 abundance of *Synechococcus* and environmental factors (Spearman's rank correlation
546 coefficient) was analyzed by using SPSS (IBM SPSS Statistics Inc., Chicago). Data with total
547 inorganic nitrogen (TIN) concentration lower than LOD (0.1 $\mu\text{mol L}^{-1}$) were set as 0.05 μmol
548 L^{-1} and with PO_4^{3-} concentration lower than LOD (0.08 $\mu\text{mol L}^{-1}$) were set as 0.04 $\mu\text{mol L}^{-1}$.

549

550 **Clone library construction and sequencing of *cpeBA* operon**

551 DNA was extracted using the enzyme/phenol-chloroform protocol described elsewhere
552 (Riemann *et al.*, 2000). Primer sets SynB3FW (5'-TCAAGGAGACCTACATCG-3') and
553 SynA1R (5'-CAGTAGTTGATCAGRCGCAGGT-3') were used to amplify the *cpeBA* operon
554 sequences (Everroad and Wood 2006). PCR reaction was carried out in 50 μL master mix
555 including 5 μL of 10 \times buffer, 2 μL of MgCl_2 (25 mM), 4 μL of dNTPs (2.5 mM), 0.2 μL of
556 Platinum Taq DNA polymerase (5 U, Invitrogen, USA), 1 μL of each primer (10 μM), 1 μL of
557 DNA template (around 20 ng/ μL) and 35.8 μL water. PCR products were purified using a
558 PureLinkTM Quick Gel Extraction Kit (Invitrogen, USA). Purified DNA sequences were
559 cloned into the PCR4.0 vector by using a TOPO TA cloning kit (Invitrogen, USA). In total,
560 twelve clone libraries were constructed (Table S3). Forty to sixty positive clones from each

561 library were purified with a PureLink quick plasmid miniprep kit (Invitrogen, USA) and
562 sequenced in the MingBo sequencing company (Shanghai, China).

563

564 **Phylogenetic and diversity analysis of *cpeBA* operon**

565 All *cpeBA* operon sequences were aligned using DNAMAN (Woffelman, 2004) and
566 trimmed to equal length (506 bp). Chimeras were checked and removed using Mothur (Schloss
567 *et al.*, 2009; Edgar *et al.*, 2011). The operational taxonomic units (OTUs) numbers were
568 calculated at the cut-off level of 95% nucleotide identity. The representative sequence of each
569 OTU was randomly extracted and then identified by using BLAST searches against the
570 National Center for Biotechnology Information (NCBI) database
571 (<http://www.ncbi.nlm.nih.gov>). OTUs for which no significant similarity (E-value>10) was
572 found in NCBI database and heterotrophic bacteria sequences were removed. Abundance of
573 left OTUs was used to calculate Shannon index using Primer 5 (Primer-E-Ltd, UK). The
574 Shannon diversity index was calculated as follows: $H = - \sum P_i \ln P_i$, where $P_i = S/N$, S= number
575 of sequences of one OTU, N= total number of sequences in the sample (Shannon, 2001).

576 Representative OTU sequences were used for phylogenetic analyses. Maximum
577 Likelihood phylogenetic tree was constructed with MEGA 6 (Kumar *et al.*, 1994) using the
578 K2+G+I model for nucleotide evolution with 200 bootstraps. The most similar reference
579 sequences were retrieved from the NCBI database. The sequences were aligned using ClustalW
580 according to codon structure (Higgins *et al.*, 1994). Strain pigment information was derived
581 from published literature (Everroad and Wood, 2012; Humily *et al.*, 2013; Humily *et al.*, 2014)
582 and from the Roscoff Culture Collection ([27](http://roscoff-culture-</p></div><div data-bbox=)

583 collection.org/strains/shortlists/taxonomic-groups/marine-synechococcus).

584

585 **PCR and 454 sequencing**

586 Amplification of the *rpoC1* gene sequences was performed as previously described
587 (Mühling *et al.*, 2006). The first round of PCR used the primer *rpoC1*-N5 and the C-terminal
588 primer *rpoC1*-C, and the PCR products were used as templates for a second round of PCR with
589 primer *rpoC1*-39F (5'-adaptor A+barcode+GGNATNGTNTGYGAGCGYTG) and *rpoC1*-
590 462R (5'-adaptor B+CGYAGRCFCTTGRTCAGCTT (Mühling *et al.*, 2006; Xia *et al.*, 2015b).
591 PCR products were gel-purified using the Qiaquick gel purification kit, as described by the
592 manufacturer (Qiagen, Germany). Library quantification was done by fluorometry using the
593 Quant-iT picoGreen dsDNA Assay Kit (Invitrogen, USA). Amplicons were mixed in equal
594 amounts and sequenced in a two-region 454 run on a GS PicoTiterPlate using a GS Junior
595 pyrosequencing system according to manufacturer instructions (Roche, 454 Life Sciences,
596 Branford, CT, USA). The number of sequences obtained from each sample is listed in Table
597 S3.

598

599 **454 Post-run Sequence analysis**

600 Analysis of *rpoC1* sequences was conducted using the microbial ecology community
601 software program Mothur (Schloss *et al.*, 2009). Raw sequences were processed by removing
602 barcodes and primers only reads with an average quality score above 20 and read lengths
603 between 300 nt and 500 nt were taken into account. Sequence denoise was carried out using
604 the command shhh.seqs with sigma value of 0.01. Chimeras were analyzed using the command

605 chimera.uchime and removed. After the above quality control, sequences were identified by
606 local Blast using BioEdit (Hall 1999) with an expectation value 1.0E-100. Reference sequences
607 of each lineage are listed in Table S4. Sequences displaying less than 80% identity to the
608 reference sequences or classified as *Prochlorococcus* and *Synechocystis* were removed. We
609 then subsampled 1,739 reads from each sample for calculating DNA distance. A “shared” file
610 was generated using Mothur’s *make.shared* routine. OTU numbers were calculated at the cutoff
611 level of 95% nucleotide identity. The Shannon diversity index was calculated as explained
612 above, then after removing OTUs containing only one sequence, the Bray-Curtis similarity
613 between samples was also computed. The Bray-Curtis similarity matrix was used to carry out
614 NMDS analysis using Primer 5 (Primer-E-Ltd, UK).

615 In order to calculate the relative abundance of each phylogenetic lineage of
616 *Synechococcus*, the representative sequence of each OTU was randomly extracted using
617 command *get.oturep* and then identified by local Blast using Bio-Edit (Hall, 1999). The
618 phylotype of each reference sequence is listed in Table S4. Since the similarity of sequences
619 within each lineage ranged from 90% to 100% (Table S5), sequences displaying less than 90%
620 identical to the reference sequences were assigned as unclassified (Xia *et al.*, 2015b). The
621 relative abundance of each lineage was calculated and used to generate a heatmap using HemI
622 (Deng *et al.*, 2014), then square root transformed in order to perform average linkage clustering
623 using Pearson correlation matrices. Clade I *rpoC1* sequences were aligned according to codon
624 structure and a Maximum Likelihood (ML) phylogenetic tree was constructed using MEGA 6
625 (Kumar *et al.*, 1994) with a K2P+G model. A heatmap showing the relative abundance of each
626 OTU was generated using iTol (Letunic and Bork, 2007).

627

628 **Redundancy and correlation analysis**

629 The relationship between measured environmental parameters (Table S7) and
630 *Synechococcus* community composition was studied by redundancy analysis (RDA) using
631 CANOCO V4.5 (Microcomputer Power, USA). Stations that did not have nutrient data were
632 not included. Data with total inorganic nitrogen (TIN) concentration lower than LOD (0.1 μmol
633 L^{-1}) were set as 0.05 $\mu\text{mol L}^{-1}$ and with PO_4^{3-} concentration lower than LOD (0.08 $\mu\text{mol L}^{-1}$)
634 were set as 0.04 $\mu\text{mol L}^{-1}$. The matrix was generated using the relative abundance of each
635 lineage transformed by square root. Environmental data were normalized using z-score
636 transformation. The significance of the eigenvalues and species-environment correlations of
637 the first three axes were determined by Monte Carlo tests (500 permutations).

638 The spearman correlation between pigment types and phylogenetic lineages was analyzed
639 using the corrplot R-package (Wei, 2011). S5.2 and clade VIII which are known to mainly
640 consist of type 1 *Synechococcus* (Fuller *et al.*, 2003; Dufresne *et al.*, 2008) were not included
641 in the analysis.

642 **Sequences submission**

643 All sequences obtained from this study have been deposited in the National Center for
644 Biotechnology Information (NCBI) Sequence Read Archive (SRA) and Genbank (Table S6).

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648

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675 **Conflict of interest:** The authors declare that there is no conflict of interest.

676

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914 Fig. 1 Localization of studied sites in the northwestern Pacific Ocean. A, Samples used for
915 pyrosequencing of the *rpoCI* gene are shown as dots and those used for constructing *cpeBA*
916 operon clone libraries as circles. Samples from different cruises were labeled in different colors.
917 B, Contour plot (created with Ocean Data View (Schlitzer, 2009)) showing *Synechococcus* cell
918 concentrations in the northwestern Pacific Ocean using Weighted-average gridding over all
919 sampling stations. Black dots indicate sampling stations. South China Sea (SCS); East China
920 Sea (ECS); western subtropical Pacific Ocean (WSTP); Tokara Strait (TS); Japan Sea/East Sea
921 (JS); Northern Pacific Current (NPC); Sea of Okhotsk (OKH); western subarctic Pacific Ocean
922 (WSAP); Bering Sea (BS); subarctic ocean (SA) including BS, OKH and WSAP.

923

924 Fig. 2 Maximum-likelihood phylogenetic tree of *cpeBA* operon sequences of *Synechococcus*
925 obtained from the western Pacific Ocean. Representative sequences of 95% OTUs containing
926 more than 2 sequences were included in the dataset. Right bars show three clades formed by
927 *cpeBA* operon sequences obtained from the western Pacific Ocean. Strain pigment information
928 derived from Everroad *et al.*, (2012) and Humily *et al.*, (2014). Bootstrap values greater than
929 50% were shown on nodes of branches. Clade names for reference strains are given after strain
930 names.

931

932 Fig. 3 Distribution of *Synechococcus* pigment types in surface waters of the northwestern
933 Pacific Ocean, as indirectly assessed using the diversity of the *cpeBA* operon (see Fig. 2).
934 Pigment type assignment was made following the nomenclature proposed by Humily *et al.*,
935 (2014).

936

937 Fig. 4 NMDS plots showing the similarity of *Synechococcus* communities based on *rpoCI*
938 gene. Samples from different cruises were labeled in different colors. A, similarity of
939 *Synechococcus* communities in the western Pacific Ocean. B, similarity of *Synechococcus*
940 community in the tropic/subtropics oceans of the western Pacific Ocean.

941

942 Fig. 5 Heatmap displaying the relative abundances of *Synechococcus* lineages for any given
943 station of the northwestern Pacific Ocean, as assigned based on *rpoCI* gene. Data were

944 transformed by square root transformation. Unclassified sequences were not included.

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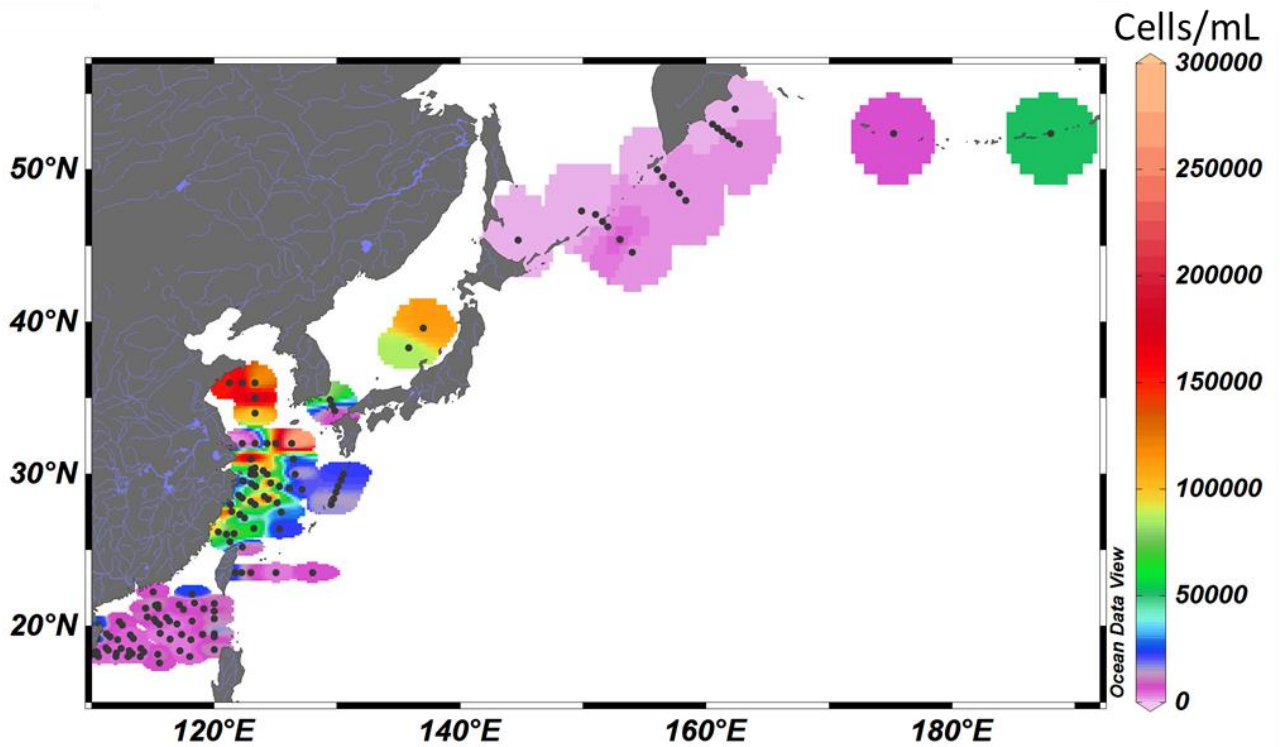
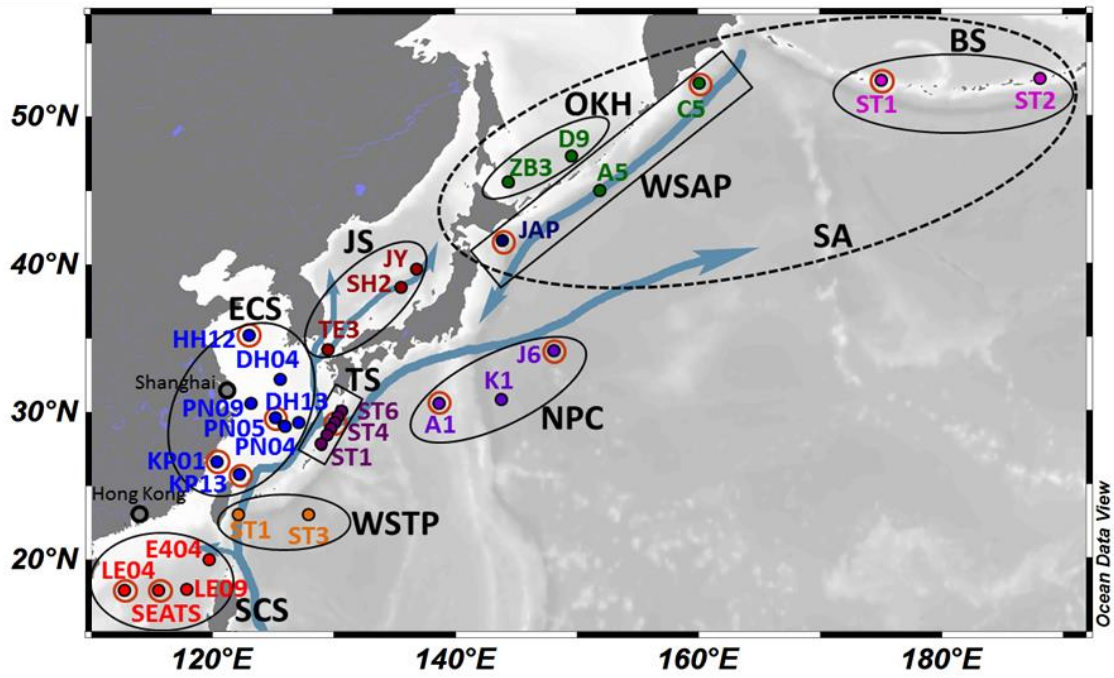
946 Fig. 6 Correlation biplots based on a redundancy analysis (RDA) depicting the relationship
947 between the environmental factors and *Synechococcus* lineages. A. All samples with
948 environmental data were analyzed. B. Samples with temperature higher than 25 °C were
949 analyzed. Relative abundance of each lineage was normalized by square root-transformation.
950 The environmental data were z-score transformed. *P<0.05, **P<0.01.

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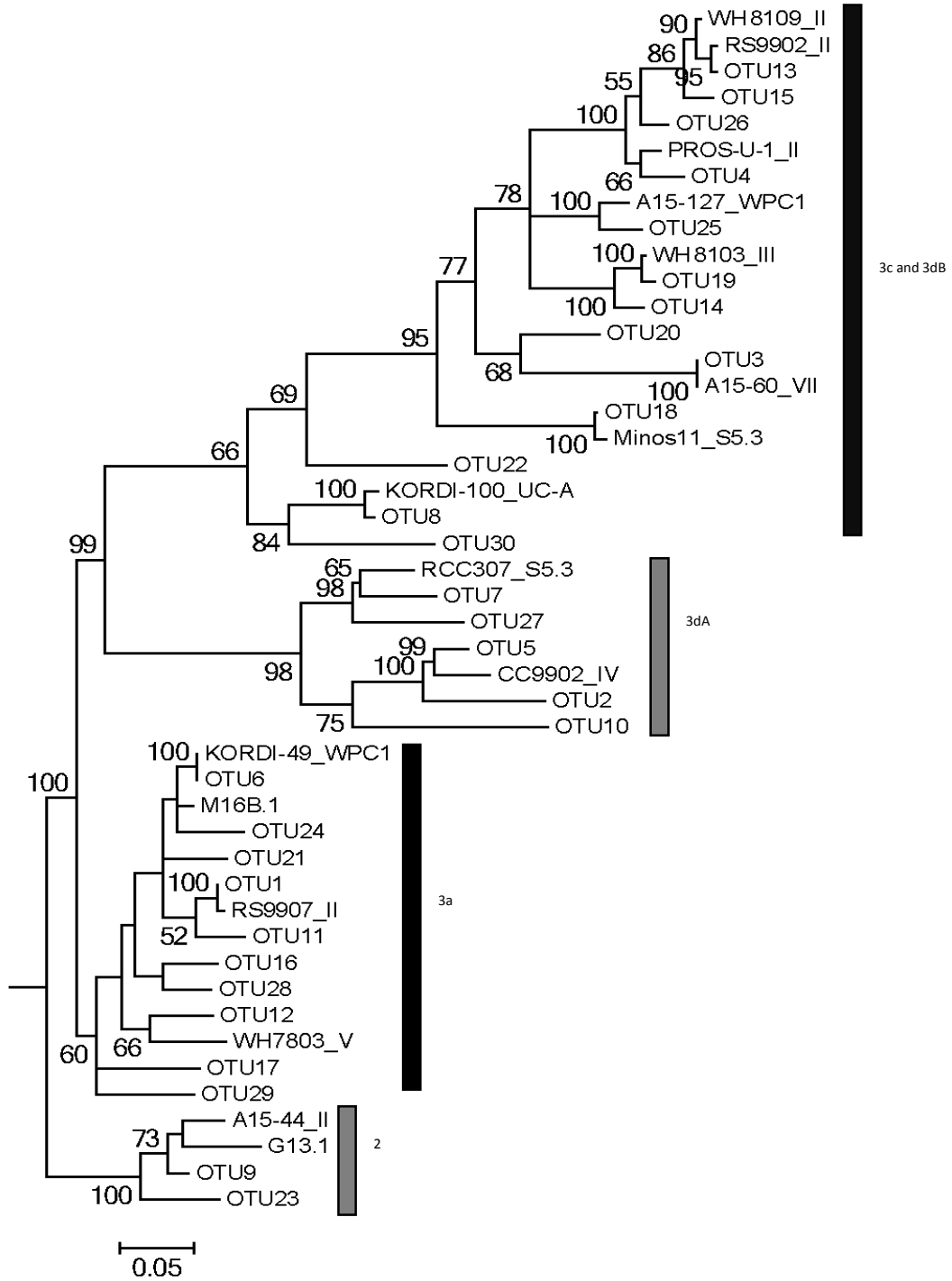
952 Fig. 7 Relative abundance of the different lineages versus latitude. A, Relative abundance of
953 the two dominant clades (I and II). B, Relative abundance of all other major lineages.

954

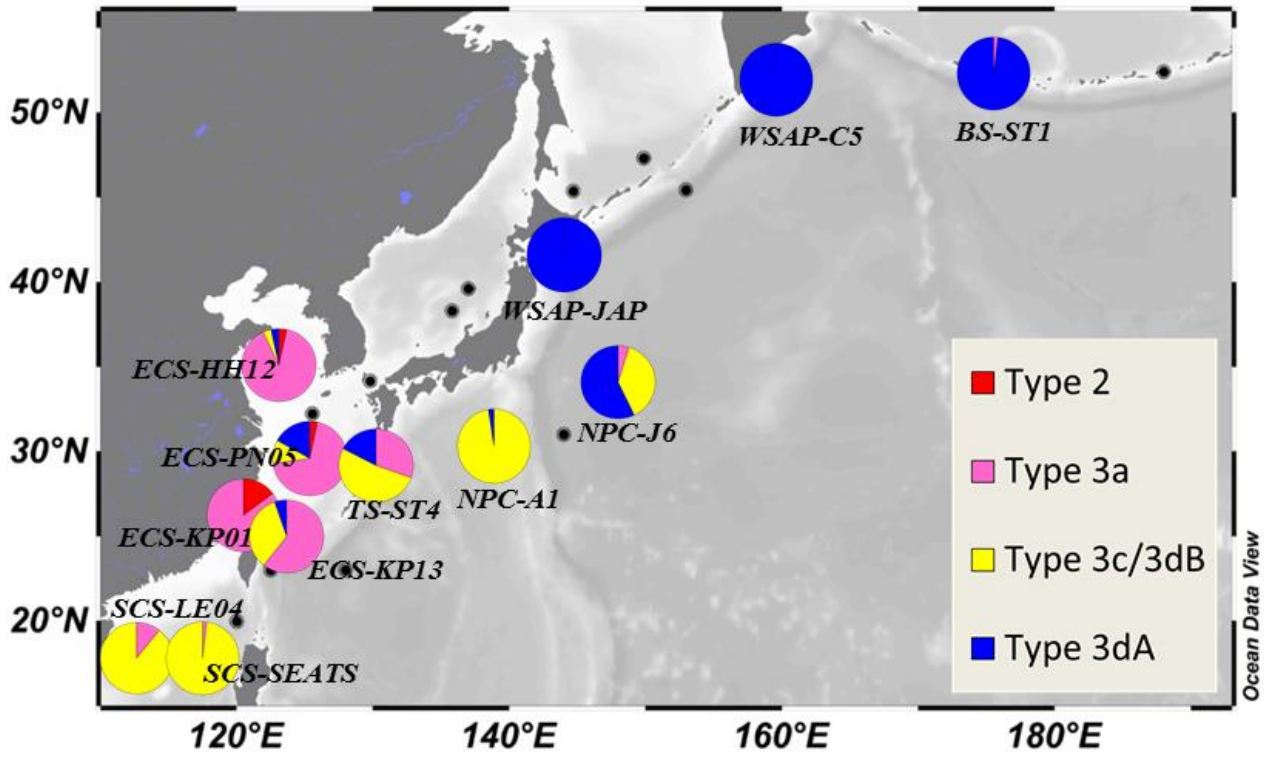
955 Fig. 8 Maximum-likelihood phylogenetic tree of clade I *Synechococcus* using the 100 most
956 abundant *rpoC1* OTUs across all samples. Heatmap on right-hand side shows the relative
957 abundance of OTUs in each library (square root transformed). Only nodes with bootstrap
958 values higher than 50% are shown. Letters A-F on right-hand side correspond to different
959 subclades within clade I.



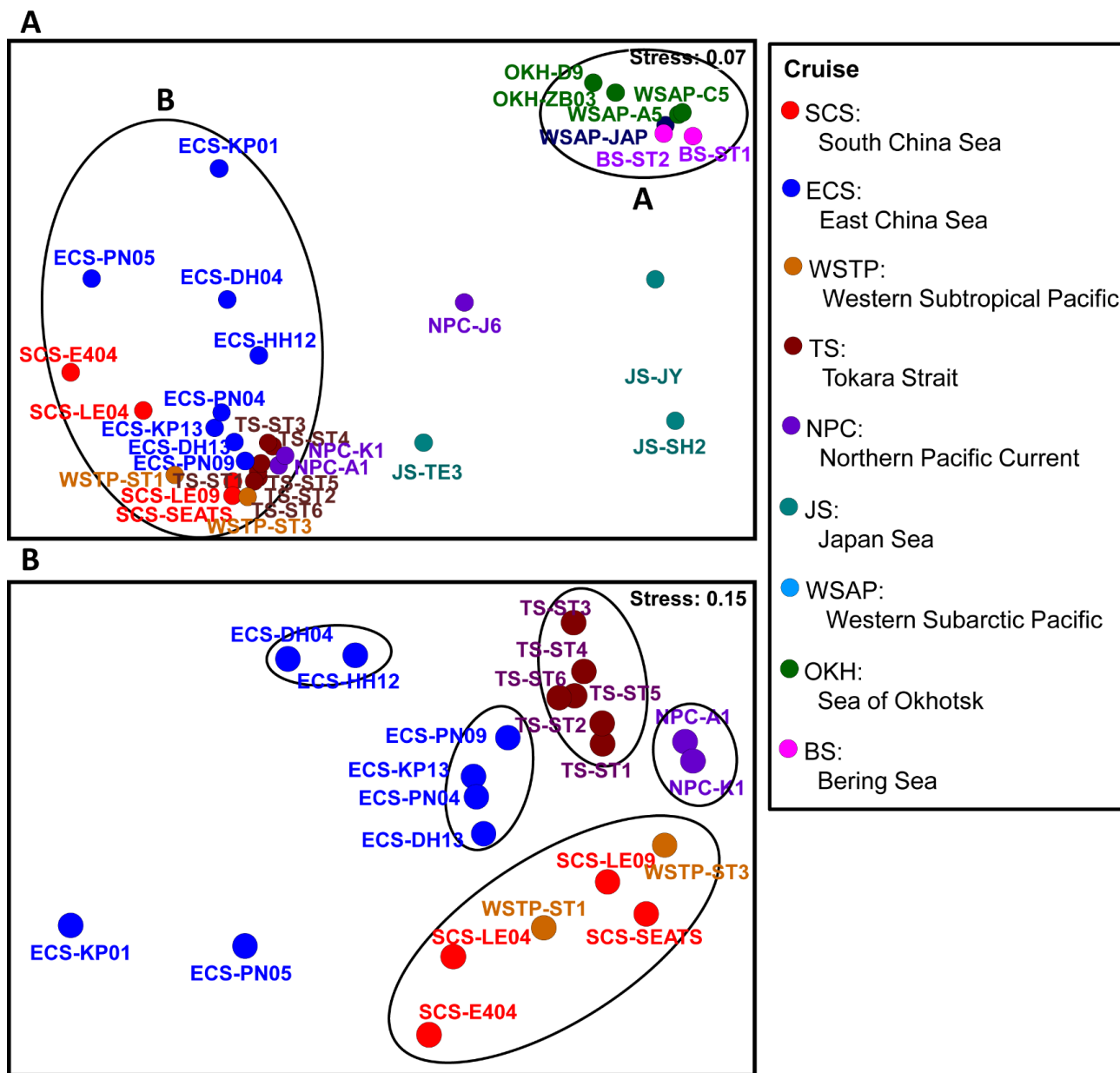
Xia et al. Fig. 1



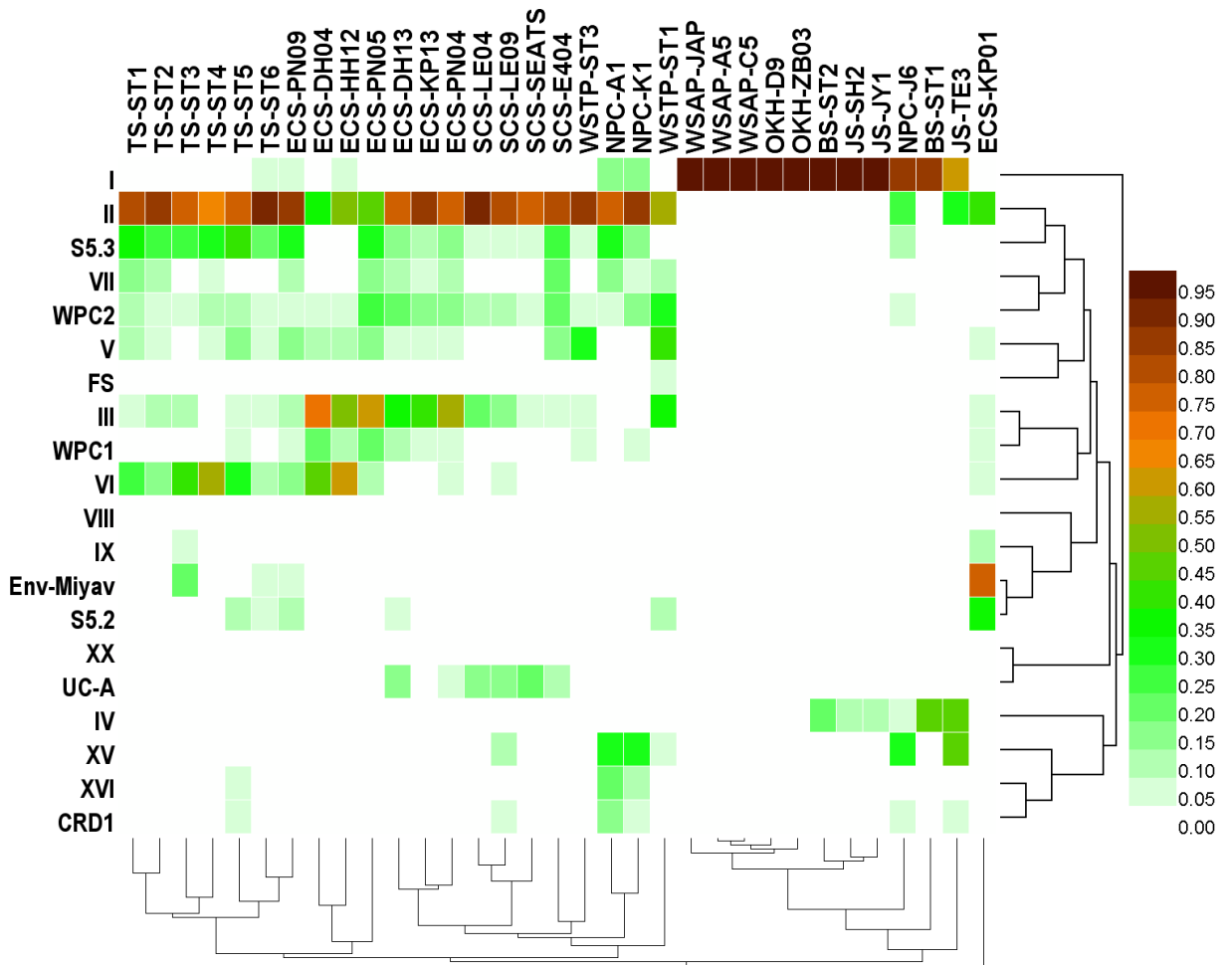
Xia et al. Fig. 2



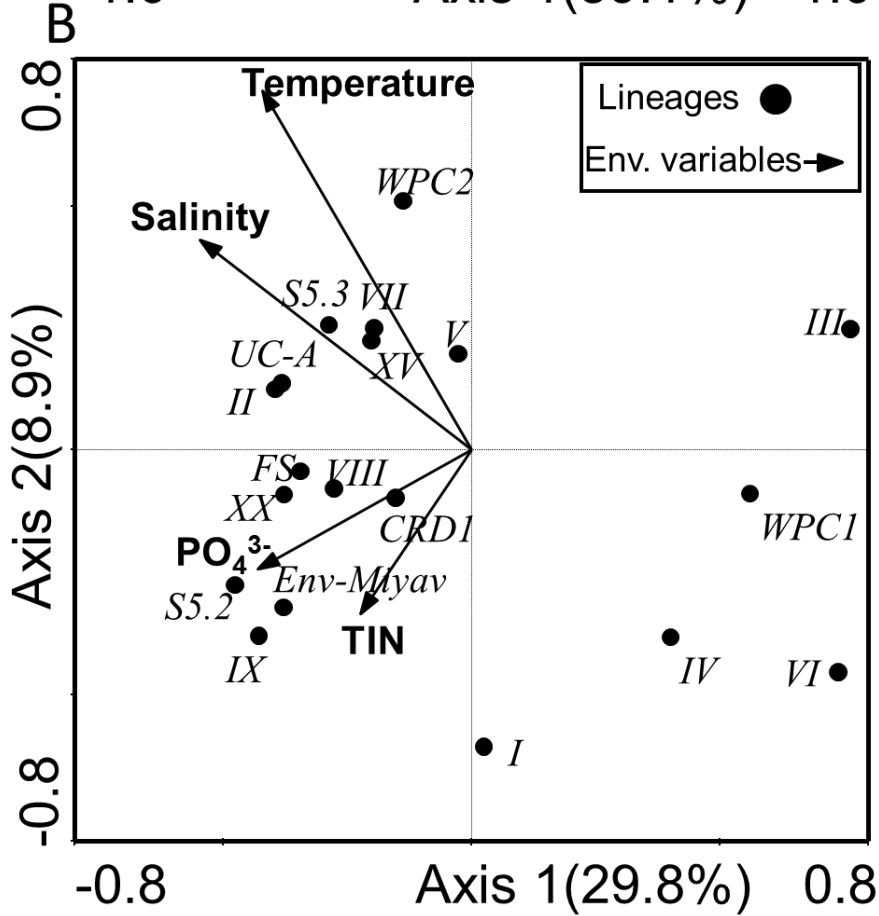
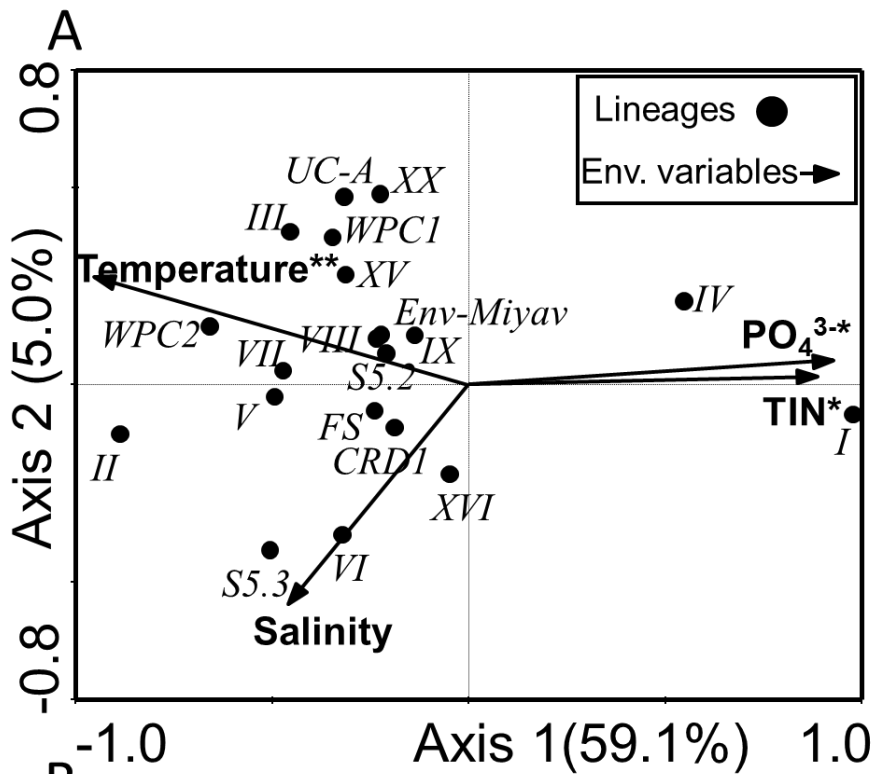
Xia et al. Fig. 3



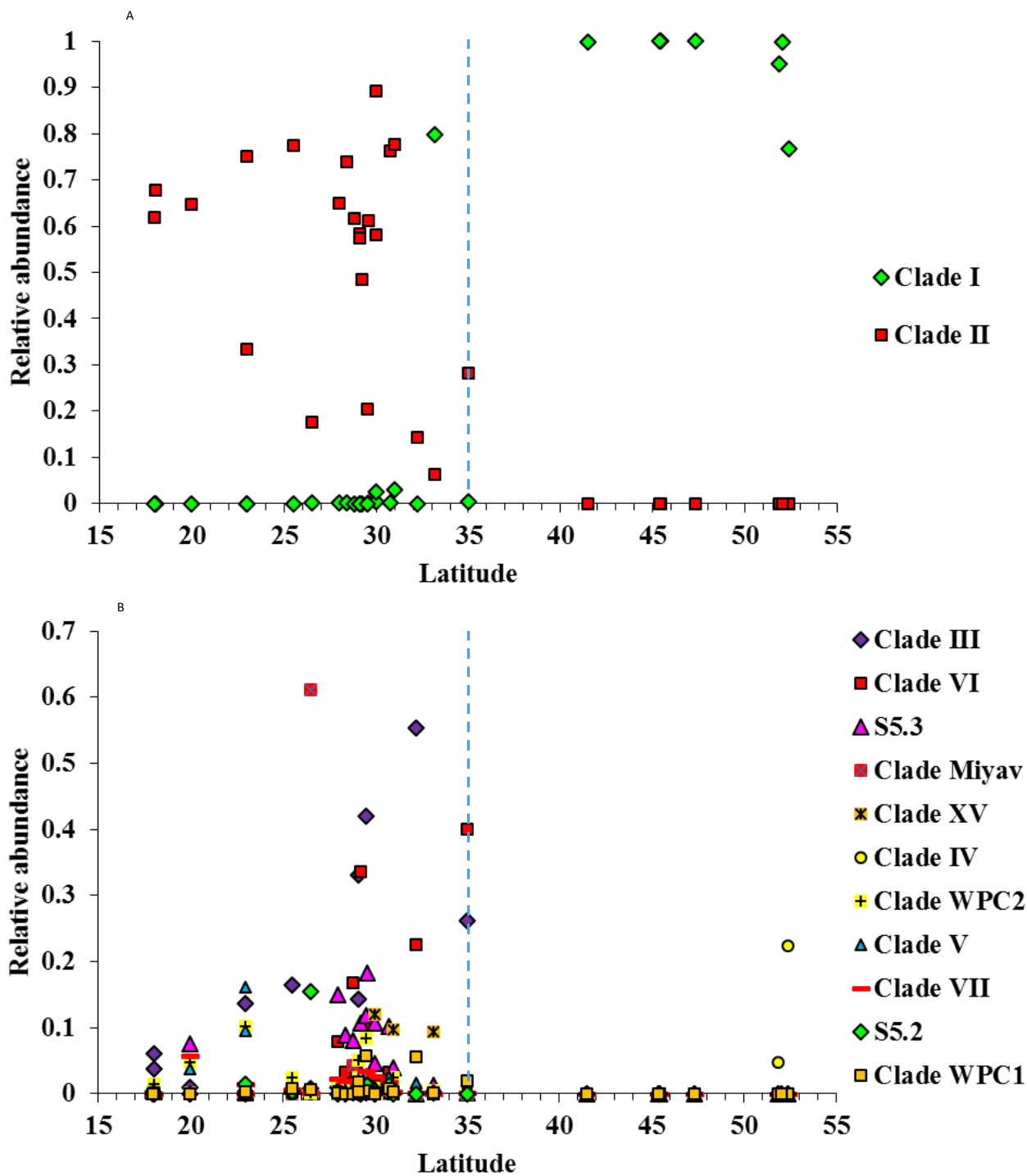
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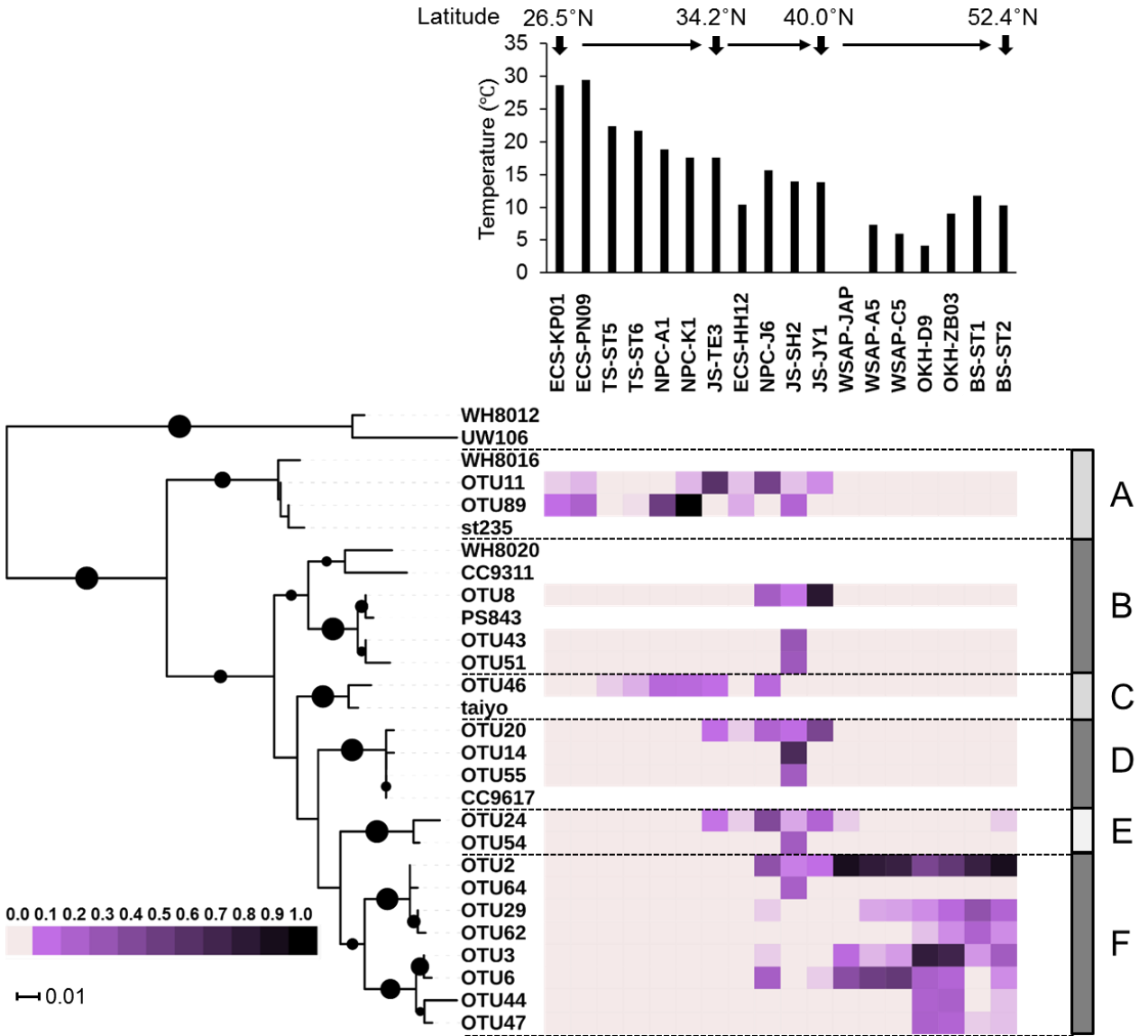
Xia et al. Fig. 5



Xia et al. Fig. 6



Xia et al. Fig. 7



Xia et al. Fig. 8