Atlas of picocyanobacteria monoclonal strains from the collection of CNR-IRSA, Italy

Cristiana Callieri,[1*] Edoardo Mandolini,[1,2] Roberto Bertoni,[1] Rosaria Lauceri,[1] Antonio Picazo,[3] Antonio Camacho,[3] Pedro J. Cabello-Yeves[4]

[1]National Research Council (CNR), Institute of Water Research (IRSA), Verbania, Italy; [2]Department of Microbiology, University of Innsbruck, Innsbruck, Austria; [3]Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain; [4]Evolutionary Genomics Group, Departamento de Producción Vegetal y Microbiología, Universidad Miguel Hernández, San Juan de Alicante, Alicante, Spain

ABSTRACT

This atlas aims to provide an overview of the picocyanobacteria cultures hold by the CNR-IRSA collection. These are monoclonal strains mostly of the genera *Synechococcus* and *Cyanobium*. These strains were mainly isolated from lakes of different trophic status around the globe and, so far, they are not available in other collections of algae and (pico)cyanobacteria. Here, we summarise the main characteristics of these strains, including some previously published data, and offer most of the strains as material available for research to every scientist upon request.

INTRODUCTION

Picocyanobacteria are tiny autotrophic cells appearing in nature both as coccoid- or rod-shaped single cells, with the minor axis shorter than 2 μm, as well as microcolonies (Callieri *et al.,* 2012). The most widespread genera are *Synechococcus* and *Cyanobium*, both belonging to the phylum of Cyanobacteria, which include some of the oldest microorganisms that played an important role to the oxygenation of the oceans and, ultimately, of Earth (Hamilton *et al.* 2016; Sanchez-Baracaldo *et al.,* 2019). The *Synechococcus* spp. are widely distributed throughout aquatic systems of the world, greatly contributing to the global primary production (Flombaum *et al.,* 2013). *Synechococcu*s cell wall is of Gram-negative type, but showing a thick peptidoglycan layer that makes the organisms particularly robust to mechanical stress. In the cytoplasm, these cells show phosphate inclusions, glycogen granules, and highly structured carboxysomes which host the enzyme ribulose-1,5-bisphosphate carboxylase/ oxygenase (RuBisCO), involved in the carbon fixation process. Furthermore, coupled to the machinery for photosynthesis, *Synechococcus* cells have a sophisticated light-harvesting system called phycobilisome (PBS). The PBS has a complex structure with an allophycocyanin (AP) core surrounded by rods made by other phycobiliproteins, specifically phycocyanin (PC) and phycoerythrin (PE). This pigment diversity allows *Synechococcus* to colonize a wide range of light niches in planktonic habitats (Stomp *et al.,* 2007; Six *et al.,* 2007; Farrant *et al.,* 2016).

Molecular analyses on *Synechococcus* genomes (Dufresne *et al.,* 2008; Scanlan *et al.,* 2009) have revealed the presence of genes for the synthesis and regulation of many processes, further emphasizing the critical role of *Synechococcus* spp. in aquatic systems (Scanlan 2012; Callieri *et al.,* 2012). New studies on the genome of non-marine *Synechococcus* recently published (Cabello-Yeves *et al.,* 2017; Di Cesare *et al.,* 2018, Cabello-Yeves *et al.,* 2018, Sanchez-Baracaldo *et al.,* 2019) emphasized the need to provide more insights on the relationships among the different clades of *Synechococcus* spp. derived from freshwater, brackish and marine environments.

The analysis of 16S rRNA from marine *Synechococcus* strains showed the presence of three sub clusters containing strains of marine origin, 5.1, 5.2, and 5.3, as well as 13 non-marine clusters (Fuller *et al.,* 2003; Callieri *et al.,* 2013). Whereas cluster 5.1 only includes marine strains, clusters 5.2 and 5.3 include marine, brackish and freshwater strains. To fully appreciate the phylogenetic relationships among all the clusters, it is of paramount importance to isolate and sequence as many new strains as possible from both freshwater and brackish environments, whose investigations are still scarce to date. Altogether, more detailed genomic, ecological, and evolutionary studies would raise awareness on the essential role that these organisms play in the biogeochemical cycles of aquatic ecosystems. Thus, in this

atlas, we present 25 monoclonal strains of picocyanobacteria currently cultured at the CNR-IRSA (Italy) cell collection. These strains, coming from a great variety of habitats of different geographical locations, are physiologically characterized. The strains have been isolated from freshwater and brackish environments, including three strains isolated from samples of the Black Sea obtained from the depths of 6.5 and 750 m (Callieri *et al.,* 2019). Some of the strains presented have their genome already sequenced and almost all have the 16S rRNA sequenced. The atlas provides data on the strains, from pictures made by epifluorescence microscopy to flow cytometry characterization, giving information of their size, volume, and absorbance spectra.

We kindly underline that most of the *Synechococcus* strains or other genera (*Cyanobium*, *Vulcanococcus*) reported in this atlas can be requested for scientific purposes to the corresponding author.

DATA DESCRIPTION

The twenty-five picocyanobacteria strains were described using epifluorescence microscopy, spectrophotometry, spectrofluorometry, flow cytometry, and genomic analyses. Detailed characteristics of the strains, together with their original location, depth of sampling, genome and 16S rRNA sequence accession numbers, are reported in Tab. 1.

The analysis under the epifluorescence microscope showed that the picocyanobacteria have a simple cell morphology, typically tending to either the coccoid or the bacillary form with an average cell volume of 0.5 μm³, comprised between strain Morenito 9A2 having the smallest average cell volume (0.06±0.08 μm³) and strain La Cruz 8H5 with the largest one (2.28±0.48 μm³). The general dimension trend is of small cells, mainly of coccoid shape, with only strains BO8801, MW73D5, ATX 6E5, *Cyanobium* Tous-M-B4, BS55D and BSA11S displaying bacillar form. The difference in shape and size could be observed well at the microscopy images taken for each organism by the epifluorescence microscope camera. In some of these pictures, it was also possible to detect some cell clusters (e.g., *Cyanobium usitatum,* AMD-g), suggesting the capacity to form microcolonies.

The pigment composition of the strains is indicated from the absorbance spectra measured by spectrophotometric analysis, allowing a first clear separation of cells in PE cell and PC cell strains. In all absorption spectra measured, we observed absorption peaks corresponding to the main cyanobacterial pigments: chlorophyll-*a* (one in vivo peak at 440 nm and a second one at 680 nm), carotenoids (peak at 500 nm), phycoerythrin (peak at 570/580 nm), phycocyanin and allophycocyanin (peaks at 620 nm and 650 nm, respectively). According to the fluorescence emitted by the two representative phycobiliproteins, phycoerythrin and phycocyanin, the substantial differences between the two

cell types (PE and PC cells) was described (Wood *et al.,* 1985; Ong and Glazer, 1991). UV-Vis absorption spectra of PE cells and PC cells are characterized by the presence or absence, respectively, of the absorbance peak between 550-570 nm, typical of phycoerythrin (Callieri *et al.,* 1996). PC cells have a prevalence of phycocyanin, showing a peak between 610 and 640 nm, less visible in the PE cells, but still detectable. In most of PE cell's absorbance spectra (e.g., *Vulcanococcus limneticus* LL) five absorption peaks of the pigments could be sequentially observed: chlorophyll-*a,* carotenoids, phycoerythrin, phycocyanin and, again, chlorophyll-*a.* Contrastingly*,* PC cells lack the phycoerythrin peak in their absorption spectra (e.g., Morenito 9A2).

PE and PC picocyanobacteria types have specific cytograms and show a dominant cytometric cloud according to their pigments. The cytograms obtained at FL2-H (phycoerythrin) *vs* FL3-H (chlorophyll-*a*), confirm the existence of two distinct footprints, depending on the pigment composition of the cells. Flow cytometer analyses, in particular the FL2-H *vs* FL3-H cytograms, allowed for the characterization of the strains, depending on their pigment type (PE or PC) and based on the intensity of the autofluorescence signal detected. The clouds of density with fluorescence values of FL2-H between 10^3 and 10^5 and FL3-H between 10^3 and 10^6 corresponded to events displaying prominent phycoerythrin fluorescence. Clouds with fluorescence values of FL2-H between 10 and 10^3 and FL3-H between 10^3 and 10^6 corresponded to events displaying dominant phycocyanin/allophycocyanin fluorescence. Events recorded with FL3-H below 10^3 were generally considered debris or dead cells (Olson *et al.,* 1989).

Spectrofluorimetric analysis was performed for a more precise characterization of the accessory pigments of the strains on three representative picocyanobacteria, BO8801 (PC), Nahuel Huapi 1G10 (PE) and Aljojuca 7D2 (PE), by recording their emission and excitation spectra (Fig. 1). Emission spectra were obtained exciting at 436 nm (chlorophyll-*a* absorbance peak) and at 520 nm (at a shorter wavelength than the phycoerythrin absorbance peak). In the first case (λ_{ex}= 436 nm) (Fig. 1B), the emission of chlorophyll-*a* was visible (around 680 nm), as well as the emission of the main accessory pigment: phycoerythrin (around 580 nm) for PE cell strains (N.Huapi 1G10 and Aljojuca 7D2) and phycocyanin (around 650 nm) for the PC cell strain (BO8801). When the excitation wavelength was set at 520 nm (outside the chlorophyll-*a* absorbance peak and nearer the phycoerythrin absorbance peak) (Fig. 1A), chlorophyll-*a* emission was greatly reduced and was no more detectable in the BO8801 strain, while phycoerythrin emission peak (580 nm) was predominant in the spectra of N.Huapi 1G10 and Aljojuca 7D2 strains. This peak was absent in the BO8801 strain, confirming that phycoerythrin was not produced by this organism. Excitation spectra were executed with λ_{em} at 680 nm (chlorophyll-*a* peak) and 580 nm (phycoerythrin peak). The contribution to the emission

at 680 nm was monitored from 400 nm to 670 nm (Fig. 1C), whereas the contribution to the emission at 580 nm was monitored from 450 nm to 570 nm (Fig. 1D). In both cases, the excitation spectra showed the presence of phycoerythrin as main accessory pigment for N.Huapi 1G10 and Aljojuca

7D2 strains (580 nm), and of phycocyanin as main accessory pigment for BO8801 strain (between 610 and 640 nm). However, the shape of the excitation spectra was quite different from that of the absorbance spectra as a consequence of the energy transfer processes that transport

Tab. 1. List of 25 picocyanobacterial monoclonal strains isolated from different aquatic systems. From left: general information on the strain, dimensions and accession number for genome sequence (Sanchez Baracaldo *et al.*, 2018) or for 16SrRNA (Callieri *et al.*, 2013), epifluorescence microscopy image (1250x, mark 2μm; PE: blue filter-set and PC: green filter-set), specific absorbance spectra (red, phycoerythrin (PE); green, phycocyanin (PC)) and cytogram (FL2 *vs* FL3). For the strains whose complete genome is available, the accession number to the Genome OnLine Database (GOLD) is included.

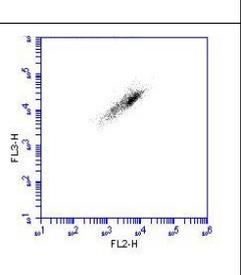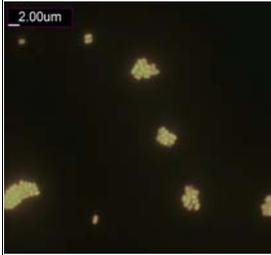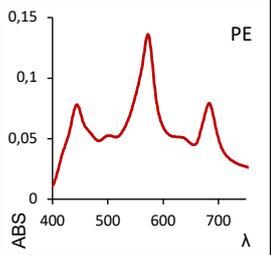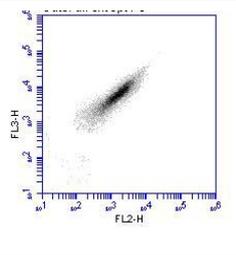|				
***Vulcanococcus limneticus* (LL)** Phycoerythrin (PE) Lake Albano, IT Mesotrophic, vulcanic lake Depth: 0.5 m	Dimensions: Ax max μm: 1.81 ± 019 Ax min μm: 1.08 ± 0.12 Vol cell μm³: 1.51 ± 0.40 *Accession n.: NQLA00000000 GOLD IDs: Gp0118741			
Morenito 9A2 Phycocyanin (PC) Lake Morenito, AR Mesotrophic, glacial lake Depth: 4 m	Dimensions: Ax max μm: 1.50 ± 0.13 Ax min μm: 0.94 ± 0.03 Vol cell μm³: 0.93 ± 0.11 Accession n.: HE805919			
Nahuel Huapi 1G10 Phycoerythrin (PE) Lake Nahuel Huapi, AR Ultraoligotrophic, glacial lake Depth: 70 m	Dimensions: Ax max μm: 0.707 ± 0.20 Ax min μm: 0.553 ± 0.12 Vol cell μm³: 0.14 ± 0.09 *Accession n.: NQKW00000000 GOLD IDs: Gp0118737			
Candia 3B3 Phycoerythrin (PE) Lake Candia, IT Eutrophic, morenic lake Depth: 0.5 m	Dimensions: Ax max μm: 1.29 ± 0.22 Ax min μm: 0.76 ± 0.14 Vol cell μm³: 0.49 ± 0.24 Accession n.: HE805933			
Candia 3F8 Phycoerythrin (PE) Lake Candia, IT Eutrophic, morenic lake Depth: 0.5 m	Dimensions: Ax max μm: 1.80 ± 0.15 Ax min μm: 0.94 ± 0.08 Vol cell μm³: 1.11 ± 0.24 Accession n.: HE805935			
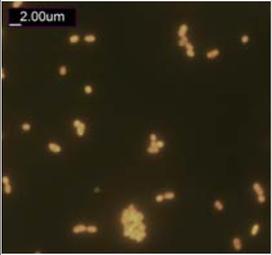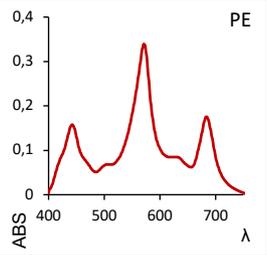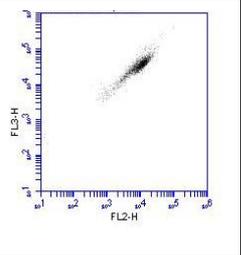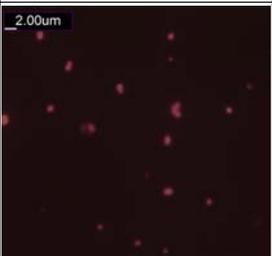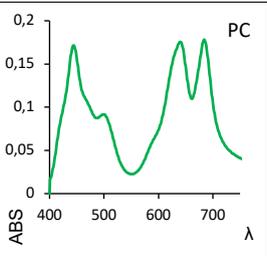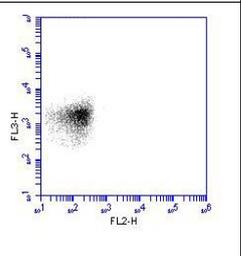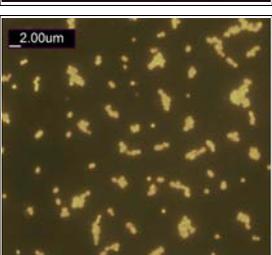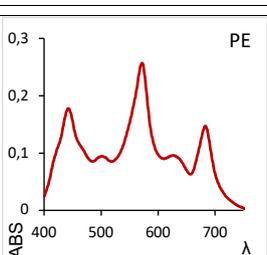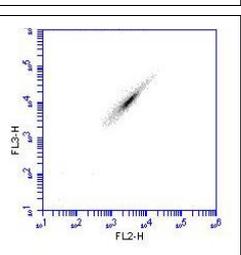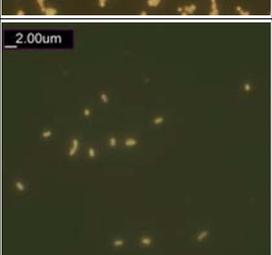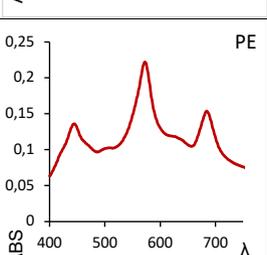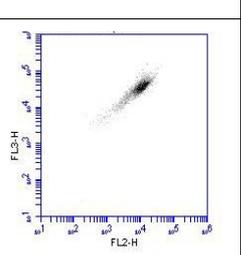
Table 1. Continued from previous page.

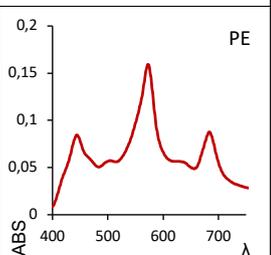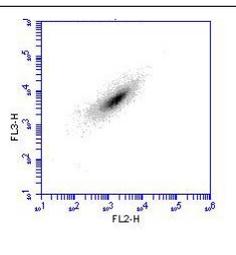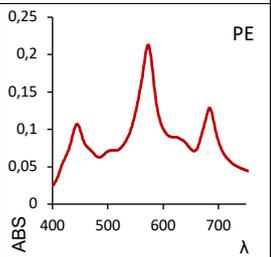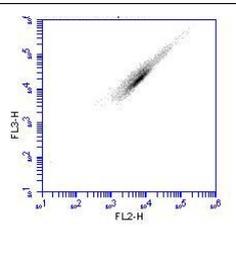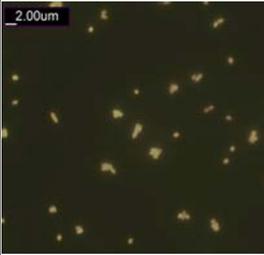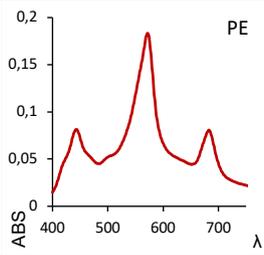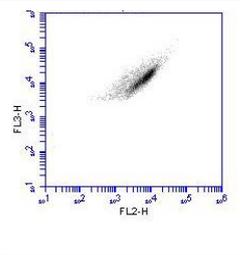|				
ATX 6A9 Phycoerythrin (PE) Lake Atexcac, MX Oligo-mesotrophic, vulcanic lake Depth: 2 m	Dimensions: Ax max µm: 1.43 ± 0.08 Ax min µm: 0.83 ± 0.06 Vol cell µm³: 0.70 ± 0.13 Accession n.: Not available			
ATX 6E5 Phycoerythrin (PE) Lake Atexcac, MX Oligo-mesotrophic, vulcanic lake Depth: 10 m	Dimensions: Ax max µm: 1.43 ± 0.12 Ax min µm: 0.64 ± 0.09 Vol cell µm³: 0.41 ± 0.09 Accession n.: HE805955			
ATX 6H9 Phycoerythrin (PE) Lake Atexcac, MX Oligo-mesotrophic, vulcanic lake Depth: 15 m	Dimensions: Ax max µm: 1.58 ± 0.36 Ax min µm: 0.90 ± 0.36 Vol cell µm³: 1.10 ± 1.11 Accession n.: HE805958			
ALC 8F6 Phycoerythrin (PE) Lake Alchichica, MX Oligotrophic, vulcanic lake Depth: 2 m	Dimensions: Ax max µm: 1.23 ± 0.14 Ax min µm: 0.84 ± 0.07 Vol cell µm³: 0.61 ± 0.12 *Accession n.: NQKZ00000000 GOLD IDs: Gp0118741			
ALC 5G6 Phycoerythrin (PE) Lake Alchichica, MX Oligotrophic, vulcanic lake Depth: 20 m	Dimensions: Ax max µm: 1.79 ± 0.21 Ax min µm: 0.85 ± 0.03 Vol cell µm³: 0.89 ± 0.1 *Accession n.: HE805950			
ALJ 7A6 Phycoerythrin (PE) Lake Aljojuca, MX Mesotrophic, vulcanic lake Depth: 10 m	Dimensions: Ax max µm: 2.45 ± 0.30 Ax min µm: 1.38 ± 0.04 Vol cell µm³: 3.27 ± 0.50 Gelatinous *Accession n.: HE805951			
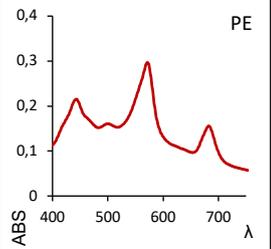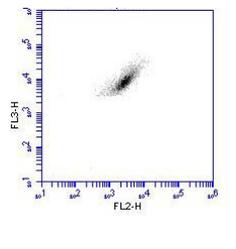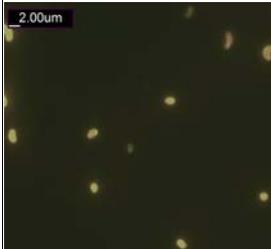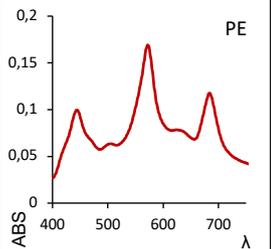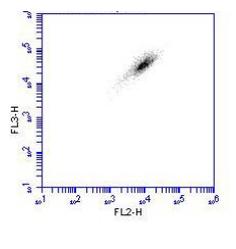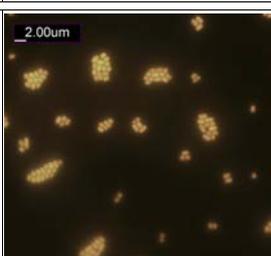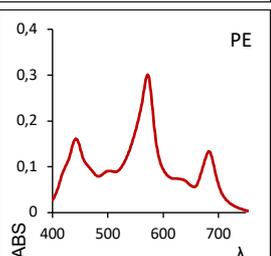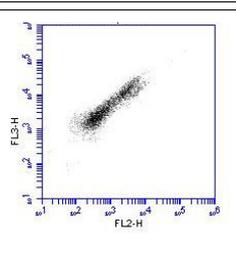
Table 1. Continued from previous page.

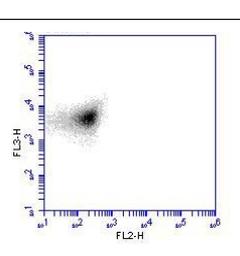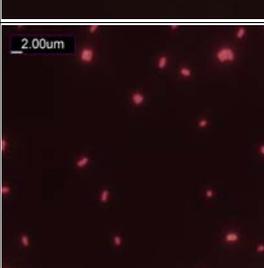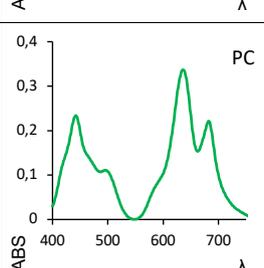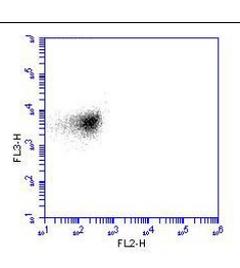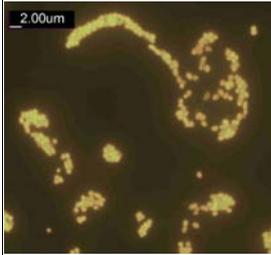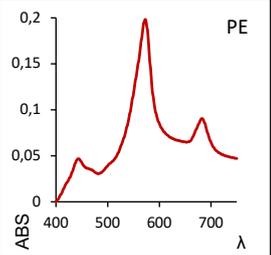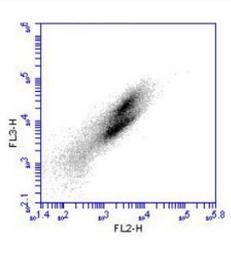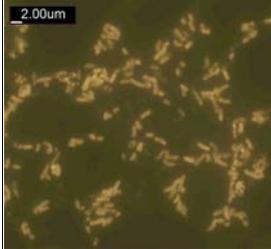|			
ALJ 7D2 Phycoerythrin (PE) Lake Aljojuca, MX Mesotrophic, vulcanic lake Depth: 15 m	Dimensions: Ax max µm: 0.69 ± 0.06 Ax min µm: 0.37 ± 0.02 Vol cell µm^3: 0.06 ± 0.01 *Accession n.: HE805953		
La Prec 7G6 Phycoerythrin (PE) Lake La Preciosa, MX Oligotrophic, vulcanic lake Depth: 5 m	Dimensions: Ax max µm: 1.42 ± 0.12 Ax min µm: 1.00 ± 0.08 Vol cell µm^3: 1.00 ± 0.20 Gelatinous *Accession n.: HE805960		
La Prec 7H9 Phycoerythrin (PE) Lake La Preciosa, MX Oligotrophic, vulcanic lake Depth: 5 m	Dimensions: Ax max µm: 1.36 ± 0.14 Ax min µm: 0.90 ± 0.04 Vol cell µm^3: 0.77 ± 0.13 *Accession n.: HE805961		
MW73D5 Phycoerythrin (PE) Lake Hallstättersee, AU Oligotrophic, glacial lake Depth: 0-20 m	Dimensions: Ax max µm: 1.60 ± 0.23 Ax min µm: 1.00 ± 0.12 Vol cell µm^3: 1.16 ± 0.41 *Accession n.: AY151241		
MW101C3 Phycocyanin (PC) Lake Mondsee, AU Mesotrophic, glacial lake Depth: 0-20 m	Dimensions: Ax max µm: 1.24 ± 0.09 Ax min µm: 0.90 ± 0.04 Vol cell µm^3: 0.69 ± 0.06 *Accession n.: NQKX00000000 GOLD IDs: Gp0118739		
BO8801 Phycocyanin (PC) Lake Constance, DE Mesotrophic, glacial lake Depth: 0-20 m	Dimensions: Ax max µm: 1.369 ± 0.28 Ax min µm: 0.854 ± 0.14 Vol cell µm^3: 0.66 ± 0.31 Gelatinous *Accession n.: NQKY00000000 GOLD IDs: Gp0118738		
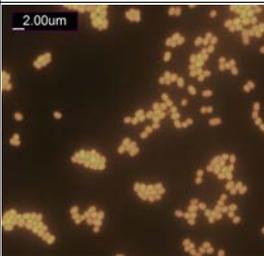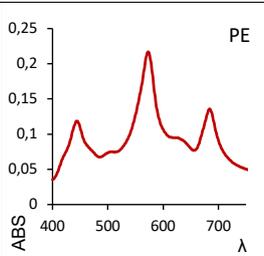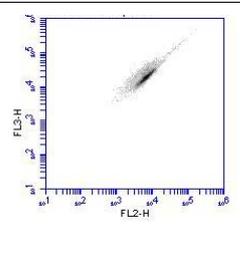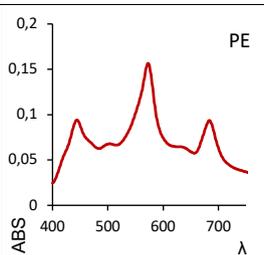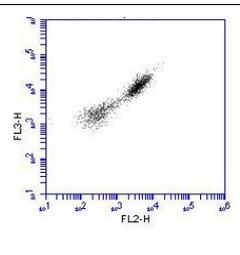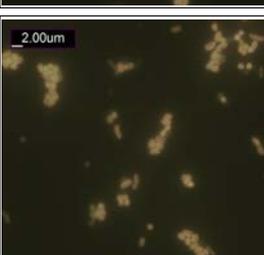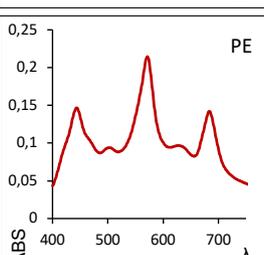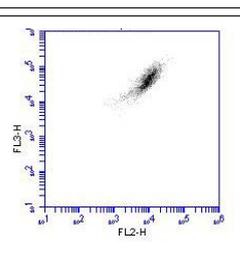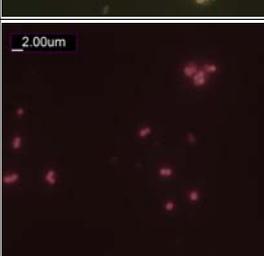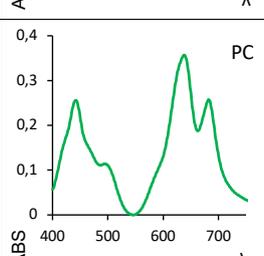
Table 1. Continued from previous page.

***Cyanobium usitatum* (Tous)** Phycoerythrin (PE) Lake Tous, ES Oligotrophic, reservoir Depth: 12 m	Dimensions: Ax max μm: 1.22 ± 0.04 Ax min μm: 0.97 ± 0.09 Vol cell μm³: 0.81 ± 0.17 *Accession n.: PXXO00000000			
Cyanobium Tous-M-B4 Phycoerythrin (PE) Tous reservoir, ES Oligotrophic, reservoir Depth: 12 m	Dimensions: Ax max μm: 1.30 ± 0.04 Ax min μm: 0.85 ± 0.06 Vol cell μm³: 0.65 ± 0.07 Accession n.: submitted			
La Cruz 8H5 Phycocyanin (PC) Lake La Cruz, ES Oligo-mesotrophic, Meromictic, doline lake Depth: 8 m	Dimensions: Ax max μm: 2.32 ± 0.27 Ax min μm: 1.18 ± 0.08 Vol cell μm³: 2.28 ± 0.48 Accession n.: submitted			
La Cruz 9H2 Phycocyanin (PC) Lake La Cruz, ES Oligo-mesotrophic, Meromictic, doline lake Depth: 12 m	Dimensions: Ax max μm: 1.65 ± 0.09 Ax min μm: 1.07 ± 0.07 Vol cell μm³: 1.31 ± 0.16 Accession n.: submitted			
AMD-g Phycocyanin (PC) Lake Amadorio, ES Mesotrophic, reservoir Depth: 12 m	Dimensions: Ax max μm: 1.48 ± 0.16 Ax min μm: 0.93 ± 0.10 Vol cell μm³: 0.89 ± 0.20 Accession n.: submitted			
BS56D Phycoerythrin (PE) Black Sea, BG Mesotrophic, meromictic Sea Depth: 750 m	Dimensions: Ax max μm: 1.84 ± 0.18 Ax min μm: 0.87 ± 0.13 Vol cell μm³: 0.98 ± 0.29 *Accession n.: PHQU00000000			
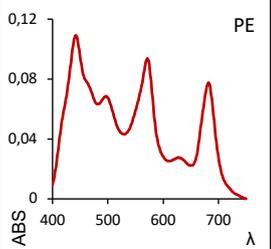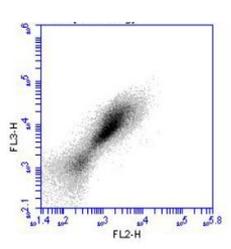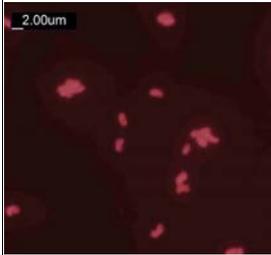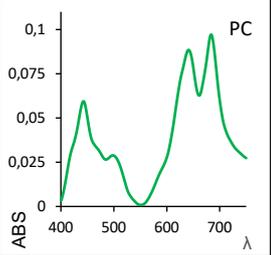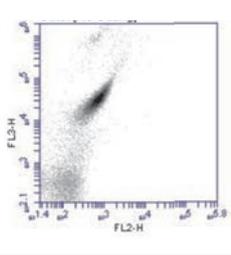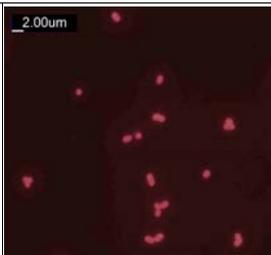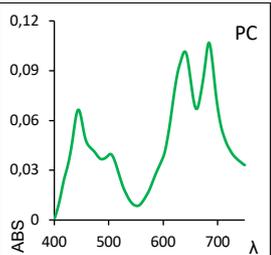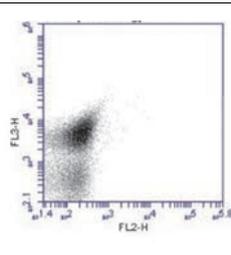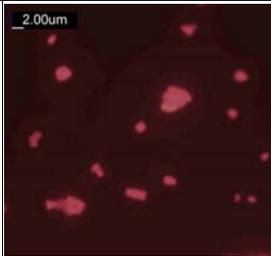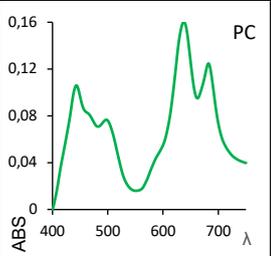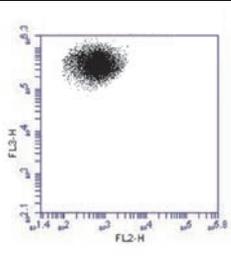
Table 1. Continued from previous page.
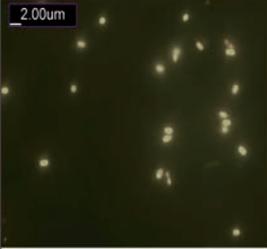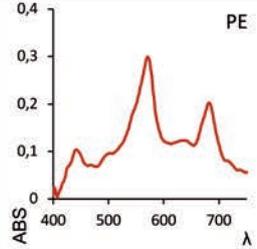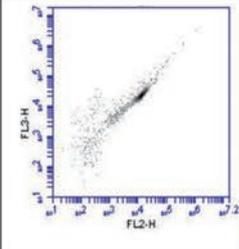
*Complete genome accession number.

excitation to the reaction centre.

The twenty-five picocyanobacterial strains that were selected from the CNR-IRSA collection represent: i) seven different 16S rRNA gene phylogenetic groups (group A, *C. gracile*; group B, Subalpine cluster I; undefined group, L. Atexcac Mexico; unnamed group; Halotolerants group; Nahuel Huapi group; group I) (Callieri *et al.,* 2013); and ii) eleven different phylogenomic groups, based on a new phylogenomic tree (Fig. 2). The tree contains previously studied freshwater representatives (Cabello-Yeves *et al.,* 2018; Sanchez-Baracaldo *et al.,* 2019) and other sequenced representatives from all known sub-clusters and clades. The majority of our strains are affiliated with the polyphyletic sub-cluster 5.2, which comprises brackish, estuarine, euryhaline and freshwater species. Candia 3F8, 3B3 and MW73D5 are strains with >99% of Average Nucleotide Identity (ANI) to *Vulcanococcus limneticus* LL (Di Cesare *et al.,* 2018). Initially, these strains were named as *Synechococcus*, and were already published in a previous 16S rRNA tree under this nomenclature (Callieri *et al*., 2013). Recently, we opened up the possibility for a new genus *Vulcanococcus* and species *limneticus* naming only for taxonomic purposes, but we acknowledge the use of both nomenclatures. There are also two marine strains coming from the Black Sea (750 m) that are affiliated to the sub-cluster 5.1A/B. These isolates are positioned near halotolerant strains WH8101 (Atlantic Ocean) and RS9916 (Red Sea) (Fuller *et al*. 2003, Doré *et al*., 2020)

MATERIALS AND METHODS

Isolation and culture media

A detailed description of sampling type, sampling collection, isolation and purification can be found in Callieri *et al.,* (2013), and Cabello-Yeves *et al.,* (2018). Briefly, water samples were filtered through a 3 µm polycarbonate membrane directly after sampling, on boat or at lake shore (or in the laboratory if nearby). Then, the filtrate water was added into a sterile tube containing BG-11 medium and kept in fresh and low light container until arrival to the laboratory, where they were cultured on a climate chamber at 18-20ºC under low light intensity (10-15 µmol photons m^{-2} s^{-1}). Finally, strain isolation was performed with flow cytometric single-cell sorting (InFlux V-GS flow cytometer, Becton Dickinson Inc.). For a more detailed description on the entire procedure and the culture media preparation, see supplementary materials.

Microscopy, cytometry and spectrophotometry

The morphology of the different picocyanobacteria strains was observed by epifluorescence microscope. Briefly, appropriate cell dilutions from cultures growing at 20°C with light intensity of 10 µmol m^2 s^{-1} were collected on 0.2 µm white polycarbonate filters (Nucleopore). The filters were mounted on microscope slides with anti-refractive immersion oil (Cargille Type FF) and visualized by epifluorescence microscope (Axioplan, ZEISS) using filter sets for blue (BP450-490, FT510, LP520) and green light excitation (LP510-560, FT580, LP590), adopted for the detection of phycoerythrin (PE) and phycocyanin (PC) autofluorescence, respectively (Callieri *et al.,* 2011). For the cell measurements, a minimum of 100 cells were counted for each strain, in at least 10 different fields at a magnification of 1250x. The final average cell dimension was calculated with the help of a semi-automated image software (Optimas) that elaborated the images taken by a highly sensible camera (Silicon intensified Target TV) equipped to the microscope.

All cultures were further analysed using a flow cytometer (Accuri C6, Becton Dickinson, HJ, USA) equipped with a 20 mW 488 nm solid state blue laser and a 14.7 mW 640 nm diode red laser. The forward (FSC) and side light scattering (SSC) signals from green (FL1 channel = 533/30 nm), orange (FL2 channel = 585/40 nm) and red fluorescence (FL3 channel >670 nm and FL4 channel 675/25) were acquired and considered for the characterization of the different strains. Cells were characterized and distinguished according to their distinct pigment composition (i.e., reflecting on different intensities of autofluorescence signals collected at the orange and red channels, Croce and van Amerongen, 2014) and size (i.e., proportionally related to light scatter signals, Tzur *et al.* 2011) using a semi-automated

software (BD Accuri™ C6) installed in the flow cytometer. With threshold values set at 1000 for the FL3 channel and at 2000 for the FL4 channel (or 7000 for FSC-H and 1500 for FL4-H) the density plots of FL2-H *vs* FL3-H and FL3-H *vs* FL4-H allowed for the optimal gating design and quantification.

Absorbance spectra (450-750nm) of the strains were obtained using a UV-mc2 spectrophotometer (Safas, Monaco). Samples were adequately diluted in a 1 cm quartz cuvette and analyzed before and after the addition of 50 µl NaClO (for removal of pigments, Ferrari and Tassan, 1999). The absorbance spectra with minimized scattering contribute were obtained by subtracting the spectrum recorded after the NaClO treatment to the one of the untreated diluted sample (Kishino *et al.,* 1985).
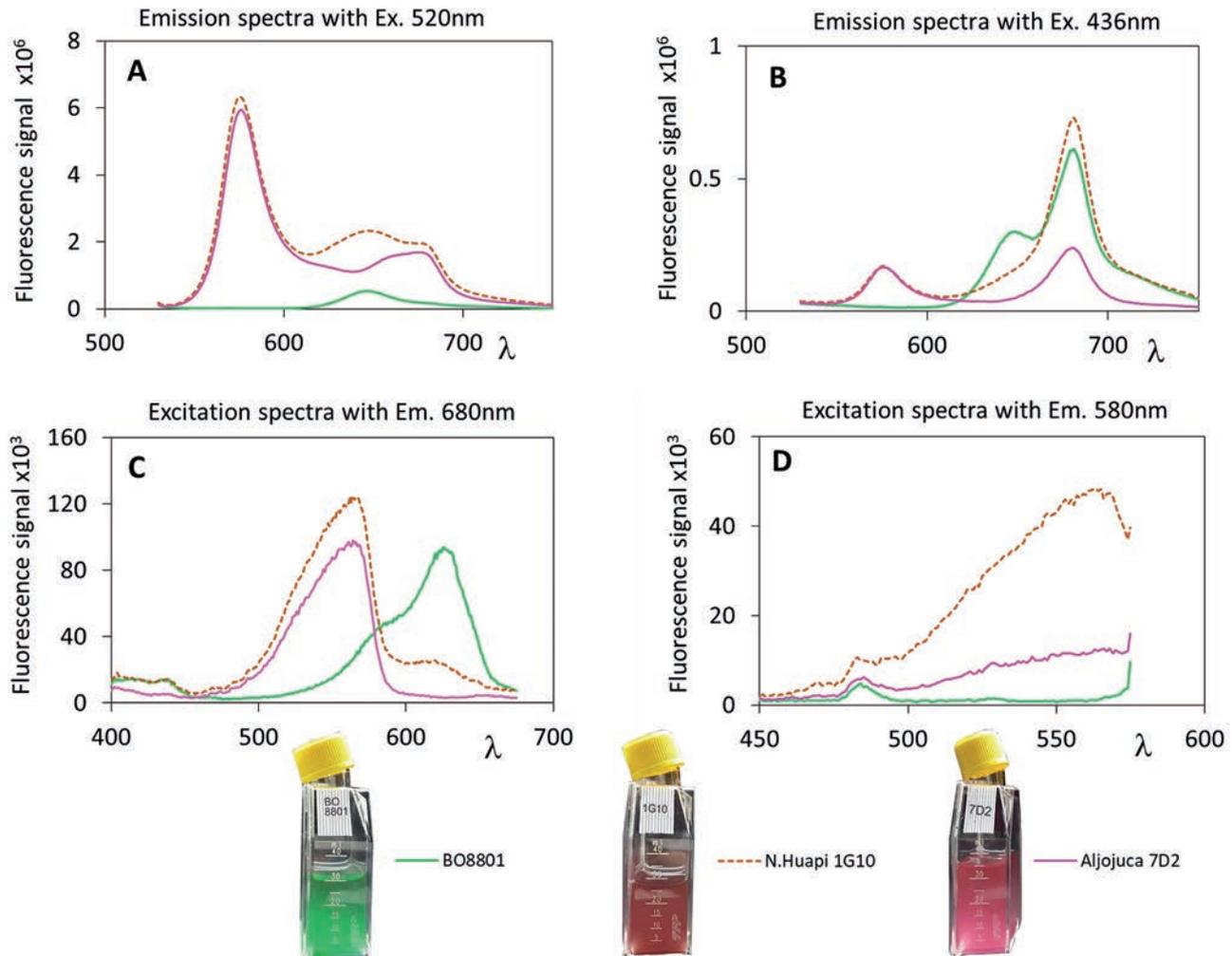
Fig. 1. Emission and excitation spectra of three *Synechococcus* strains with different accessory pigments BO8801 (PC, green), Nahuel Huapi 1G10 (PE, orange) and Aljojuca 7D2 (PE, pink). Emission spectra with excitation at 520 nm (A) and 436 nm (B). Excitation spectra with emission at 680 nm (C) and 580 nm (D). The native cultures for each strain is also included, displaying the typical colour given by their unique pigmentation.
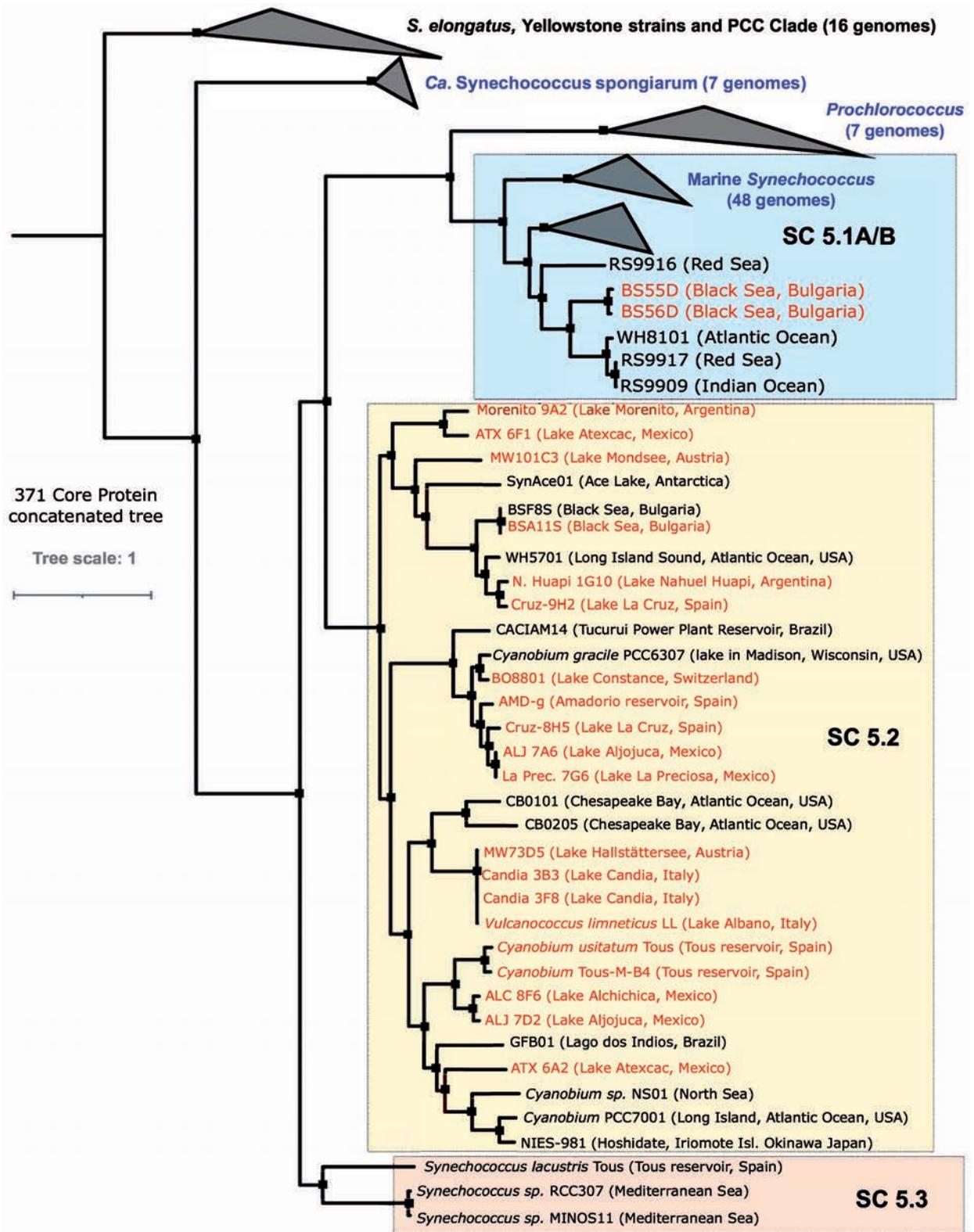
Fig. 2. Protein-concatenated phylogenomic tree constructed with 371 core proteins with PhyloPhlAn tool. The tree includes all culture-derived picocyanobacteria from *Synechococcus* and *Cyanobium* genera inside sub-clusters 5.1, 5.2 and 5.3, a few *Prochlorococcus* representatives and Ca. *Synechococcus spongiarum*. The tree was rooted at the *S. elongatus* and PCC clade. All sequenced cultures derived from this collection and included in Tab. 1 are red coloured. Bootstrap values higher than 0.95 are marked as black squares on nodes. The scale bar represents the number of mutations/site.

Spectrofluorimetric analysis

Emission and excitation spectra for selected strains, BO8801 (PC), Nahuel Huapi 1G10 (PE) and Aljojuca 7D2 (PE), were carried out with a FluoroMax-4 spectrofluorometer (HORIBA Scientific, Irvine, CA, USA) to well characterize their representative content in phycobiliproteins, highly fluorescent molecules.

Excitation spectra were executed setting the emission wavelength (λ_{em}) at 680 nm (chlorophyll-*a* emission peak) and 580 nm (phycoerythrin emission peak). On the other hand, emission spectra were executed at an excitation wavelength (λ_{ex}) of 436 nm (one of the chlorophyll-*a* absorbance peak) and 520 nm (shorter wavelength than the phycoerythrin absorbance peak). Excitation spectra at $\lambda_{em} = 680$ and 580 nm and emission spectra at $\lambda_{ex} = 436$ and 520 nm were chosen to compare them with already published works that evaluate the colour differences between PE cells and PC cells (Callieri *et al.,* 1996). The fluorescence spectra of the cultures were measured in a 1 cm quartz cuvette, with an excitation and emission slits of 10 nm and scan speed of 1 nm s^{-1}. Samples' spectra were corrected by subtracting the spectrum of the medium BG-11 (blank).

DNA extraction, sequencing and phylogenomics

DNA extraction, sequencing and phylogenomic analyses of the strains were already described in detail in previous works (Cabello-Yeves *et al.,* 2018; Di Cesare *et al.,* 2018; Sanchez-Baracaldo *et al.,* 2019). Briefly, 2 ml of highly concentrated culture, in order to obtain a sufficient pellet to extract DNA, were centrifuged. DNA was extracted from medium-free pellets using the Ultra-Clean Microbial DNA Isolation kit (MOBio Laboratories, Inc., Carlsbad, CA, USA) according to manufacturer's instruction. E.Z.N.A.® Soil DNA Kit (Omega Bio-tek) and phenol-chloroform DNA extraction methodologies were also applied for some strains (Cabello-Yeves *et al.,* 2018). Strains were sequenced with Illumina HiSeq or MiSeq and draft genomes were obtained from an assembly step with SPAdes assembler following ´auto´ parameters (Bankevich *et al.,* 2012). A 371 protein-concatenated phylogenomic tree was constructed using the tool PhyloPhlAn (Segata *et al.,* 2013), which comprised all culture-derived picocyanobacteria from *Synechococcus* and *Cyanobium* genera inside sub-clusters 5.1, 5.2 and 5.3. A FastTree with 100 bootstraps was constructed with the above mentioned tool. For the construction of the tree, no sequences were available of strains ATX 6A9 and ATX 6H9. Instead, they were represented in the phylogenomics by their closely related picocyanobacteria ATX 6A2 and ATX 6F1, respectively. The universal markers of strains *Cyanobium* Tous-M-B4, La Cruz 8H5, La Cruz 9H2 and AMD-g were gently granted and used for the

phylogenomic tree, but were not yet published in databases by the time this atlas was written.

REFERENCES

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19:455-477.

Cabello-Yeves PJ, Haro-Moreno JM, Martin-Cuadrado AB, Ghai R, Picazo A, Camacho A, Rodriguez-Valera F, 2017. Novel *Synechococcus* genomes reconstructed from freshwater reservoirs. Front. Microbiol. 8:1151.

Cabello-Yeves PJ, Picazo A, Camacho A, Callieri C, Rosselli R, Roda-Garcia JJ, et al., 2018. Ecological and genomic features of two widespread freshwater picocyanobacteria. Environ. Microbiol. 20:3757-3771.

Callieri C, Amicucci E, Bertoni R, 1996. Fluorometric characterization of two picocyanobacteria strains from lakes of different underwater light quality. Int. Revue Ges. Hydrobiol. 81:13–23.

Callieri C, Lami A, Bertoni R, 2011. Microcolony formation by single-cell *Synechococcus* strains as a fast response to UV radiation. Appl. Environ. Microbiol. 77:7533-7540.

Callieri C, Cronberg G, Stockner J, 2012. Freshwater picocyanobacteria: single cells,microcolonies and colonial forms, p. 229-269. In: B. Whitton (ed.), Ecology of Cyanobacteria: Their Diversity in Time and Space. Springer.

Callieri C, Coci M, Corno G, Macek M, Modenutti B, Balseiro E, Bertoni R, 2013. Phylogenetic diversity of nonmarine picocyanobacteria. FEMS Microbiol. Ecol. 85:293-301.

Callieri C, Slabakova V, Dzhembekova N, Slabakova N, Peneva E, Cabello-Yeves PJ. et al., 2019. The mesopelagic anoxic Black Sea as an unexpected habitat for *Synechococcus* challenges our understanding of global "deep red fluorescence". ISME J. 13(7):1676-1687

Croce R, Van Amerongen H, 2014. Natural strategies for photosynthetic light harvesting. Nature Chem. Biol. 10:492-501.

Di Cesare A, Cabello-Yeves PJ, Chrismas NAM, Sánchez-Baracaldo P, Salcher MM, Callieri C, 2018. Genome analysis of the freshwater planktonic *Vulcanococcus limneticus* sp. nov. reveals horizontal transfer of nitrogenase operon and alternative pathways of nitrogen utilization. BMC Genomics. 19:259

Doré H, Farrant GK, Guyet U, Haguait J, Humily F, Ratin M, et al., 2020. Evolutionary mechanisms of long-term genome diversification associated with niche partitioning in marine picocyanobacteria. Front. Microbiol. 11:567431.

Dufresne A, Ostrowski M, Scanlan DJ, Garczarek L, Mazard S, Palenik BP, et al., 2008. Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. Genome Biol 9: R90.

Farrant GK, Dore H, Cornejo-Castillo FM, Partensky F, Ratin M, Ostrowski M, et al., 2016. Delineating ecologically significant taxonomic units from global patterns of marine picocyanobacteria. Proc. Natl. Acad. Sci. U S A 113:E3365–E3374.

Ferrari GM, Tassan S, 1999. A method using chemical oxidation

to remove light absorption by phytoplankton pigments. J. Phycol. 35:1090-1098.

Flombaum P, Gallegos JL, Gordillo A, Rincon J, Zabala LL, Jiao N, et al., 2013. Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. Proc. Natl. Acad. Sci. USA 110:9824–9829.

Fuller NJ, Marie D, Partensky F, Vaulot D, Post AF, Scanlan D, 2003. Clade-specific 16S Ribosomal DNA oligonicleotides reveal the predominance of a single *Synechoocccus* clade throughout a stratified water column in the red sea. Appl. Environ. Microbiol. 69:2430-2443.

Hamilton TL, Bryant DA, Macalady JL, 2016. The role of biology in planetary evolution: cyanobacterial primary production in low oxygen Proterozoic oceans. Environ. Microbiol. 18:325-340.

Kishino M, Takahashi M, Okami N, Ichimura S, 1985. Estimation of the spectral absorption coefficients of phytoplankton in the sea. Bull. Mar. Sci. 37:634-642.

Olson RJ, Zettler ER, Anderson OK, 1989. Discrimination of eukaryotic phytoplankton cell types from light scatter and autofluorescence properties measured by flow cytometry. Cytometry. 10:636–643.

Ong LJ, Glazer AN, 1991. Phycoerythrins of marine unicellular cyanobacteria .3. Sequence of a Class-II Phycoerythrin. J. Biological Chem. 266:9535–9539.

Sanchez-Baracaldo P, Bianchini G, Di Cesare A, Callieri C, Chrismas NA, 2019. Insights into the evolution of picocyanobacteria and phycoerythrin genes (mpeBA and cpeBA). Front. Microbiol 10:45.

Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, et al., 2009. Ecological genomics of marine picocyanobacteria. Microbiol. Mol. Biol. Rev. 73: 249–299.

Scanlan D, 2012. Marine Picocyanobacteria, p. 503-533. In: B. Whitton (ed.), Ecology of Cyanobacteria: Their Diversity in Time and Space. Springer.

Segata N, Börnigen D, Morgan XC, Huttenhower C, 2013. PhyloPhlAn is a new method for improved phylogenetic and taxonomic placement of microbes. Nature Comm. 4:1-11.

Six C, Thomas JC, Garczarek L, Ostrowski M, Dufresne A, Blot N, et al., 2007. Diversity and evolution of phycobilisomes in marine *Synechococcus* spp.: a comparative genomics study. Genome Biol. 8:R259.

Stomp M, Huisman J, Vörös L, Pick FR, Laamanen M, Haverkamp T, Stal LJ, 2007. Colorful coexistence of red and green picocyanobacteria in lakes and seas. Ecol. Lett. 10:290-298.

Tzur A, Moore JK, Jorgensen P, Shapiro HM, Kirschner MW, 2011. Optimizing optical flow cytometry for cell volume-based sorting and analysis. PloS One 6:e16053.

Wood MA, Horan PK, Muirhead K, et al., 1985. Discrimination between types of pigments in marine *Synechococcu*s spp. by scanning spectroscopy, epifluorescence microscopy, and flow cytometry. Limnol. Oceanogr. 30:1303-1315.