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Comparative thermophysiology of marine *Synechococcus* CRD1 strains isolated from different thermal niches in iron-depleted areas

1 Mathilde Ferrieux¹, Louison Dufour¹8, Hugo Doré¹8, Morgane Ratin¹, Audrey Guéneuguès², Léo

2 Chasselin², Dominique Marie¹, Fabienne Rigaut-Jalabert³, Florence Le Gall¹, Théo Sciandra¹,

3 Garance Monier¹, Mark Hoebeke⁴, Erwan Corre⁴, Xiaomin Xia⁵, Hongbin Liu⁶, David J.

4 Scanlan⁷, Frédéric Partensky¹ and Laurence Garczarek^{1,8}

- ¹ Sorbonne Université, CNRS, UMR 7144 Adaptation and Diversity in the Marine Environment
 (AD2M), Station Biologique de Roscoff (SBR), Roscoff, France.
- ² Sorbonne Université, CNRS, UMR 7621 Laboratoire d'Océanographie Microbienne (LOMIC),
 Observatoire Océanologique de Banyuls/mer, Banyuls, France.
- ³ Sorbonne Université, CNRS, Fédération de Recherche FR2424, Station Biologique de Roscoff,
 29680, Roscoff, France.
- ⁴ CNRS, FR 2424, ABiMS Platform, Station Biologique de Roscoff (SBR), Roscoff, France
- 12 ⁵ Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of
- 13 Oceanology, Chinese Academy of Sciences, Guangzhou 510220, China.
- ⁶ Department of Ocean Science, The Hong Kong University of Science and Technology, Hong Kong.
- ⁷ University of Warwick, School of Life Sciences, Coventry CV4 7AL, UK.
- 16 ⁸ CNRS Research Federation (FR2022) *Tara* Océans GO-SEE, Paris, France.
- 17 & Current address: Department of Ecology, Evolution and Marine Biology; University of California,
- 18 Santa Barbara, USA
- 19
- 20 * Correspondence:
- 21 Corresponding Author: L. Garczarek
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- 23 adaptation
- 24

25 Abstract

26 Marine Synechococcus cyanobacteria are ubiquitous in the ocean, a feature likely related to their 27 extensive genetic diversity. Amongst the major lineages, clades I and IV preferentially thrive in 28 temperate and cold, nutrient-rich waters, whilst clades II and III prefer warm, nitrogen or phosphorus-29 depleted waters. The existence of such cold (I/IV) and warm (II/III) thermotypes is corroborated by 30 physiological characterization of representative strains. A fifth clade, CRD1, was recently shown to 31 dominate the Synechococcus community in iron-depleted areas of the world ocean and to encompass 32 three distinct ecologically significant taxonomic units (ESTUs CRD1A-C) occupying different thermal 33 niches, suggesting that distinct thermotypes could also occur within this clade.

34 Here, using comparative thermophysiology of strains representative of these three CRD1 ESTUs we 35 show that the CRD1A strain MITS9220 is a warm thermotype, the CRD1B strain BIOS-U3-1 a cold 36 temperate thermotype, and the CRD1C strain BIOS-E4-1 a warm temperate stenotherm. Curiously, the 37 CRD1B thermotype lacks traits and/or genomic features typical of cold thermotypes. In contrast, we 38 found specific physiological traits of the CRD1 strains compared to their clade I, II, III and IV 39 counterparts, including a lower growth rate and photosystem II maximal quantum yield at most 40 temperatures and a higher turnover rate of the D1 protein. Together, our data suggests that the CRD1 41 clade prioritizes adaptation to low-iron conditions over temperature adaptation, even though the 42 occurrence of several CRD1 thermotypes likely explains why the CRD1 clade as a whole occupies 43 most iron-limited waters.

44

45 INTRODUCTION

46 Marine picocyanobacteria contribute to the biogeochemical cycling of various elements, most notably 47 carbon, contributing ~25% of ocean net primary productivity, of which the Synechococcus genus alone 48 is responsible for about 16% (Flombaum et al., 2013). The large geographic distribution of these 49 organisms, extending from the equator to subpolar waters, is largely attributable to their extensive 50 genetic and functional diversity (Zwirglmaier et al., 2008; Farrant et al., 2016; Doré et al., 2020). 51 Amongst the nearly 20 clades within subcluster (SC) 5.1, the most abundant and diversified 52 Synechococcus lineage in oceanic ecosystems (Dufresne et al., 2008; Scanlan et al., 2009; Ahlgren and 53 Rocap, 2012), only four (clades I, II, III and IV) were thought to largely dominate in situ. Clades I and 54 IV mainly thrive in temperate and cold, nutrient-rich waters, while clades II and III reside in warm, 55 oligotrophic or mesotrophic areas (Zwirglmaier et al., 2008; Mella-Flores et al., 2011), suggesting the 56 existence of cold (I/IV) and warm (II/III) Synechococcus 'thermotypes'. This hypothesis was 57 subsequently confirmed by work demonstrating that strains representative of these different clades 58 exhibit distinct thermal preferenda (Mackey et al., 2013; Pittera et al., 2014; Breton et al., 2020; Six et 59 al., 2021), a feature notably linked to differences in the thermostability of light-harvesting complexes 60 (Pittera et al., 2017), lipid desaturase gene content (Pittera et al., 2018) and the ability of some strains 61 to induce photoprotective light dissipation at colder temperatures using the orange carotenoid protein 62 (OCP; Six et al., 2021). Field studies using global ocean datasets have allowed to refine the respective 63 ecological niches of the different thermotypes, with clade I extending further north than clade IV 64 (Paulsen et al., 2016; Doré et al., 2022) and clades II and III predominating in N- and P-depleted waters, 65 respectively, but also to highlight the importance of a fifth clade within SC 5.1, the CRD1 clade 66 (Farrant et al., 2016; Sohm et al., 2016; Kent et al., 2019). Initially thought to be limited to the Costa 67 Rica dome area (Saito et al., 2005; Gutiérrez-Rodríguez et al., 2014), the latter clade was recently found 68 to be a major component of Synechococcus communities in Fe-depleted areas (Farrant et al., 2016; 69 Sohm et al., 2016; Ahlgren et al., 2020). Furthermore, analysis of the global distribution of these 70 organisms using high-resolution marker genes has highlighted large within-clade microdiversity 71 associated with niche differentiation in marine Synechococcus (Farrant et al., 2016; Larkin and Martiny, 2017; Xia et al., 2019), as also observed in Prochlorococcus (Kashtan et al., 2014; Larkin et 72 73 al., 2016). Using the *petB* gene encoding cytochrome b_6 , Farrant et al. (2016) showed that most major 74 clades encompassed several Ecologically Significant Taxonomic Units (ESTUs), i.e. genetically 75 related subgroups within clades occupying distinct oceanic niches. This is notably the case for ESTU 76 IIB that occupies a cold thermal niche in sharp contrast with IIA, the dominant ESTU within clade II 77 that occupies warm, mesotrophic, and oligotrophic iron (Fe)-replete waters. Similarly, three distinct 78 ESTUs with distinct thermal niches were identified within the CRD1 clade and the co-occurring clade 79 EnvB (a.k.a. CRD2; Ahlgren et al., 2020): i) CRD1B/EnvBB are found in cold mixed waters in co-80 occurrence with ESTUs IA, IVA and IVC, ii) CRD1C/EnvBC dominate in warm, high-nutrient low-81 chlorophyll (HNLC) regions such as the central Pacific Ocean, and iii) CRD1A/EnvBA are present in 82 both environments and thus span a much wider range of temperatures than CRD1B and C (Farrant et 83 al., 2016). This suggests that these three CRD1 ESTUs could correspond to different thermotypes.

In order to test this hypothesis, we used strains representative of each of the three CRD1 ESTUs to determine the fundamental thermal niches of these organisms as compared to typical cold (clades I and IV) and warm (clades II and III) thermotypes. Furthermore, given the strong influence of temperature 87 on optimal functioning of the photosynthetic apparatus in marine *Synechococcus* (Pittera et al., 2014, 88 2017; Guyet et al., 2020), we also examined the effect of temperature acclimation on the 89 photophysiology of CRD1 ESTUs compared to their clade I and IV counterparts and show that CRD1 90 thermotypes actually differ more strongly in this respect to members of clades I-IV than from each 91 other.

92 MATERIALS AND METHODS

93 Strains and growth conditions

94 The eight Synechococcus spp. strains used in this study were retrieved from the Roscoff Culture 95 Collection (RCC; https://roscoff-culture-collection.org/), including representative strains of the three 96 known CRD1 ESTUs (CRD1A - C) and one or two of each of the four dominant clades in the field 97 (clades I – IV) used as controls (Table 1 and Supplementary Fig. 1). Cells were grown in 50 mL flasks 98 (Sarstedt, Germany) in PCR-S11 culture medium (Rippka et al., 2000) supplemented with 1 mM 99 sodium nitrate. Cultures were acclimated for at least two weeks in temperature-controlled chambers 100 across a range of temperatures dependent on the thermal tolerance of each strain and under a continuous light of 75 µmol photons m⁻² s⁻¹ (hereafter $\mu E m^{-2} s^{-1}$) provided by a white-blue-green LED system 101 (Alpheus, France). For each experiment cultures were grown in triplicate, inoculated at an initial cell 102 103 density of $\sim 3 \times 10^6$ cells mL⁻¹, and samples harvested daily to measure growth rate and fluorescence 104 parameters as described below.

105 In order to compare the capacity of strains to repair the D1 subunit of photosystem II (PSII; see 106 'Measurement of PSII repair rate' section), cultures grown in 250 ml flasks at 75 µE m⁻² s⁻¹ were acclimated at 18, 22 and 25°C, temperatures at which all strains were able to grow, were subjected to 107 108 high light stress (375 μ E m⁻² s⁻¹). Exponentially growing cultures were sampled at T0 and after 15, 30, 109 60, and 90 min of stress, before shifting cultures back to the initial light conditions and then sampling 110 again after 15, 30, 60 min and 24h of recovery (R). While D1 repair measurements were performed at 111 all time points, cell concentrations were measured by flow cytometry only at T0, T30min, T90min, 112 R30min and R24h and liposoluble pigment content was determined only at T0.

113 Flow cytometry

Culture aliquots (200 µl) sampled for flow cytometry were fixed using 0.25% (v/v) glutaraldehyde (grade II, Sigma Aldrich, USA) and stored at -80°C until analysis (Marie et al., 1999). Cell concentrations were estimated using a Guava easyCyte flow cytometer (Luminex Corporation, USA)

117 and maximum growth rates (μ_{max}) were calculated as the slope of the linear regression of ln(cell

- 118 density) vs. time during the exponential growth phase. Synechococcus cells were identified based on
- their red (695 nm) and orange (583 nm) fluorescence, proxies for their chlorophyll *a* and phycoerythrin
- 120 content, respectively. Fluorescence, forward scatter and side scatter values were normalized to that of
- 121 standard 0.95 µm beads using Guavasoft software (Luminex Corporation, USA).

122 Fluorescence measurements

123 The maximum PSII quantum yield (F_v/F_M) was estimated using a Pulse Amplitude Modulation 124 fluorimeter (Phyto-PAM II, Walz, Germany) during the exponential growth phase after 10 min dark 125 acclimation followed by addition of 100 μ M of the PSII blocker 3-(3,4-dichlorophenyl)-1,1-126 dimethylurea (DCMU, Sigma-Aldrich, USA; Campbell et al., 1998).

127 The PSII quantum yield was calculated as:

128
$$F_V/F_M = (F_M - F_0)/F_M$$

129 where F_0 is basal fluorescence, F_M maximal fluorescence level and F_V variable fluorescence (Campbell 130 et al., 1998; Six et al., 2007).

Fluorescence excitation (with emission set at 580 nm) and emission (with excitation set at 530 nm) spectra were generated using a LS-50B spectrofluorometer (Perkin-Elmer, USA) as described in Six et al. (2004). The fluorescence excitation ratio (Exc_{495:550nm}) was used as a proxy for the PUB:PEB ratio. Phycobilisome (PBS) rod length and the degree of coupling of the PBS to PSII reaction center chlorophylls was then assessed using fluorescence emission spectra by calculating the phycoerythrin (PE, $F_{max} = 565-575$ nm) to phycocyanin (PC, $F_{max} = 645-655$ nm) ratio as well as the PC to PBS terminal acceptor (TA; $F_{max} = 680$ nm) ratio, respectively (Pittera et al., 2017).

138 **Pigment analyses**

- 139 Triplicate cultures were harvested during the exponential phase when F_v/F_M was maximum for each
- 140 temperature condition. Cultures (50 mL) were subjected to centrifugation in the presence of 0.01%
- 141 (v/v) pluronic acid (Sigma-Aldrich, Germany) at 4°C, 14,000 x g for 7 min, using an Eppendorf 5804R
- 142 (Eppendorf, France). Pellets were resuspended and transferred to 1.5 ml Eppendorf tubes and
- 143 centrifuged at 4°C, 17,000 x g for 2 min using an Eppendorf 5417R centrifuge (Eppendorf, France).
- 144 Once the supernatant was removed samples were stored at -80 °C until further analysis. Pigment

- 145 content was subsequently assessed using calibrated high-performance liquid chromatography (HPLC
- 146 1100 Series System, Hewlett Packard, St Palo Alto, CA), as previously described (Six et al., 2005).

147 Measurement of the photosystem II repair rate

Each culture acclimated to 75 µE m⁻² s⁻¹ and 18, 22 or 25 °C was split into two new 50 mL flasks 148 149 (Sarstedt Germany) with one used as a control and the other flask supplemented with lincomycin (0.5 150 mg mL⁻¹ final concentration, Sigma-Aldrich, USA) in order to inhibit protein synthesis and thus D1 151 repair (Guyet et al., 2020). Both sub-cultures were then subjected to light stress by exposing cultures 152 to 375 μ E m⁻² s⁻¹ continuous light (at the same temperature), and F_V/F_M measured at different time points as described above. The PSII repair rate for each strain at each temperature was determined from 153 154 the coefficient differences between the exponential curves fitted over the 90 min time course of F_V/F_M 155 measurements for control and +lincomycin samples. This light stress experiment was replicated on

156 four independent cultures.

157 Determination of the realized environmental niches of major Synechococcus ESTUs

158 The realized niches of CRD1 and clades I-IV ESTUs were determined using *petB* reads extracted from 159 metagenomic data from the Tara Oceans and Tara Polar circle expeditions, the Ocean Sampling Day 160 (OSD; June 21st 2014) campaign, and *petB* metabarcodes from i) various oceanographic cruises (CEFAS, BOUM, Micropolar, RRS Discovery cruise 368 and several in the northwestern Pacific 161 162 Ocean as detailed in Xia et al., 2017), ii) two individual sampling sites in the Mediterranean Sea 163 (Boussole, Point B) as well as iii) a bi-monthly sampling at the long-term observatory site SOMLIT 164 ("Service d'Observation en Milieu Littoral")-Astan located 2.8 miles off Roscoff between July 2009 165 and December 2011 (Supplementary Table 1).

petB metagenomic recruitment using the *Tara* Oceans and OSD datasets was performed as described previously (Farrant et al., 2016). *Synechococcus petB* sequences from both metagenomes and metabarcodes were used to define operational taxonomic units (OTUs) at 97% identity using Mothur v1.34.4 (Schloss et al., 2009) that were then taxonomically assigned using a *petB* reference database (Farrant et al., 2016). OTUs encompassing more than 3% of the total *Synechococcus* reads for a given sample were grouped into ESTUs and used to determine the whole temperature range occupied by each of the five major *Synechococcus* ESTUs.

173

174 Comparative genomics

175 The Cyanorak v2.1 information system (http://www.sb-roscoff.fr/cyanorak/; Garczarek et al., 2021)

176 was used to compare the phyletic pattern i.e., the presence/absence pattern of each cluster of likely

177 orthologous genes (CLOG) in each strain, for CRD1 strains and their clades I-IV counterparts for a

178 number of selected genes potentially involved in adaptation to low temperature based on previous

- 179 literature (see results).
- 180

181 **RESULTS**

182 The fundamental thermal niches of CRD1 vs. clades I to IV strains

183 In order to determine the temperature optima and boundary limits of the different CRD1 strains and to 184 compare them to those of typical cold and warm Synechococcus thermotypes, representative strains of 185 each of the three CRD1 ESTUs and strains of clades I, II, III and IV were grown over a range of 186 temperatures from 6 to 36°C. The growth responses of all strains to temperature followed a typical 187 asymmetric bell-shaped curve over the selected temperature range (Fig. 1), with a progressive rise in 188 growth rate (μ) until T_{opt} (the temperature associated with maximum μ : μ_{max}) was reached, and a sharp 189 decline above T_{opt}. BIOS-U3-1 (CRD1-B) was able to grow between 12 and 29°C with a T_{opt} at 25°C 190 $(\mu_{max} = 0.78 \pm 0.02 \text{ d}^{-1})$, a growth pattern most similar to that of the clade IV strain BL107, while the 191 clade I strain MVIR-18-1 was able to grow at much lower temperatures, down to 8°C but could not 192 grow above 25°C (Fig. 1A). MITS9220 (CRD1-A) and BIOS-E4-1 (CRD1-C) displayed thermal 193 growth characteristics more similar to the clade II (A15-62 and M16.1) and III (WH8102) strains, 194 representatives of warm thermotypes (Fig. 1B). While most strains in this category displayed a minimal 195 growth temperature of 16°C, large variations between strains were observed at the highest thermal 196 boundary limit (T_{max}). Maximum growth temperature was obtained for M16.1 (II; T_{max} : 34°C), then 197 A15-62 (II) and WH8102 (III; both with T_{max} at 32°C), MITS9220 (CRD1-A; T_{max} : 31°C) and finally 198 for BIOS-E4-1 (CRD1-C; T_{max}: 30°C). The latter strain also displayed the highest minimal growth 199 temperature (T_{min} : 18°C) and thus possesses the narrowest temperature range for growth of all the 200 strains studied (12°C vs. 15-18°C). It is also worth noting that CRD1 strains display a lower maximum 201 growth rate and more generally lower growth rates at most temperatures than their clade I, II, III and 202 IV counterparts.

203

204 The environmental realized niches of CRD1 vs. clades I to IV strains

205 We then compared the fundamental thermal niches of all studied strains, i.e. the whole temperature 206 range over which they can grow in a laboratory setting in the absence of biotic interactions (e.g. 207 competition or predation), with environmental realized niches (sensu Pearman et al., 2008) of the 208 corresponding ESTUs. For this, we determined the distribution limits of each of these ESTUs along 209 the Tara Oceans and Tara Polar circle transects, 203 samples from OSD2014 and additional 210 oceanographic cruises and individual sampling sites, altogether encompassing 413 samples worldwide 211 covering a wide range of temperature conditions (Fig. 1, Supplementary Fig. 2, Supplementary Table 212 1). This made it possible to have much finer estimates of the limits of the thermal niches of the different 213 ESTUs than in the study performed by Farrant et al. (2016), in particular for the cold adapted ESTUs, 214 which were poorly represented in the initial Tara Oceans dataset (Supplementary Fig. 2).

This analysis showed that the CRD1B ESTU displayed a reduced thermal tolerance range in the environment (14 to 24.5°C) compared to the BIOS-U3-1 strain in culture (12 to 29°C), while the typical cold thermotypes colonized larger thermal niches *in situ* than their representative strains (Fig. 1A). Environmental realized niches indeed ranged from 2.5 to 24°C for ESTU IA (compared to 8 to 25°C for MVIR-18-1) and from 8.5 to 25°C for ESTU IVA (compared to 12 to 29°C for BL107). Interestingly, the median temperature of the CRD1B ESTU is 3°C higher than that observed for ESTUs IA and IVA.

222 As concerns warm thermotypes, CRD1C displayed a fairly narrow thermal tolerance range in situ 223 (22 to 29.5°C), which, similar to the cold thermotype CRD1B, was even narrower than for its 224 representative strain BIOS-E4-1 (18 to 30°C; Fig. 1B). Comparatively, the CRD1A ESTU was detected 225 across a wider temperature range (14 to 30.5°C) than the other two CRD1 ESTUs and also slightly 226 larger than the corresponding strain in culture (MITS9220, 16 to 31°C). Still, the most extended 227 temperature range was observed for ESTU IIA and IIIA (12 to 32°C) that reached significantly lower 228 temperature limits than the corresponding clade II (16 to 32-34°C) and III (16 to 32°C) strains. Of note, 229 although both IIA and IIIA ESTUs displayed a similar temperature range, the median temperature of 230 ESTU IIA (25°C) was about 3°C higher than that of ESTU IIIA (22°C) and the maximum median 231 temperature was surprisingly observed for the CRD1C ESTU (26.5°C). In this context, it is also worth 232 mentioning that although clade II strains are clearly both warm thermotypes, M16.1 displays a

significantly higher temperature limit for growth than A15-62 and more generally than all other strains.

234 This suggests that ESTU IIA may encompass two distinct ESTUs, but such a high temperature niche

(>32 °C) where they could be discriminated is exceptional and not available in our dataset
(Supplementary Table 1).

237 Comparative genomics

238 In order to assess whether the cold, temperate thermotype BIOS-U3-1 (CRD1B) exhibits similar 239 adaptation mechanisms to those previously described for the cold-adapted clades I and/or IV, we 240 examined a number of clusters of likely orthologous genes (CLOGs) from all Synechococcus genomes 241 belonging to clades I-IV and CRD1 present in the Cyanorak v2.1 information system (Garczarek et al., 242 2021). First, we looked for the occurrence of two amino-acid substitutions in phycocyanin α - (RpcA) 243 and β -subunits (RpcB), which were shown to differ between cold- (Gly in clades I and IV for RpcA43; 244 Ser in RpcB42) and warm-thermotypes (Ala in clades II and III for RpcA43; Asp in RpcB42), these 245 substitutions being potentially responsible for the differential thermotolerance of this phycobiliprotein 246 between thermotypes (Pittera et al., 2017). In all three CRD1 strains, both sites displayed the warm-247 type residue (Supplementary Fig. 3A-B), suggesting that in contrast to typical cold and warm 248 thermotypes, the molecular flexibility of this phycobiliprotein does not differ between CRD1 249 thermotypes. We then looked at fatty acid desaturases that are essential for regulating membrane 250 fluidity and thus the activity of integral membrane proteins, including photosynthetic complexes 251 (Mikami and Murata, 2003; Pittera et al., 2018; Breton et al., 2020). All three CRD1 strains surprisingly 252 possess in addition to the core $\Delta 9$ -desaturase gene desC3, a second $\Delta 9$ -desaturase, desC4 253 (Supplementary Table 2), previously thought to be specific to cold-adapted strains as well as the $\Delta 12$ -254 desaturase desA3 found in both cold-adapted clades I and IV as well as in clade III, a warm thermotype 255 subjected to much stronger seasonal variability than its (sub)tropical clade II counterparts (Pittera et 256 al., 2018). Furthermore, BIOS-U3-1 also possesses desA2, thought to be specific to warm 257 environments, while this gene is in contrast absent from the other two CRD1 warm-adapted strains. 258 Thus, CRD1 strains exhibit a different desaturase gene set and potentially display a larger capacity to 259 regulate membrane fluidity than typical cold- or warm-adapted thermotypes. Finally, while all clades 260 I, III and IV genomes possess the ocp operon, involved in the protection of PSII against 261 photoinactivation through the dissipation of excess light energy (Kirilovsky, 2007) and which was 262 recently shown in marine Synechococcus to play a key role at low temperature (Six et al., 2021), none 263 of the three CRD1 genomes possess this operon.

265 **Photosynthetic activity and pigment content**

266 PSII quantum yield (F_V/F_M) , used as a proxy of photosynthetic activity, was measured for each strain 267 over their whole temperature growth range. Most strains displayed a decrease in this parameter at both 268 low and high boundary limits of their growth temperature range and this effect was particularly striking 269 for BIOS-U3-1, reaching values down to 0.11 at 14°C and 0.32 at 28°C (Fig. 2A). Besides MVIR-18-270 1 that exhibited a quite constant F_V/F_M over its whole temperature range, the decrease in F_V/F_M at high 271 temperature was stronger for cold than warm thermotypes that are able to maintain a quite high F_V/F_M 272 in the warmest growth conditions (Fig. 2B). Finally, as for growth rate, CRD1 strains exhibited lower 273 F_V/F_M at all temperatures than clade I to IV strains.

274 The Exc495:550nm fluorescence excitation ratio, used as a proxy for PUB:PEB ratio, was consistent 275 with the pigment type of each strain (Humily et al., 2013; Table 1; Supplementary Fig. 4A). This ratio 276 remained pretty constant over the whole temperature range for all strains except for the chromatic 277 acclimator BL107 (pigment type 3dA), for which a sharp increase was observed at its maximal growth 278 temperature (28°C) to reach a value (1.35) intermediate between that typically observed in green light 279 (or white light; 0.6-0.7) and blue light (1.6-1.7). This suggests that the chromatic acclimation process 280 could be affected by growth temperature, at least in this strain. The phycobilisome (PBS) rod lengths 281 and the degree of coupling of PBS to PSII reaction center chlorophylls, as estimated from PE:PC and 282 PE:TA ratios respectively, showed fairly limited variations over the temperature range, indicating that 283 the phycobiliprotein composition of PBS is quite stable over the growth temperature range of each 284 strain (Supplementary Fig. 4B-C). One notable exception was a rise in both ratios for strain A15-62 at 285 its minimal growth temperature, likely attributable to the partial decoupling of individual 286 phycobiliproteins and of the whole PBS from PSII, a phenomenon typically observed under stressful 287 conditions (Six et al., 2007; Guyet et al., 2020). It is also worth noting that MITS9220 and to some 288 extent BIOS-E4-1, exhibited a significantly higher PE:PC ratio than the five other strains, potentially 289 indicating a different phycobiliprotein composition and/or length of PBS rods.

In terms of liposoluble pigments, the β -carotene/chlorophyll *a* (β -car/Chl *a*) ratio tended to increase with temperature in BIOS-E4-1 and MITS9220, as observed for the other warm thermotypes, whilst this ratio was more stable in the cold thermotypes BIOS-U3-1 and BL107, and seemed to slightly increase in the lower part of the thermal range for the clade I strain MVIR-18-1 (Fig. 3). For all strains, 294 these ratios result from a concomitant increase with temperature of Chl a and β -car content per cell 295 (Supplementary Fig. 5), indicating an enhancement of the surface of thylakoids per cell at higher 296 temperatures that was particularly marked for BIOS-E4-1 and A15-62, whilst this variation was fairly 297 limited in the other two CRD1 strains. As these two pigments are present in different proportions in 298 PSI and II (Umena et al., 2011; Xu and Wang, 2017), the higher β -car/Chl a ratio measured in clades 299 I and IV strains also suggests that they may have a higher PSII:PSI ratio than all other strains, including 300 BIOS-U3-1, and that this ratio might be more strongly affected by temperature in warm than cold 301 thermotypes.

302 As concerns the zeaxanthin/chlorophyll a (Zea/Chl a) ratio, although an increase in this ratio was 303 measured at low temperature for all strains, the amplitude was globally larger for cold than for warm 304 thermotypes, with BIOS-U3-1 behaving very similarly to the clade IV strain BL107 that exhibits the 305 largest variation in this ratio (Fig. 3). Changes in this ratio likely originate partially from the decrease 306 in Chl a content in response to cold, a strategy typically used by cells to regulate light utilization under 307 slow growth conditions (Inoue et al., 2001). However, several strains also displayed an increase in their 308 Zea content per cell at low temperature, a response particularly striking in BIOS-U3-1 and A15-62, but 309 that also seems to occur in M16.1 and in the two other CRD1 strains BIOS-E4-1 and MITS9220 310 (Supplementary Fig. 5). Thus, although Zea has been hypothesized to be involved in the 311 photoprotection of cold-adapted strains by dissipating excess light energy under low temperature 312 conditions (Kana et al., 1988; Breton et al., 2020), this process seems to be present in both cold and 313 warm-adapted CRD1 strains and in most warm thermotypes as well. In this context, it is also worth 314 noting that the two clade II strains, A15-62 and M16.1, displayed fairly distinct temperature-induced 315 variations in their Zea:Chl a ratios and individual pigment contents, possibly linked to their different 316 isolation temperatures (see discussion below).

317 **Photosystem II repair capacity**

The ability of the different strains to repair PSII in response to light stress (375 μ E m⁻² s⁻¹) was determined in cultures acclimated to 18, 22 and 25°C by measuring changes in F_V:F_M over time after adding the protein synthesis inhibitor lincomycin, or not (Supplementary Fig. 6). While a decrease in F_V:F_M ratio during the 90 min light stress period was observed in both cultures supplemented with lincomycin and controls, this ratio only re-increased back up to initial F_V:F_M values, after shifting cultures back to standard light conditions (75 μ E m⁻² s⁻¹), in the control group in most strains and

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temperature conditions. Thus, all studied strains were able to recover from this light stress, as long as the D1 repair cycle was not inactivated by inhibition of protein synthesis. Yet, a fast decrease in $F_V:F_M$ was observed for all three CRD1 cultures supplemented with lincomycin, while the +/- lincomycin curves overlapped during the first 15-30 min of light stress in most other strains and conditions. This suggests that the initial decrease in $F_V:F_M$ in clades I-IV strains was not due to D1 damage but rather to dissipation of light energy as heat through non-photochemical quenching (Campbell et al., 1998), whilst the damage and hence repair of D1 proteins only occurred later on.

331 The PSII repair rate (R_{PSII}), as calculated from the time course of F_V:F_M with and without 332 lincomycin, increased with temperature in most strains, except for BIOS-U3-1 that displayed its highest 333 rate at 22°C (Fig. 4). Strikingly, all three CRD1 strains displayed significantly higher R_{PSII} than clade 334 I-IV strains at all three tested temperatures, a difference ranging from 3- to nearly 40-fold at the lowest 335 common temperature (18°C). Furthermore, CRD1 strains displayed fairly limited variation in RPSII with 336 temperature (ranging from 1.33 to 1.87-fold) compared to the other strains, the strongest increase in 337 RPSII being observed for the clade I strain MVIR-18-1 (21.5-fold) and the clade III strain WH8102 (5.5-338 fold). This indicates that CRD1 strains exhibit a constitutively high level of PSII repair compared to 339 the other strains whatever the growth temperature and only trigger a moderate increase in R_{PSII} in 340 response to temperature variations.

341

342 **DISCUSSION**

343 Temperature constitutes one the strongest driving factors that have shaped genetic diversification and 344 niche partitioning in marine cyanobacteria (Scanlan et al., 2009; Flombaum et al., 2013; Biller et al., 345 2015) and phytoplankton at large (Sunagawa et al., 2015; Delmont et al., 2020). While temperature has 346 caused one major diversification event in Prochlorococcus, resulting in the divergence of the cold-347 adapted HLI from the warm-adapted HLII clades (Johnson et al., 2006; Kettler et al., 2007), several 348 independent temperature-related diversification events also occurred in the Synechococcus SC 5.1 349 radiation, leading to the emergence of clades I and IV (Dufresne et al., 2008; Zwirglmaier et al., 2008). 350 Here, determination of the temperature optimum and boundary limits (i.e., the fundamental niche) of 351 strains representative of the three CRD1 ESTUs identified in the field (Farrant et al., 2016) showed 352 that different thermotypes can also be delineated within the CRD1 clade, which dominates the 353 Synechococcus populations in low-Fe areas of the world Ocean. Comparison with representative strains

of the cold-adapted *Synechococcus* ecotypes (clades I and IV) on the one hand, and warm-adapted ecotypes (clades II and III) on the other, made it possible to classify i) the CRD1A strain MITS9220, isolated from the equatorial Pacific Ocean, as a warm thermotype, ii) the CRD1B strain BIOS-U3-1, isolated from the Chilean upwelling, as a cold temperate thermotype, and finally iii) the CRD1C strain BIOS-E4-1, isolated from the edge of the South Pacific gyre, a stable, warm, Fe-depleted oceanic region (Claustre et al., 2008), as a warm temperate stenotherm.

360 As expected from theory (Pearman et al., 2008), the realized environmental thermal niches of 361 CRD1 ESTUs were narrower than their fundamental niches (or similar for MITS9220). In contrast, for 362 ESTUS IA to IVA, the realized environmental niche was significantly more extended towards the low 363 thermal limit than the fundamental niches of their representative strains, this expansion being 364 particularly marked for ESTU IA (Fig. 1). This could be due to passive transport of Synechococcus 365 populations by currents into water masses colder than their temperature limits for growth. 366 Alternatively, these ESTUs may exhibit a greater microdiversity than previously assessed (Farrant et 367 al., 2016) and could be subdivided into distinct ESTUs occupying slightly different thermal niches 368 from the current ones, although representative strains to test this hypothesis remain to be isolated. In 369 agreement with the latter hypothesis Paulsen et al. (2016) measured a positive growth rate of 370 Synechococcus natural populations dominated by clade I in waters as cold as 2°C in the vicinity of the 371 Svalbard island. Thus, CRD1 ESTUs appear to be strongly outcompeted by their ESTU IA to IVA 372 counterparts at their lower temperature limits. Consistent with this, comparison of their gene content 373 showed that CRD1 ESTUs, including the cold thermotype CRD1B, lack the main adaptation 374 mechanisms reported so far for typical cold thermotypes. Indeed, all CRD1 strains examined in this 375 study i) exhibit warm-type substitutions in their α and β -phycocyanin subunits, influencing the 376 thermotolerance of this phycobiliprotein (Pittera et al., 2017; Supplementary Fig. 3A-B); ii) possess a 377 different set of desaturase genes, involved in regulation of membrane fluidity (Pittera et al., 2018), than 378 typical warm and cold thermotypes (Supplementary Table 2) and iii) lack the OCP system, involved 379 in the protection of PSII against photoinactivation, which seemingly plays a key role at low temperature 380 (Kirilovsky, 2007; Six et al., 2021). Still, we cannot exclude that CRD1 strains could use alternative 381 strategies to cope with temperature variations and notably to deal with the generation of reactive 382 oxygen species, known to be generated by a variety of factors including low and high temperature 383 (Nishiyama et al., 2006; Latifi et al., 2009). For instance, all CRD1 strains possess the srxA gene 384 encoding sulfiredoxin catalyzing the reduction of 2-Cys peroxiredoxin involved in H₂O₂ detoxification

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(Findlay et al., 2005; Guyet et al., 2020) as well as *isiA* that, besides its role in increasing the lightharvesting efficiency of PSI under conditions of Fe-limitation, was also shown to provide photoprotection to PSII by dissipating excess light energy under oxidative stress conditions (Yeremenko et al., 2004; Ihalainen et al., 2005; Supplementary Table 2).

389 The ability of cyanobacteria to grow over a large temperature range largely relies on their capacity 390 to optimize the functioning of their photosynthetic apparatus, notably at low temperature that induces 391 a general slowing down of cell metabolism (Murata et al., 2007; Pittera et al., 2014, 2017). For this 392 reason, we also compared the photophysiology of CRD1 and clades I-IV strains at three growth 393 temperatures common to all strains. These analyses showed that all three CRD1 strains exhibit a lower 394 growth rate at most temperatures than clade I to IV strains (Fig. 1), possibly explaining why they are 395 easily outcompeted by other taxa when iron is no longer limiting, as observed for instance around the 396 Marquesas Islands (Caputi et al., 2019). Moreover, CRD1 strains also display a lower PSII maximum 397 quantum yield (Fig. 2), suggesting that PSII is partially photoinactivated, that is their D1 repair cycle 398 does not fully compensate damage to this protein, even under optimal growth temperatures. Consistent 399 with this, the very high turnover rate of the D1 protein measured in all CRD1 strains indicates that their 400 PSII is much more sensitive to light stress than other strains and can only trigger a moderate increase 401 in R_{PSII} in response to both light and temperature variations, possibly indicating that they are adapted 402 to live deeper in the water column than clades I to IV. This sensitivity could be partially linked to the 403 abovementioned absence of the OCP, potentially reducing their ability to dissipate excess light energy, 404 although it must be noted that the clade II strain A15-62 also lacks the OCP system. Interestingly in 405 this context, all cold thermotypes including the CRD1B strain BIOS-U3-1, possess more copies of the 406 D1:2 isoform (3-6 copies, average: 3.9 ± 1.1) than warm thermotypes (2-3 copies, average: 2.2 ± 0.4), 407 this isoform providing a lower quantum yield but higher PSII resistance to photoinhibition than D1:1 408 (Supplementary Table 2; Clarke et al., 1993a, 1993b; Campbell et al., 1995; Garczarek et al., 2008). 409 Moreover, A15-62 is one of the only Synechococcus strains to possess two complete copies of the D1:1 isoform, a duplication which could partly explain why, despite the absence of OCP, this atypical clade 410 411 II strain is able to maintain a high PSII quantum yield over its whole growth temperature range with 412 fairly low D1 repair rates. Interestingly, CRD1 strains also possess a paralog of *psbN*, which was found 413 to be required for the assembly of the PSII reaction centre in Nicotiana tabacum and would play an 414 important role in the D1 repair cycle (Torabi et al., 2014).

415 Taken together, both comparative genomics and photophysiological analyses highlighted a 416 number of specificities of CRD1 strains compared to their clade I-IV counterparts, rather than them 417 possessing traits distinctive of cold or warm thermotypes. In this context, it is worth noting that 418 although strains representative of ESTUs IVA and CRD1B exhibit a similar fundamental thermal niche 419 in culture, the higher median temperature of CRD1B in the field indicates that it preferentially thrives 420 in temperate waters (about 18°C, Fig. 1), where energetically costly temperature adaptation 421 mechanisms might not be essential. This suggests that for members of the CRD1 clade, adaptation to 422 low-Fe conditions likely prevails over adaptation to temperature variations, and/or that adaptation 423 mechanisms to temperature variations might be more complex and diversified than previously thought. 424 Still, in terms of a realized environmental thermal niche, the occurrence of several CRD1 thermotypes 425 likely explains why the CRD1 clade as a whole occupies most Fe-limited areas, a vast ecosystem 426 constituting about 30% of the world Ocean (Moore et al., 2013; Bristow et al., 2017). A notable 427 exception is the Southern Ocean, for which from the little available data shows that Synechococcus is 428 scarce south of the polar front (Wilkins et al., 2013; Farrant et al., 2016), consistent with the fairly high 429 low-temperature limit (14°C) of the CRD1B environmental realized niche (Fig. 1), while low-Fe 430 availability likely limits the growth of clades I and IV in this area. In contrast, CRD1 growth does not 431 currently appear to be limited by warm temperatures since most oceanic waters display a temperature 432 below 30°C (Supplementary Fig. 2B; Supplementary Table 1). However, one cannot exclude that with 433 global change, some areas of the world Ocean could become warmer than the highest limits determined 434 here for representative strains of CRD1A and C, i.e. 31°C and 30°C respectively. In this context, it is 435 worth mentioning that in the dataset used for this study, several coastal stations, sampled during the 436 OSD campaign reached 31.5 °C (Supplementary Table 1). Thus, although biogeochemistry global 437 models predict that *Synechococcus* could be one of the winners of the phytoplankton community in a 438 future world Ocean (Flombaum et al., 2013; Schmidt et al., 2020; Visintini et al., 2021), it might well 439 not be able to survive in the warmest low-Fe areas, an ecological niche that is currently expanding 440 (Polovina et al., 2008). Although a few studies have started to analyze the genomic bases of adaptation 441 of Synechococcus cells to Fe-limitation in the field (Ahlgren et al., 2020; Garcia et al., 2020), further 442 comparative genomic and physiological studies are still needed to decipher the specific capacity of 443 CRD1 clade members to deal with Fe-limitation which should help predict the future distribution and 444 dynamics of Synechococcus taxa in the world Ocean.

445

446 DATA AVAILABILITY STATEMENT

Unpublished metabarcoding data supporting the conclusions of this article are available as raw data
(SRA accession numbers) and processed data (number of *petB* reads per ESTU) in Supplementary
Table 1. The latter Table also encompasses the description of all environmental samples used in this
study.

451

452 **CONFLICT OF INTEREST**

453 The authors declare that the research was conducted in the absence of any commercial or financial

454 relationships that could be construed as a potential conflict of interest.

455

456 **AUTHOR CONTRIBUTIONS**

MF, HD and LG designed the experiments. MF, LD, HD, MR, AG, FRJ, TS, LC and GM collected the
samples and performed the physiological measurements. MF and DM ran the flow cytometry analyses.
FL isolated several CRD1 strains used in this study. HD, XX, DJS, HL and LG performed sequencing
and bioinformatics analyses of metabarcodes. MH, EC, FP and LG developed and refined the Cyanorak *v2.1* database. MF, HD, FP and LG made the figures. MF, LD, HD, FP and LG interpreted results. All
the authors contributed to the preparation of the manuscript, read and approved the final manuscript.

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468

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480

481 SUPPLEMENTARY MATERIAL

482 Supplementary Table 1 | Environmental samples used in this study for the determination of the
483 realized environmental niches of the major *Synechococcus* ESTUs

484 Supplementary Table 2 | Phyletic pattern of CRD1 and clade I-IV genes mentioned in this study
485 retrieved from the Cyanorak v2.1 database

486 Supplementary Figure 1. Isolation sites of the Synechococcus strains used in this study. Isolation 487 site of each strain is indicated on the map by a bubble arrow colored according to their corresponding 488 ESTUs indicated below the strain name. Longhurst provinces (Longhurst A., 2007, Ecological 489 Geography of the Sea, Academic Press, London) are shown as a colored background shown in the 490 insert. Only provinces from which at least one strain has been isolated are indicated on the map using 491 the following abbreviations: PEQD (Pacific equatorial divergence, Pacific, Trade wind), CHIL (Chile-492 Peru, current coastal province, Pacific, Coastal), NAST W (Northwest Atlantic subtropical gyral, 493 Atlantic, Westerly), CARB (Caribbean, Atlantic, Trade wind), MEDI (Mediterranean Sea, Atlantic, 494 Westerly), SARC (Atlantic sub-Arctic, Atlantic, Polar).

Supplementary Figure 2. Oceanwide environmental data used in this study to determine the
environmental realized thermal niches of the main ESTUs from clades CRD1 and I to IV. (A)
Map of the sampling sites, (B) Relative abundance of *Synechococcus* ESTUs IA to IVA and CRD1A

- 498 to C. (C) Temperature distribution of the sampling sites. The inserts specify (A, C) the name of
- 499 campaigns or datasets analyzed and **(B)** the *Synechococcus* ESTUs.
- 500 Supplementary Figure 3. Alignment of RpcA and RpcB, encoding phycocyanin α- and b-
- 501 subunits, from CRD1 and clades I-IV Synechococcus strains. (A) RpcA. (B) RpcB. Substitutions
- 502 potentially involved in thermotolerance are shown by a red rectangle in the alignment.
- 503 Supplementary Figure 4. Variation with growth temperature of phycobilins and 504 phycobiliproteins fluorescence excitation and emission ratios. (A) Average PUB:PEB ratios. (B)
- 505 Average phycoerythrin (PE) to phycocyanin (PC) ratios. (C) Average PC to terminal acceptor (TA)
- 506 ratios. The insert indicates the strain names, their corresponding ESTU (*sensu* Farrant et al., 2016) and
- 507 pigment type (*sensu* Humily et al., 2013) between brackets.
- 508 Supplementary Figure 5. Variation with growth temperature of the three main liposoluble
- 509 pigments per cell for CRD1 vs. clade I and IV strains. (A, D) Chlorophyll (Chl) *a* content (fg/cell).
- 510 (**B**, **E**) Zeaxanthin (Zea) content (fg/cell). (**C**, **F**) β-carotene (β-car) content (fg/cell). (**A-C**) CRD1-B
- 511 strain BIOS-U3-1 vs. cold thermotypes. (D-F) CRD1-A strain MITS9220 and CRD1-C strain BIOS-
- 512 E4-1 vs. warm thermotypes. Inserts indicate the strain names and their corresponding ESTU (sensu
- 513 Farrant et al., 2016) between brackets.
- 514 Supplementary Figure 6. Time course of photosystem II quantum yield (F_V/F_M) following light
- 515 stress in the presence or absence of lincomycin for CRD1 and clade I-IV strains acclimated to
- 516 **different temperatures.** Cultures acclimated to 75 μ E m⁻² s⁻¹ were shifted to 375 μ E m⁻² s⁻¹ at T0 for
- 517 90 min, then shifted back to the initial light conditions for 24h as indicated by a vertical dashed line on
- 518 each figure. Strain names and their corresponding ESTU between brackets (*sensu* Farrant et al., 2016)
- 519 are indicated on the right-hand side, acclimation temperatures are indicated on the top, whilst line
- 520 colour indicates the lincomycin treatment (i.e. -/+ linco).
- 521
- 522

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 doi:https://doi.org/10.1111/j.1462-2920.2007.01440.x.
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TABLES

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Strains name	MVIR-18-1	A15-62	M16.1	WH8102	BL107	BIOS-E4-1	BIOS-U3-1	MITS9220
RCC # ¹	2385	2374	791	539	515	2534	2533	2571
Subcluster ²	5.1	5.1	5.1	5.1	5.1	5.1	5.1	5.1
Clade ²	Ι	II	II	III	IV	CRD1	CRD1	CRD1
ESTU ²	IA	IIA	IIA	IIIA	IVA	CRD1C	CRD1B	CRD1A
Pigment type ³	3aA	3dB	3a	3c	3dA	3cA	3dA	3dA
Ocean	Atlantic	Atlantic	Atlantic	Atlantic	Med. Sea	Pacific	Pacific	Pacific
Region	North Sea	Offshore Mauritania	Gulf of Mexico	Caribbean Sea	Balearic Sea	South East Pacific	Chile upwelling	Equatorial Pacific
Isolation latitude	61°00' N	17°37' N	27°70' N	22°48' N	41°72' N	31°52' S	34°00' S	0°00' N
Isolation longitude	1°59' E	20°57' W	91°30' W	65°36' W	3°33' E	91°25' W	73°22' W	140°00' W

730 TABLE 1 | Characteristics of the Synechococcus strains used in this study

731 ¹Roscoff Culture Collection, ²Farrant et al. (2016), ³Humily et al. (2013).

732

733 **FIGURE LEGENDS** 734 735 FIGURE 1 | Fundamental thermal niches of CRD1 vs. clade I, II, III and IV strains and environmental 736 realized niches of the corresponding ESTUs. (A) CRD1-B strain BIOS-U3-1 vs. cold thermotypes. (B) 737 CRD1-A strain MITS9220 and the CRD1-C strain BIOS-E4-1 vs. warm thermotypes. The insert indicates 738 the strain names and their corresponding ESTU (sensu Farrant et al., 2016) between brackets. Each 739 data point is the average of at least 3 biological replicates. Environmental realized niches are indicated 740 as horizontal boxplots for each ESTU above each graph with the whiskers corresponding to 1.5-fold 741 the interquartile range and outliers being plotted as individual points. 742 743 FIGURE 2 | Variation with growth temperature of photosystem II quantum yield (F_V/F_M) for CRD1 744 vs. clade I, II, III and IV strains. (A) CRD1-B strain BIOS-U3-1 vs. cold thermotypes. (B) CRD1-A strain 745 MITS9220 and the CRD1-C strain BIOS-E4-1 vs. warm thermotypes. The insert indicates the strain

names and their corresponding ESTU (*sensu* Farrant et al., 2016) between brackets.

747

FIGURE 3 | Variation with growth temperature of cellular mass pigment ratios of CRD1 vs. clade I, II, III and IV strains. (A, B) β -carotene (β -car) to chlorophyll *a* (Chla) ratio. (C, D) Zeaxanthin (Zea) to Chl*a* ratio with (A, C) CRD1-B strain BIOS-U3-1 vs. cold thermotypes and (B, D) CRD1-A strain MITS9220 and the CRD1-C strain BIOS-E4-1 vs. warm thermotypes. Inserts indicate strain names and their corresponding ESTUs (*sensu* Farrant et al., 2016) between brackets.

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FIGURE 4 | Variation with growth temperature of cumulative photosystem II repair rate (RPSII) of
 CRD1 vs. clade I, II, III and IV strains. The insert indicates the strain names and their corresponding
 ESTU (sensu Farrant et al., 2016) between brackets.



Ferrieux et al., Figure 1



Ferrieux et al., Figure 2



Ferrieux et al., Figure 3



Ferrieux et al., Figure 4

Ferrieux et al. Supplementary Figures





Supplementary Figure 1. Isolation sites of the *Synechococcus* strains used in this study. Isolation site of each strain is indicated on the map by a bubble arrow colored according to their corresponding ESTUs indicated below the strain name. Longhurst provinces (Longhurst A., 2007, Ecological Geography of the Sea, Academic Press, London) are shown as a colored background shown in the insert. Only provinces from which at least one strain has been isolated are indicated on the map using the following abbreviations: PEQD (Pacific equatorial divergence, Pacific, Trade wind), CHIL (Chile-Peru, current coastal province, Pacific, Coastal), NAST W (Northwest Atlantic subtropical gyral, Atlantic, Westerly), CARB (Caribbean, Atlantic, Trade wind), MEDI (Mediterranean Sea, Atlantic, Westerly), SARC (Atlantic sub-Arctic, Atlantic, Polar).





Supplementary Figure 2. Oceanwide environmental data used in this study to determine the environmental realized thermal niches of the main ESTUs from clades CRD1 and I to IV. (A) Map of the sampling sites, (B) Relative abundance of *Synechococcus* ESTUs IA to IVA and CRD1A to C. (C) Temperature distribution of the sampling sites. The inserts specify (A,C) the name of campaigns or datasets analyzed and (B) the *Synechococcus* ESTUs.

A. RpcA

	1 10	20	30 4	40 5	0 60	70	80 90	100 11	0	120 130	140	150 163
1. CC9311 Ia IA	MKTPLTEAVAAAD	SQGR F L SNTEVQAA	SGRENRAKASLD	AAKGLTAKADA	LVSSATQAVYTKFP	YTTQMEGPNYSAT	SEGKAKCSRD I GYYLRMI	TYCL VAGGTGPMDD Y	LIAGLDEI	NRTFELSPSWYVEALKE	IQSNHGL SGDA/	ATEANSY INYA INALT*
2. WH8020 Ia IA	MKTPLTEAVAAAD	OGRELSNTEVQAA	SGRENRAKASLE	AAKGLTAKADS	LVSSATQAVYTKFP	YTTQMEGPNYSAT	SEGKAKCSRD I GYYLRMI	TYCL VAGGTGPMDDY	LIAGLDEI	NRTFELSPSWYVEALKE	IQSNHGL SGDA/	ATEANSY INYA INALT*
3. MVIR-18-1 lb IA	MKTPLTEAVAAAD	SQGRFLSNTEVQAA	SGRENRAQASLE.	AAKGLTAKAD	LVSGATQAVYTKFP	YTTQMEGPNYSTT	PEGKAKCSRD I GYYLRMV	TYCLVAGGTGPMDDY	LIAGLDET	NRTFELSPSWYVEALKE	IKSNHGLSGDA/	ATEANSY INYA INALT*
4 PROS-9-1 Ib IA	MKTPLTEAVAAAD	OGREL SNTEVOAA	SGRENRAKASLE	AAKGLTSKAET	LVNSAAQAVYTKEP	FTTOMEGTNYAST	SEGKAKCSRD I GYYLRMI	TYCLVAGGTGPMDDY	LIAGLAET	NRTFELSPTWYVEALRN	KSNHGL SGDA	ATEANSY IDYAINALI*
5 POS9604 IN IA	MKTPL TEAVAAAD	SOGREL SNTEVOAA	SCRENRAKASLE	AAKGI TAKADA	UVNGAADAVYTKEP	VITOMOGSNVSTT	SEGKAKCARD LGYVLRMI	TYCI VAGGTGPMDDY	LAGIDEL	NETEELSESWAVEALKY	IKANHGI SONA	STEANSY IDVAINAL I*
6 SVN20 Ib IA	MKTRLTEAVAAAD	SOGREL SNTEVOAA	SCRENDAKASLE	AAKCI TAKADS	I VNGAAOAVYTKEP	YTTOMOGSNYSTT	PECKAKCAPDICYVIPMI	TYCL VAGGTGPMDDY	LAGEDEL	NOTEFI SPSWVVFALKE	KANHGI SONA	GTEANSY IDVAINAL I*
	NIK TPETEAVAAAAD	OCDEL SNTEVQAA	SCRENDAKASLE.	AAKGL TAKAD		111QWQG5N1511	FEGRARCARD I GITTERMI	TICEVAGGTGFMDDI	LIAGEDET	INTERL COCHARGE AL KY	IKANHGE SGNA	GTEANSTIDIAINALI.
7. WH8016_ID_IB	MKTPLTEAVAAAD	SQGRELSNIEVQAA	SGRENRARASLE.	AAKGLTAKAD	LVNGAAQAVTIKEP	TTTQMQGSNYSTT	TEGRARCARDIGTTERMI	TYCEVAGGTGPMDDY	LINGLUEI	NRIFELSPSWYVEALKY	I KANHGE SGNA	GTEANSTIDTAINALT*
8. BL107_IVa_IVA	MKTPLTEAVAAAD	SQGRELSNIEVQAA	SGRENRAKASLE	AAKGLISKAEA	AL VNGAAQAVY I KEP	FILQMEGINYASS	PEGKAKCSRDVGYYLRMI	TYCEVAGGTGPMDDY	LIAGLSEI	NRIFELSPSWYVEALKE	TK INHGESGDA	ATEANSY IDYATNSLI*
9. CC9902_IVa_IVA	MKTPLTEAVAAAD	SQGR F L SNTEVQAA	SGRENRAKASLE	AAKGLTSKADA	LVNGAAQAVYSKEP	YTTQMDGANYAST	PEGKAKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLSEI	NRTFELSPSWYVEALKE	IKANHGL SGDA/	ATEANSY I DYAVNSLT*
10. A15-44_IIa_IIA	MKTPLTEAVAAAD	SQGR F L SNTE I QGA	FGRENRAKAALE	AAKALTTKADI	LVNGAAQAVYSKFP	YTTQMQGPTYAST	PEGKAKCSRDIGYYLRMV	TYCLVAGGTGPMDDY	LIAGLDEI	NRTFELSPSWYVEALKY	IKANHGL SGEA/	ATEANNYFDYAINALV*
11. KORDI-52_IIa_IIA	MKTPLTEAVAAAD	SQGR F L SNTEVQAA	SGRENRAKASLE	AAKALTSKADS	LVNGAAQAVYTKFP	YTTQMAGPNYSAS	PEGKAKCSRDVGYYLRMI	TYCL VAGGTGPMDD Y	LIAGLDEI	NRTFELSPSWYVEALKE	IKANHGLSGDA/	ATEANSY IDYATNALT*
12. M16.1 IIa IIA	MKTPLTEAVAAAD	OGRELSNTEVQAA	SGRENRAKASLE	AAKALTSKADS	LVNGAAQAVYTKFP	YTTQMEGSNYSAT	AEGKAKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLDET	NRTFELSPSWYVEALKE	IKANHGL SGDA/	ATEANSY IDYAINALI*
13 RS9902 IIa IIA	MKTPLTEAVAAAD	OGREL SNTEVOAA	SGRENRAKASLE	AAKALTSKADS		YTTOMAGPNYSAT	PEGKAKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLDET	NRTFELSPSWYVEALKE	IKANHGLSGDA	ATEANSY IDYAINALI*
14 PS9007 IIa IIA	MKTPLTEAVAAAD	OGREL SNTEVOAA	SGRENRAKASLE	AAKAL TSKADS	I VNGAAOAVYTKEP	YTTOMEGSNYSAT	AEGKAKCSRDVGVVLRMI	TYCL VAGGTGPMDDY	LIAGUDEL	NRTEEL SPSWYVEALKE	IKANHGI SGDA	ATEANSY IDVAINAL I*
15 TAK0803 Un UA	METRITEAVAAAD	OCREL SNTEVOAA	SCRENDAKASI E	AAVALTSKADS	UVNGAAOAVVTKER	VITOMEGENIVEAT	AEGKAKCSRDVGVVI PMI	TYCL VACGTORMODY	LINGLDEL	NOTE EL SOSWAVE AL KE	LKANHGI SODA	ATEANSY IDVALNAL I*
15. TAK9602_IIA_IIA	NIK TPETEAVAAAD	OCDEL SNTEVQAA	SCRENDAKASLE.	AAKAL TOKAD		VITTOME GONVEAT	ALGRARCSRDVGTTERMT	TYCL VAGGTGFMDD	LIAGEDET	NRTFEESF SHIVEALKE	IKANUGLOGDA	ATEANSY IDVALUAL I
16. WH8109_IIa_IIA	MKTPLTEAVAAAD	SQGRELSNIEVQAA	SGRENRARASLE.	AARALISKAD	LVNGAAQAVTAKEP	TTTQMAGPINTSAT	PEGRARCSRDVGTTLRMI	TYCEVAGGTGPMDDY	LINGLUEI	NRIFELSPSWYVEALKP	INANHGE SGDA	ATEANST IDTAINAL I*
17. A15-62_IIc_IIA	MKTPLTEAVAAAD	SQGR F L SNTEVQAA	SGRENRAKASLE	AAKALTSKADS	LVNGAAQAVYTKEP	YTTQMAGPNYSAT	PEGKAKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLDEI	NRTFELSPSWYVEALKE	KANHGL SGDA/	ATEANSY IDYA I NAL I *
18. CC9605_IIc_IIA	MKTPLTEAVAAAD	SQGRFL SNTEVQAA	SGRENRAKASLE	AAKALTGKADS	LVNGAAQAVYTKFP	YTTQMAGPNYSAT	PEGKAKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLAEI	NRTFELSPSWYVEALKE	TKANHGL SGDA/	ATEANSY IDYA INALT*
19. PROS-U-1_IIh_IIB	MKTPLTEAVAAAD	SQGR F L SNTEVQAA	SGRENRAKASLE	AAKALTSKADS	LVNGAAQAVYTKFP	YTTQMAGPNYSAT	PEGKAKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLAEI	NRTFELSPSWYVEALKE	IKSNHGLSGDA/	ATEANSY IDYATNAL I *
20. A15-24 IIIa IIIA	MKTPLTEAVAAAD	SQGR F L SNTEVQAA	SGRENRAKASLE	AAKALTSKADS	LVNGAAQAVYTKFP	YTTQMEGPNYSAT	PEGKAKCSRDVGYYLRMI	TYCL VAGGTGPMDD Y	LIAGLAET	NRTFELSPSWYVEALKE	IKSNHGLSGDA/	ATEANSY IDYA I NALT*
21. A18-40 IIIa 3IIIA	MKTPLTEAVAAAD	SQGRFLSNTEVQAA	SGRENRAKASLE	AAKALTSKADS	LVNGAAQAVYSKEP	YTTQMEGSNYSAT	PEGKAKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLDET	NRTFELSPSWYVEALKE	IKANHGL SGDA/	ATEANSY IDYAINALI*
22 A18-46 1 IIIa IIIA	MKTPLTEAVAAAD	OGREL SNTEVOAA	SGRENRAKASLE	AAKALTSKADS	LVNGAAQAVYSKEP	YTTOMEGSNYSAT	PEGKSKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLDET	NRTFELSPSWYVEALKE	IKANHGLSGDA	ATEANSY IDYAINALI*
22. POLIM119 UID UIA	MKTPLTEAVAAAD	OGREL SNTEVOAA	SGRENRAKASLE	AAKAL TSKADS	I VNGAAOAVYSKEP	YTTOMEGSNYSAT	PEGKAKCSRDVGYYLRMI	TYCL VAGGTGPMDDY	LIAGUDEL	NRTEEL SPSWYVEALKE	IKANHGI SGDA	ATEANSY IDVAINAL I*
23. DOOMTTO_IIIA_IIIA	METRITEAVAAAD	OCREL SNTEVOAA	SCRENDAKASI E	AAVALTSKADS	U VNGAAOAVVSKED	VTTOMEGENVEAT	DECKAKCS RDVGVVI PMI	TYCL VAGGTGRMDDY	LINGLDEL	NOTEEL COCHAVEAL KE	LKANHGI SGDA	ATEANSY IDVALNAL I*
24. K59915_IIId_IIIA	NIK TPETEAVAAAD	OCDEL SNTEVQAA	SCRENDAKASLE.	AAKAL TOKAD		VITTOME CONVERT	PEGKARCSRDVG11ERMI	TYCL WASGTGPWDD	LIAGLACI	NRTFEESF SHIVEALKI	IKANIIGE SODA	ATE ANGY I DYA I NALTA
25. WH8102_IIIa_IIIA	MKTPLTEAVAAAD	SQGRELSNIEVQAA	SGRENRARASLE.	AARALISKAD	LVNGAAQAVTIKEP	TTTQMEGPINTSAT	PEGKSKCSRDVGTTERMI	TYCEVAGGTGPMDDT	LIAGLAET	NRIFELSPSWIVEALKE	INSINHGE SGDA	ATEANSTIDTAINALI*
26. WH8103_IIIa_IIIA	MKTPLTEAVAAAD	SQGRELSNIEVQAA	SGRENRAKASLE	AAKALISKADS	LVNGAAQAVYSKEP	YTTQMEGSNYSAT	PEGKAKCSRDVGYYLRMI	TYCEVAGGTGPMDDY	LIAGEDET	NRTFELSPSWYVEALKE	TRANHGE SGDA	ATEANSY IDYATNALT*
27. A15-28_IIIb_IIIA	MKTPLTEAVAAAD	SQGR F L SNTEVQAA	SGRENRAKASLE	AAKALTSKADA	LVNGAAQAVYSKEP	YTTQMEGSNYSAT	PEGKAKCSRDVGYYLRMI	TYCLVAGGTGPMDDY	LIAGLDEI	NRTFELSPSWYVEALKE	IKANHGL SGDA/	ATEANSY IDYA I NAL I *
28. MITS9220_CRD1_CRD1A	MKTPLTEAVAAAD	SQGR F L TNAE VQAA	SGRETRAKASLE	AAKALTSKGDA	LVNGAAQAVYTKFP	FTTQMEGSNYAST	TEGKAKCSRD I GYYLRMV	TYCLVAGGTGPMDDY	LIAGLAEI	NRTFELSPTWYVEALKE	IKANHGL SGDA/	ATEANSYLDYAINALI*
29. BIOS-U3-1_CRD1_CRD1B	MKTPLTEAVAAAD	AQGR F L SNTEVQAA	SGRFIRAKASLE.	AAKAL SAKADT	LVNGAAQAVYTKFP	YTTQMEGANYSAT	TEGKAKCSRDVGYYLRMV	TYCL VAGGTGPMDD Y	LIAGLDEI	NRTFELSPSWYVEALKE	IKANHGLSGDA/	ATEANSYLDYATNALT*
30. BIOS-E4-1 CRD1 CRD1C	MKTPLTEAVAAAD	OGRELTNAEVQAA	SGRETRAKASLE	AAKALTAKGDA	LVNSAAQAVYTKEP	FTTQMEGSNYAST	TEGKAKCSRD I GYYLRMV	TYCLVAGGTGPMDDY	LIAGLAET	NRTFELSPTWYVEALKE	IKANHGL SGDA/	ATEANSYLDYAINALI*
B. RpcB												
B. RpcB	1 10	20	30 40	50	60	70 80	90 100	110	120	130 140	150	160 173
B. RpcB			30 40	50			90 100		120	130 140	150 DEAGLESSOCAS	160 173
B. RpcB	1 10 MEDAFTKVVAQADA	20 RGQFI SASEIDALAA	30 40 MVSGSNKRLDAV	50 SR I SNNAST I VA	60 NAARELFAQQPALIS	70 80 PGGNAYTSRRMAAC	90 100 LIRDME I I LRYVTYSAFTGG	110 ASVLEDRCLNGLRETT	120 LALGTPGT	130 140 SVATGVNLMKDAALSI VN	150 DSAGI SSGDCASI	160 173 LSSE IGTYFDRAAASVA*
B. RpcB	1 10 MFDAFTKVVAQADAI MFDAFTKVVAQADAI	20 RGQF I SASE I DALAA RGQF I STSE I DALAA	30 40 MVSGSNKRLDAV MVSGSNKRLDAV	50 SR I SNNAST I VA SR I SNNAST I VA	60 NAARELFAQQPALIS SAARELFAQQPALIS	70 80 PGGNAYTSRRMAAC PGGNAYTSRRMAAC	90 100 LRDME I LRYVTYSAFTGC LRDME I LRYVTYSAFTGC	110 ASVLEDRCLNGLRETT ASVLEDRCLNGLRETT	120 LALGTPGT LALGTPGT	130 140 SVATGVNLMKDAAL SI VN SVAAGVNLMKDAAL SI VN	150 DSAGTSSGDCAS DRAGTSNGDCAS	160 173 LSSE I GTYFDRAAASVA* LSSE I GTYFDRAAASVA*
B. RpcB 1. cc9311_la_IA 2. WH8020_la_IA 3. MWR-18-1_lb_IA	1 10 MFDAFTKVVAQADAI MFDAFTKVVAQADAI MFDAFTKVVAQADAI	Z0 RGQF I SASE I DALAA RGQF I STSE I DALAA RGQF I SANE I DALAA	30 40 MVSGSNKRLDAV MVSGSNKRLDAV MVSGSNKRLDAV	50 SR I SNNAST I VA SR I SNNAST I VA SR I SNNAST I VA	60 NAARELFAQQPALIS SAARELFAQQPALIS NAARQLFAQQPALIS	70 80 PGGNAYTSRRMAAC PGGNAYTSRRMAAC	90 100 LIRDME I LIRVYTYSAFTGD LIRDME I LIRVITYSAFTGD	110 ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY	120 LALGTPGT LALGTPGT LALGTPGA	130 140 SVATGVNLMKDAAL STVN SVAAGVNLMKDAAL STVN SVATGVNLMKDAAL STVN	150 DSAGI SSGDCASI DRAGI SNGDCASI DSAGI SSGDCASI	160 173 LSSE I GTYFDRAAASVA* LSSE I GTYFDRAAASVA*
B. RpcB 1. CC9311_la_IA 2. WH8020_la_IA 3. MVIR-18-1_lb_IA 4. PR05-9-1_lb_IA	1 10 MFDAFTKVVAQADAI MFDAFTKVVAQADAI MFDAFTKVVAQADAI MFDAFTKVVAQADAI	20 RGQF I SASE I DALAA RGQF I SASE I DALAA RGQF I SANE I DALAA RGQF I SASE I DALAA	30 40 MVSGSNKRLDAV MVSGSNKRLDAV MVSGSNKRLDAV MVSGSNKRLDAV	50 SR I SNNAST IVA SR I SNNAST IVA SR I SNNAST IVA SR I SSNAST IVA	60 NAARELFAQQPALIS SAARELFAQQPALIS NAARQLFAQQPALIS NAARELFAQQPALIS	70 80 PGGNAYTSRIMAAC PGGNAYTSRIMAAC PGGNAYTSRIMAAC PGGNAYTSRIMAAC	90 100 LRDME I LRYVTYSAFTGU LRDME I LRYVTYSAFTGU LRDME I LRYTYSAFTGU LRDME I LRY I TYSAFTGU	110 ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLEDRCLNGLRET	120 'LALGTPGT 'LALGTPGT 'LALGTPGA 'LALGTPGA	130 140 SVATGVNLMKDAAL S I VN SVASVVLMKDAAL S I VN SVATGVULMKDAAL S I VN SVATGVLMKDAAL S I VN	150 DSAGI SSGDCASI DSAGI SSGDCASI DSAGI TNGDCASI DSAGI TNGDCASI	160 173 LSSE IGTYFDRAAASVA* LSSE IGTYFDRAAASVA* LSSE IGTYFDRAAASVA* LSSELGTYFDRAAASVA*
B. RpcB 1. CC9311_la_IA 2. WH8020_la_IA 3. WWR-18-1_lb_IA 4. PROS-9-1_lb_IA 5. ROS8604_lb_IA	1 MFDAFTKVVÁQADA MFDAFTKVVAQADA MFDAFTKVVAQADA MFDAFTKVVAQADA	20 RGQF I SASE I DALAA RGQF I SASE I DALAA RGQF I SASE I DALAA RGQF I SASE I DALAA RGQF I SSSE I DALSA	30 40 MVSGSNKRLDAV MVSGSNKRLDAV MVSGSNKRLDAV MVSDSDKRLDAV	50 SRISNNASTIVA SRISNNASTIVA SRISNNASTIVA SRISSNASTIVA SRISSNASTIVA	60 NAARELFAQOPALIS SAARELFAQOPALIS NAARELFAQOPALIS NAARELFAQOPALIS NAARELFAQOPALIA	70 80 PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC	90 100 LIROME I LIRVYTYSAFTG LIROME I LIRVYTYSAFTG LIROME I LIRVYTYSAFTG LIROME I LIRVYTYSAFTG	110 ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLDDRCLNGLRETY	120 'LALGTPGT 'LALGTPGT 'LALGTPGA 'LALGTPGA 'LALGTPGA	130 140 SVATGVNLMKDAALS I VN SVATGVNLMKDAALS I VN SVATGVSLMKDAALS I VN SVATGVSLMKDAALS I VN	150 DSAG I SSGDCASI DSAG I SNGDCASI DSAG I SSGDCASI DSAG I TNGDCASI DKAG I SSGDCSSI	160 173 LSSE IGTYFDRAAASVA* LSSE IGTYFDRAAASVA* LSSE IGTYFDRAAASVA* LSSE IGTYFDRAAASVA*
B. RpcB 1. CC9311_la_IA 2. WH8020_la_IA 3. MVIR-18-1_lb_IA 4. PR053-9-1_lb_IA 5. ROS8604_lb_IA 6. SYN20_Jb_IA	1 10 MEDAF TKVVAQADAI MEDAF TKVVAQADAI MEDAF TKVVAQADAI MEDAF TKVVAQADAI MEDAF TKVVAQADAI	20 RGQFI SASEI DALAA RGQFI STSEI DALAA RGQFI SASEI DALAA RGQFI SASEI DALSA RGQFI SSSEI DALSA	30 40 MVSGSNKRLDAVS MVSGSNKRLDAVS MVSGSNKRLDAVS MVSGSNKRLDAVS MVSDSKRLDSVS	50 SR I SNNAST I VA SR I SNNAST I VA SR I SNNAST I VA SR I SSNAST I VA SR I SSNAST I VA SR I SSNAST I VA	60 NAARELFAQOPALIS SAARELFAQOPALIS NAAROLFAQOPALIS NAAROLFAQOPALIA NAAROLFAQOPALIA NAAROLFAQOPALIA	70 80 PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC	90 100 LRDMETTLRVYTYSAFTG LRDMETTLRVYTYSAFTG LRDMETTLRVTYSAFTG LRDMETTLRVTYSAFTG LRDMETTLRVTYSAFTG LRDMETTLRVTYSAFTG	110 ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLDDRCLNGLRETY	120 LALGTPGT LALGTPGT LALGTPGA LALGTPGA LALGTPGA	130 140 SVATGVNLMKDAALS I VN SVATGVNLMKDAALS I VN SVATGVNLMKDAALS I VN SVATGVSLMKDAALS I VN SVATGVSLMKDAALS I VN SVAGVSLMK FAALA I N	150 DSAGI SSGDCASI DRAGI SNGDCASI DSAGI SSGDCASI DSAGI TNGDCASI DKAGI SSGDCSSI DKAGI SSGDCSSI	160 173 LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAAAVA+
B. RpcB 1. CC9311_la_lA 2. WH8020_la_lA 3. WJR-B4_lb_lA 4. PROS-91_lb_lA 4. PROS-91_lb_lA 5. ROS8604_lb_lA 6. SYN20_lb_lA	1 10 MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI	20 RGQFI SASE I DALAA RGQFI STSE I DALAA RGQFI SASE I DALAA RGQFI SASE I DALSA RGQFI SSSE I DALSA RGQFI SSSE I DALSA	30 40 MVSGSNKRLDAV MVSGSNKRLDAV MVSGSNKRLDAV MVSDSDKRLDAV MVSDSDKRLDSV MVSDSKRLDSV	50 SRISNNASTIVA SRISNNASTIVA SRISNNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA	60 NAARELFAQOPALIS SAARELFAQOPALIS NAARELFAQOPALIS NAARELFAQOPALIA NAAROLFAQOPALIA NAAROLFAQOPALIA	70 80 PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC	90 100 LRDME I LRYVYTSAFTGC LRDME I LRYVYTSAFTGC LRDME I LRY I TYSAFTGC LRDME I LRY I TYSAFTGC LRDME I LRYVTSFSAFTGC LRDME I LRYVTSFSAFTGC	110 ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLDDRCLNGLRET ASVLDDRCLNGLRET SVLDDRCLNGLRET	120 LALGTPGT LALGTPGT LALGTPGA LALGTPGA LALGTPGA LALGTPGA	140 140 SVAT GVINLIM DAALS I VIN SVAT GVINLIM DAALS I VIN SVAT GVINLIM DAALS I VIN SVAT GVINLIM DAALS I VIN SVAAGVINLIM EAALA I VIN SVAAGVINLIM EAALA I VIN	150 DSAGI SSGDCASI DRAGI SNGDCASI DSAGI SSGDCASI DSAGI SSGDCSSI DKAGI SSGDCSSI DKAGI SSGDCSSI	160 173 LSSE IGTYFDRAAASVA* LSSE IGTYFDRAAASVA* LSSE IGTYFDRAAASVA* LSSE IGTYFDRAAASVA* LTSE IGTYFDRAAAVA* LTSE IGTYFDRAAAVA*
B. RpcB 1. cc9311_la_IA 2. wHe020_la_IA 3. MVIR-18-1 lb_IA 4. PPG5-9-1 lb_IA 5. ROS8604_lb_IA 6. SYN20_Jb_IA 7. WH8016_lb_IA 8. BL107_JVA_JVA	1 10 MEDAF TKUVAQADA MEDAF TKUVAQADA MEDAF TKUVAQADA MEDAF TKUVAQADA MEDAF TKUVAQADA MEDAF TKUVAQADA	20 GQF I SASE I DALAA GQF I SASE I DALAA GQF I SASE I DALAA GQF I SASE I DALSA GQF I SSSE I DALSA GQF I SSSE I DALSA GQF I SSSE I DALAA	30 40 MVSGSNKRLDAVS MVSGSNKRLDAVS MVSGSNKRLDAVS MVSDSKRLDSV MVSDSKRLDSV MVSDSKRLDSV VVSDSNRLDSV	50 SRISNIASTIVA SRISNIASTIVA SRISNIASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA	60 NAARELFAQOPALIS SAARELFAQOPALIS NAARCLFAQOPALIS NAARQLFAQOPALIA NAARQLFAQOPALIA NAARQLFAQOPALIA NAARQLFAQOPALIA	70 80 PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC	90 100 LRDMETILRVYTSAFTG LRDMETILRVYTSAFTG LRDMETILRVTSAFTG LRDMETILRVYTSAFTG LRDMETILRVYTSAFTG LRDMETILRVYTSAFTG LRDMETILRVYTSAFTG	110 ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLDDRCLNGLRETY ASVLDDRCLNGLRETY ASVLDDRCLNGLRETY	120 'LALGTPGT 'LALGTPGA 'LALGTPGA 'LALGTPGA 'LALGTPGA 'LALGTPGA	130 140 SVATGVNLMKDAAL SLVN SVATGVNLMKDAAL SLVN SVATGVSLMKDAAL SLVN SVAGVSLMKEAAL SL SVAGVNLMKEAAL SL SVAGVNLMKEAAL SL	150 DSAGI SSGDCASI DSAGI SSGDCASI DSAGI SSGDCASI DSAGI SSGDCSSI DKAGI SSGDCSSI DKAGI SSGDCSSI DKAGI SSGDCSSI DKAGI SSGDCSSI	160 173 LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAAAVA+ LSSE IGTYFDRAAAAVA+ LSSE IGTYFDRAAAAVA+ LSSE IGTYFDRAAASVA+
B. RpcB 1. CC9311_la_IA 2. WH8020_la_IA 3. WWR-18-1_lb_IA 4. PROS-91_lb_IA 5. ROS&604_lb_IA 6. SYN20_lb_IA 7. WH8016_lb_IA 8. BL107_IVA_IVA	1 10 MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI	20 RGQ FI SASE I DALAA RGQ FI STSE I DALAA RGQ FI SASE I DALAA RGQ FI SASE I DALSA RGQ FI SSSE I DALSA RGQ FI SSSE I DALSA RGQ FI STSE I DALSA	30 40 WVSGSNKRLDAV WVSGSNKRLDAV WVSGSNKRLDAV WVSGSNKRLDAV WVSDSDRLDSV WVSDSNRLDAV VVSDSNRLDAV	50 SRISNNASTIVA SRISNNASTIVA SRISNNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA	60 NAARELFAQOPALIS SAARELFAQOPALIS NAAROLFAQOPALIS NAAROLFAQOPALIA NAAROLFAQOPALIA NAAROLFAQOPALIA NAAROLFAQOPALIA	70 80 PGGNAYTSRIMAAC PGGNAYTSRIMAAC PGGNAYTSRIMAAC PGGNAYTSRIMAAC PGGNAYTSRIMAAC PGGNAYTSRIMAAC PGGNAYTSRIMAAC	90 100 LRDME I LRYVTYSAFTGC LRDME I LRIVTYSAFTGC LRDME I LRVTYSAFTGC LRDME I LRVTYSAFTGC LRDME I LRVTYSAFTGC LRDME I LRVTYSAFTGC LRDME I LRVTYSVFTGC LRDME I LRVTYSVFTGC	110 ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLEDRCNGLRET ASVLDDRCNGLRET ASVLDDRCLNGLRET ASVLDDRCLNGLRET ASVMEDRCLNGLRET	120 LALGTPGT LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA	130 140 SVATGVNLIMCDAALS I VN SVATGVNLIMCDAALS I VN SVATGVNLIMCDAALS I VN SVATGVSLIMCDAALS I VN SVAGVSLIMC BALALI VN SVAGVNLIMC BALALI VN SVAGVNLIMCDAAL BMVN	150 DSAG I SSGDCASI DRAG I SNGDCASI DSAG I SSGDCASI DSAG I SSGDCASI DSAG I SSGDCASI DKAG I SSGDCSSI DKAG I SSGDCSSI DKAG I SSGDCSSI DKAG I SSGDCSSI DKAG I SSGDCSSI DKAG I SSGDCSSI	160 173 LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+
B. RpcB 1. cc9311_la_A 2. wr8020_ls_LA 3. wr8020_ls_LA 4. procs-91_lb_LA 4. procs-91_lb_LA 5. srx20_lb_LA 7. wr8016_lb_LA 8. BL107_IVa_IVA 9. cc29902_IVa_IVA 10. 415-44_lla_ILA	1 10 MEDAETIK WAQADAI MEDAETIK WAQADAI MEDAETIK WAQADAI MEDAETIK WAQADAI MEDAETIK WAQADAI MEDAETIK WAQADAI MEDAETIK WAQADAI MEDAETIK WAQADAI	20 ROOFISASEIDALAA ROOFISASEIDALAA ROOFISASEIDALAA ROOFISASEIDALSA ROOFISSSEIDALSA ROOFISSSEIDALSA ROOFISSSEIDALAA ROOFISTSEIDALAA	30 40 MVSGSNRLDAV MVSGSNRLDAV MVSGSNRLDAV MVSDSNRLDAV MVSDSNRLDAV VVSDSNRLDAV VVSDSNRLDAV VVSDSNRLDAV	50 SRISNNASTIVA SRISNNASTIVA SRISNNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA	60 NAARELFACOPALIS SAARELFACOPALIS NAAROLFACOPALIS NAAROLFACOPALIA NAAROLFACOPALIA NAAROLFACOPALIA NAAROLFACOPALIA NAAROLFACOPALIA	70 80 PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC PGGNAYTSRRMAAC	90 100 LEDME ILLRYVTYSAFTOC LEDME ILLRYVTYSAFTOC LEDME ILLRYTYSAFTOC LEDME ILLRYTYSAFTOC LEDME ILLRYVTYSAFTOC LEDME ILLRYVTYSAFTOC LEDME ILLRYVTYSFTOC LEDME ILLRYVTYSFTOC	110 ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLEDRCLNGLRETY ASVLDDRCLNGLRETY ASVLDDRCLNGLRETY ASVLDDRCLNGLRETY ASVMEDRCLNGLRETY ASVMEDRCLNGLRETY	120 'LALGTPGT 'LALGTPGA 'LALGTPGA 'LALGTPGA 'LALGTPGA 'LALGTPGA 'LALGTPGA 'LALGTPGA	130 140 SVAT GWILINKDAAL 5 I VI SVAT GWILINKDAAL 5 I VI SVAT GWILINKDAAL 5 I VI SVAT GWILINKDAAL 5 I VI SVAAGVILINK EAALAI I NI SVAAGVILINK EAALAI I NI SVAAGVILINK BAALAI MIN SVAS GWILINKDAAL BINNI SVAS GWILINKDAAL BINNI	150 DSAGI SSGDCASI DSAGI SNGDCASI DSAGI SSGDCASI DSAGI SSGDCSSI DKAGI SSGDCSSI DKAGI SSGDCSSI DRNG I TAGDCASI DRSGI TAGDCASI	160 173 LSSE IGTYFDRAMSVA+ LSSE IGTYFDRAMSVA+ LSSE IGTYFDRAMSVA+ LSSE IGTYFDRAMSVA+ LSSE IGTYFDRAMAVA+ LSSE IGTYFDRAMAVA+ LSSE IGTYFDRAMSVA+ LSSE IGTYFDRAMAVA+
B. RpcB 1. CC9311_la_IA 2. WH8020_la_IA 3. WIXF.18-1_Ib_IA 4. PROS-9-1_Ib_IA 5. ROS&60A_Ib_IA 6. SYN20_lb_IA 7. WH8016_Ib_IA 8. BL107_IVA_IVA 9. CC9902_IVA_IVA 10. A15-44_IIa_IIA 11. KORPDE52_IIIa_IIA	1 MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI MEDAFTKVVAQADAI	20 RGQF I SASE I DALAA RGQF I SASE I DALAA RGQF I SASE I DALAA RGQF I SSSE I DALSA RGQF I SSSE I DALSA RGQF I STSE I DALAA RGQF I STSE I DALAA RGQF I STSE I DALAA RGQF I DALAA	20 40 WYSGSNKRLDAW WYSGSNRRLDAW WYSGSNRRLDAW WYSDSDRLDSW WYSDSDRRLDSW WYSDSDRRLDSW WYSDSNRLDAW WYSDSNRLDAW WYSDSNRLDAW	50 SR I SNIAST I VA SR I SNIAST I VA SR I SNIAST I VA SR I SNIAST I VA SR I SSNAST I VA SR I SSNASS I VA	60 NAARELFAQOPALIS SAARELFAQOPALIS NAAROLFAQOPALIS NAAROLFAQOPALIA NAAROLFAQOPALIA NAAROLFAQOPALIA NAAROLFAQOPALIA SAAROLFAQOPALIA DAAROLFAQOPALIA	70 80 PGGIAYTSRIMAAC PGGIAYTSRIMAAC PGGIAYTSRIMAAC PGGIAYTSRIMAAC PGGIAYTSRIMAAC PGGIAYTSRIMAAC PGGIAYTSRIMAAC PGGIAYTSRIMAAC	90 100 LRDME I LRYVTYSAFTGC LRDME I LRYVTYSAFTGC LRDME I LRYTYSAFTGC LRDME I LRYTYSAFTGC LRDME I LRYVTYSAFTGC LRDME I LRYVTYSAFTGC LRDME I LRYVTYSFTGC LRDME I LRYVTYSFTGC LRDME I LRYVTYSFTGC	110 ASVLEDRCLNGLRETM ASVLEDRCLNGLRETM ASVLEDRCLNGLRETM ASVLEDRCLNGLRETM ASVLDDRCLNGLRETM ASVLDDRCLNGLRETM ASVMEDRCLNGLRETM ASVMEDRCLNGLRETM	120 LALGTPGT LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA	130 140 SVATGVNLIMCDAALS I VN SVATGVNLIMCDAALS I VN SVATGVNLIMCDAALS I VN SVATGVSLIMCDAALS I VN SVATGVNLIMC BALAI VN SVAAGVNLIMC BALAI VN SVASGVNLIMCDAAL BINN SVASGVNLIMCDAAL BINN SVASGVNLIMCDAAL BINN	150 DSAG I SSGDCASI DSAG I SNODCASI DSAG I SNODCASI DSAG I TNODCASI DKAG I SSGDCSSI DKAG I SSGDCSSI DKAG I SAGDCASI DKAG I SAGDCASI	160 173 LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+ LSSE IGTYFDRAAASVA+
B. RpcB 1. CC9311_la_IA 2. WH8020_la_IA 3. WJR184-l_b_IA 4. PROS-9-1_lb_IA 5. ROS8604_lb_IA 6. SYN20_lb_IA 6. SYN20_lb_IA 8. BL107_VA_IVA 9. BL107_VA_	1 10 MEDAETKVVAQADA MEDAETKVVAQADA MEDAETKVVAQADA MEDAETKVVAQADA MEDAETKVVAQADA MEDAETKVVAQADA MEDAETKVVAQADA MEDAETKVVAQADA	20 GOF I SASE I DALAA GOF I SASE I DALAA GOF I SASE I DALAA GOF I SSSE I DALSA GOF I SSSE I DALSA GOF I SSSE I DALSA GOF I STSE I DALAA GOF I STSE I DALAA GOF I STSE I DALAA GOF I STSE I DALAA	30 40 MVSGSNKRLDAV MVSGSNKRLDAV MVSGSNKRLDAV MVSDSDKRLDSV MVSDSDKRLDSV VVSDSNKRLDAV VVSDSNKRLDAV MVSDSNKRLDAV	50 SELSINASTIVA SELSINASTIVA SELSINASTIVA SELSINASTIVA SELSINASTIVA SELSINASTIVA SELSINASTIVA SELSINASTIVA SELSINASSIVA SELSINASTIVA	60 NAARELFAOQPALIS SAARELFAOQPALIS NAAROLFAOQPALIS NAAROLFAOQPALIS NAAROLFAOQPALIA NAAROLFAOQPALIA NAAROLFAOQPALIA SAAROLFAOQPALIA SAAROLFAOQPALIA	70 80 PGGNAYTSRRIMAAC PGGNAYTSRRIMAAC PGGNAYTSRRIMAAC PGGNAYTSRRIMAAC PGGNAYTSRRIMAAC PGGNAYTSRRIMAAC PGGNAYTSRRIMAAC PGGNAYTSRRIMAAC PGGNAYTSRRIMAAC	90 100 LEDKE IL RWY 15 AFTG LEDKE IL RWY 15 AFTG	110 ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVLEDRCLNGLRET ASVMEDRCLNGLRET SSVMEDRCLNGLRET SSVMEDRCLNGLRET	120 LALGTPGT LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA	120 140 SVAT GYNLINKDAAL 5 IVX VARGYNLINKDAAL 5 IVX SVAT GYNLINKDAAL 5 IVX SVAT GYNLINKDAAL 5 IVX SVAGYNLINK FAALAI IN SVAGYNLINK FAALAI INX SVAGYNLINK FAALAI INX SVAGYNLINK FAALAI INX SVAGYNLINK FAALAI INX SVAGYNLINK FAALAI INX SVAGYNLINK FAALAI INX	150 DSAGI SSOCASI DSAGI SSOCASI DSAGI SSOCASI DSAGI TNGCASI DKAGI SSOCSI DKAGI SSOCSI DRNGI TAGCASI DKAGI TAGCASI DKAGI TAGCASI DKAGI TAGCASI	190 173 LSSE IGTYFORAMSVA LSSE IGTYFORAMSVA LSSE IGTYFORAMSVA LSSE IGTYFORAMSVA LSSE IGTYFORAMSVA LSSE IGTYFORAMSVA LSSE IGTYFORAMSVA LSSE IGTYFORAMSVA LSSE IGTYFORAMSVA
B. RpcB 1. CC9311_la_lA 2. WH8020_la_lA 3. WIR-18-1_lb_lA 4. PROS-91_lb_lA 5. ROS&604_lb_LA 6. SYN20_lb_LA 7. WH8016_lb_LA 8. BL107_IVA_IVA 9. CC9902_IVA_IVA 10. A15-44_lla_lIA 11. KORD-52_lla_lIA 12. M16.1_IVA_IVA	1 10 MEDAFTKVVACADA MEDAFTKVVACADA MEDAFTKVVACADA MEDAFTKVVACADA MEDAFTKVVACADA MEDAFTKVVACADA MEDAFTKVVACADA MEDAFTKVVACADA MEDAFTKVVACADA	20 GGOF ISASE IDALAA GGOF ISASE IDALAA GGOF ISASE IDALAA GGOF ISASE IDALSA GGOF ISSSE IDALSA GGOF ISSSE IDALSA GGOF ISSSE IDALSA GGOF ISSSE IDALAA GGOF ISSSE IDALAA GGOF ISSSE IDALAA GGOF ISSSE IDALAA GGOF ISSSE IDALAA	20 40 WYSGSNRIDAW WYSGSNRIDAW WYSGSNRIDAW WYSDSNRIDAW WYSDSNRIDAW WYSDSNRIDAW WYSDSNRIDAW WYSDSNRIDAW WYSDSNRIDAW	50 SRISNNASTIVA SRISNNASTIVA SRISNNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA SRISSNASTIVA	60 SAARELEFAQOPALIS SAARELEFAQOPALIS NAARELEFAQOPALIS NAARELEFAQOPALIS NAARELEFAQOPALIA NAARELFAQOPALIA NAARELFAQOPALIA SAARELFAQOPALIA SAARELFAQOPALIS SAARELFAQOPALIS SAARELFAQOPSLIS	70 80 PGGNAYTSRRIMAC PGGNAYTSRRIMAC PGGNAYTSRRIMAC PGGNAYTSRRIMAC PGGNAYTSRRIMAC PGGNAYTSRRIMAC PGGNAYTSRRIMAC PGGNAYTSRRIMAC PGGNAYTSRRIMAC	90 100 LEDME I LRVYTYSAFTO LEDME I LRVTYSAFTO LEDME I LRVTYSAFTO	110 ASVL EDRCLINGLRET ASVL EDRCLINGLRET ASVL EDRCLINGLRET ASVL EDRCLINGLRET ASVL EDRCLINGLRET ASVL EDRCLINGLRET ASVL EDRCLINGLRET ASVL EDRCLINGLRET ASVL EDRCLINGLRET ASVL EDRCLINGLRET	120 LALGTPGT LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA	130 140 SVATGVNLIMCDAAL 51 VN SVATGVNLIMCDAAL 51 VN SVATGVNLIMCDAAL 51 VN SVATGVSLIMCDAAL 51 VN SVATGVSLIMCBAAL 51 VN SVAGVSLIMCBAAL 51 VN SVAGVSLIMCBAAL 51 VN SVAGVNLIMCBAAL 51 VN SVAGVNLIMCBAAL 51 VN SVAGVNLIMCBAAL 51 VN SVAGVNLIMCBAAL 51 VN SVAGVNLIMCBAAL 51 VN	150 DSAG I SSGDCASI DSAG I SNGDCASI DSAG I SNGDCASI DSAG I TNGDCASI DKAG I SSGDCSSI DKAG I SSGDCSSI DKAG I SAGDCASI DKAG I SAGDCASI DKAG I SAGDCASI DKAG I SAGDCASI	160 173 LSSE (GTYPDRAASSVA= LSSE (GTYPDRAASSVA= LSSE (GTYPDRAASSVA= LSSE (GTYPDRAASSVA= LSSE (GTYPDRAASVA= LSSE (GTYPDRAASVA= LSSE (GTYPDRAASVA= LSSE (GTYPDRAASVA= LSSE (GTYPDRAASVA= LSSE (GTYPDRAASVA=
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B. RpcB 1. CC9311_la_IA 2. WH8020_la_IA 3. WJR-18-1_lb_IA 4. PROS-9-1_lb_IA 5. ROS8604_lb_IA 6. SYN20_lb_IA 7. WH8016_lb_IA 8. BL107_V0_JNA 9. CC9902_IV0_JNA 10. A15-04_lb_IA 11. KO16-05_IA 14. RS9902_lb_IA 14. RS9902_lb_IA 14. RS9902_lb_IA 15. TAK9802_lb_IA 15. TAK9802_lb_IA 16. WH8109_Ib_IA 18. CC9605_IC_IA 19. PROS-U-1_IIh_IB 20. A15-24_IIb_IIA 21. A18-40_IIb_IIA 21. A18-40_IIb_IIA 21. A18-40_IIb_IIA	1 10 INERA FT VVA CARA INERA F	20 GGPE13A5E1DALAA GGP13T5E1DALAA GGP13A5E1DALAA GGP13A5E1DALAA GGP13A5E1DALAA GGP1355E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA GGP13T5E1DALAA	20 40 MVSGSNRILDAW MVSGSNRILDAW MVSGSNRILDAW MVSDSDRILDSW MVSDDRILDSW MVSDDRRLDSW MVSDDRRLDSW MVSDSNRILDAW	50 51 SINA 31 V/ 51 V/ 51 SINA 51 V/ 51 V/ 5	EQ SAATELFACOPALIS SAATELFACOPALIS SAATELFACOPALIS SAATELFACOPALIS SAATELFACOPALIS SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA SAATELFACOPALIA	70 80 P GGIAYT SRRMAC D GGIAYT SRRMAC	90 100 LEDKE IL RYV1Y-SAFTOC LEDKE IL RYV1Y-SAFTOC	110 AVLE DRCLNGLRET AVLEDRCLNGLRET AVLEDRCLNGLRET AVLEDRCLNGLRET AVLDRCLNGLRET AVLDRCLNGLRET SWIEDRCLNGLRET SSWIEDRCLNGLRET SSWIEDRCLNGLRET SSWIEDRCLNGLRET SSWIEDRCLNGLRET ASWIEDRCLNGLRET ASWIEDRCLNGLRET ASWIEDRCLNGLRET ASWIEDRCLNGLRET ASWIEDRCLNGLRET ASWIEDRCLNGLRET ASWIEDRCLNGLRET	120 LALGTPGT LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA LALGTPGA	120 140 SVAT GYNLINKDAAL SI VN YAAGYNLINKDAAL SI VN SVAT GYNLINKDAAL SI VN SVAT GYNLINKDAAL SI VN SVAT GYNLINKDAAL SI VN SVAGYNLINK BALLAI IN SVAGYNLINK BALLAI IN SVAGYNLINK BALLAI NN SVAGYNLINK BALLAI NN	150 D SAG I S S CDCAS I D SAG I S S CDCAS I D SAG I S CDCAS I D SAG I S CDCAS I D SAG I TACCAS I D SAG I S CDCS SI D SAG I S CDCAS I D S C	160 173 LSSE IGTYFDRAMSVA- LSSE IGTYFDRAMSVA-
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Supplementary Figure 3. Alignment of RpcA and RpcB, encoding phycocyanin a- and β -subunits, from CRD1 and clades I-IV Synechococcus strains. (A)

RpcA. (B) RpcB. Substitutions potentially involved in thermotolerance are shown by a red rectangle in the alignment.



Supplementary Figure 4. Variation with growth temperature of phycobilins and phycobiliproteins fluorescence excitation and emission ratios. (A) Average PUB:PEB ratios. (B) Average phycoerythrin (PE) to phycocyanin (PC) ratios. (C) Average PC to terminal acceptor (TA) ratios. The insert indicates the strain names, their corresponding ESTU (sensu Farrant et al., 2016) and pigment type (sensu Humily et al., 2013) between brackets.



Supplementary Figure 5. Variation with growth temperature of the three main liposoluble pigments per cell for CRD1 vs. clade I and IV strains. (A, D) Chlorophyll (Chl) a content (fg/cell). (B, E) Zeaxanthin (Zea) content (fg/cell). (C, F) β -carotene (β -car) content (fg/cell). (A-C) CRD1-B strain BIOS-U3-1 vs. cold thermotypes. (D-F) CRD1-A strain MITS9220 and CRD1-C strain BIOS-E4-1 vs. warm thermotypes. Inserts indicate the strain names and their corresponding ESTU (*sensu* Farrant et al., 2016) between brackets.



Supplementary Figure 6. Time course of photosystem II quantum yield (F_v/F_M) following light stress in the presence or absence of lincomycin for CRD1 and clade I-IV strains acclimated to different temperatures. Cultures acclimated to 75 µE m⁻² s⁻¹ were shifted to 375 µE m⁻² s⁻¹ at T0 for 90 min, then shifted back to the initial light conditions for 24h as indicated by a vertical dashed line on each figure. Strain names and their corresponding ESTU between brackets (*sensu* Farrant et al., 2016) are indicated on the right hand side, acclimation temperature are indicated on the top, whilst line colour indicates the lincomycin treatment (i.e. -/+ linco).