Marine cyanobacteria

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Chapter 3. Marine cyanobacteria

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Abstract Although numerous marine prokaryotes dwelling in the sunlit layer of oceans can exploit solar energy, cyanobacteria are the only ones to perform oxygenic photosynthesis and to produce organic carbon, a critical process that sustains the whole marine trophic web. Here, we review the recent advances on marine cyanobacteria, with a special focus on the two most abundant genera of the ocean: *Prochlorococcus* and *Synechococcus,* which have been studied at all scales of organization from the gene to the global ocean. Both display a wide genetic and functional diversity intimately related to the ecological niches in which they thrive. Another ecologically important group of marine cyanobacteria are diazotrophs that, by their ability to fix atmospheric dinitrogen, constitute a major source of new nitrogen for the microbial community. Diazotrophic cyanobacteria are polyphyletic and display a remarkably large range of morphologies and lifestyles. These include both multicellular cyanobacteria, such as the colonial *Trichodesmium* or the heterocyst-forming *Calothrix*, *Richelia* and *Nodularia*, and unicellular cyanobacteria belonging to three major taxa: the symbiotic species *Candidatus* Atelocyanobacterium thalassa (UCYN-A) and the free-living genera *Crocosphaera watsonii* (UCYN-B) and *Crocosphaera subtropica* (previously known as *Cyanothece* sp., UCYN-C). Whereas some of these organisms can form immense blooms (*Nodularia*, *Trichodesmium*), others such as UCYN-C can also have a significant ecological impact, even though they represent only a minor fraction of the phytoplanktonic community. After several billions of years of evolution, which led them to colonize most marine niches reached by solar light, cyanobacteria appear as truly fascinating organisms that constitute a major component of the marine microbial communities and are the matter of a thriving field of research. The considerable amount of omics data now available on both isolates and natural populations of marine cyanobacteria provides a solid basis for investigating their molecular ecology, contribution to biogeochemical cycles, as well as use for data mining and potential biotechnological applications, such as e.g. production of hydrogen, ethanol, alkanes or fluorophores.

3.1 Introduction

A large proportion of bacteria thriving in the upper lit layer of oceans is able to use solar light as an energy source, including aerobic anoxygenic photosynthetic bacteria (Béjà et al. 2002; Boeuf et al. 2013; Koblížek 2015; Lehours et al. 2018; Auladell et al. 2019; Yurkov and Hughes 2107) and proteorhodopsin-containing bacteria (Béjà et al. 2001; Boeuf et al. 2016; Pinhassi et al. 2016; Giovannoni 2017). Yet, cyanobacteria are the only prokaryotes capable of performing 'oxygenic photosynthesis', a capacity they share with eukaryotic microalgae and plants. The latter process requires two photosystems (PSI and PSII), connected via an electron transfer chain (Falkowski and Raven 2007). Photons are collected by antenna complexes coupled to photosystems and transferred to special chlorophylls (Chls) located in the core of both photosystems. Most often, like in red algae, the light-harvesting antennae of cyanobacteria are large membrane-extrinsic, water-soluble complexes called phycobilisomes that consist of phycobiliproteins binding numerous chromophores (phycobilins), and the terminal acceptors in photosystems are Chl *a* molecules (Sidler 1994; Bar-Eyal et al. 2018). Notable exceptions to this rule are the atypical marine cyanobacteria *Acaryochloris*, *Prochloron* and *Prochlorococcus*, which possess integral membrane light-harvesting complexes and often use unusual Chls as terminal acceptors, such as Chl *d* or divinyl-Chl *a* (for reviews, see e.g., Partensky and Garczarek 2003; Green 2019). Photon energy is used to break water molecules, a reaction that releases oxygen as a by-product, and produce adenosine triphosphate (ATP) as well as reducing power in the form of reduced ferredoxin and nicotinamide adenine dinucleotide phosphate (NADPH). NADPH and ATP are ultimately used to synthesize organic carbon from atmospheric CO_2 via the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) and the Calvin-Benson-Bassham cycle (Falkowski and Raven 2007). *Cyanobacteria* have recently been suggested to share a common ancestor with two non-oxyphototrophic bacterial lineages, called *Melainabacteria* and *Sericytochromatia* (Soo et al. 2014; Shih et al. 2017; Soo et al. 2017). Based on this phylogenetic relatedness, Soo et al. (2017) proposed that the three lineages should be gathered into a single phylum called '*Cyanobacteria*', and that members of the monophyletic branch capable of oxygenic photosynthesis should be renamed '*Oxyphotobacteria*'. However, this proposal is the matter of a vivid debate within the scientific community and a number of leading scientists have strongly rejected the idea to call '*Cyanobacteria*' organisms that are *not* capable of oxygenic photosynthesis (Garcia-

Pichel et al. 2020). Instead they support the idea that *Cyanobacteria* should remain a separate phylum and reaffirmed that the correct definition of *Cyanobacteria* is the following: “organisms in the domain *Bacteria* able to carry out oxygenic photosynthesis with water as an electron donor and to reduce CO_2 as a source of carbon, or those secondarily evolved from such organisms.”

In this chapter, we will make an overview of recent advances on the biology, ecology, evolution and exploitability of marine cyanobacteria *sensu* Garcia-Pichel et al. (2020). Besides their ability to use solar energy to synthesize organic carbon, which places them at the basis of the marine food web, they also perform several other important functions within the marine ecosystem. For instance, many marine cyanobacteria are diazotrophs, i.e., capable of fixing atmospheric dinitrogen (N_2) and transforming it into ammonium that is rapidly transferred to other members of the microbial community (Berthelot et al. 2016). The latter can be either free-living planktonic microbes, benthic organisms co-occurring with cyanobacteria in microbial mats or eukaryotic partners in symbioses. Such mutually beneficial associations are frequently encountered in nutrient-depleted areas of the ocean (Foster and Zehr 2019). Some cyanobacteria, such as the marine genus *Trichodesmium* or the brackish water genus *Nodularia*, can develop large blooms that can be seen from space (McKinna 2015; Rousset et al. 2018) and may be toxic to other members of the planktonic community (Sivonen et al. 1989; Sacilotto Detoni et al. 2016).

Marine cyanobacteria also constitute a unique resource for biotechnological applications. Notable examples are *Crocosphaera subtropica* (formerly *Cyanothece* sp.; cf. section 3.5.3) strains that have a natural potential for high yield hydrogen production (Bandyopadhyay et al. 2010; Melnicki et al. 2012), the discovery of a biosynthetic pathway for short-to-medium chain alkanes in *Prochlorococcus* (Schirmer et al. 2010; Lea-Smith et al. 2015), ethanol production in *Synechococcus* sp. PCC 7002 by simultaneous inactivation of glycogen synthesis pathways and introduction of ethanologenic cassettes (Wang et al. 2020), or several strains producing beneficial metabolites (Jones et al. 2011; Mevers et al. 2014; Salvador-Reyes and Luesch 2015; Shao et al. 2015).

3.2 Marine cyanobacteria and the next generation sequencing revolution

The availability of complete genome sequences is of utmost importance to understand the molecular bases of the biochemical and ecological potential of marine cyanobacteria. Thanks to their small genomes and large abundance in oceanic ecosystems, the first marine representatives of this phylum to be sequenced were three strains of *Prochlorococcus* (Dufresne et al. 2003; Rocap et al. 2003) and one of *Synechococcus* (Palenik et al. 2003). Since these pioneer studies, there has been a steady increase in the number of available genome sequences of marine cyanobacteria. Isolates of all major species of marine and brackish cyanobacteria known to date have been sequenced in recent years (Fig. 3.1), including i) the planktonic, free-living *Crocosphaera watsonii* (also called "unicellular cyanobacteria group B" or "UCYN-B"; Montoya et al. 2004), *Cyanothece*-like members of the UCYN-C group recently renamed *Crocosphaera subtropica* (Mareš et al. 2019), *Cyanobium sp.*, *Nodularia spumigena* and *Trichodesmium erythraeum*, ii) a few benthic species such as *Acaryochloris marina*, *Microcoleus chtonoplastes* and *Rivularia* sp., but also iii) a number of symbionts, including *Candidatus* Atelocyanobacterium thalassa (UCYN-A; Thompson et al. 2012), as well as *Calothrix*, *Prochloron* and *Richelia* spp. (the latter genus is missing from Fig. 3.1, but see e.g., Hilton et al. 2013).

Sequencing projects so far have exhibited a large bias toward *Prochlorococcus* and *Synechococcus*, which have the advantage to exhibit few DNA repeats and transposase genes, considerably facilitating their assembly and closure compared to many other cyanobacteria (Palenik et al. 2006; Kettler et al. 2007; Dufresne et al. 2008; Biller et al. 2014a; Lee et al. 2019; Doré et al. 2020; Garczarek et al. 2021). Yet, the genomic diversity of a few other marine genera such as *Crocosphaera*, which contain hundreds of transposase genes, has also been well studied, with ten different strains sequenced to date (Bench et al. 2013; Bombar et al. 2014). It must be stressed that the true genomic diversity of marine picocyanobacteria exceeds vastly the information obtained from laboratory strains, which is nowadays largely complemented by genomic analyses of single amplified genomes (SAGs) obtained from flow cytometry-sorted cells (see e.g., Malmstrom et al. 2013; Kashtan et al. 2014; Berube et al. 2018; Pachiadaki et al. 2019) and metagenome-assembled genomes (MAGs; see e.g.,

Rusch et al. 2010; Shi et al. 2011; Delmont and Eren 2018; Tully et al. 2018; Engelberts et al. 2020). For instance, sequencing of *Prochlorococcus* SAGs by Kashtan et al. (2014) revealed the occurrence of hundreds of coexisting *Prochlorococcus* subpopulations with distinct genomic backbones (cf. section 3.4.5.1 for more details).

The tremendous power of next generation sequencing technologies coupled with rapid advancements in bioinformatics and data processing is currently revolutionizing our view of the genetic and functional diversity of marine cyanobacteria, notably by allowing scientists to integrate comparative genomics, transcriptomics and/or meta-omics information and to decipher their association with environmental factors using network approaches (see e.g., Guidi et al. 2016; Garcia et al. 2020; Guyet et al. 2020; Doré et al. 2020).

3.3 Cyanobacterial origin and evolution

3.3.1 The advent of Cyanobacteria *and oxygenic photosynthesis*

Cyanobacteria are generally considered to be the most ancient microorganisms capable of oxygenic photosynthesis and held responsible for the 'Great Oxidation Event' (GOE), i.e. the first sharp rise of atmospheric O_2 concentration in Earth history that occurred 2.3 ± 0.1 billion years (Gy) ago (Lyons et al. 2014). Yet, there is still a vivid controversy about when oxygenic photosynthesis arose, with estimates based on geological or geochemical evidence spanning from 3.7 (Rosing and Frei 2004) to 2.3 Gy ago (Kirschvink and Kopp 2008). For instance, a study of the distribution of chromium isotopes and redox-sensitive metals in paleosols indicated that there were already notable levels of atmospheric oxygen (i.e., 3×10^{-4} times present levels) about 3.0 Gy ago, suggesting that ancestral cyanobacteria might have evolved by this time (Crowe et al. 2013). A similar controversy also exists about timing the advent of the *Cyanobacteria* phylum based on molecular analyses. For instance, by using cross-calibrated Bayesian relaxed molecular clock analyses of slowly evolving core proteins, Shih et al. (2017) suggested a fairly recent advent of this group about 2.0 Gy ago, i.e. *after* the GOE, a hypothesis that they explained by the occurrence of nowadays extinct stem lineages that would have evolved oxygenic photosynthesis after their divergence

from *Melainabacteria* and would have sourced the O_2 fluxes that led to the GOE. In sharp contrast, by combining fossil record data and relaxed molecular clock phylogeny based on sequences of the UV-A sunscreen scytonemin, Garcia-Pichel et al. (2019) estimated the advent of the *Cyanobacteria* phylum at 3.6 ± 0.2 Gy, i.e. well *before* the GOE.

3.3.2 Evolutionary history of marine cyanobacteria

Besides their age, other intriguing questions about early cyanobacteria deal with their morphology and habitat: did they first occur on land or in the ocean? The oldest microfossil interpreted with certainty as a cyanobacterium, *Eoentophysalis belcherensis*, dates back ~1.9 Gy and was discovered in silicified stromatolites originally located in intertidal mudflats (Hofmann 1976; Demoulin et al. 2019). Its modern counterpart, the coccoid cyanobacterium *Entophysalis major*, is one of the main mat-forming microorganisms (Golubic and Abed 2010). Yet, in many phylogenetic and phylogenomic studies on cyanobacterial evolution, trees are rooted using the rock-dwelling *Gloeobacter violaceus*, which has the unique 'primitive' characteristics to lack thylakoids (Fig. 3.1; Blank and Sánchez-Baracaldo 2010; Larsson et al. 2011; Mareš et al. 2013; Shih et al. 2013). Even if early cyanobacteria likely had a coccoid morphotype, filamentous forms must have evolved soon after, possibly during or just after the GOE, since they were prominent components of microbial mats during most of the Proterozoic Eon (2.5–0.54 Gy; Knoll and Semikhatov 1998). The fact that marine planktonic cyanobacteria lineages are not monophyletic within the cyanobacterial radiation but dispersed among terrestrial, freshwater and marine benthic species in phylogenetic trees (Fig. 3.1; see also e.g. Shih et al. 2013) strongly suggests that ancestral cyanobacteria appeared first either in lakes or intertidal habitats and that the marine pelagic environment was colonized much later, through several independent colonization events (Blank and Sánchez-Baracaldo 2010; Larsson et al. 2011; Sánchez-Baracaldo and Cardona 2020). The first marine planktonic lineages of cyanobacteria were likely diazotrophs and evolved only during the Neoproterozoic (0.54-1.0 Gy; Sánchez-Baracaldo 2015), possibly explaining why the open ocean was anoxic during most of the Proterozoic Eon (Reinhard et al. 2013).

3.3.3 Adaptation to salinity

Comparative genomics studies have brought important insights into how cyanobacteria have adapted to the marine habitat. In particular, they showed that adaptation to salinity has been made possible by the development of specific machineries allowing cells to actively export inorganic ions (the “salt-out-strategy”) and to increase the cellular osmolarity by accumulating small organic molecules, called compatible solutes (Pade and Hagemann 2014). The nature of these compatible solutes strongly varies depending on habitats and/or lineages, with i) glucosylglycerol, trehalose and/or sucrose found in brackish species, ii) glycine betaine in halophiles and stromatolite-forming cyanobacteria, iii) sucrose and glucosylglycerate in *Prochlorococcus*, and iv) trehalose in *Crocosphaera watsonii* (Scanlan et al. 2009; Klähn et al. 2010; Teikari et al. 2018b). While the marine *Synechococcus* model strain WH7803 was shown to be able to accumulate glucosylglycerol and possibly glucosylglycerate, and to take up glucosylglycerol, sucrose and glycine betaine (Scanlan et al. 2009), these compounds appear to be involved in osmoregulation at different times of the cell cycle and to respond to distinct environmental conditions (Guyet et al. 2020). For *Trichodesmium*, no known compatible solute biosynthesis genes had been identified during a first screening of the *Trichodesmium* genome (Pade and Hagemann 2014). Yet, in a strategy using NMR and liquid chromatography coupled to mass spectroscopy, the quaternary ammonium compound N,N,N-trimethyl homoserine (or homoserine betaine), a previously unknown compatible solute, was identified as the main compatible solute in cultures and natural populations of *Trichodesmium* (Pade et al. 2016). The structure of this compound made it likely that it was synthesized by stepwise, salt-regulated methylation of homoserine. Indeed, a transcriptomic analysis identified a single methyltransferase gene that was upregulated with increasing salinity, and biochemical assays of the recombinant protein, using L-homoserine as the precursor and S-adenosylmethionine as the methyl group donor, showed that this enzyme produced homoserine betaine (Pade et al. 2016).

3.3.4 Adaptation to nitrogen depletion

Another environmental factor that strongly influenced the evolution of marine cyanobacteria is the availability of nutrients. Nitrogen (N) limits marine productivity in the upper layer of ca. 75% of the global ocean, while limitation by phosphorus (P) or iron (Fe) prevails in other areas (Moore et al. 2001; Moore et al. 2013; Bristow et al. 2017). Cyanobacteria have developed three main strategies to deal with inorganic N deprivation: i) to fix atmospheric N_2, ii) to decrease their cell and genome size in order to limit their cellular requirement for N, and iii) to use organic N sources.

The first strategy has led to many different physiological and morphological adaptations aimed at circumventing the main drawback of N_2 fixation, i.e. the oxygen sensitivity of nitrogenase, an inhibition that might have hindered the rise of atmospheric oxygen levels during the Proterozoic Eon (Allen et al. 2019). The endosymbiotic, filamentous genera *Richelia* and *Calothrix*, which fix N_2 during the day (Fig. 3.2), both form heterocysts, i.e., cells devoid of PSII and specialized in N_2 fixation. These heterocysts occur at the basis of the trichomes of *Richelia* and *Calothrix* and are morphologically different from typical CO_2-fixing cells, their thick wall providing a physical barrier against oxygen (Fig. 3.1; Hilton et al. 2013). The non-heterocystous *Trichodesmium* genus also primarily fixes N_2 during the day (Fig. 3.2), but this process is both spatially and temporally sequestered from oxygenic photosynthesis. Indeed, nitrogenase is localized in 'non-granulated' cell types of the trichomes called 'diazocytes', the frequency of which is lower at dawn and increased toward noon and is negatively regulated by the presence of inorganic N (Bergman et al. 2013). The unicellular *Crocosphaera watsonii* displays a much more stringent temporal separation than *Trichodesmium* between photosynthesis and N_2 fixation, these processes being restricted to day and night, respectively (Fig. 3.2; Compaoré and Stal 2010; Shi et al. 2010). At last, the symbiotic *Candidatus* Atelocyanobacterium thalassa (UCYN-A) literally lost its PSII, making it the sole cyanobacterium *sensu* Garcia-Pichel et al. (2020) known so far to be incapable of oxygenic photosynthesis, while it is an efficient N_2-fixer during the day (Fig. 3.2; Zehr et al. 2008; Tripp et al. 2010). Interestingly, all non-heterocystous N_2-fixing cyanobacteria were found to synthesize specific hopanoid lipids that may limit O_2 diffusion across their membranes, thus protecting nitrogenase

from oxidation in O_2-saturated surface oceanic waters (Cornejo-Castillo and Zehr 2019).

The second strategy, consisting in a drastic decrease in cell size, a process that confers cells an advantage for nutrient uptake by increasing their surface to volume ratio, is observed in the marine picocyanobacteria *Synechococcus* (average cell diameter ~1 µm) and is pushed to its limits in most *Prochlorococcus* lineages that have an average cell diameter of only about 0.7 µm (Chisholm et al. 1988; Waterbury et al. 1979). This decrease in cell size went hand in hand with a drastic genome streamlining, since *Prochlorococcus* genomes decreased in size by about 30% compared to the size of their ancestor via an extensive streamlining process (cf. section 3.4.6; Dufresne et al. 2005; Kettler et al. 2007; Ting et al. 2007; Scanlan et al. 2009; Partensky and Garczarek 2010; Doré et al. 2020).

The third strategy, often combined with one of the two others, is to assimilate organic forms of N, such as urea, amino acids or amino sugars. Indeed, although marine cyanobacteria were long considered to have a strict photoautotrophic lifestyle, there is more and more evidence that they are in fact mixotrophs, since they are capable of assimilating organic forms of nutrients and carbon via a light-stimulated process called 'photoheterotrophy' (Zubkov 2009; Gómez-Pereira et al. 2013; Muñoz-Marín et al. 2013; Yelton et al. 2016; Duhamel et al. 2018; Muñoz-Marín et al. 2020).

3.3.5 Adaptation to spectral niches

A third factor that primitive cyanobacteria had to deal with over the course of their adaptation to the marine habitat is the wide range of light qualities encountered in the water column, the vibrational modes of the H_2O molecule delineating five underwater spectral niches, extending from the red niche found in turbid coastal waters to the violet niche in the nutrient-poorest open ocean waters (Kirk 1994; Stomp et al. 2007; Holtrop et al. 2020). Most cyanobacteria possess large, extrinsic light-harvesting complexes, called phycobilisomes, constituted of an allophycocyanin core surrounded by six to eight rods with variable phycobiliprotein composition, namely phycocyanin (PC) alone or in combination with phycoerythrocyanin (PEC), phycoerythrin-I (PEI) and/or phycoerythrin-II (PEII; Sidler 1994; Six et al. 2007; Bar-Eyal et al. 2018). These

phycobiliproteins exhibit distinct absorption properties depending upon the nature and relative proportions of covalently bound phycobilins, which can be phycourobilin (PUB; A_{max}: 495 nm), phycoerythrobilin (PEB; A_{max}: 550 nm), phycoviolobilin (PVB; A_{max}: 590 nm) and/or phycocyanobilin (PCB; A_{max}: 620 nm). While all phycobilisomes contain PCB, PVB is specific to PEC, a pigment common in cyanobacteria thriving in soils, freshwater, hot springs and marine benthic habitats; PEB is the major phycobilin in cyanobacteria living in habitats where green light is predominant, such as coastal or mesotrophic marine waters; finally, PUB is the dominant cyanobacterial chromophore in blue pelagic waters (Lantoine and Neveux 1997; Grébert et al. 2018; Holtrop et al. 2020). Both PCB and PEB derive from biliverdin IXa and exist as free pigments, whereas PVB and PUB necessarily result from the binding and isomerization of PCB and PEB, respectively (Zhao et al. 2000; Blot et al. 2009; Shukla et al. 2012; Sanfilippo et al. 2019). Interestingly, although PVB and PUB occur in different organisms, the lyase-isomerases PecE-F and RpcG that catalyze the binding and isomerization reactions at the equivalent binding site (α-84) of PEC and PC, respectively, are chemically similar and phylogenetically closely related. This is a nice example of adaptation to blue light likely resulting from lateral gene transfer followed by a change in gene function (Blot et al. 2009). All oceanic, free-living cyanobacteria possess PE-rich phycobilisomes characterized by high (*Crocosphaera*) or intermediate PUB:PEB ratios (*Trichodesmium*), while algal symbionts such as *Richelia* have a low PUB:PEB ratio, possibly because this better complements the absorption properties of their host pigments (Ong and Glazer 1991; Neveux et al. 1999; Neveux et al. 2006; Six et al. 2007). *Synechococcus* is likely the most diversified group of marine cyanobacteria with regard to its pigment content, with some PEII-containing strains exhibiting constitutively low, medium or high ratios of PUB:PEB, while others called Type IV chromatic acclimaters are capable of varying this ratio depending on the ambient light color (Palenik 2001; Everroad et al. 2006; Six et al. 2007; Shukla et al. 2012; Humily et al. 2013; Sanfilippo et al. 2019). This ability likely confers them a strong fitness advantage, since they were recently shown to represent more than half of all *Synechococcus* cells of the world ocean (Grébert et al. 2018). In this context, *Prochlorococcus* constitutes an exception among marine planktonic cyanobacteria in that it has no phycobilisomes but instead possesses membrane-intrinsic antenna complexes (Pcb) binding divinyl derivatives of Chl *a* and *b*, a unique pigment

complement allowing this microorganism to collect, with even more efficacy than PUB, the blue light prevailing at the bottom of the photic layer in open ocean waters (Goericke and Repeta 1992; Morel et al. 1993; Garczarek et al. 2000; Ting et al. 2002). Interestingly, *Prochlorococcus* has nevertheless retained a minimal set of phycobiliprotein genes from which small amounts of a chromophorylated phycoerythrin (PEIII) is produced (Hess et al. 1996; Hess et al. 1999; Hess et al. 2001). PEIII was shown to play a minor role in light harvesting in *P. marinus* SS120 and might rather act in light sensing in these cells, even though this hypothesis has not been experimentally validated yet (Steglich et al. 2005). A few other marine cyanobacteria have also lost their phycobilisomes either entirely, like *Prochloron didemni*, a symbiont of ascidians (Lewin 1984), despite the presence in its genome of a *cpcBA* operon (Donia et al. 2011), or partially, like some strains of the benthic cyanobacterium *Acaryochloris marina* that contain phycobiliprotein aggregates (Miyashita et al. 2003). When present, these aggregates are localized in the inter-thylakoidal space and can efficiently transfer photon energy to PSII (Marquardt et al. 1997; Hu et al. 1999; Petrasek et al. 2005). Structurally, they are composed of phycocyanin and a vestigial allophycocyanin (APC) constituted of the sole β-APC subunit (Partensky et al. 2018). Like for *Prochlorococcus*, the main light-harvesting complexes —and even the sole in *Prochloron* and in *A. marina* strains CCME5410 and HCIR111A that lack phycobiliprotein aggregates (Chan et al. 2007; Partensky et al. 2018)— are Pcb proteins binding either Chl *a* and *d* at a molar ratio of ca. 0.06 in most *Acayochloris* species (Miyashita et al. 1997) or Chl *a* and *b* at a molar ratio of ca. 6 in *Prochloron didemni* and *A. thomasi* (Withers et al. 1978; Partensky et al. 2018). The occurrence of such large amounts of the near-infrared light absorbing Chl *d* (A_{max}: 705 nm) in most marine *Acaryochloris* species known so far is likely an adaptation to their shaded benthic niches. For instance, *A. marina* cells have been observed underneath *Prochloron*-containing didemnid ascidians, a habitat enriched in far-red light, since most visible light was filtered out (Kühl et al. 2005).

After several billions of years of evolution, which led them to colonize any single niche of the marine habitat reached by solar light, cyanobacteria appear as truly fascinating organisms and are the matter of an ebullient research area. The following paragraphs will detail recent advances on the main marine cyanobacterial genera.

3.4 *Prochlorococcus* and *Synechococcus*

3.4.1 Interest as model organisms in marine biology and ecology

Despite their fairly recent discovery in the late 20th century, the non-diazotrophic, marine unicellular cyanobacteria *Prochlorococcus* (Chisholm et al. 1988; Chisholm et al. 1992) and *Synechococcus* (Waterbury et al. 1979) are nowadays the best known marine cyanobacteria at all scales of organization from the gene to the global ocean (Coleman and Chisholm 2007; Scanlan et al. 2009; Biller et al. 2015; Sohm et al. 2015; Farrant et al. 2016; Kent et al. 2019; Flombaum et al. 2020; Garcia et al. 2020; Doré et al. 2020). Indeed, they have a number of advantages that make them particularly relevant model organisms for ecological, physiological and evolutionary studies, including: i) their abundance and ubiquity and thence strong contribution to global marine chlorophyll biomass and primary productivity (Partensky et al. 1999b; Partensky et al. 1999a; Buitenhuis et al. 2012; Flombaum et al. 2013; Flombaum et al. 2020), ii) their culturability (Waterbury and Willey 1988; Moore et al. 2007; Morris et al. 2008; Hunter-Cevera et al. 2016) that allowed refined comparative physiology analyses of representative isolates (Moore and Chisholm 1999; Mella-Flores et al. 2012; Humily et al. 2013; Krumhardt et al. 2013; Paz-Yepes et al. 2013; Bagby and Chisholm 2015; Pittera et al. 2015; Berube et al. 2015; Pittera et al. 2018; Breton et al. 2019; Guyet et al. 2020), as well as iii) their small genome sizes, which favored the sequencing of a large number of genomes, SAGs and MAGs within the last decade (Malmstrom et al. 2013; Biller et al. 2014a; Kashtan et al. 2014; Berube et al. 2018; Pachiadaki et al. 2019; Engelberts et al. 2020; Doré et al. 2020; Garczarek et al. 2021).

3.4.2 Global abundance and distribution

With cell densities of up to 3×10^5 cells mL^{-1} in the upper layer of warm, nutrient-poor central gyres, and a distribution area extending between 40°S and 45°N, *Prochlorococcus* is undoubtedly the most abundant photosynthetic organism on Earth,

its annual mean global abundance being estimated at $2.9 \pm 0.1 \times 10^{27}$ cells (Partensky et al. 1999b; Buitenhuis et al. 2012; Flombaum et al. 2013). It always co-occurs with *Synechococcus*, but the latter is even more widespread, since its distribution extends to sub-polar areas and to brackish waters, such as the Baltic Sea or estuary zones (Partensky et al. 1999a; Haverkamp et al. 2008; Cottrell and Kirchman 2009; Larsson et al. 2014; Xia et al. 2015; Paulsen et al. 2016; Xia et al. 2017b; Xia et al. 2017a; Hunter-Cevera et al. 2020). *Synechococcus* abundance is typically two orders of magnitude lower than *Prochlorococcus* in warm, central oceanic gyres, but it often outcompetes *Prochlorococcus* in nutrient-rich regions and can even reach cell densities above 10^6 cells mL^{-1} in the Costa Rica Dome, likely due to the high concentrations of cobalt and iron in this area (Saito et al. 2005; Ahlgren et al. 2014). Although *Synechococcus* is globally less abundant than *Prochlorococcus*, with an estimated mean global abundance of $7.0 \pm 0.3 \times 10^{26}$ cells, its contribution to the oceanic net production is estimated to be twice higher (16.7% vs. 8.5%, respectively) due to their larger cell sizes and higher CO_2 fixation rates (Li 1994; Flombaum et al. 2013). *Synechococcus* and its phages have also been shown to be key players of the plankton networks that drive carbon export and thence the oceanic biological pump (Guidi et al. 2016).

3.4.3 Phylogeny

Marine *Synechococcus* and *Prochlorococcus* are phylogenetically closely related and together with *Cyanobium* form a monophyletic branch, called ‘Cluster 5’ (Herdman et al. 2001), which long diverged from all other cyanobacteria, including several freshwater *Synechococcus* species. So, the *Synechococcus* ‘genus’ is clearly polyphyletic (Fig. 3.1; Scanlan et al. 2009; Shih et al. 2013; Sánchez-Baracaldo 2015; Salazar et al. 2020; Sánchez-Baracaldo and Cardona 2020; Doré et al. 2020). All members of Cluster 5 share the presence of α-carboxysomes encapsulating a Form-IA RuBisCo (Badger and Price 2003). These proteins are phylogenetically more closely related to those of chemoautotrophic proteobacteria, such as thiobacilli, than to the β-carboxysomes and Form-IB RuBisCO found in all other cyanobacteria. Yet, a comparative physiology study of strains possessing one or the other RuBisCo type

could not find any obvious functional differences between these two types of carboxysomes (Whitehead et al. 2014).

The marine *Synechococcus/Cyanobium* radiation is divided into three deep-branching groups, called sub-clusters (SC) 5.1 to 5.3 (Dufresne et al. 2008). All SC 5.1 lineages except one (clade VIII) are strictly marine and this SC largely predominates in open ocean waters (Figs. 3.3b and 3.3d; see also Zwirglmaier et al. 2008; Sohm et al. 2015; Farrant et al. 2016; Ahlgren et al. 2020). SC 5.2 encompasses halotolerant species thriving in estuaries and near coastal areas (Chen et al. 2006; Xia et al. 2015; Hunter-Cevera et al. 2016; Xia et al. 2017a) but also strictly freshwater species, such as *Cyanobium gracile* PCC 6307 or *Vulcanococcus limneticus* (Shih et al. 2013; Di Cesare et al. 2018). Finally, SC 5.3 comprises both strictly marine members, locally constituting a significant component of *Synechococcus* communities in P-limited areas, such as the Mediterranean Sea or the Gulf of Mexico (Fig. 3.3d; see also Huang et al. 2012; Sohm et al. 2015; Farrant et al. 2016), and freshwater members that can be very abundant in lakes (Cabello-Yeves et al. 2017; Cabello-Yeves et al. 2018). A major diversification event seemingly occurred in SC 5.1 soon after its divergence from SC 5.2 and 5.3 (Urbach et al. 1998) so that, depending on the genetic marker, this SC nowadays accounts between 10 to 20 distinct clades (Penno et al. 2006; Scanlan et al. 2009; Ahlgren and Rocap 2012; Huang et al. 2012; Gutiérrez-Rodríguez et al. 2014; Xia et al. 2019), which can be further split into a number of sub-clades (Mazard et al. 2012).

3.4.4 The wide genomic diversity of marine picocyanobacteria and its taxonomic implications

Genomes of marine picocyanobacteria sequenced thus far exhibit a tremendous diversity of both nucleotide sequences and gene content (Kettler et al. 2007; Dufresne et al. 2008; Lee et al. 2019; Doré et al. 2020). Average amino acid identity (AAI) between pairs of genomes is ranging from 53.2 to 98.9% and intra-clade AAI is on average 91.0% (Doré et al. 2020). This large genomic diversity led one research team to split the *Prochlorococcus* branch into 5 distinct ‘genera’ (Tschoeke et al. 2020) and the *Synechococcus* SC 5.1, 5.2 and 5.3 into 2, 3 and 2 genera,

respectively, based on a ‘genus’ delimitation of ≥ 70% AAI, with further refinement using phylogenetic analyses. Yet, these taxonomic delineations were made without any attempt to match SC, clades and/or sub-clades previously defined by other teams (see below for details). Using 53 (including 32 previously unpublished) marine or brackish *Synechococcus/Cyanobium* genomes, Doré et al. (2020) drew a plot of AAI vs. 16S rRNA identity for the different pairs of genomes (Fig. 3.4) and showed that while there was a continuum of 16S rRNA identities ranging from 95.5 to 100%, two major discontinuities could be defined based on the AAI: the first one at ca. 80% AAI discriminated (with a few exceptions) pairs of strains belonging to the same clade from pairs of strains belonging to different clades; the second one at 65% AAI set apart *Synechococcus* strains of the same SC from strains of different SC. However, there was *no* discontinuity at 70% AAI, suggesting that this cut-off is irrelevant to define genera in this particular group (Fig. 3.4). The same holds true for the 95% AAI cut-off often used to delineate bacterial species (see e.g., Konstantinidis and Tiedje 2005) that, when blandly applied to marine picocyanobacteria, leads to a myriad of species with no apparent ecological, biochemical or physiological relevance (e.g., at least 137 species within the set of *Prochlorococcus* genomes analyzed by Tschoeke et al. 2020). Clearly, the taxonomy of *Cyanobacteria* Cluster 5 *sensu* Herdman et al. (2001) needs to be revised, but in order to define meaningful and consensual families, genera and species within this group, it is important not only to take into account *natural* genomic delineations using key parameters such as the AAI, but also use solid phylogenies and have a good knowledge about the physiology and ecological niches occupied by the candidate taxa.

3.4.5 Role of environmental factors in genetic and functional diversification

3.4.5.1 *Prochlorococcus*

The evolutionary history of marine picocyanobacteria has been strongly influenced by environmental factors (Scanlan et al. 2009; Biller et al. 2015; Martiny et al. 2015). For *Prochlorococcus*, Moore and co-workers were the first to demonstrate the key role played by light in the vertical niche partitioning of high-light (HL) adapted

ecotypes in the upper lit layer and low-light (LL) adapted ecotypes further down the euphotic layer (Moore et al. 1998). Yet, more recent studies have considerably refined this simplistic picture, by showing that there is a multiplicity of HL and LL ecotypes. Most LL clades (LLII-VII) are confined to the bottom of the euphotic layer, with LLV-VI being specific of oxygen minimum zones (Lavin et al. 2010), while the LLI clade is more photo-tolerant (Johnson et al. 2006; Malmstrom et al. 2010; Partensky and Garczarek 2010; Huang et al. 2012). Indeed, in warm, stratified oligotrophic waters, LLI cells are most abundant at the base of the upper mixed layer and in temperate mixed waters they can even reach the surface, suggesting that they are capable to stand a temporary exposure to high irradiance (Johnson et al. 2006; Malmstrom et al. 2010; Thompson et al. 2018).

The genetic diversification of *Prochlorococcus* HL clades has been influenced by different environmental factors than for LL lineages. Indeed, Fe availability has likely conditioned the differentiation between the HLI-II clades, which thrive in Fe-replete waters, and HLIII-IV clades that co-occur in warm, Fe-depleted areas, notably in high-nutrient low-Chl (HNLC) areas of the Pacific Ocean (Fig. 3.3c; Rusch et al. 2010; West et al. 2011; Farrant et al. 2016). HNLC waters have been extensively explored during the *Tara* Oceans expedition, explaining the large proportion of HLIII-IV clades observed in this global metagenome dataset, even though HLII remains globally the most abundant clade (Fig. 3.3a). Temperature has seemingly favored the separation between the HLI ecotype, which predominates in temperate waters at high latitudes and the HLII ecotype that preferentially thrives in warm, (sub)tropical, oligotrophic waters, though it is worth noting that the minor ecotype in each thermal niche is never outcompeted to extinction (Johnson et al. 2006; Zinser et al. 2007; Chandler et al. 2016). Shifts between HLI- and HLII-dominated *Prochlorococcus* communities sometimes occur over short geographical distances, e.g., between the Mediterranean Sea and the Red Sea (Farrant et al. 2016) or along the temperature gradient between the core and the outside of ‘Agulhas rings’, these huge anticyclonic eddies formed in the southern Indian Ocean and which then drift across the South Atlantic Ocean (Fig. 3.3c; Villar et al. 2015). Lastly, P and N availability have also played a key role in the more recent evolutionary history of the *Prochlorococcus* genus. A number of studies have indeed shown that *Prochlorococcus* HL populations thriving in P-depleted areas possess many more genes involved in P metabolism or its regulation than populations

(or strains isolated) from P-replete areas (Martiny et al. 2006; Martiny et al. 2009a). A similar phenomenon, explained by vertical inheritance, gene loss, and homologous recombination, has also been observed for genes involved in nitrite and/or nitrate assimilation, the frequency of cells capable of assimilating these oxidized forms of nitrogen being positively correlated with decreased N availability (Martiny et al. 2009b; Astorga-Eló et al. 2015; Villar et al. 2015; Berube et al. 2015; Berube et al. 2016; Berube et al. 2019; Aldunate et al. 2020). Despite this apparent relationship between *Prochlorococcus* clades and community structure, several recent studies have shown the occurrence of a large genetic within-clade microdiversity. For instance, Kashtan et al. (2014) showed that *Prochlorococcus* HLII populations collected at three seasons at the BATS station off Bermuda islands were composed of hundreds of closely related subpopulations possessing distinct genomic 'backbones', each consisting of a different set of core genes associated with a defined set of accessory genes. These discrepancies were sufficient to allow differentiated responses of these sub-populations to seasonal changes in the environment. Occurrence of within-clade sub-populations exhibiting distinct ecological niches has also been observed in other *Prochlorococcus* clades. Using the high resolution marker *petB*, Farrant et al. (2016) have defined 'ecologically significant taxonomic units' (ESTUs) as within-clade operational taxonomic units (OTUs) at 94% nucleotide identity that occupied distinct niches along the *Tara* Oceans expedition transect. For instance, a minor *Prochlorococcus* LLI sub-population (ESTU LLIB) was found to be adapted to Fe-limited surface waters, like the major HLIIIA-IVA ESTUs, whereas a minor HLI sub-population (ESTU HLIC) was shown to thrive not only in cold temperate waters, as do typical HLI cells, but also in warm subtropical waters, thus extending the global niche occupied by these clades (Fig. 3.3c). Similarly, Larkin et al. (2016) observed that four different HLI sub-ecotypes had distinct seasonal and spatial patterns of diversity across latitudes. All these observations constitute strong pieces of evidence that genomic diversification is still ongoing within *Prochlorococcus* clades.

3.4.5.2 *Synechococcus*

The temporal succession of physico-chemical parameters that have driven the evolution of the *Synechococcus/Cyanobium* radiation is more difficult to establish, because the rapid diversification of lineages that occurred within SC 5.1 (Urbach et al. 1998) has resulted in a fuzzier relationship between phylogeny and adaptation to specific environmental factors than for *Prochlorococcus*. Yet, temperature has clearly also played a major selective role, since clades I and IV mainly occur in cold, nutrient-rich waters, while clades II and III preferentially thrive in warm, oligotrophic waters (Zwirglmaier et al. 2008). Like for *Prochlorococcus* HLI and HLII clades, these differences in realized thermal niches can be explained by the distinct thermophysiologies of these phylotypes, as demonstrated by comparing temperature growth ranges and optima of representative isolates in culture (Mackey et al. 2013; Pittera et al. 2014; Varkey et al. 2016; Breton et al. 2019). Yet, it must be stressed that clade I and IV strains fall in distantly related branches in phylogenies made using 16S rRNA or other core gene markers (see e.g., Dufresne et al. 2008; Scanlan et al. 2009; Tai and Palenik 2009; Doré et al. 2020). Furthermore, a study of the global distribution of *Synechococcus* at high taxonomic resolution (Fig. 3.3d) showed that a minor ESTU within clade II (ESTU IIB) was able to colonize cold niches (Farrant et al. 2016). Like for *Prochlorococcus*, several recent studies have indicated that Fe availability could also be an important driving factor of the composition of *Synechococcus* communities. Indeed, CRD1 and EnvB clades —the latter, defined with the *petB* marker, corresponds to the CRD2 clade, as defined with ITS (Ahlgren et al. 2020)— were shown to co-dominate in Fe-depleted areas (Ahlgren et al. 2014; Sohm et al. 2015; Farrant et al. 2016). Finally, P availability also seems to have influenced *Synechococcus* genetic diversification since both ESTUs IIIA and 5.3A appear to be adapted to P-limited areas, such as the Mediterranean Sea and Gulf of Mexico (Fig. 3.3d), as confirmed by comparative genomics (Doré et al. 2020). The co-occurrence in the same niche of *Synechococcus* genotypes belonging to phylogenetically distant clades indicates that adaptation to specific environmental parameters (temperature, Fe and/or P availability) likely happened several times independently during the evolution of the *Synechococcus* radiation through convergent evolution.

3.4.6 Prochlorococcus *genome streamlining*

The evolutionary history of the *Prochlorococcus* genus is also characterized by a major genome streamlining event that has affected most lineages (Dufresne et al. 2003; Kettler et al. 2007; Partensky and Garczarek 2010; Biller et al. 2015; Doré et al. 2020). While no comparable decrease in genome size has occurred during the evolution of marine *Synechococcus*, it is worth mentioning that members of one sub-clade of clade II, representative of the most abundant *Synechococcus* population of the ocean, possess the smallest genomes known to date in this 'genus' (2.14 ± 0.05 Mbp), suggesting that some genome reduction occurred in this subclade relative to the rest of the *Synechococcus* population (Lee et al. 2019). In *Prochlorococcus*, the streamlining process has led to an overall decrease of up to about one third of the genome size —corresponding to about 930 genes— compared to its most recent common ancestor with *Synechococcus*, with a concomitant drop of the GC content down to ca. 30% in HL clades (Dufresne et al. 2005; Kettler et al. 2007; Partensky and Garczarek 2010). It is important to note that while many gene gains and losses have taken place in all *Prochlorococcus* lineages, this gene flow was balanced in the basal LLIV lineage, whose members have genome sizes around 2.56 Mbp —i.e. in the range of *Synechococcus* SC 5.1 genomes: 2.11 to 3.31 Mbp— but strongly unbalanced toward losses in all other lineages, which have genome sizes between 1.48 and 1.92 Mbp (Kettler et al. 2007; Biller et al. 2014a; Doré et al. 2020). Yet, only about one hundred protein-coding genes are shared between all complete *Prochlorococcus* LLIV and *Synechococcus* genomes but lacking from all streamlined *Prochlorococcus* genomes (according to 'phyletic pattern' searches in the Cyanorak v2.1 database; www.sb-roscoff.fr/cyanorak). This indicates that the different *Prochlorococcus* lineages have retained or lost different sets of genes.

For a free-living phototroph, *Prochlorococcus* genomes encode only a small set of regulatory proteins, encompassing on average five different sigma factors, five to six two-component systems and eight types of other transcriptional regulators (Scanlan et al. 2009; Lambrecht et al. 2020). Although this appears to be a minimal regulatory system, it seems to be sufficient to address all relevant stress situations that *Prochlorococcus* cells have to face in the huge but relatively stable warm, open ocean environment in which they thrive. Furthermore, riboswitches and RNA-based

regulation also likely play a significant role in the regulation of gene expression in *Prochlorococcu*s (for a review, see Lambrecht et al. 2020).

3.4.7 Core, accessory and pangenomes

The core genome of *Prochlorococcus* and *Synechococcus/Cyanobium* encompasses 1,346 and 1,713 genes, respectively —these numbers correspond to the 'large core' genome, i.e. genes shared by more than 90% of members of the considered genus or group; Doré et al. (2020)—, while their accessory genome has a highly variable size (Kettler et al. 2007; Dufresne et al. 2008; Scanlan et al. 2009). A large fraction of accessory and unique genes, most of which of unknown function, is localized in hypervariable regions called genomic islands, which are likely involved in adaptation to the local environment and/or the resistance against grazers or phages (Coleman et al. 2006; Palenik et al. 2006; Dufresne et al. 2008; Avrani et al. 2011). The recent comparison of 81 non-redundant picocyanobacterial genomes has revealed that closely related strains shared many more island genes than distantly related ones and that only a few gene exchanges had occurred between distantly related clades (Doré et al. 2020). By building a network of islands shared between different strains, these authors also showed that *Prochlorococcus* HL strains shared a large number of genomic regions that were already present in the common ancestor of all HL strains, then vertically transferred to all descendants, much like the phycobilisome region is shared by all *Synechococcus* strains (Six et al. 2007; Dufresne et al. 2008), while other islands are shared only by a subset of strains. As mentioned above, *Prochlorococcus* isolated from P-depleted areas possess a number of specific genes involved in organic P acquisition, which are located in genomic islands (Martiny et al. 2006; Martiny et al. 2009a). In *Synechococcus*, several strains possess island genes coding for 'giant' proteins (>1 MDa) that owe their huge size to a high number of repeats (Dufresne et al. 2008). This includes the cell wall protein SwmB that, together with SwmA, is required for a unique form of swimming motility (McCarren and Brahamsha 2005; McCarren and Brahamsha 2007). Interestingly, although this ability was known to be restricted to clade III members, Doré et al. (2020) have suggested that not all clade III strains are motile since two out of eight strains of this clade did not

possess *swmA* and *swmB* genes. Instead, they contained a 3-gene cluster composed of a *nfeD* homolog and two flotillin-like genes (*floT1-2*) that might be involved in the production of lipid rafts.

While the core genome of picocyanobacteria is quite small, their pangenome is huge, since each new sequenced genome contains, on average, 277 new genes (Baumdicker et al. 2010). Using a quantitative, evolutionary model for the distributed genome, Baumdicker et al. (2012) predicted that the pangenome is finite and would contain 57,792 genes, but in a more recent study based on more genomes this number raised to 84,872 genes (Biller et al. 2015). Yet, this vast 'collective genome' might still miss some important gene functions, since *Prochlorococcus* cells were shown to depend upon free-living 'helper' bacteria from their immediate environment, such as *Alteromonas*, e.g. to mitigate oxidative stress using exogenous catalases (Morris et al. 2008). Interestingly, this exchange might be reciprocal since *Prochlorococcus* cells can themselves release lipid vesicles containing proteins and nucleic acids that might be used for transferring material to their 'helper' bacteria, but also possibly be used as lures for cyanophages (Biller et al. 2014b).

3.4.8 Potential biotechnological value

Another advantage of the wealth of *Prochlorococcus*, *Synechococcus* and *Cyanobium* genomes currently available (Biller et al. 2014a; Garczarek et al. 2021) is to inform us about the ability of these picocyanobacteria to synthesize products of potential biotechnological interest. Due to their small genome size, they possess relatively few genes involved in the biosynthesis of secondary metabolites compared to their larger sized marine, freshwater or extremophile counterparts (Mandal and Rath 2015). Yet, picocyanobacteria not only possess and express alkane biosynthesis genes (Klähn et al. 2014), but also produce and accumulate hydrocarbons, which may constitute between 0.02 and 0.37% of their dry cell weight (Schirmer et al. 2010; Lea-Smith et al. 2015). Thus, at the global ocean scale, picocyanobacteria would produce about 540 million tons of hydrocarbons annually. A number of genes are involved in cell defense mechanisms, including antibiotic resistance genes (Hatosy and Martiny 2015), and genes involved in the biosynthesis of microcin C, a 'Trojan horse' antibiotic

that was shown to have a role in allelopathy between *Synechococcus* strains (Paz-Yepes et al. 2013). Some strains of *Prochlorococcus* and *Synechococcus* are prolific producers of prochlorosins, a particular class of lanthipeptides, ribosomally derived and posttranslationally modified peptide secondary metabolites. Lanthipeptides display diverse bioactivities (e.g., antifungal, antimicrobial, and antiviral) and therefore possess great potential for bioengineering and synthetic biology (Hetrick et al. 2018). Prochlorosins were discovered in *Prochlorococcus* sp. MIT9313, which possesses 29 *procA* genes encoding the peptide skeletons, which then are transformed by a single promiscuous enzyme into a library of highly diverse prochlorosins (Li et al. 2010). All 29 homologs were demonstrated to be transcribed (Voigt et al. 2014). Interestingly, several *procA* homologs contain a second transcriptional start site within the coding region, pointing at potential additional diversity due to a transcriptional mechanism (Voigt et al. 2014). The capacity for the production of prochlorosins is frequent in the LLIV clade of *Prochlorococcus* but also found in representatives of *Synechococcus* belonging to SC 5.1 clades I and IX (Cubillos-Ruiz et al. 2017). Genome sequencing of marine cyanobacteria from environmental samples led to the discovery of 1.6 million open reading frames encoding these lanthipeptides (Cubillos-Ruiz et al. 2017). Despite their impressive distribution and variability, the natural functions of prochlorosins have remained enigmatic thus far.

Other high value products are phycobiliproteins that have a great potential for nutraceutical, pharmaceutical, cosmetic, feed and food industries (Pagels et al. 2019). Due to their high quantum yield and large Stokes shift, phycobiliproteins are also widely used as fluorophores in fluorescence biotechnologies, notably in antibody conjugates for surface labeling in flow cytometry and enzyme-linked immunosorbent assay (ELISA; Giepmans et al. 2006). In this context, although to our knowledge it has not been exploited for such purpose yet, it is noteworthy that PEII, a marine *Synechococcus*-specific phycobiliprotein with six chromophores per $\alpha\text{–}\beta$ monomer, is the most fluorescent of all phycobiliproteins and thus appears as a promising compound for fluorescence biotechnologies, especially PEII forms that bind large amounts of PUB whose optical properties match well with blue lasers (Ong et al. 1984). The fact that most enzymes involved in PEI and/or PEII biosynthesis have been characterized in the last two decades (Mahmoud et al. 2017; Sanfilippo et al. 2019; Carrigee et al. 2020a; Carrigee et al. 2020b; Grébert et al. 2021) should now make it

possible to produce these fluorophores using heterologous coexpression systems (Biswas et al. 2010). Also noteworthy, a small genomic island involved in Type IV chromatic acclimation, which exists in two configurations (CA4-A and -B; Humily et al. 2013), was recently characterized in marine *Synechococcus* (Shukla et al. 2012; Sanfilippo et al. 2016; Grébert et al. 2021) and could potentially be used in heterologous systems to control the expression of recombinant genes in a reversible manner by changes in light color (blue to green light shifts or reciprocally) rather than by the usual irreversible chemical induction.

Finally, metabolic modeling is an approach with great potential for better understanding cyanobacterial physiology and for optimizing the productivity of cultures in the perspective of biotechnological applications (Zavřel et al. 2019; Toyoshima et al. 2020). Although it has not been widely applied yet to marine cyanobacteria, it is worth noting that a genome-scale metabolic model using a multi-omic machine learning pipeline has been proposed recently for *Synechococcus* sp. PCC 7002, a strain thought to have a large potential for renewable biofuels production (Vijayakumar et al. 2020).

3.5 Nitrogen-fixing cyanobacteria

3.5.1 Ecological role and significance of diazotrophy in marine ecosystems

Nitrogen (N) is the predominant limiting nutrient for primary productivity in the upper sunlit layer throughout much of low-latitude oceans (Falkowski 1997; Canfield et al. 2010; Moore et al. 2013; Bristow et al. 2017). By assimilating dinitrogen (N_2), the simplest and most abundant N form in the atmosphere and in seawater, microbial diazotrophs constitute an important source of bioavailable N to oceanic surface waters, and even the most important external N source before atmospheric and riverine inputs (Deutsch et al. 2007; Grokopf et al. 2012). Diazotrophy is a crucial marine biogeochemical process that compensates bioavailable N losses due to denitrification and anaerobic ammonium oxidation and sustains new primary production (Karl et al. 1997; Karl et al. 2002; Zehr and Kudela 2011). Most observations of marine diazotroph abundances have been made in the N-depleted waters of the tropical Atlantic and

western tropical Pacific oceans, with sparse observations in the Indian and eastern Pacific oceans (Tang and Cassar 2019). However, two recent studies have revealed the occurrence of N_2 fixation in the cold, nutrient-rich waters of the Arctic Ocean (Harding et al. 2018; Shiozaki et al. 2018). Some oceanic zones constitute hot spots of N_2 fixation, such as the western tropical South Pacific Ocean, with average fixation rates of 570 µmol $N.m^{-2}.d^{-1}$ over the whole area (Bonnet et al. 2017).

Even though cyanobacteria are not the sole N_2-fixers in marine ecosystems, the contribution of heterotrophic diazotrophs, such as some Gammaproteobacteria and Deltaproteobacteria, is only starting to be elucidated (see e.g., Cornejo-Castillo and Zehr 2020), and it is generally considered that the marine N_2 fixation is mainly driven by diazotrophic cyanobacteria (Zehr and Kudela 2011; Turk-Kubo et al. 2014; Tang et al. 2019; Zehr and Capone 2020). A significant fraction of the N_2 they fix is rapidly released in seawater as dissolved inorganic N and transferred to non-diazotrophic components of the microbial community (Berthelot et al. 2016; Knapp et al. 2016). The main genera of N_2-fixing marine cyanobacteria are *Trichodesmium, Nodularia, Richelia, Calothrix, Candidatus* Atelocyanobacterium (UCYN-A) and *Crocosphaera* (UCYN-B and -C; Fig. 3.2). Comparative genomic analyses showed that genes encoding the N_2 fixation machinery are generally clustered in a specific genomic region which, in some cases, may have been transferred laterally, causing diazotrophy to occur in taxa that are otherwise not known to fix nitrogen, such as in *Microcoleus chthonoplastes* (Bolhuis et al. 2010) or in *Acaryochloris* sp. HICR111A (Pfreundt et al. 2012).

A recent data-driven model of the biogeography of the major diazotrophic cyanobacteria (Tang and Cassar 2019) predicted that i) *Trichodesmium* is prevalent in the 30°N-30°S latitudinal range, dominating in the tropical Atlantic, western Indian and western Pacific oceans, ii) UCYN-B exhibits a similar distribution but with smaller depth-integrated abundances, iii) UCYN-A distribution extends from tropical areas to temperate and polar regions, and iv) *Richelia* shows a relatively homogeneous distribution in the tropical ocean, with hot spots in the Amazon River plume, the central North Pacific and in the Mediterranean Sea. Abundance data for UCYN-C are still too scarce to get a global view of their distribution. Yet, local studies show that they can have a substantial impact even though they only represent a small share of the diazotrophic cells in the total microbial population. For instance, during the VAHINE

mesocosm experiment performed in 2013 in the shallow water of the New Caledonia lagoon (Bonnet et al. 2016b), N_2 fixation rates reached >60 nmol N L^{-1} d^{-1}, which are among the highest rates reported for marine waters (Luo et al. 2012; Bonnet et al. 2016a). N_2-fixing cyanobacteria of the UCYN-C type were found to dominate the diazotroph community in the mesocosms (Turk-Kubo et al. 2015). However, based on relative 16S rRNA gene copy numbers that were normalized by comparison against the flow cytometry counts of abundant marine picocyanobacteria, a maximum of only 500 cells mL^{-1} was calculated for these diazotrophs (Pfreundt et al. 2016). This matched reasonably well to the maximum of 100 UCYN-C *nifH* copies mL^{-1} determined for the same population (Turk-Kubo et al. 2015), especially when taking into account that representative UCYN-C isolates, such as ATCC 51142 or TW3, usually contain two to three 16S rRNA gene copies per cell compared to the single-copy gene *nifH* (Taniuchi et al. 2008). In contrast, the total number of bacteria (heterotrophs and picocyanobacteria) was between 5 and $7x10^6$ cells mL^{-1}, hence UCYN-C diazotrophs had a share of <0.01% in the total microbial population but impacted its biogeochemical properties in a profound way.

Like for *Prochlorococcus* and *Synechococcus* (cf. section 3.4.5), accumulating evidence from genomic analyses now suggests that these groups also consist of genetically diverse, physiologically and morphologically distinct sub-lineages that represent ecotypes adapted to specific conditions (Turk-Kubo et al. 2017; Henke et al. 2018; Zehr and Capone 2020).

3.5.2 Filamentous marine diazotrophs

The major genera of multicellular, filamentous marine diazotrophs include: i) the non-heterocystous, colony-forming *Trichodesmium* (Capone 1997; Karl et al. 2002), ii) the heterocyst-forming *Richelia*, which lives in symbiosis with the diatoms *Rhizosolenia* (a diatom-diazotroph association also referred to as Het-1) or *Hemiaulus* (Het-2; Villareal 1991; Villareal 1992; Villareal 1994; Janson et al. 1999; Foster and Zehr 2006; Zeev et al. 2008; Goebel et al. 2010; Foster and Zehr 2019; Pyle et al. 2020), and iii) the heterocystous *Calothrix* (Het-3), which is commonly associated with the diatom *Chaetoceros* (Carpenter and Foster 2002; Foster and Zehr 2006; Goebel et al. 2010).

These diatom-diazotroph associations are thought to be highly host specific, one symbiont type associating with a single host genus, though the driver of this specificity remains to be elucidated.

Other filamentous diazotrophs are found in coastal and reef environments, such as the non-heterocystous genus *Lyngbya* (Omoregie et al. 2004; Woebken et al. 2015). Heterocystous cyanobacteria belonging to the genus *Nodularia* thrive in brackish water environments, such as the Baltic Sea (Ploug et al. 2011).

3.5.2.1 *Trichodesmium*

Diazotrophic cyanobacteria of the genus *Trichodesmium* can form huge surface blooms of tens of thousands of km^2 in the tropical and subtropical ocean (Dupouy et al. 1988), constituting an important source of new N to these oligotrophic environments (Capone 1997; Mahaffey et al. 2005; Davis and McGillicuddy Jr. 2006). *Trichodesmium* is taxonomically close to filamentous cyanobacteria of the genus *Oscillatoria* (Larsson et al. 2011). It possesses the unique property among diazotrophic filamentous cyanobacteria to express the oxygen-sensitive nitrogenase and to fix N_2 during the day (Dugdale et al. 1961) concomitantly with photosynthetic oxygen evolution (Fig. 3.2). Nickel (Ni) availability seems to play a key role in regulating this process, since Ni-replete *Trichodesmium* cells can fix N_2 throughout most of the light:dark cycle (including the dark period) and fixation rates are several-fold higher in high-Ni than the low-Ni cultures (Rodriguez and Ho 2014). Whereas other diazotrophs such as *Calothrix*, *Nodularia* or *Anabaena* develop heterocysts, i.e. differentiated cells specialized for nitrogen fixation (Muro-Pastor and Hess 2012), *Trichodesmium* uses a different, not terminally differentiated cell type for this purpose, called diazocyte (Berman-Frank et al. 2001; El-Shehawy et al. 2003; Sandh et al. 2009; Sandh et al. 2012). In addition, *Trichodesmium* can form colonies or multicellular aggregates of surprisingly varying morphologies, including threads (trichomes), radial puffs, vertically aligned fusiform tufts, and bowties (Post et al. 2002; Webb et al. 2007; Hynes et al. 2012; Olson et al. 2015). Although the physiological relevance of these morphologies is not well understood (Eichner et al. 2019), there is clear evidence for true multicellular

behavior of *Trichodesmium* colonies, e.g., in the acquisition of mineral-rich dust particles (Rubin et al. 2011; Kessler et al. 2020).

Different factors have been discussed to influence termination of *Trichodesmium* blooms, including bacteriophage-induced lysis (Ohki 1999; Hewson et al. 2004) or grazing by copepods (O'Neil and Roman 1994). An important factor seems to be the activation of an autocatalytic mechanism leading to programmed cell death, resembling apoptosis in metazoans. While this process is well documented in marine phototrophs (Bidle and Falkowski 2004; Franklin et al. 2006), details of the involved mechanisms have remained enigmatic. In *Trichodesmium*, programmed cell death may be triggered by several environmental factors, such as P or Fe starvation, high irradiance, or oxidative stress (Berman-Frank et al. 2004; Berman-Frank et al. 2007) and involves the activation of caspase-like activity (Berman-Frank et al. 2004). Analyses on *Trichodesmium* from the South Pacific Ocean demonstrated the stimulation of metacaspase expression and activity and the connection to programmed cell death induced mortality (Spungin et al. 2016; Spungin et al. 2019).

With a size of 7.75 Mbp and 4,451 annotated genes, the genome of the reference strain *Trichodesmium erythraeum* IMS101 belongs to the larger cyanobacterial genomes known to date (Larsson et al. 2011; Shih et al. 2013). In sharp contrast with other free-living cyanobacteria genomes, which have an average coding capacity of ~85 %, only 64 % of the *T. erythraeum* genome encodes proteins (Larsson et al. 2011). This unusually high non-coding genome share is supported by metagenomic datasets (Walworth et al. 2015), hence it is typical for *Trichodesmium*. Transcription from such non-coding genome space can produce non-coding RNAs (sRNAs), which frequently have regulatory functions in cyanobacteria (Georg et al. 2014; Klähn et al. 2015; Kopf and Hess 2015). Indeed, the analysis of the primary transcriptome of *Trichodesmium erythraeum* IMS101, i.e. the sequencing of enriched nascent transcript starts, revealed that at least 40% of all promoters that are active under standard laboratory conditions produce non-protein-coding transcripts and that these accumulate in much larger amounts than mRNA (Pfreundt et al. 2014).

Amongst those non-coding transcripts is an actively splicing twintron (Pfreundt & Hess 2015), and a diversity generating retroelement (DGR). DGRs are widely distributed in the genomes of bacteria (Yan et al. 2019). They can generate mutations in their target genes and hence induce diversification of the encoded proteins, which

likely is beneficial. In analogy to retrons, another class of retroelements in bacteria, it might be speculated that these have defense-related functions (Millman et al. 2020). However, experiments on the precise role of DGRs in natural populations are still lacking. DGRs consist of two components, a non-coding RNA called template repeat RNA that serves as a template for the second element, an error-prone reverse transcriptase that converts this template into cDNA for recombination into the protein-coding region of the target gene(s) (Doulatov et al. 2004; Guo et al. 2008). Although there was previous evidence for the existence of this mechanism in *Trichodesmium* (Doulatov et al. 2004), the target genes remained unknown. The primary transcriptome enabled the exact definition of the DGR components in *Trichodesmium* and revealed 12 putative target genes (Pfreundt et al. 2014), an unprecedented potential for *in vivo* protein diversification in bacteria. Although none of these genes possesses a clear functional assignment, some appear connected to putative signaling proteins (kinases), possibly constituting their receptor component. It can be speculated that at least some of the targeted proteins are involved in the defense against bacteriophages by systematic variation of a surface receptor. It is further possible that this system is involved in generating phenotypic variability by diversifying a few key genes. This could play a role, for instance, in the cell-cell recognition required for multicellular behavior and colony formation.

Transcriptomic analyses was also the key to establish the biosynthetic pathway for the previously unknown compatible solute N,N,N-trimethyl homoserine (homoserine betaine) via a step-wise methylation from L-homoserine and using S-adenosylmethionine as the methyl group donor (Pade et al. 2016; see also section 3.3.3). To balance the typical open-ocean salinity of ~35-37 grams per liter, the cells accumulate substantial amounts of this compound and hence require also substantial amounts of fixed carbon. S-adenosylmethionine is involved as the methyl group donor in many different reactions, including DNA methylation. In a seven-year evolution experiment, higher DNA methylation levels in *Trichodesmium* correlated with phenotypic adaptation to enhanced CO_2, and increased N_2 fixation rates (Hutchins et al. 2015). In a follow-up analysis, it was concluded that long-term m5C methylome modifications correlated with phenotypic adaptation to CO_2 (Walworth et al. 2020). It is truly fascinating that in *Trichodesmium* the presence of massive amounts of a trimethylated compatible solute, epigenetic modification, i.e, DNA methylation, and

adaptation to enhanced CO_2 fixation rates, coincide with each other. Future research will show whether this is purely coincidental or linked in more intricate ways.

3.5.2.2 *Nodularia*, a bloom-forming cyanobacterium specifically adapted to salinity gradients

Almost every summer, massive blooms of toxic cyanobacteria occur in the central regions of the Baltic Sea. These cyanobacteria cope well with the salinity gradient and brackish conditions that characterize the Baltic Sea. The dominating genera within these blooms are the filamentous, N_2-fixing *Aphanizomenon*, *Dolichospermum* and *Nodularia. Dolichospermum* and *Nodularia* can produce toxins, such as microcystins or the hepatotoxin nodularin (Mazur-Marzec et al. 2012; Fewer et al. 2013) and protease inhibitors of the pseudoaeruginosin family (Liu et al. 2015). Excess P combined with low N concentrations in surface brackish waters are thought to favor the growth and bloom formation of diazotrophic cyanobacteria in summer (Sellner 1997), particularly under stably stratified warm water conditions. Additionally, gas vesicles that provide buoyancy to *Nodularia* and related cyanobacteria lead to the formation of large surface scums in the absence of mixing.

Nodularia appears to have a selective advantage under the brackish conditions of the Baltic Sea (Möke et al. 2013). The question of what these advantages could be was addressed by genomic and transcriptomic analyses of representative strains. The genome sequence of *Nodularia* UHCC 0039 is 5.38 Mbp and contains 5,108 protein-coding genes (Teikari et al. 2018b). From these, 52.8% (2,699) were annotated to encode hypothetical proteins, illustrating the uniqueness of this group and that deeper functional analyses are required. Comparison to draft genome sequences of two other *Nodularia* isolates revealed a core genome consisting of 3,627 genes. Among the shared and strongly conserved genes is a gene cluster for the biogenesis of chaperone usher fimbriae, pointing at an unknown role of this type of fimbriae, possibly in adhesion, colony formation or during bloom development. *Nodularia* is known for its higher tolerance to periods of increased P starvation (Degerholm et al. 2006). Therefore, it is of interest that *Nodularia* genomes possess *phn* gene clusters potentially enabling the uptake, degradation and use of phosphonates as an alternative

P source (Voss et al. 2013; Teikari et al. 2018a). Indeed, the analysis of axenic *Nodularia* strains revealed that they can utilize methylphosphonates as the sole P source and concomitantly release methane (Teikari et al. 2018a). Together with earlier observations on *Trichodesmium* (Beversdorf et al. 2010) and other cyanobacteria (Bižić et al. 2020), these results suggest that certain marine cyanobacteria can release methane through the degradation of methylphosphonates, hence contributing to the oceanic methane paradox that posits that methane concentrations in surface waters can be above the atmospheric equilibrium (Repeta et al. 2016).

Further insights were gained from transcriptome analyses after exposure of *Nodularia* cultures to high light and oxidative stress, mimicking the extreme environmental conditions occurring in the surface layer of the Baltic Sea in summer (Kopf et al. 2015). The observed up-regulation of genes encoding enzymes for the biosynthesis of toxins implied that these compounds may play an important role in the acclimation of cells to conditions of bloom formation, consistent with similar observations for the freshwater cyanobacterium *Microcystis* (Zilliges et al. 2011). The photosynthetic activity of *Nodularia spumigena* CCY9414 trichomes remained high also at the highest irradiances of 1,200 µmol photons m^{-2} s^{-1}, and there were signs of an increase in photorespiratory flux (Kopf et al. 2015). This observation is of interest for understanding the acclimation of cyanobacterial trichomes to the combination of high light, high O_2 partial pressure and low nutrients, including low Fe and carbonates concentration in the surface layer. Indeed, in cyanobacteria, photorespiration cooperates with Mehler-like reactions catalyzed by flavodiiron proteins to dissipate excess absorbed energy (Hackenberg et al. 2009; Allahverdiyeva et al. 2011; Allahverdiyeva et al. 2013). The observed activation of photorespiratory flux was also consistent with the observation of many up-regulated genes encoding photorespiratory enzymes and flavodiiron proteins. Finally, the identification of many additional stress-induced genes encoding proteins of unknown functions (Voss et al. 2013; Kopf et al. 2015; Teikari et al. 2018a) further suggests the existence of stress-related mechanisms in surface cyanobacterial blooms occurring in the Baltic Sea, which remain to be identified.

3.5.2.3 *Richelia and Calothrix*

Whereas *Richelia* is an obligatory symbiont, *Calothrix* are epiphytic on the surface of *Chaetoceros* cells (Carpenter and Foster 2002) and can even display a free-living lifestyle (Foster et al. 2010). These differences between obligatory or facultative interaction with their respective diatom host match distinct differences in the genetic capacity of these two cyanobacteria in a dramatic way. Indeed, the genome of the marine *Calothrix rhizosoleniae* strain SC01, isolated from outside the frustule of *Chaetoceros,* is at least 6.0 Mbp, a size similar to those of free-living heterocyst-forming cyanobacteria, whereas *Richelia intracellularis* HH01, isolated from inside the siliceous frustule of *Hemiaulus,* has a genome size of only 3.2 Mbp. Moreover, the *Richelia* genome lacks genes for ammonium transporters, nitrate/nitrite reductases and glutamine:2-oxoglutarate aminotransferase, showing clear signs of adaptation as an obligate symbiont (Hilton et al. 2013). Modelling estimated that i) the rate of N_2 fixation in these diatom-diazotroph associations is 5.5 times higher than without N transfer to the host, ii) 25% of fixed C from the host diatom is transferred to the symbionts to support the high rate of N_2 fixation, and iii) 82% of the N fixed ends up in the host (Inomura et al. 2020). The high degree of integration between symbiont and host was further demonstrated in the metatranscriptomic analysis of surface samples from the North Pacific Subtropical Gyre, revealing the coordination of gene expression in the diatom-*Richelia* symbiosis over day–night transitions (Harke et al. 2019).

Despite some evidence of genome streamlining, it is currently unknown whether *Richelia* species that are found in the diatom-diazotroph association with *Rhizosolenia* (Het-1) are obligate symbionts as well (Villareal 1992; Hilton et al. 2013).

3.5.3 Unicellular marine diazotrophs

Marine unicellular cyanobacterial diazotrophs are phylogenetically divided into three groups (Fig. 3.1): i) the yet uncultivated unicellular cyanobacteria group A (UCYN-A), provisionally called *Candidatus* Atelocyanobacterium thalassa (Zehr et al. 2001; Zehr et al. 2008; Tripp et al. 2010), which lives in association with unicellular haptophyte algae (Zehr and Capone 2020), ii) *Crocosphaera watsonii* (UCYN-B), and iii) the

UCYN-C group, for which several representative isolates exist in culture, including CCY0110, ATCC 51142, ATCC 51472 and TW3 (Reddy et al. 1993; Taniuchi et al. 2012). The taxonomy of the UCYN-C group was until recently particularly confusing since this group encompassed both strains named *Cyanothece* spp. and *Gloeocapsa* spp. (Taniuchi et al. 2012), so Mareš et al. (2019) have revised it and proposed that ATCC 51142 and 51472 be renamed *Crocosphaera subtropica* and CCY0110 be renamed *Crocosphaera chwakensis* (TW3 was not included in their analysis).

UCYN-A is a major player in marine biogeochemical cycles, and particularly in the nitrogen cycle (Montoya et al. 2004; Goebel et al. 2010; Jardillier et al. 2010; Zehr and Kudela 2011; Cabello et al. 2015). Measurements in the tropical North Atlantic showed that it contributed to the total N_2 fixation approximately as much as *Trichodesmium* (Martínez-Pérez et al. 2016). In pioneering work, the streamlined genome of UCYN-A was sequenced after isolating cells by flow cytometry cell sorting (Zehr et al. 2008; Tripp et al. 2010). The lack of all genes encoding the photosystem II complex, the Calvin-Benson-Bassham cycle, as well as other pathways that are normally essential, such as the tricarboxylic acid cycle, led these authors to hypothesize that UCYN-A lives in symbiotic interaction, an assumption that was later confirmed (Thompson et al. 2012; Hagino et al. 2013; Thompson et al. 2014). Many insights into the relevance of symbiotic interactions were further gained from the analysis of their association with their eukaryotic host (Krupke et al. 2014). Indeed, in this mutualistic relationship, UCYN-A provides fixed N to its host in exchange for fixed carbon. Yet, before any carbon is transported from the haptophyte to UCYN-A, transfer of N is required from UCYN-A to its host (Krupke et al. 2015). The UCYN-A group consists of at least four distinct clades (Thompson et al. 2014; Farnelid et al. 2016; Cornejo-Castillo et al. 2019), called UCYN-A1, UCYN-A2, UCYN-A3 and UCYN-A4 and the whole group is monophyletic within the clade that also includes UCYN-B and UCYN-C species (Bombar et al. 2014). With regard to the symbiotic hosts, the small-sized UCYN-A1 sublineage is associated with an open ocean picoplanktonic prymnesiophyte, while the larger-sized UCYN-A2 lives in a symbiosis with the coastal, nanoplanktonic coccolithophore *Braarudosphaera bigelowii* (Thompson et al. 2012; Hagino et al. 2013; Thompson et al. 2014; Cabello et al. 2020). Surprisingly, while UCYN-A2 shares 1,159 of the 1,200 UCYN-A1 protein-coding genes (96.6%) with high synteny, the average amino-acid sequence identity between these orthologs is only

86% (Bombar et al. 2014). These authors suggested that these two variants may reflect adaptation of their respective prymnesiophyte hosts to two different niches, namely coastal and open ocean habitats. In fact, phylogenomic and Bayesian relaxed molecular clock analyses suggested that UCYN-A has diverged and coevolved with its prymnesiophyte host for ~91 My, since the late Cretaceous (Cornejo-Castillo et al. 2016).

The UCYN-B group gathers *Crocosphaera watsonii* and related cyanobacteria, which are mostly free-living diazotrophs, but are also capable of colonial aggregation (Foster et al. 2013) and to form symbioses with the diatom *Climacodium frauenfeldianum* (Carpenter and Janson 2000; Foster et al. 2011). Otherwise, it is a typical N_2-fixing cyanobacterium in the sense that it is photosynthetic during the day and fixes N_2 during the night (Fig. 3.2), and the peak of nitrogenase gene expression occurs just prior to the dark period (Wilson et al. 2017). Currently, there are genome sequences of ten *Crocosphaera watsonii* strains available, with sizes ranging from 4.55 Mbp for WH0401 to 6.24 Mbp for WH8501 (Webb et al. 2009; Bench et al. 2013).

The ecology and genetic diversity of the UCYN-C group are less well known, except that this group seems to be adapted to warmer waters compared to its UCYN-A and -B counterparts (Berthelot et al. 2017). However, one strain representative of this group, ATCC 51142 (recently renamed to *Crocosphaera subtropica;* Mareš et al. 2019), gained much attention as a platform strain in biotechnology and synthetic biology. Sequence analysis revealed a particular genome organization consisting of one major, circular and one linear chromosome and four small plasmids (Welsh et al. 2008) The presence of two chromosomes from which one is linear is unique among cyanobacteria. Information on the 5.46 Mbp ATCC 51142 genome and its 5,304 predicted protein-coding genes enabled a systems biology analysis of this UCYN-C group representative and popularized its use for applied research alike. Its transcriptome composition was addressed during diurnal cycles (Stöckel et al. 2008; Toepel et al. 2008; Elvitigala et al. 2009; McDermott et al. 2011) as was the dynamics of the cellular protein complement (Stöckel et al. 2011; Aryal et al. 2018). Other analyses targeting the proteome were performed under constant light conditions (Aryal et al. 2012) or under conditions supporting enhanced hydrogen production (Aryal et al. 2013). Studies addressing the metabolome were performed comparing mixotrophic and photoheterotrophic growth conditions (Feng et al. 2010). Variations were studied

in the rhythms of respiration and nitrogen fixation (Bandyopadhyay et al. 2013) and a temperature-dependent ultradian metabolic rhythm was discovered (Červený et al. 2013).

Findings that ATCC 51142 can produce considerable amounts of hydrogen (Min and Sherman 2010) boosted a series of studies in this direction describing it as one of the most prolific natural producers of hydrogen (Bandyopadhyay et al. 2010) and advancing the design of custom-built photobioreactors (Melnicki et al. 2012). The availability of multiple omics datasets facilitated modelling of light-driven reductant partitioning and carbon fluxes (Vu et al. 2012), of batch culture growth (Sinetova et al. 2012) and of the relation between oxygenic photosynthesis and nitrogenase-mediated hydrogen production (Bernstein et al. 2015) More specialized work targeted α-glucan branching enzymes (Hayashi et al. 2015), the enzyme chlorophyllase as a biocatalyst (Chou et al. 2015), a novel α-dioxygenase suitable for the biotechnological production of odor-active methyl-branched aldehydes (Hammer et al. 2020) and a highly active extracellular carbonic anhydrase (Kupriyanova et al. 2019). ATCC 51142 has also served as a donor of genetic information. Already during the whole genome analysis, the strain was recognized to contain the largest intact contiguous cluster of nitrogen fixation-related genes (Welsh et al. 2008). This arrangement allowed engineering nitrogen fixation into a normally non-fixing cyanobacterium via transfer of this gene cluster (Mueller et al. 2016; Liu et al. 2018), providing promise for the engineering of more complex organisms, including crop plants (Thiel 2019).

3.6 Concluding remarks

Numerous studies on various aspects of marine cyanobacteria have been published since the previous version of this chapter (Hess et al. 2016), illustrating the strong and continued interest of the scientific community in these fascinating microorganisms. Like most fields of biology, research on marine cyanobacteria has strongly benefited from the ever-increasing distribution and application of (meta)omics approaches. Thus, tremendous progress has notably been made in: i) elucidating the molecular bases of adaptations and regulatory circuitries used by marine cyanobacteria to deal with salinity or temperature gradients, high light or UV stress, light color variations, as well

as N, P and/or Fe limitation, which are the main abiotic factors that these photosynthetic microorganisms have to face in the marine environment, ii) better understanding biotic interactions with their predators (phages, phagotrophs, etc.), symbiotic hosts or co-occurring bacteria, and iii) deciphering the distribution patterns of the major phylotypes of cyanobacteria and how they relate to ecological niches.

Given their ubiquity and natural abundance, marine cyanobacteria largely contribute to the biogeochemical cycles of two major elements, C and N, through their photosynthetic and, for many of them, diazotrophic abilities. It must be stressed that a number of studies modelling the effects of the ongoing human-induced global change on phytoplanktonic communities predicted that *Synechococcus* and free-living diazotrophs should have higher growth rates and relative abundances in the future warmer (predicted temperature increase range: +1 to +8°C) and more acidic world Ocean (predicted pH decrease: -0.2 to -0.3 units), while adverse effects were predicted for eukaryotic microphytoplankton, such as diatoms or coccolithophores (Flombaum et al. 2013; Dutkiewicz et al. 2015; Schmidt et al. 2020). Interestingly, while Fe starvation is known to limit the growth of diazotrophs and therefore their occurrence in Fe-depleted areas, it was recently shown that *Trichodesmium* cellular Fe content tends to decrease as temperature rises and that the optimum growth temperature of Fe-limited cells is ~5 °C higher than for Fe-replete cells (Jiang et al. 2018). Thus, the projections made by these authors predict that Fe use efficiencies of N_2 fixers could increase by ~76% by 2100 and potentially alleviate the prevailing Fe limitation. The situation is different for *Prochlorococcus*, since a doubling of the present-day CO_2 concentration combined with higher temperature (+4°C) had no effect on *Prochlorococcus* cell division and photosynthetic rates, whereas in the same conditions *Synechococcus* grew more than twice faster and exhibited four times higher photosynthetic rates than the controls (Fu et al. 2007). This suggests that *Prochlorococcus* could have a competitive disadvantage compared to *Synechococcus* under the predicted year 2100 CO_2 and temperature regimes. Yet, this study was performed on uni-algal cultures and did not take into account a possible mutualistic effect between these two picocyanobacteria. Such an effect was recently evidenced by co-culturing experiments, in which the growth rate of *Prochlorococcus* correlated positively with the *Synechococcus* cell density at high CO_2 concentrations (Knight and Morris 2020). Thus, as also previously demonstrated by the need of *Prochlorococcus* cells for

catalases synthesized by ‘helper’ heterotrophic bacteria to mitigate oxidative stress (Morris et al. 2008), it seems that *Prochlorococcus* strongly depends upon organisms co-occurring in its immediate vicinity, a probable result of the extensive genome streamlining that has affected most lineages.

Interactions with other members of the microbial community have been recognized as an important factor in the lifestyle of other marine cyanobacteria as well. *Trichodesmium* has a complex associated microbiome (Gradoville et al. 2017), with which it coordinates the regulation of gene expression (Frischkorn et al. 2018). However, when a *Trichodesmium* bloom collapses, previously numerically underrepresented bacteria such as *Alteromonas* spp. may flourish (Hou et al. 2018). More generally, interactions occurring between cyanobacteria and other members of the marine planktonic community are only starting to be understood. They range from mutualistic interactions to saprophytism on the C and N fixed by cyanobacteria. In particular, the release of extracellular membrane vesicles into the marine environment, demonstrated for *Prochlorococcus* and *Synechococcus* (Biller et al. 2014b), is likely a general phenomenon in cyanobacteria and may play a role in carbon cycling, gene transfer, and viral defense, complementing more classical secretion modes. In this context, the analysis of marine *Synechococcus* exoproteome revealed the occurrence of transport systems for inorganic nutrients and of a large array of strain-specific exoproteins likely involved in mutualistic or hostile interactions (e.g., hemolysins, pilins, adhesins), as well as exoenzymes with a potential mixotrophic goal (e.g., exoproteases and chitinases; Christie-Oleza et al. 2015). Given the remarkable variety of lifestyles exhibited by marine cyanobacteria, it is very likely that the majority of the interactions they have forged with other members of the microbial community still awaits to be discovered, and this is clearly one of the next major challenges in this thriving research field.

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Legends to Figures

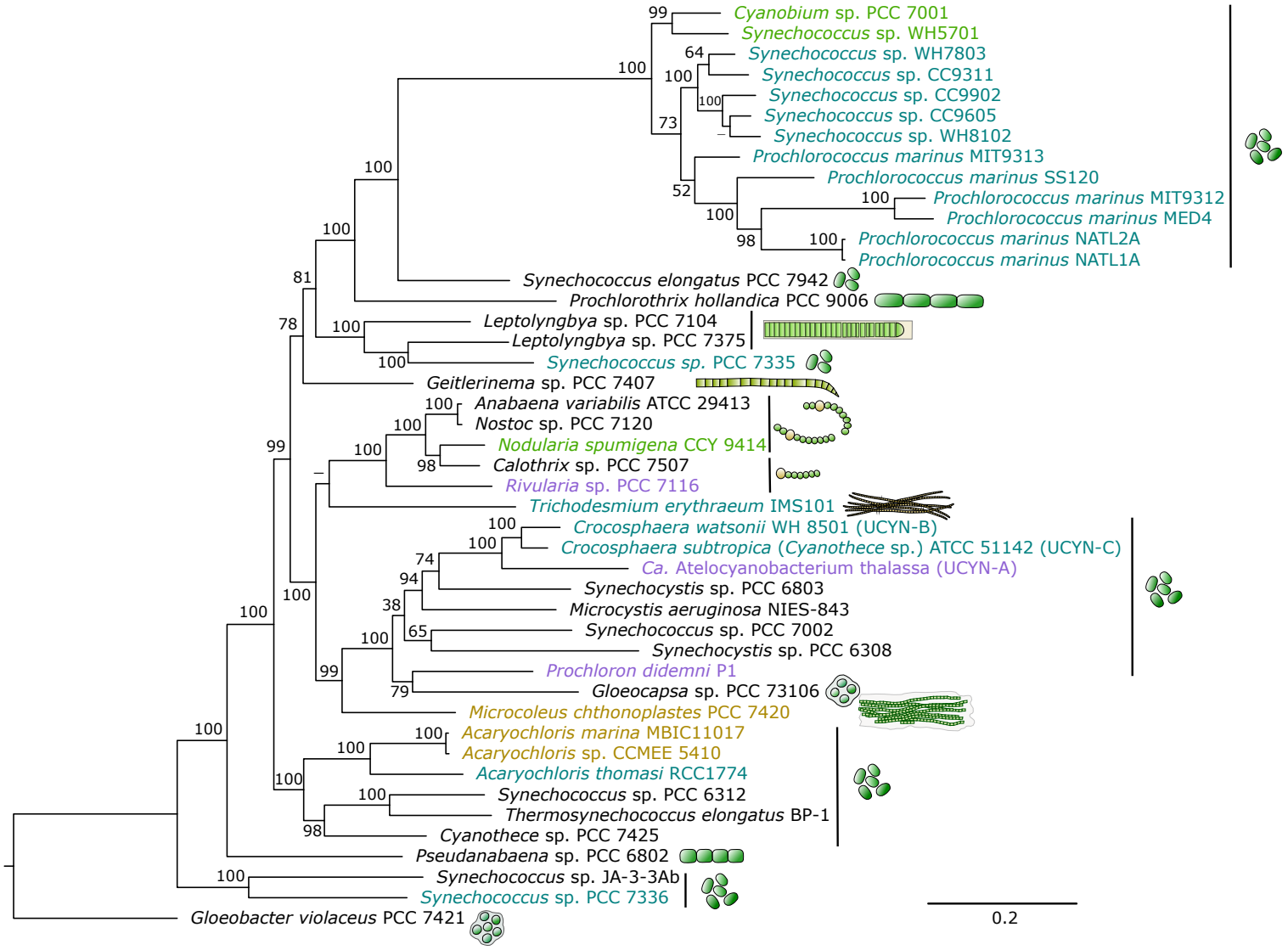
Fig. 3.1 Maximum-likelihood phylogenomic tree of selected sequenced cyanobacteria based on 29 concatenated core markers. Species names are color-coded as follows: strictly marine planktonic strains (i.e., with obligate requirement for high salinities) are shown in lagoon blue, halotolerant planktonic strains (i.e., capable to grow over a large range of salinities) in green, marine benthic strains in beige and symbiotic strains in purple. All other strains (freshwater, soils, etc.) are shown in black. *Cyanothece* sp. ATCC 51142, a representative of the unicellular cyanobacteria Group C (UCYN-C), has recently been renamed *Crocosphaera subtropica* by Mareš et al. (2019). Only nodes supported with a bootstrap value higher than 60% are indicated. Morphologies are indicated for each strain or group of strains. Adapted with permission from Supplementary Fig. S1 in Partensky et al. (2018).

Fig. 3.2 Major types of open-ocean N_2-fixing cyanobacteria showing distinct physiological differences in the daily cycles of C and N metabolism, O_2, and nutrients. Cyanobacteria differ in whether they fix N_2 in the light or in the dark and have different adaptations for obtaining non-N nutrients that have implications for ecological distributions and magnitude of N_2 fixation. Adapted with permission from Fig. 3 in Zehr & Capone (2020).

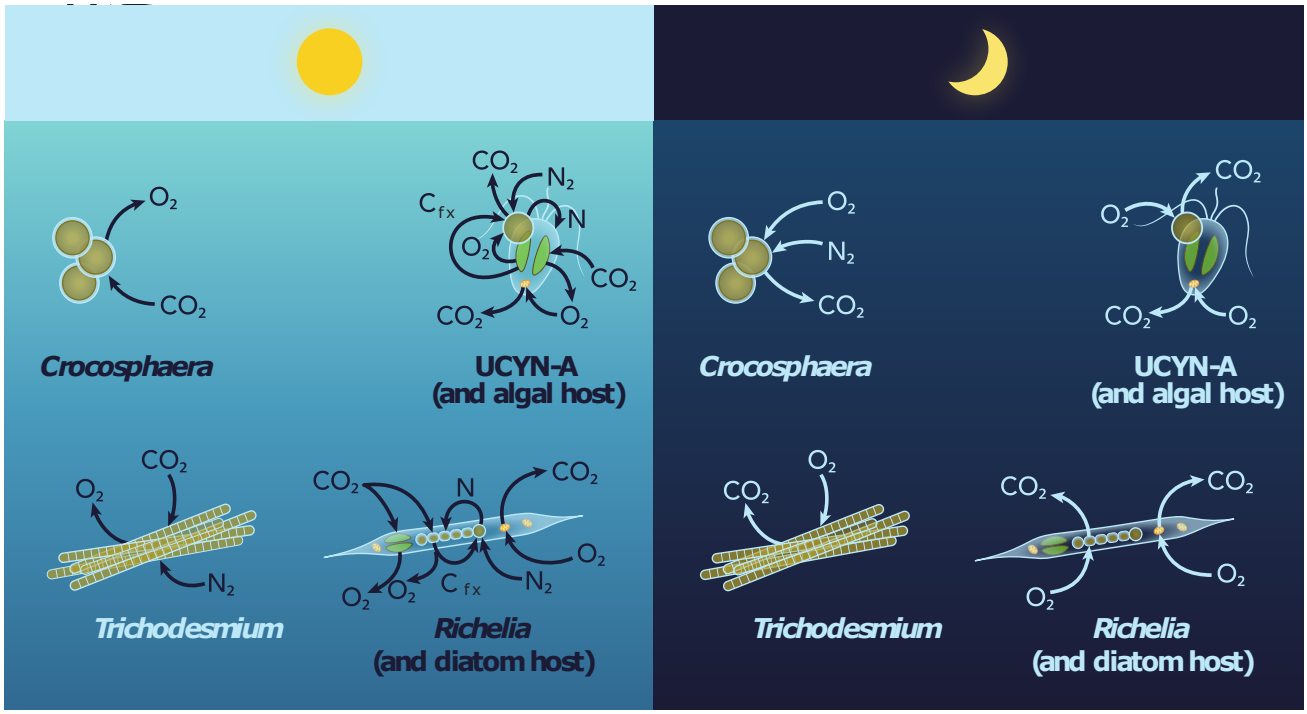
Fig. 3.3 Relative abundance of *Prochlorococcus* and *Synechococcus* clades in surface (A-B) and global distribution along the *Tara* Oceans expedition transect of ecologically significant taxonomic units (ESTUs, defined as within-clade 94% OTUs sharing a similar distribution pattern; C-D). The size of circles is proportional to the number of *Prochlorococcus* or *Synechococcus* reads at the corresponding station. Reproduced with permission from Farrant et al. 2016 (A-B) or drawn using data from this study (C-D).

Fig. 3.4 Relationships between 16S rRNA identity, AAI, and taxonomic information for *Synechococcus/Cyanobium* (A) and *Prochlorococcus* (B) genomes.

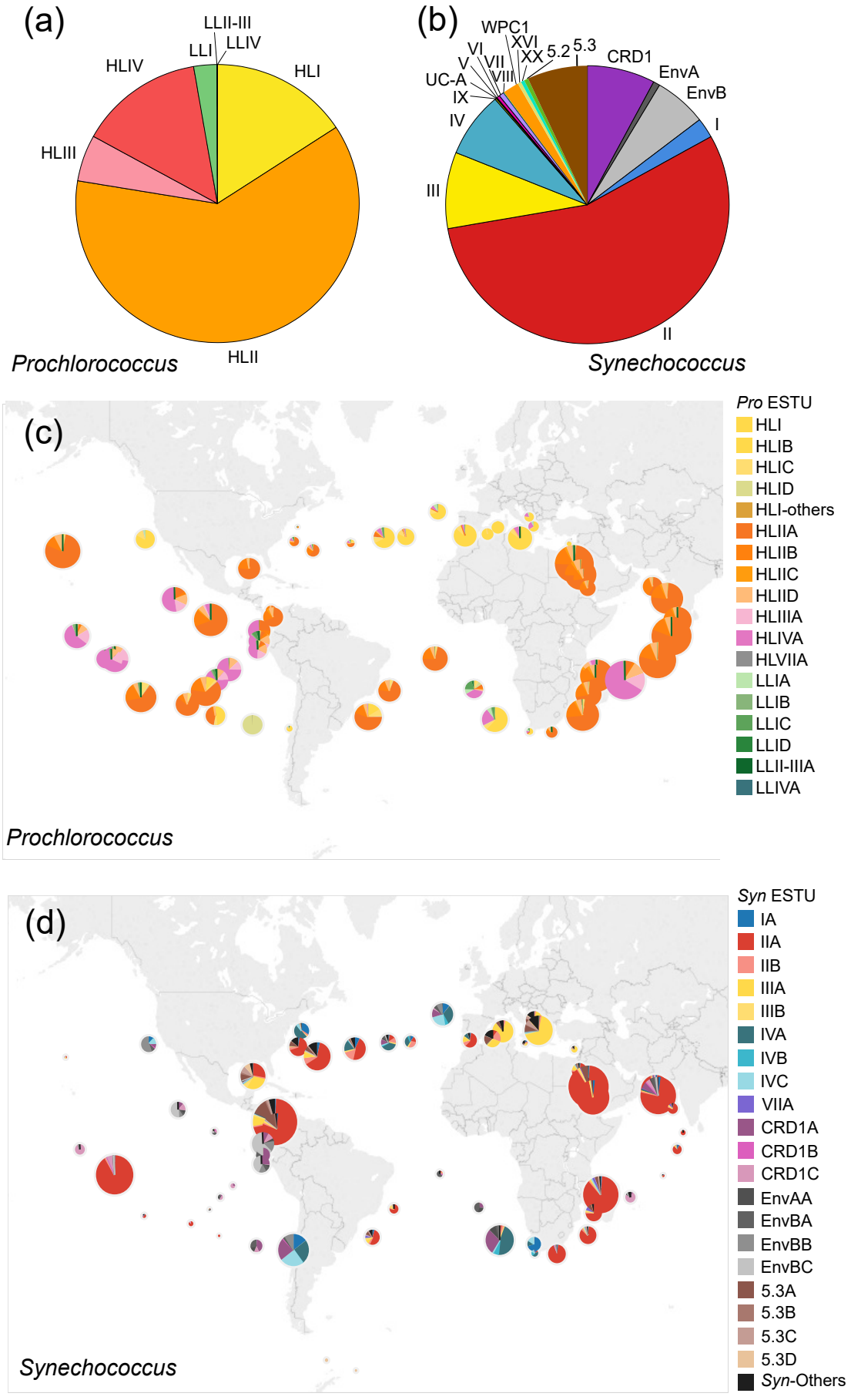
Dots correspond to comparisons between pairs of genomes belonging to the same clade, triangles between pairs of genomes belonging to the same SC (or phototype, HL or LL, for *Prochlorococcus*) but different clades and squares between pairs of genomes belonging to different SC or phototype. Continuous vertical lines correspond to the 'natural' AAI discontinuities mentioned by Doré et al. (2020), while the vertical dashed lines correspond to the limits for genus (70% AAI) and species (95% AAI) used by Tshoeke et al. (2020) for *Prochlorococcus* and Salazar et al. (2020) for the *Synechococcus/Cyanobium* group. Adapted from Fig. 3B in Doré et al. (2020).



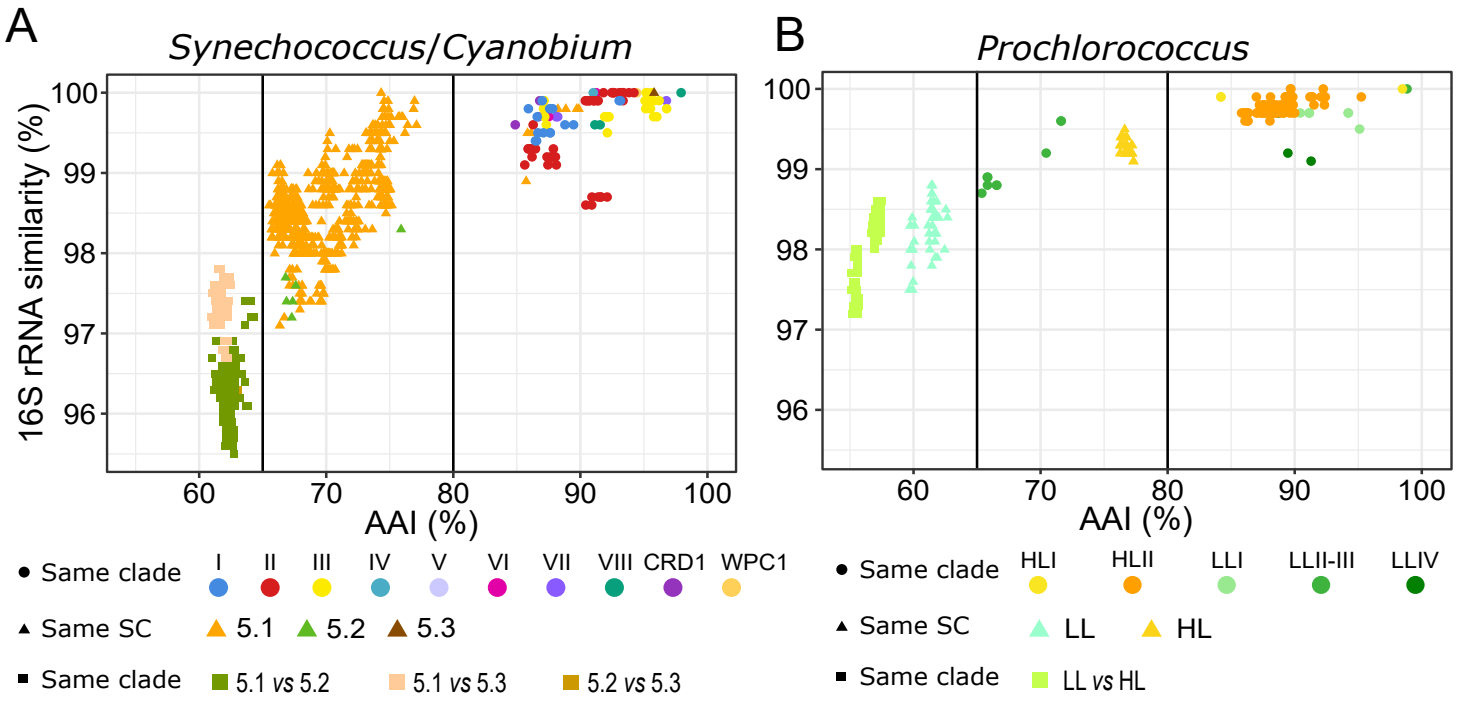
Partensky et al. Figure 1



Partensky et al. Figure 2



Partensky et al. Figure 3



Partensky et al. Figure 4