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Phylogeny and taxonomy of *Synechococcus*–like cyanobacteria

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Abstract: Unicellular cyanobacteria constitute a substantial, ecologically important part of freshwater and marine microflora. Solitary, elongated cyanobacterial cells without apparent slime envelopes and dividing in a single plane perpendicular to the longitudinal axis were traditionally classified into the genus *Synechococcus*. The type species originates from a freshwater benthic habitat while a number of other *Synechococcus*–like species were described from diverse environments. Morphologically similar, unicellular populations of "*Synechococcus*" belong to the most abundant oxygen evolving prokaryotes inhabiting freshwater and oceanic picoplanktic communities. Other species from extreme thermal habitats were described from temperatures over 70 °C. Recent molecular analyses, particularly the 16S rRNA gene and other multi–locus gene based phylogenies of *Synechococcus*–like species reveal the polyphyly and phylogenetic diversity of such simple cyanobacteria emphasizing the necessity of their accurate polyphasic taxonomic re–classification. To better understand the phylogeny of different *Synechococcus*–like species, we constructed a genome–derived phylogenetic tree using 133 cyanobacterial and eubacterial genomes as well as a 16S rRNA gene phylogenetic tree. Both trees indicate existence of deep splits between individual *Synechococcus* lineages corresponding with indistinct ecological and cytomorphological differences. The generic units derived from the traditional concept of the genus *Synechococcus* based on the combined traits and markers must be defined and validly described. The comparative taxonomic classification of phylogenetic clusters corresponding to various morphotypes and genotypes similar to the genus *Synechococcus* (*Cyanobium, Parasynechococcus, Picosynechococcus, Prochlorococcus, Thermosynechococcus, Thermostichus*) are discussed in the article.

Key words: cyanobacteria, picoplankton, phylogeny, *Synechococcus*–like genera, taxonomy

Introduction

Prokaryotic cyanobacteria are an indispensable component of the Earth´s biosphere. The ~3400 My of their existence provided sufficiently long time for their evolution and diversification (Tomitani et al. 2006). Today, they play a prominent role among the phototrophic microbial communities in almost all ecosystems. Cyanobacterial picoplankton (solitary cells smaller than 2–3 μm in diameter) inhabits all eutrophic freshwater (Komárková 2002a, b; Ernst et al. 2003; Garcia–Pichel et al. 2003; Callieri et al. 2012) and marine habitats (Partensky et al. 1999; Zeidner et al. 2003; Pérez & Carrillo 2005; Six et al. 2007; Mella–Flores et al. 2012; Scanlan 2012), where it often dominates in the biomass. The morphologically similar species living in solitary cells or in colonies flourish also in the extreme environments of thermal springs, cold lakes, and deserts of Antarctica (Priscu et al. 1998; Komárek 1999; Miller & Castenholz 2000; Stibal et al. 2015).

The small unicelled picoplanktic cyanobacteria were traditionally identified as members of the genera *Synechocystis* Sauvageau (1892) (with spherical cells and division in two subsequent perpendicular planes), or *Synechococcus* Nägeli (1849) (with elongated oval or rod–like cells with one crosswise, perpendicular division plane). *Synechococcus* was commonly classified as a rod–like cyanobacterium of benthic freshwater origin in Nägeli´s original concept (Nägeli 1849). However, wider ecological range was later attributed to this morphologically simple genus with

additional species descriptions that were included in numerous monographs (Geitler 1932; Elenkin 1938; Desikachary 1959; Starmach 1966). Members of the genus *Synechococcus* were classified from many habitats during most of the 20th century, but the introduction of phylogenetic analyses of DNA sequences into cyanobacterial classification (Komárek 1996; Katoh et al. 2001; Herrero & Flores 2008; Coutinho et al. 2016a,b; Dvořák 2017), combined with ecological characteristics, enabled the more detailed revision of the diversity within this polyphyletic genus. With polyphasic approach it became also evident that the structure of several traditional cyanobacterial *Synechococcus*–like taxa is variable and the corresponding units had to be taxonomically revised, including picoplanktic species (Komárek et al. 2014).

The minute, very simple, solitary, oceanic cyanobacteria were recognized as belonging to different genera. This set of taxa includes *Prochlorococcus* (Chisholm et al. 1992) or invalidly described *Parasynechococcus* (Coutinho et al. 2016 a,b), which both are genetically related to the freshwater *Cyanobium gracile* (Rippka & Cohen–Bazire 1983). The oceanic members of *Parasynechococcus (Synechococcus marinus)* and *Prochlorococcus* are very abundant small coccoid cyanobacteria, both in numbers of individual cells and amount of biomass in planktic communities (Partensky et al. 1999; Palenik et al. 2003; Six et al. 2007; Flombaum et al. 2013; Dvořák et al. 2014a, 2017). Several of these populations have already been described in detail by numerous authors, particularly their metabolic and ecophysiological functions (Rabouille et al. 2007; Hess 2008; Swingley et al. 2008; Wiethaus et al. 2010; Bragg 2011; Hughes et al. 2011; Mazard et al. 2012; Scanlan 2012; Ward et al. 2012; Dvořák et al. 2014a, Mena et al. 2016) as well as their genomic characteristics (Coutinho et al. 2016a,b; Dvořák 2017).

Synechococcus–like populations were described also as intracellular symbionts (Hindák 2013) and 16S rRNA gene and multilocus phylogenies revealed that *Synechococcus*–like cyanobacteria from hot–springs occur in different phylogenetic clades (e.g. Hess 2008; Swingley et al. 2008; Schirrmeister et al. 2013; Dvořák et al. 2014a,b; Dvořák 2017; Mareš et al. 2019). The thermophilic genus *Thermosynechococcus* Katoh et al. (2001, nom. inval.), was distinguished from the typical, traditional *Synechococcus* mainly by ecophysiological and biochemical features (Katoh et al. 2001), because all strains and species were known to thrive in thermal waters at high temperatures, possibly up to 70 °C. Katoh et al. (2001) proposed the name "*Thermosynechococcus*", but they did not describe the genus validly according to either the bacteriological or the botanical codes of nomenclature (ICNP and ICN, respectively). Other challenging thermophilic "*Synechococcus"* species originate from a hot spring in Yellowstone National Park, USA (Copeland 1936; Bhaya et al. 2007). Several strains currently recognized as "*Synechococcus*" (e.g. JA–33Ab and JA–23Ba) and found at sites with temperatures possibly up to ~ 70 °C formed a separate clade in overall cyanobacterial phylogeny (e.g., Miller & Castenholz 2000; Schirrmeister et al. 2013; Shih et al. 2013) and were found related to the Bulgarian strain RUP–VU–1 (Strunecký et al. 2019).

We have applied, therefore, the polyphasic approach (Komárek & Anagnostidis 1998) for evaluation of the traditional genus *Synechococcus* sensu lato in this paper. We propose the introductory taxonomic revision and classification of the phylogenetically separate clades as various genera, which can be delimited by a combination of phylogenetic, cytomorphological and ecological markers. We honor those who recognized the clades as separate by using their proposed names and describe new taxa. They will be validly described in this paper under the rules of the ICN. The review of corrected genera is presented in a new proposed classification and discussed in the context of the higher cyanobacterial taxa.

Material and Methods

This paper is based on the study of natural populations respecting the data of previous authors, together with molecular evaluation of numerous isolated strains.

rRNA operon sequencing and phylogenetic analyses. Total genomic DNA was isolated following the modified xanthogenate–SDS buffer extraction protocol with addition of 3% polyvinylpolypyrrolidone (PVPP) and PEG–$MgCl_2$ precipitation (Yilmaz et al. 2009). A section of the rRNA operon containing the partial 16S rRNA gene and the 16S–23S intergeneric spacer (ITS) was amplified using the primers 359F (Nübel et al. 1997) and 23S30R (Wilmotte et al. 1993) and a PCR protocol as previously described (Mareš et al. 2013a). Sequencing was performed commercially at Macrogen (Amsterdam, Netherlands) using primers 359F, 23S30R, 1492R, and 810R (Bohunická et al. 2015). The newly acquired sequences were deposited to NCBI GenBank database under accession numbers MG207959 – MG207960. Forty–four sequences of *Synechococcus* like cyanobacteria were used for 16S rRNA gene phylogeny. One hundred and seventy–six sequences of cyanobacterial strains outside of the *Synechococcus* group were added as outgroup taxa for inferring congruent phylogeny as used elsewhere (Mareš et al. 2013a; Bohunická et al. 2015), and the tree was rooted with a representative of *Candidatus Melainabacter,* the heterotrophic prokaryotic group sister to Cyanobacteria. Sequences were aligned in MAFFT (www.mafft.cbrc.jp) (Katoh & Standley 2013) and visually checked in BioEdit 7.0.1 (Hall 1999).

Bayesian inference (BI) and maximum likelihood (ML) analyses were performed using partial 16S rRNA gene sequences containing a maximum of 1195 characters including nucleotides and indels. Bayesian inference was conducted with MrBayes XSDE V3.2.6 (Ronquist et al. 2012) through the CIPRES Science Gateway, applying the GTR+G+I model of nucleotide substitutions, the model indicated as "best"

model in jModeltest2, also run on the CIPRES Science Gateway. A total of 84 million generations were run. The BI analysis had an estimated sample size (ESS) exceeding 5000 for all parameters (average ESS ranging 5104– 52,570), well above the average of 200 typically accepted as sufficient by phylogenetists (Drummond et al. 2006). The final average standard deviation of split frequencies was <0.01. The potential scale reduction factor (PSRF) value for all the estimated parameters in the BI analysis was 1.00, indicating that convergence of the MCMC chains was statistically achieved (Gelman & Rubin 1992). The ML analysis with rapid bootstrapping was conducted using RAxML–HPC v.8 on XSDE V8.2.10 (Stamatakis 2014), also through the CIPRES Science Gateway, applying the GTR+G+ I model of nucleotide substitutions, with 1000 bootstrap iterations. Bootstrap values for the ML analysis were mapped on to the BI analysis tree, the phylogeny figured in this publication.

A data set comprehensively covering the complete genomes of cyanobacteria was generated using publicly available genomes from NCBI (ftp://ftp.ncbi.nih.gov/genomes/Bacteria/). A representative set of 117 cyanobacterial genomes supplemented with 8 outgroup bacterial genomes were used for analyses (Figs 1, 2). Data for multi–locus alignment were mined from a custom BLAST database compiled from the 125 genomes. Protein homologs were harvested by TBLASTN searches with an E–value of 1e–10 performed using BLAST+ 2.2.28 (Camacho et al., 2009). *Synechococcus lividus* PCC 6715 (NZ_CP01809) was selected as a reference organism due to its well–annotated genome and intermediate phylogenetic position among cyanobacteria. Amino acid sequences of all of its annotated proteins were BLASTed against the constructed BLAST database. The obtained 2548 sets of amino acid sequences were aligned and reordered using MAFFT with – localpair – reorder – maxiterate 1000 settings (Katoh & Standley 2013). Whenever multiple BLAST hits for a single locus were retrieved from a particular genome, only the most similar hit was kept for subsequent analysis. Obtained alignments were further filtered so that only those containing more than 120 cyanobacterial sequences were kept for phylogenetic analysis. The resulting 260 alignments were considered to represent ubiquitous housekeeping loci and they were concatenated providing a 313,388 amino acid alignment. Custom PERL scripts were applied for the BLAST hit filtering and the concatenation of alignments. The validity of the 260 selected protein alignments was tested using OD–seq (Jehl et al. 2015) and Guidance v. 2.02 (Sela et al. 2015) detecting no sequence outliers. All positions in the alignment with less than 80% site coverage were eliminated, providing a total of 156,767 sites in the final dataset. The tree inference was performed using the RAxML–HPC v.8 on XSDE V8.2.10 with rapid bootstrapping and with NJ under JTT model and 500 bootstrap (BP) repetitions (due to exceptional time requirements for analysis of such a long alignment) in MEGA 10.0.4 (Kumar et al. 2018).

Morphological and ultrastructural observations. The morphology of 9 strains traditionally classified to *Synechococcus* were particularly studied. Strains were observed by light microscopy; the observations were repeated through all stages of the batch cultures using an Olympus BX 51 optical microscope equipped with Nomarski DIC optics and an Olympus DP71 digital camera. Extracellular polysaccharides (EPS) in the cultured strains were visualized by adding a drop of shellac–based drawing ink (Koh–i–noor, Czech Republic). Cultured strains were prepared for transmission electron microscopy (TEM) observation.

Living cells from liquid samples (semicultures) were prepared by fixation in glutaraldehyde and osmium tetroxide (Kellenberger et al. 1958) for 3 hours at 4 °C. Glutaraldehyde (3%; w/v) was applied in a 100mM cacodylate buffer, pH 7.3. The cells were postfixed in 1% (w/v) osmium tetroxide in the same buffer for 2 hours at 4 °C. Following fixation, cells were washed with the buffer overnight at 4 °C. Thereafter, they were instilled in 1.3% agar and dehydrated by a series of ethanol concentrations increasing from 70% to 99.9% (20 min each) and, thereafter, by pure acetone. Cells were then infiltrated with Durcupan epoxide resin and, in this condition, encapsulated and polymerized using UV light at 4 °C for 3 days. Ultrathin (50 – 60nm) sections were cut using a Leica EM UC6 ultramicrotome. Sections placed on supporting grids were contrasted by 2.5% (w/v) uranyl acetate and Reynolds solution (3%, w/v, lead citrate) for 6 min. Sections were observed and photographed in an FEI Morgagni 268D digital transmission electron microscope. Osmium fixation was started by application of 1% (w/v) osmium tetroxide in 0.7% (w/v) veronal–acetate buffer, pH 6.5, with traces of sodium chloride and calcium chloride. All preparation procedures and photographic documentation following the preparation techniques were the same as the glutaraldehyde fixation.

Results and Discussion

The polyphyletic genus *Synechococcus* was divided into distinct, genetically and taxonomically separated lineages (genera) based on phylogenetic analyses (Figs 1, 2). The 16S rRNA gene phylogeny includes more sequences due to their greater availability in GenBank (www.ncbi.nlm.nih.gov), however it is congruent with the phylogenetic relationship based on multi locus analysis of 260 concatenated genes. *Gloeobacter* (Rippka et al. in Mareš et al. 2013), without intracellular thylakoidal membranes is located morphologically also near this cluster of cyanobacteria, but it differs clearly by phylogenetic position and by cell structure. *Synechococcus*–like, highly thermophilic *Thermostichus* gen. nov. (Figs 1, 2) is located close to the root of all cyanobacterial lineages, in the position between *Gloeobacter* and filamentous *Pseudanabaena* where represents evidently an individual taxonomic unit.

The separate node containing *Prochlorococcus, Parasynechococcus* (= marine "*Synechococcus*"), and *Cyanobium* (type strain) forms a separate cluster (Prochlorococcaceae). This clade is a sister to the clade containing *Synechococcus* sensu stricto both in the 16S rRNA gene and multilocus analyses (Figs. 1, 2). Morphological characters that are commonly used in the characterization of distinct and delimited morphogenera fail within this clade because the particularly freshwater taxa defined herein are mostly morphologically cryptic.

The thermophilic *Thermosynechococcus* is more closely related to filamentous Pseudanabaenaceae and

represents a special family in the 16S rRNA gene derived phylogeny (Fig. 1), but it formed a slightly more separated clade in the multi–locus phylogenetic tree (Fig. 2). The last described genus *Picosynechococcus* is positioned distantly from the previously described genera within the family Aphanothecaceae.

Order Thermostichales, family Thermostichaceae
Four simple, highly thermophilic strains were located in our phylogenetic evaluation close to *Gloeobacter* (Fig. 1). They had elongated cells with few (2–3) ± parietal thylakoids (Fig. 5B). According to the molecular analyses, it is necessary to classify these strains into the new genus *Thermostichus*, which represents a special unit on the level of a separate family and order (Figs 1, 2, 5B, 6; Tables 1, S1, S2).

The genus *Thermostichus* represents a distinct cluster with strains JA 33Ab, JA 23Ba and RUP–VU–1. In the phylogenomic tree of Walter et al. (2017) they designated these strains as *Synechococcus yellowstonii/Leptococcus yellowstonii* (JA 33Ab) and *Synechococcus springii/Leptococcus springii* (JA 23Ba) without further characteristics. They were also located close to the genus *Gloeobacter* and distantly from strains of *Synechococcus elongatus.* However, the generic name "*Leptococcus*" was proposed only in the text and in Table 1, but not described according to either the botanical or prokaryotic codes of nomenclature, and is consequently an invalid name. The species epithets *yellowstonii* and *springii* are likely later synonyms of species described by Copeland (1936). This cluster contains the extremely thermophilic strains, growing and surviving in the range of temperatures 23.5–85.2(?) °C and corresponding probably to several species, described by Copeland (1936) under the generic name *Synechococcus* from Yellowstone National Park, USA. The morphological similarity of these thermophilic strains to the genus *Synechococcus* (Copeland 1936) and *Thermosynechococcus* (cf. Katoh et al. 2001) is evident, but they differ genetically (Figs 1 and 2). We propose as type species of this extremely thermophilic cluster the *Thermostichus vulcanus*. As compared with *Thermosynechococcus*, the extremely high thermophilic *Thermostichus* clade (Figs 1,2) is genetically different to such a degree that its separation as a special genus and classification in different families is justified. This whole group is also more or less morphologically uniform and therefore can be defined clearly as a separate genus. Very interesting character occurring in the genus *Thermostichus* is also the obligatory position of one or groups of cyanophycin granules in terminal parts of cells, which look as dark polar points in elongated cells in optical microscope. The presence of these granules is characteristic for all studied specimens in this simple cyanobacterial order.

Thermostichales Komárek et Strunecký ordo nova
Description: Unicellular and colonial cyanobacteria. Cells elongated, without prominent mucilaginous envelopes with few parietal thylakoids. Thermophilic (up to over 80 °C).

Thermostichaceae Komárek et Strunecký fam. nov.
Description: With characters of the order.

***Thermostichus* Komárek et Strunecký gen. nov. (Figs 1, 2, 6, Table S1)**
Description: Cyanobacterial cells solitary, in irregular agglomerations, or in short, few–celled indistinct rows, narrow, with rounded ends, usually several times longer than wide, straight, strictly cylindrical or slightly curved or arcuated, without prominent slime envelopes. Cell division perpendicular in one plane in subsequent generations, symmetrical or slightly asymmetrical, with daughter cells usually separating soon after division, less frequently forming short rows of a few cells. Cytoplasm sometimes with visible narrowed peripheral chromatoplasma (= few, ± parietal thylakoids), usually pale or intensely olive–green or blue–green, without aerotopes, usually with solitary granules situated in the terminal parts of cells. – Ecology: Mostly in thermal springs from 20–40 °C (up to possibly over 80 °C, comp. Copeland 1936 under *Synechococcus;* other habitats not yet confirmed); in such localities probably a cosmopolitan genus.

Type species: *Thermostichus vulcanus* (Copeland) Komárek et Strunecký comb. nov. (basionym: *Synechococcus vulcanus* Copeland, Ann. N. Y. Acad. Sci. 1936: 61, 1936).
Figura typica: figura nostra 6e. Because this established genus is based on Copeland´s (1936) characteristics and a holotype was not selected and deposited by the original author, we select a neotype from our available material.
Neotype here designated: CBFS A–092–1, dried material of the strain "*Thermostichus vulcanus* –Rupite" (RUP–VU–1), deposited in the herbarium collection of the University of South Bohemia.
Etymology: *Synechococcus*–like cyanobacteria with short cylindrical cells, growing in thermal waters.

This thermophilic genus includes probably other morphospecies, which were, however, not yet confirmed genetically (cf. Figs 5B, 6, Table S1):
– *Thermostichus amphigranulatus* (Copeland) Komárek et Strunecký comb. nov. (Basionym: *Synechococcus elongatus* var. *amphigranulatus* Copeland, Ann. N. Y. Acad. Sci. 1936: 59, 1936);
– *Thermostichus arcuatus* (Copeland) Komárek et Strunecký comb. nov. (Basionym: *Synechococcus arcuatus* Copeland, Ann. N. Y. Acad. Sci. 1936: 63, 1936);
– *Thermostichus bigranulatus* (Skuja) Komárek et

Strunecký comb. nov. (Basionym: *Synechococcus bigranulatus* Skuja, Fedde Repert. Sp. Nov. 31: 7, 1933);
– *Thermostichus lividus* (Copeland) Komárek et Strunecký comb. nov. (Basionym: *Synechococcus lividus* Copeland, Ann. N. Y. Acad. Sci. 1936: 56, 1936);
– *Thermostichus praelongus* (Emoto et Yoneda) Komárek et Strunecký comb. nov. (Basionym: *Synechococcus praelongus* Emoto et Yoneda, Ecol. Rev. 66: 267, 1940);
– *Thermostichus sublividus* (Emoto et Yoneda) Komárek et Strunecký comb. nov. (Basionym: *Synechococcus sublividus* Emoto et Yoneda, Jap. J. Bot. 17: 704–720, 1941);
– *Thermostichus sulphuricus* (Dor) Komárek et Strunecký comb. nov. (Basionym: *Synechococcus sulphuricus* Dor, Sea Fish Res. St. Haifa 48: 12–13, 1967);
– *Thermostichus vescus* (Copeland) Komárek et Strunecký comb. nov. (Basionym: *Synechococcus vescus* Copeland, Ann. N. Y. Acad. Sci. 1936: 55, 1936);
– *Thermostichus viridissimus* (Copeland) Komárek et Strunecký comb. nov. (Basionym: *Synechococcus viridissimus* Copeland, Ann. N. Y. Acad. Sci. 1936: 60, 1936).

Other thermophilic species, which contain prominent polar granules in their cells probably also belong to this genus (Fig. 6). Within the genus *Thermostichus* possibly a few other endemic taxa will be recognized on the species level, to which belong, e.g., *Synechococcus*

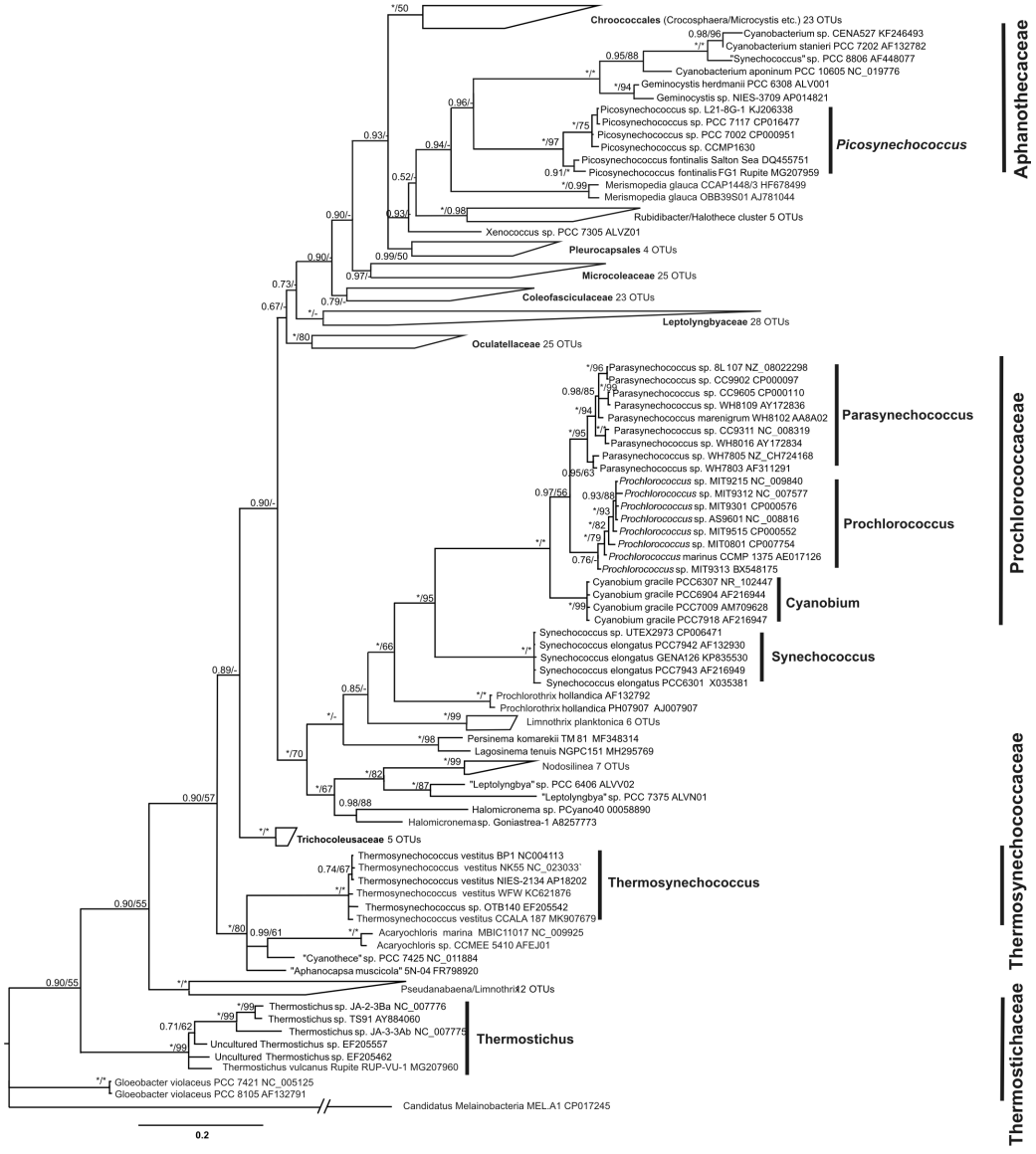

Fig. 1. Bayesian inference with the available "*Synechococcus*"–like strains, based on 16S rRNA gene sequences derived from our work and NCBI Genbank accessions. Nodes defining clades are noted with these codes. Support values at the nodes represent Bayesian posterior probabilities/ML bootstrap support. The diacritical characters of the families (Aphanothecaceae, Gloeobacteraceae, Prochlorococcaceae, Synechococcaceae, Thermosynechococcaceae) are based also on important qualitative markers, not included in this tree (presence of thylakoids, composition of assimilation pigments).

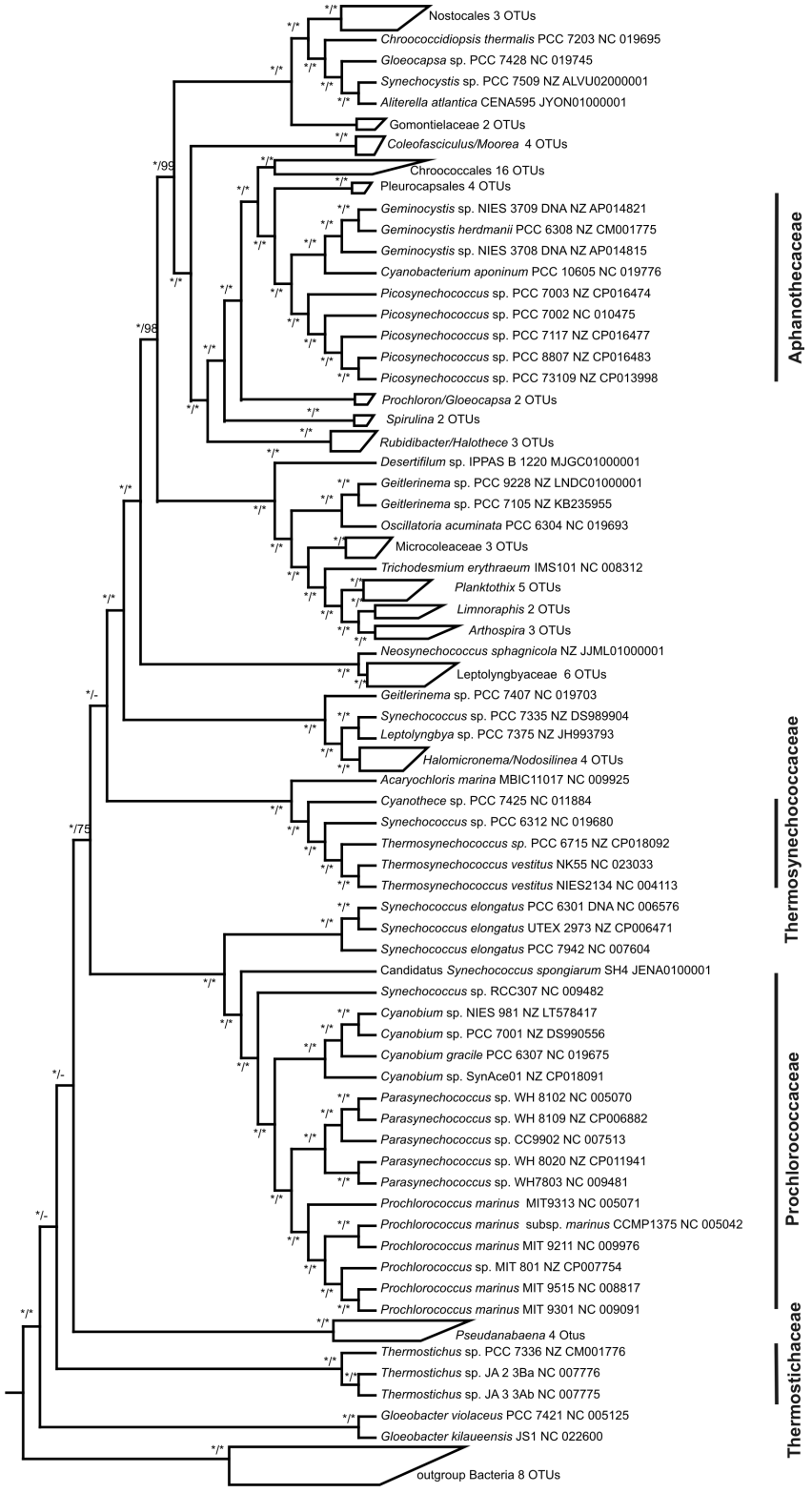

Fig. 2. Multi–locus phylogenetic analysis based on 260 ubiquitous concatenated housekeeping loci. Support values at the nodes represent maximum likelihood and neighbor joining bootstrap support, asterisk indicate the value of 100.

caldarius OKADA (1939), or a few varieties described within *Synechococcus lividus*.

Order Synechococcales, family Synechococcaceae

A wide variety of *Synechococcus*–like strains were identified in the phylogenetic trees (Figs 1, 2, 4A), which are isolated mostly from benthic or planktic habitats of inland freshwaters (comp. e.g. ERNST et al. 2003), or from the thermal springs' habitats. They were characterized by distinctly elongated cells living solitary or in agglomerations. Previously, Synechococcales has been broadly conceived to contain most of the genera with

Table 1. Short taxonomic review of the simplest phototrophic cyanobacterial orders (partly without thylakoids – Gloeobacterales) and unicellular and filamentous genera with mostly parietal thylakoids in cells (Synechococcales, Pseudanabaenales) (state of 2018). Only genera are included, discussed in the article. The order Chroococcales contains mostly the types with ± irregularly arranged thylakoids in cells.

Gloeobacterales	Gloeobacteraceae	Gloeobacter Rippka el al. ex Mareš et al. 2013
Thermostichales	Thermostichaceae	Thermostichus Komárek et al. (this paper)
Synechococcales	Prochlorococcaceae	Cyanobium Rippka et Cohen-Bazire 1983
Cryptogenera		Parasynechococcus Coutinho et al. 2016, validation
		Prochlorococcus Chisholm et al. 1992
Free unicellular and colonial types; cells divide perpendicular to the long cell axis	Synechococcaceae (18 genera)	Synechococcus Nägeli 1849
	Merismopediaceae (10 genera)	
	Prochloraceae (1 genus)	
	Coelosphaeriaceae (6 genera)	
	Acaryochloridaceae (1 genus)	
	Chamaesiphonaceae (5 genera)	
Pseudanabaenales	Thermosynechococcaceae (1 genus)	Thermosynechococcus Katoh et al. 2001, validation
Mostly filamentous types	Romeriaceae (4 genera)	
	Prochlorotrichaceae (4 genera)	
	Pseudanabaenaceae (11 genera)	
	Leptolyngbyaceae (23 genera)	Neosynechococcus Dvořák et al. 2014
	Heteroleibleiniaceae (3 genera)	
	Schizothrichaceae (3 genera)	
	Oculatellaceae (10 genera)	
Chroococcales	Aphanothecaceae (15 genera)	Picosynechococcus Komárek et al. (this paper)
Mostly coccoid types	Cyanobacteriaceae (1 genus)	
	Chroococcaceae (17 genera)	
	Microcystaceae (5 genera)	
	Cyanothrichaceae (1 genus)	
	Gomphosphaeriaceae (1 genus)	
	Chroococcaceae (17 genera)	
	Stichosiphonaceae (1 genus)	
	Entophysalidaceae (8 genera)	

parietal thylakoids (Komárek et al. 2014) but the later analyses showed the presence of parietal thylakoids through the whole cyanobacterial phylogeny (Mareš et al. 2019). However, based on phylogenetic position and distinct ecological constraints of studied strains, it is possible to classify such similar morphotypes in different genera.

The type species of the genus *Synechococcus, S. elongatus* Nägeli (1849), was described from a mud bed of the Katzensee, a freshwater lake near Zürich in Switzerland (47°25'57"N, 8°29'34"E), but holotype material is unavailable. The elongated cells are 2.2–4.5 μm long and 1.5 μm wide with homogeneous pale cytoplasm. This taxon is commonly known from wet soils, subaerophytic or benthic substrates and from the littoral of freshwater inland water bodies (Nägeli 1849; Geitler 1932). Due to the impossibility to study the phylogenetic position of the original type material, we have prepared neotype material from the strain that is morphologically, cytologically and ecologically a close match to Nägeli´s freshwater epipelic type. The revised genus *Synechococcus* is in a clade distant in position from other *Synechococcus*–like strains (Fig. 1).

Synechococcus sp. PCC 6301 was recommended by Rippka & Cohen–Bazire (1983) as the reference strain for *Synechococcus elongatus*, and this recommendation was repeated in Bergey's Manual (Herdman et al. 2001). We accept this proposal due to the close match with Nägeli's original description. The strains corresponding closely to *Synechococcus* sensu stricto evidently include strains PCC 6301, PCC 7942, PCC 7943, UTEX 2973, and CENA 126. The traditionally defined species as *S. mundulus, S. nidulans, S. rhodobaktron* belong very probably also to this genus (Table 1), but require molecular confirmation. All strains included in *S. elongatus* clade had high genetic identities (>99.9%). Coutinho et al. (2016b) published a separated cluster (with support of phylogenetic position), designated by the name *Synechococcus elongatus* with the strains PCC 6301 and PCC 7942 and a sister clade *Synechococcus* sp. with seven other strains. The ultrastructure of all other members of the genus *Synechococcus* has not yet been described in detail, but the position of the thylakoids is not strictly parietal, at least in the type species of *S. nidulans* (see fig. 14a in Komárek & Anagnostidis 1986, that showed rather irregularly organized thylakoids in cells). The list of the common *Synechococcus* species is included in Table S1.

For *Synechococcus elongatus* Nägeli (1849) we designate the following neotype: CBFS A–102–1, herbarium material preserved by drying biomass of the reference strain, PCC 6301, deposited in the herbarium collection of the University of South Bohemia. *Synechococcus elongatus* is a freshwater species, periphytic or living on wet soil or mud. PCC 6301 was collected from Waller Creek, Austin, Texas, isolated 1st January 1952. The reference strain *Synechococcus elongatus* PCC 6301. Additional strains belonging to this species are PCC 7942, PCC 7943, UTEX 2973 and CENA 126.

Order Synechococcales, family Prochlorococcaceae
A distinct clade, which contains coccoid genera *Prochlorococcus, Parasynechococcus* and *Cyanobium,* represents an isolated cluster (family Prochlorococcaceae; Figs 1, 2, Table 1) with solitary, slightly elongated cells. This group of three genera is phylogenetically separated from other coccoid and filamentous cyanobacterial taxa (including Gloeobacteraceae and Synechococcaceae) based on the phylogeny constructed from 260 concatenated genes (Fig. 2) and must be described as a special family of Synechococcales.

Prochlorococcaceae Komárek et Strunecký fam. nov.
Description: Unicellular photosynthetic prokaryotes with elongated cells and with 1–4 parietally arranged thylakoids, without gelatinous envelopes. Reproduction by binary fission of cells, perpendicular to the long axis of the cell. At present this family comprises the genera *Prochlorococcus*, *Parasynechococcus* and *Cyanobium*, which differ mainly by phylogenetic markers, by modifications in arrangement of thylakoids in cells and partly by ecology (marine vs. freshwater ecosystems).
Type genus: *Prochlorococcus.*

***Prochlorococcus* Chisholm, Frankel, Goericke, Olson, Palenik, Waterbury, West–Johnsrud et Zettler gen. nov.**
Description: Oxygenic photoautotrophs possessing divinyl chlorophylls–a and –b as their principal photosynthetic pigments. Cells lacking mucilage, spherical to rod shaped just prior to division, cells individual or in pairs, dividing by transverse binary fission, with peripheral photosynthetic thylakoids closely appressed to one another.
Ecology: mostly marine, planktic species.
Type species: *Prochlorococcus marinus*.
Etymology: Latin *Pro*=before, *chlorococcus*=green coccoid, named for the presence of chlorophyll–b, and idea that this taxon could be the predecessor of chloroplasts in green algae.

***Prochlorococcus marinus* Chisholm, Frankel, Goericke, Olson, Palenik, Waterbury, West–Johnsrud et Zettler sp. nov. (Chisholm et al. 1988; Chisholm et al. 1992) (Fig. 3B)**
Description: Cells 0.6 to 0.8 μm in diameter, 1.2 to 1.6 μm in length just prior to division. – Habitat: Open ocean waters in subsurface chlorophyll maximum layer.
Type locality: The reference strains for this species (upon which the holotype is based) were isolated from the Sargasso Sea in May 1988 (28°58.9'N, 64°21.5'W).

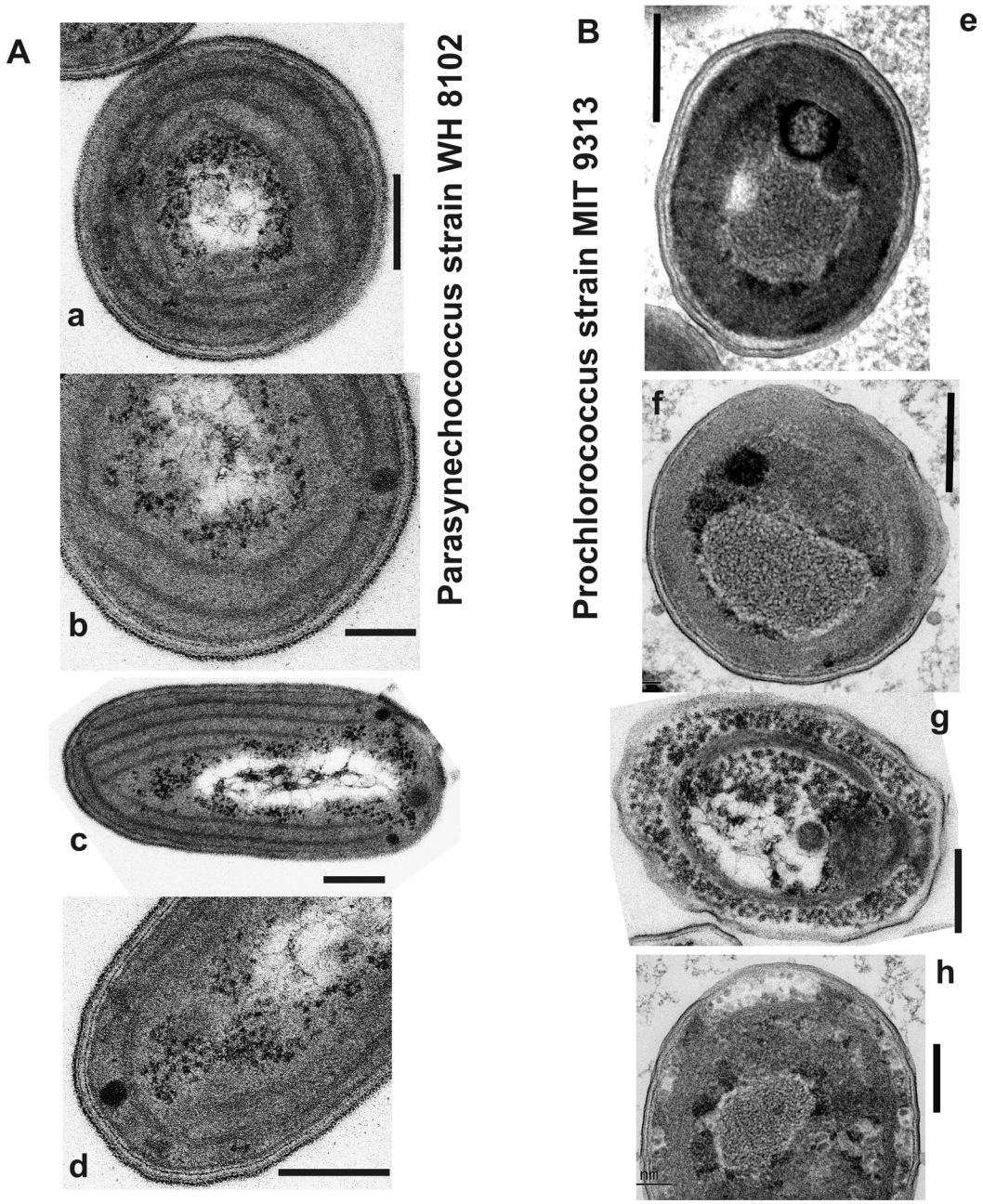

Fig. 3. Comparison of the ultrastructure of cells: (A) *Parasynechococcus marenigrum* (a–d, strain WH 8102); (B) *Prochlorococcus marinus* (e–h, strain MIT 9313). Scale bars 100 nm (b), 200 nm (a,c–h).

Holotype here designated: CBFS A–122–1, herbarium material preserved by drying biomass of the strain MIT 9313 is deposited at the Herbarium of the University of South Bohemia.
Additional strains: *Prochlorococcus marinus* CCMP–1375, CCMP–1378.
Etymology: *marinus* = *marine.*

Prochlorococcus is a picoplanktic and oceanic autotrophic cyanobacterium originally found by Chisholm et al. (1992) from the Sargasso and Mediterranean Seas and cited also in Bergey´s Manual. The only described species is *Prochlorococcus marinus* (in spite of the divarication in phylogenetic schemes, e.g., in Fig. 2, which suggest at least two taxa). Its ecotypes and ecological variability were already characterized by Charpy & Blanchot (1999), Furnas & Crosbie (1999), Hess & Schön (1999), Jiao & Yang (1999), Partensky et al. (1999), Rocap et al. (1999), and others. The ultrastructure has some specific features in comparison to the morphologically similar *Parasynechococcus* (Fig. 3A). Strain CCMP–1375 was designated as the type strain by Chisholm et al. (1992), but in her protologue Chisholm indicates that neither CCMP–1375 or CCMP–1378 were axenic, meaning they could not serve as types under the ICNP (Rule 18a). Also, the descriptions were not published in IJSEM nor accepted to the list of names in IJSEM, a requirement for

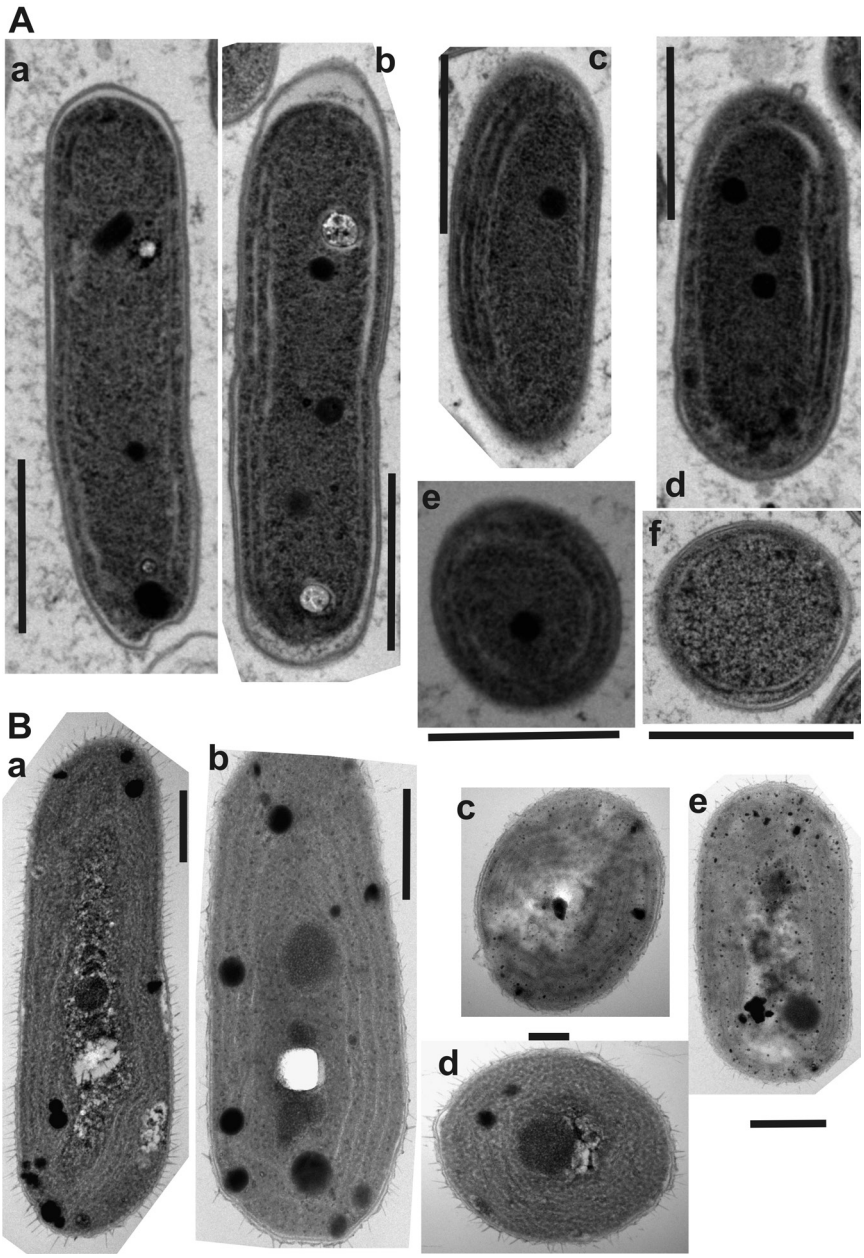

Fig. 4. Ultrastructure of cells: (A) *Synechococcus elongatus* strains PCC 6301 (CCALA 188), scale bars 1000 nm; (B) *Picosynechococcus fontinalis* strain FG–1, scale bars 500 nm (a,b,e), 200 nm (d).

valid publication under the ICNP (Rule 27). A strain cannot serve as holotype under the ICN (Article 8.4), unless it is immobilized (chemical preservation, drying, lyophilisation, or cryopreservation). Their description also violated Article 44.1, which requires a Latin description or diagnosis between December 31, 1958 and December 31, 2011. For these reasons, *Prochlorococcus* was not validly published under either nomenclatural code. We describe *Prochlorococcus* as a new genus here so that it will be nomenclaturally valid, serving the requirement in both codes for higher level taxa to be rooted to a genus name. *Prochlorococcus* was never considered to belong to *Synechococcus* as its unique pigmentation was recognized soon after it was isolated into culture.

The eight strains of *Prochlorococcus* in our analysis (Fig. 1) had variable genetic identities (97.8– 100%). According to Yarza et al. (2014), the threshold sequence identity for recognition is ≤98.7%, indicating that more than one species could be described even they are morphologically cryptic. Interestingly, the original reference strain, *P. marinus* CCMP–1375, is below the 98.7% threshold in comparison to all other strains. Strains MIT 9313 and MIT 0801 are also below the threshold in comparison to the rest of the strains, with the other MIT strains forming a tight group with all being ≥ 99.7% similar to each other. The TEM images of *P. marinus* CCMP–1375 show 3–5 concentric rings of thylakoids (Chisholm et al. 1988; Chisholm et al. 1992) in comparison to only 2 concentric rings in

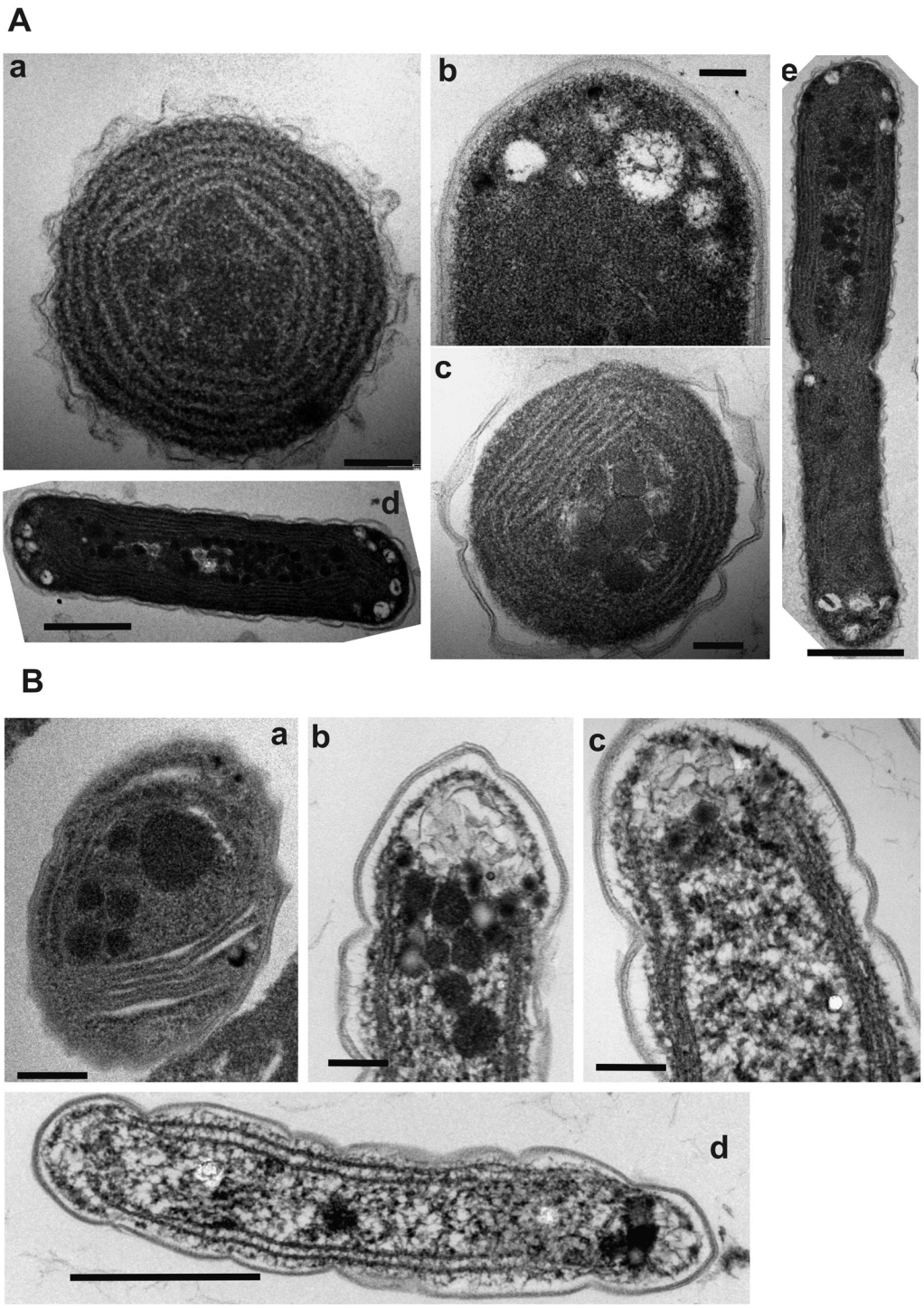

Fig. 5. Ultrastructure of cells: (A) *Thermosynechococcus vestitus* strain NIES 2133, scale bars 200nm (a,b,c), 1 µm (d,e); (B) *Thermostichus vulcanus* strain RUP –VU– 1, scale bars 200 nm (a,b,c), 1 µm (d).

Prochlorococcus sp. MIT 9313 (Fig. 3B). This coarse analysis would be consistent with the recognition of four species in *Prochlorococcus*, but defining these species at this point is beyond the scope of this paper.

Parasynechococcus (Coutinho et al. 2016; PeerJ 4:e1522, nomen inval.; *Synechococcus* sensu auctorum – Partensky et al. 1999; Partensky & Garczarek 2010; Scanlan 2012; Six et al. 2007; non *Synechococcus* Nägeli 1849) was isolated from marine (oceanic) picoplanktic populations of "*Synechococcus*" and is different from specimens of freshwater habitats according to molecular analyses (Brahamsha 1999), morphology (they are relatively smaller) and according to details of ultrastructure. These populations must be classified in the taxonomic group together with *Prochlorococcus* and typical *Cyanobium* (Figs 1, 2).

The genomic phylogenetic and ultrastructural

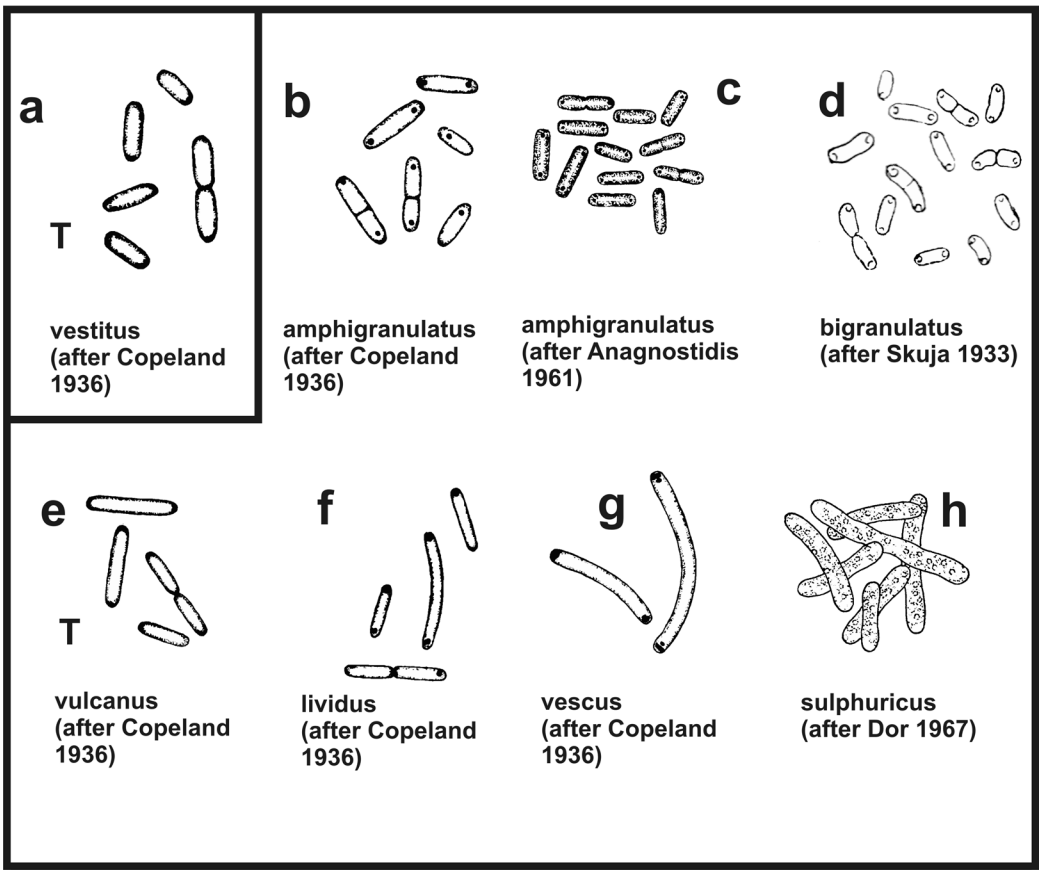

Fig. 6. Morphology of selected characteristic species of extremely thermophilic "*Synechococcus*"–types with solitary cylindrical cells and ± with polar granules, which belong mostly to the thermophilic genera *Thermosynechococcus* (a = *T. vestitus*, type of the genus) and *Thermostichus* (*T. vulcanus* marked by "T" – 6e is a type species of the genus). Selected from various authors.

comparison was made between strains of the *Prochlorococcus marinus* strain MIT9313 and *Cyanobium* strain WH5701 (Fig 1, Table S2) and the formal descriptions of the separate genera of marine picoplanktic *Synechococcus*–like populations are therefore necessary. Coutinho et al. (2016 a, b) published the separate cluster with support of phylogenetic position and used the name *Parasynechococcus* for oceanic "*Synechococcus*" populations, but this description is not valid according to both the bacteriological and botanical Codes. Therefore, we validate this genus with the names, proposed by the original authors, who kindly expressed their agreement with this validation.

***Parasynechococcus* Coutinho, Tschoeke, Thompson et Thompson gen. nov. (Fig. 3A)**

Description: Planktic, marine, solitary picocyanobacterial cells without prominent slime envelopes, subspherical up to slightly elongated and short rod–like, maximally 2.5 μm long, with 1–4 parietally arranged thylakoids, without gas vesicles. Reproduction by ± symmetrical binary fission, perpendicular to the longer axes of the cells, in one division plane in the following generations.

Type species: *Parasynechococcus marenigrum* Coutinho, Tschoeke, Thompson et Thompson.

Etymology: Gr. *Para* = similar to; *Parasynechococcus* = similar to *Synechococcus*.

***Parasynechococcus marenigrum* Coutinho, Tschoeke, Thompson et Thompson sp. nov. (Fig. 3A)**

Syn.: *Synechococcus marinus* C.–C. Jao 1948 nom. illeg., non *Synechococcus marinus* Ercegović 1932.

Description: Cells planktic, marine, solitary, (0.4)0.6–1.2(2.1) μm long, 0.4–0.8 μm wide. Mucilaginous envelopes absent or facultatively forming a very narrow, diffluent and indistinct layer around individual cells. Cytoplasm with (1)2–4 parietal thylakoids, each a little distant from one another, with white bodies between thylakoids in the cells probably corresponding to polyphosphate inclusions, with a few small cyanophycin granules and numerous ribosomes in the centroplasma (Fig. 3A). Cyanophages sometimes seen adsorbed on the cell wall.

Habitat: Marine/oceanic picoplankton from tropical to subpolar zones (type locality: Sargasso Sea). – Holotype here designated: CBFS A–094–1, herbarium material preserved by drying biomass of the reference strain, WH 8102, deposited in the herbarium collection of the University of South Bohemia.

Reference strain: *Parasynechococcus marenigrum*

WH 8102, previously known as *Synechococcus* sp.
Etymology: referring to the dark water of open ocean in the Sargasso Sea.

The isolated marine (oceanic) picoplanktic populations of "*Synechococcus*" are different from specimens of freshwater habitats according to molecular analyses (Brahamsha 1999), morphology (they are relatively smaller), and details of ultrastructure. The reference strain upon which our designated holotype is based has been the subject of numerous papers on the ecology and biochemistry of this taxon (Moore et al. 2005; Su et al. 2006; Iancu et al. 2007; Six et al. 2007; Tai & Palenik 2009; Tetu et al. 2009; Post et al. 2011, etc.). The genome for WH 8102 was sequenced by Palenik et al. (2003), and is presented in NCBI as *Synechococcus* sp. WH 8102 (NC_005070.1).

The cellular structure of oceanic "*Synechococcus*" (*Parasynechococcus*) was described by Scanlan (2012). The ultrastructural markers and differences between oceanic *Parasynechococcus* and *Prochlorococcus* are in the cell size, cell wall structure, and thylakoid position (Fig. 3). The ecology of the type species *Parasynechococcus marenigrum* is diagnostic; it forms a more or less homogeneous suspension in upper layers of oceanic water.

Walter et al. (2017) proposed new generic names (not validly described) for several species of Prochlorococcaceae, but without clear markers and justification (*Parasynechococcus*: *Pseudosynechococcus, Regnicoccus, Magnicoccus, Inmanycoccus; Prochlorococcus*: *Eurycolium, Prolificoccus, Thaumococcus).* The acceptance of these generic names (and many associated species) needs surely more detailed study and formal descriptions according to nomenclatural rules.

Coutinho et al. (2016b) described 15 species of *Parasynechococcus*, all of which were invalid due to typification issues under both codes of nomenclature. Their identification is problematic as these species were identified based on genetic data only, with insufficient morphological characterization. They must be confirmed and validated in future studies. Most of the taxa, which they recognized as separate species based on genomic data were above the threshold sequence value (98.7% similar) for recognition of separate species. Other species will probably be defined within the genus *Parasynechococcus*. *Synechococcus carcerarius* and several described *Cyanobium* species also possibly belong to this genus.

The genus *Cyanobium* was proposed in 1983 and the position of the type species (separated from *Synechococcus*) was confirmed by later phylogenetic as well as our analyses. However, numerous other species were described or combined based only on simple morphology (Komárek & Anagnostidis 1998, Komárek 1999) and more than 25 distinguishable taxa are mentioned in the literature. The final position of later *Cyanobium*–taxa must be confirmed and revised; several of them have a specialized ecology (several marine species belong possibly to the genus *Parasynechococcus*). This concerns also several taxa defined only morphologically, such as *Synechococcus ferrunginosus* or *S. vantieghemii*.

***Cyanobium* Rippka et Cohen–Bazire**
Emended description: Cells solitary, lacking sheaths, without prominent gelatinous envelopes, but producing a thin layer of diffluent mucilage visible with staining by India ink, shortly oval to rod–shaped; cells occurring singly or in pairs, pale blue–green, with homogenous cell content (to 2–4 μm long), dividing by perpendicular symmetrical transverse binary fission, with few parietal photosynthetic thylakoids, not closely appressed to one another, with accessory pigments zeaxanthin and α–carotene.
Type species: *Cyanobium gracile.*

***Cyanobium gracile* Rippka et Cohen–Bazire (Figs 1,2; Table 1)**
Emended description: Cells solitary, without enveloping sheaths, but with thin layer of diffluent mucilage, with homogenous pale blue–green cytoplasm, 0.4–2.4 μm long, 0.2–1.0 μm in diameter.
Habitat: *C. gracile* thrive probably in freshwater lakes, but it was also found in brackish waters.
Type locality: lake in Wisconsin, USA, sampled in 1949.
Reference strain: *Cyanobium gracile* PCC 6307 (=UTEX 1548), additional strains: *Cyanobium* sp. PCC 6904, PCC 7009, PCC 7918, NIES 981.
Etymology: *gracile* = thin, slender.

Order Pseudanabaenales, family Thermosynechococcaceae
Phylogenetic analyses separated the thermophilic, *Synechococcus*–like genus *Thermosynechococcus* (also with rod–like cells) from the Synechococcaceae (Figs 1, 2). The position of this type is closer to the family Pseudanabaenaceae in our 16S rRNA gene phylogeny (Fig. 1), but belongs to a clade isolated from both the Pseudanabaenales and Synechococcales in our multi–locus phylogenetic tree (Fig. 2). We provisionally follow its placement into an order using more common 16S rRNA gene phylogeny, however we expect its possible change after inclusion of additionally sequenced genomes in future. It represents evidently a special family Thermosynechococcaceae, characterised by solitary cells, which form very rarely short filaments (composed from 2–4 connected cells).

Thermosynechococcaceae Komárek, Strunecký et Johansen fam. nov.
Description: Unicellular photosynthetic prokaryotes with rod–like cells and with 1–4 parietally arranged thylakoids, without or with very slight, colourless and

diffluent gelatinous envelopes. Reproduction by crosswise, sometimes asymmetrical cell division. Living solitary or in irregular groups. The family is currently comprised of only one genus, *Thermosynechococcus* gen. nov.

The genus *Thermosynechococcus* (Figs 1, 2, 5A, 6a; Tables 1, S1, S2) was proposed by Katoh et al. (2001) and separated from the traditional *Synechococcus sensu stricto,* mainly according to ecological criteria (Katoh et al. 2001), because all strains and species were known from thermal waters with higher temperatures. This group was found usually at 20–40(–70) °C and it was characterized also by specific ecophysiological and biochemical features (Katoh et al. 2001). However, the thermophilic "*Synechococcus*"–group contains at least two distinct clusters in the phylogenetic schemes, and therefore must be described in two generic taxa, *Thermosynechococcus* and *Thermostichus* (see earlier treatment in Thermostichaceae; Figs 1 and 6). The genus *Thermosynechococcus* was not validly published (no description, no type, etc.) and must be validated (with agreement of original authors).

***Thermosynechococcus* Katoh, Itoh, Shen et Ikeuchi gen. nov. (Figs 1,6a)**

Syn.: *Thermosynechococcus* Katoh et al. 2001, nom. invalid.

Generic description (validation): Cells solitary or in irregular groups (or strata on the substrate), without prominent slime envelopes, oval, cylindrical, straight or slightly curved, with ± homogeneous content, rounded or truncated at the ends, (3.0)4.0–17.0(32.0) µm long and 1.1–3.0(4.8) µm wide, with parietal thylakoids. The cell length is variable, sometimes also within the same population. The cells sometimes remain in short, few–celled, filamentous–like formations. Reproduction by binary fission, perpendicular to the long axis of the cells, occasionally in non–morphologically equal parts (asymmetrically).

Habitat: periphytic in thermal localities, benthic, usually in ± 20 up to 65(–75.8) °C.

Type species: *Thermosynechococcus vestitus* (syn. *"Thermosynechococcus elongatus"* sensu Katoh et al. 2001).

Etymology: *Thermo*: living in thermal waters.

***Thermosynechococcus vestitus* (Copeland) Komárek et Strunecký comb. nov.**

Basionym: *Synechococcus elongatus* var. *vestitus* Copeland, Ann. N.Y. Acad. Sci. 1936: 60, 1936 (Fig 5A)

Neotype here designated: Copeland 1936, p. 59, fig. 24 (under *Synechococcus elongatus* var. *vestitus*). Because Copeland (1936) and Katoh et al. (2001) did not deposit any type material, we designate Copeland´s figures (1936, fig. 24) as neotype (created prior to 1st January 2007).

Epitype here designated: CBFS A–120–1, dried material of the strain BP–1 (NIES 2133), deposited in the herbarium collection of the University of South Bohemia.

Reference strain: *T. vestitus* BP–1.

Katoh et al. (2001) designated the type species as „*Thermosynechococcus elongatus*", based on the fact that the strain was originally identified as *Synechococcus elongatus*, but was found to not belong to that genus based on phylogenetic evidence. This would confuse the typification of the genus, as the type species for *Synechococcus* cannot serve as a basionym for the type species of *Thermosynechococcus*. For this reason we do not make a new combination for *S. elongatus*, but rather have chosen Copeland's species *S. vestitus* as the basionym and neotypify and epitypify the species here.

The genus *Thermosynechococcus* may include (as distinct species) several varieties of various "*Synechococcus*" taxa from moderately thermophilic and saline habitats, or unsatisfactorily known species not occurring in extremely thermophilic habitats, such as *S. amethystinus* Copeland, *S. elongatus* f. *thermalis* Geitler, *S. brunneolus* Rabenhorst, *S. koidzumii* Yoneda, *S. marinus* Jao, *S. minutissimus* Negoro, *S. rayssae* Dor, or *S. sigmoideus* (Moore et Carter) Komárek. The position of morphologically similar freshwater and planktic *Synechococcus capitatus* Bailey–Watts et Komárek described from lakes in Scotland is taxonomically still unclear.

Order Chroococcales, family Aphanothecaceae

Few strains, morphologically similar to the genus *Synechococcus*, represent an isolated cluster near the family Chroococcaceae. The ultrastructure (irregular position of thylakoids) corresponds also to members of this family. Based on ultrastructural and genetic features this group must be therefore described as a separate genus (*Picosynechococcus*, Fig. 1). The family Aphanothecaceae belongs into this order by molecular position and ultrastructure, and contains mainly genera with elongated cells.

Picosynechococcus genus novum (not benthic and not oceanic planktic "*Synechococcus*"– like species) (Figs 1, 2, 4B; Tables S1, S2) has a cryptic character in comparison with other *Synechococcus*–like genera. It contains several freshwater, but not benthic strains, originally classified (according to simple morphology of unicells) to Synechococcaceae (e.g., PCC 7002, PCC 7117, CCMP 1630, FG–1, and others), but genetically situated in the vicinity of various species from Chroococcales. It must be described taxonomically as a distinct generic entity (Figs 1, 2; Table S2) with characteristic (=type) species, *Picosynechococcus fontinalis.* Strains from this clade are characterized by solitary, small and slightly elongated cells and prefer (according to known data) a higher content of mineral salts in their environment (possibly occur rarely also in slightly saline waters). The strain PCC 7002 was

designated by Walter et al. (2017) as a special genus *Enugrolinea* (and the species *E. euryhalinus*), but without valid description.

Picosynechococcus **Komárek, Johansen et Strunecký gen. nov. (Figs 1, 2, 4B; Tables 1, S1, S2)**
Description: Cells solitary, without prominent mucilaginous envelopes, slightly elongated and oval, rounded at the ends, sometimes very slightly curved, with homogeneous, blue–green or greenish content, motile, with ± parietal, a little irregularly organized and waved thylakoids, without gas vesicles. The cells are connected rarely freely in short (few–celled) filamentous formations. Reproduction by cell binary fission in one plane, perpendicular to the longer axis of the cell.
Habitat: Planktic and in periphyton in watery localities in mineral springs, sometimes with higher content of salts, rarely also in the littoral of seas.
Type species: *Picosynechococcus fontinalis.*

Picosynechococcus fontinalis **Komárek, Johansen et Strunecký spec. nov. (Figs 1, 2, 4B)**
Description: planktic, periphytic, or metaphytic , solitary, slightly oval cyanobacterial cells with few parietal thylakoids and ± homogeneous content, 1.2–3.0 × 0.8–2.0 μm.
Locus classicus: Bulgaria, Rupite, in small, freshwater bodies in the area of warm, mineral pools.
Holotype here designated: strain FG–1 (type and reference strain), original dried herbarium specimen, deposited in herbarium collection of the University of South Bohemia, under the designation CBFS A–093–1.
Etymology: pico = extremely small *Synechococcus*–like type from springs.

Summary

Picocyanobacteria living as solitary cells have increasingly been observed in various planktic habitats often contributing significantly to their primary production appeared in the last few decades, in both freshwaters (Komárek 1996; Becker et al. 2004; Callieri et al. 2012) and marine waters (Rabouille et al. 2007; Zwirglmaier et al. 2008; Martiny et al. 2009; Flombaum et al. 2013), particularly in tropical ecosystems.

They represent a few diversified groups, and their correct evaluation and taxonomic designation had to be revised and modified in agreement with combined cytomorphological, molecular, ecological, metabolic and physiological studies (polyphasic evaluation). Similar situation exists within morphologically similar thermophilic species which have been placed originally in *Synechococcus*.

Additional methods including molecular analyses and ecological characteristics were introduced into the taxonomic classification of cyanobacteria during the last few decades of the 20th century (Komárek 1996; Herrero & Flores 2008; Dvořák 2017; and others). Respecting the phylogenetic and ecological criteria, morpho– and ecotypes corresponding to the traditional taxonomic definition of the genus *Synechococcus* belong to several different genera (Fig. 2, Table 1) and evidently also to various higher taxonomic categories (families, orders). The recent genetic characterization of many *Synechococcus*–like species raised the necessity for correcting their taxonomic position. The generic name *Synechococcus* must be used only in accordance with the type species of this genus (which is the freshwater, benthic type from Switzerland, based on *S. elongatus*) and related taxa. All other, morphologically similar, but genetically (and often also ecologically) distant populations must be separated from this genus (e.g., *Neosynechococcus* was already described and belongs to the family Leptolyngbyaceae).

The genera *Cyanobium, Prochlorococcus* and *Parasynechococcus* form a lineage quite distinct from other cyanobacterial lineages based on multilocus analysis (Figs 1, 2). These three similar, simple, unicellular and mostly picoplanktic genera with minute solitary cells, which develop often under oligotrophic to slightly eutrophised conditions in oceans, are here classified as an isolated family of simple cyanobacteria (Prochlorococcaceae, Fig. 1, Table 1).

The specificity of a marine "*Synechococcus*" was mentioned already by Waterbury & Rippka (1989), who characterized this cluster by simple solitary picoplanktic, often motile cells. In these types was recognized sometimes reddish cell content and chromatic adaptation (Six et al. 2007). The fact that marine *Prochlorococcus* (Chisholm et al. 1992), *Parasynechococcus* (Coutinho et al. 2016) and freshwater *Cyanobium* (the original strain) are phylogenetically closely related one to another and separated from the typical *Synechococcus*, was predicted already by Castenholz (2001) and also confirmed previously by multi–genomic analysis of Schirrmeister et al. (2011), who designated this group as a separate, extensive phylogenetic cluster. Members of Prochlorococcaceae are some of the most abundant cyanobacteria found on Earth. Marine picocyanobacteria dominate and are well adapted to the spatial, light and trophic conditions of large areas of the seas and oceans (Rabouille et al. 2007; Zwirglmaier et al. 2008; Martiny et al. 2009; Flombaum et al. 2013). Due to their importance in oceanic ecosystems, many efforts have been carried out to understand their function and adaptations. Hence, while the taxonomic validity and particularly the morphological generic classification of marine "*Synechococcus*" was problematic, this name was widely used in the descriptions of almost all marine picocyanobacteria (Lindell et al. 1999; Yin & Henley 1999; Castenholz 2001; Herrero & Flores 2008). However, the ecophysiological and genetic

markers of "marine *Synechococcus*" were described also by many other authors (Moore & Chisholm 1999; Partensky et al. 1999; Rocap et al. 1999; Palenik et al. 2003; Rabouille et al. 2007; Zwirglmaier et al. 2008; Martiny et al. 2009; Mena et al. 2016), who also support the separation of these types from the typical *Synechococcus.*

In comparison with other cyanobacteria, the picocyanobacterial strains of *Prochlorococcus* have the shortest cyanobacterial genome with a range from 1.64–2.68 Mbp (Kettler et al. 2007; Biller et al. 2014) and an upper limit of ~ 3 Mb (Dufresne et al. 2003; Ting et al. 2009) while other genomes of other sequenced cyanobacteria are slightly larger and span from 4 to 11 Mb. Other genera from Prochlorococcaceae have probably more genes in their slightly larger genomes: *Parasynechococcus* (= marine *"Synechococcus*": 2.22–2.86 Mbp) and *Cyanobium gracile* (PCC 6307: 2.83–3.34 Mbp). They also belong to the smallest cyanobacteria in terms of cell size. Minimalization of the genome is considered to be an adaptation of *Prochlorococcus* to the oligotrophic conditions of the ocean, where this plankter is found (Partensky & Garczarek 2010). Small cell size brings other physical advantages, such as a smaller sedimentation rate and larger surface to volume ratio, both worthwhile properties in aquatic and highly competitive microbial environments. Oceanic *Prochlorococcus* and *Parasynechococcus,* specialized for marine oligotrophic environment (Shibata et al. 2001; Dufresne et al. 2003; Garcia–Fernandez & Diez 2004; Ting et al. 2009; Rae et al. 2013) are characterized also by specific metabolic processes and physiological and biochemical modifications (e.g., carbon fixation and nitrogen metabolism pathways). Various other features are outside the main scope of this paper, such as the presence of different type of carboxyzomes than in the other cyanobacteria, different metabolism of amino acids, different membrane ion channels, different ATP synthase etc. (Chisholm et al. 1992; Dufresne et al. 2003; Zhang & Bryant 2011; Yu et al. 2012; Mena et al. 2016). Several morphologically similar and taxonomically validly described picocyanobacteria from both marine and freshwater habitats, which were earlier included by Komárek & Anagnostidis (1998) into to genus *Cyanobium* (*Cyanobium waterburyi, C. oceanicum, C. bacillare,* etc.) also belong probably to this group, but their detailed taxonomic (especially molecular) evaluation is still mostly lacking. We consider the genomic phylogeny more reliable than the 16S rRNA gene phylogeny (Fig. 1), which places the Prochlorococcaceae next to the Synechococcaceae (Table 1).

The genus *Gloeobacter*, lacking thylakoids and considered to be the most primitive cyanobacterium due to its cytomorphological simplicity (Rippka & Cohen–Bazire 1983; Mareš et al. 2013b), is morphologically also similar to Prochlorococcaceae. It can be found also at the base of 16S rRNA trees, more or less parallel to the Prochlorococcaceae (Fig. 2), however, the absence of thylakoidal membranes classifies it taxonomically outside of this group. It is interesting that a new discovered, highly thermophilic genus *Thermostichus,* in cells of which were detected few parietal thylakoidal membranes, belongs genetically to the vicinity of *Gloeobacter.* However, the phylogenetic and cytomorphological differences between both these genera are so important that *Thermostichus* must be classified in a specially family and order.

The freshwater genus *Picosynechococcus* contains also very small planktic picocyanobacteria (Fig. 1), which were often overlooked in floristic studies probably due to small size of solitary living cells and relatively low numbers in small water bodies. It must be compared with populations described as "*Synechococcus* spp." from large inland lakes (Ernst et al. 2003; Becker et al. 2004), and this genus occurs also, e.g., in relatively undisturbed mineral and hot springs and pools in Rupite, Bulgaria, where it lives in temperatures between 20 to 40 °C. Ecology and other physiological features of such types should be studied more in the future.

The ecologically interesting thermophilic *Synechococcus*–like cyanobacteria with cylindrical cells have been divided into several phylogenetic groups (Figs 1, 2) and validated. The first problem was that the already defined genus *Thermosynechococcus* was not validly described in Katoh et al. (2001) and the authors did not define clearly the type species. Moreover, *Thermosynechococcus* sensu Katoh et al. (2001), growing in the temperature range ± 20(?)–65(75,8) °C is different phylogenetically from the other extremely high thermophilic types (e.g., strains JA 33Ab and JA 23Ba) with long, strictly cylindrical cells growing possibly (according to Copeland 1936) up to more than in 80 °C (comp. the genus *Thermostichus* , Figs 1, 5B, 6). We validate therefore the genus *Thermosynechococcus* in the sense of Katoh et al. (2011) with necessary selection of the type species, with respect to the insufficient description and typification of the genus in the original paper of Katoh et al. (2001) and describe *Thermostichus* as a new generic entity. The genus *Thermosynechococcus* was studied by several authors under *"Synechococcus"* (e.g., the complete genome structure of the strain BP1 was described by Nakamura et al. 2002). The authors of this generic entity (Katoh et al. 2001, p. 600) write that they re–named "*Synechococcus vulcanus* from Japanese hot springs in Yanomine as *Thermosynechococcus vulcanus*", but also they mention the "thermophilic cyanobacterium *Thermosynechococcus* (formerly *Synechococcus*) *elongatus* strain BP–1 from a hot spring in Beppu, Japan", and with the species *Thermosynechococcus vulcanus* and "*T. elongatus*" sensu Katoh, which belong in separate clades. However, *Synechococcus elongatus*, as the type species of the genus *Synechococcus*

is not thermophilic and "*Synechococcus elongatus* sensu Yamaoka et al. (1978), strain BP–1" (and sensu Katoh et al. 2001) must be classified as a separate species of the genus *Thermosynechococcus*, different from Nägeli´s (1849) type (cf. Fig. 2), in spite of the similar specific epithet ("*elongatus*"). We validate therefore the genus *Thermosynechococcus* in the sense of Katoh et al. 2011 (with his kind agreement) and Copeland (1936, under the generic name *"Synechococcus*"), with necessary selection of a described species for the type (*T. vestitus*) with respect to the insufficient description and typification of the genus in the original paper of Katoh et al. (2001).

Both thermophilic genera *Thermosynechococcus* and *Thermostichus* (phylogenetically different and classified in different orders) belong probably to the phototrophic organisms (cyanobacteria), adapted to the highest possible temperature acceptable for phototrophic life in the biosphere and could be used therefore as convenient ecological models for experimental studies. However, the adaptations and limits to high temperatures must be studied more in detail, both in nature and in culture; it is possible that their metabolism is very different in various populations and strains.

This paper is the next step in the taxonomic characterization and classification of *Synechococcus*–like cyanobacteria and briefly describes their phylogenetic relations to other cyanobacterial groups. The cyanobacterial genus *Synechococcus* is morphologically simple, and was thought to be broadly distributed in diverse habitats. Phylogenetic analyses and physiological studies indicate that the genus in the present concept must be actually classified at least in seven different genera with fairly specific ecological requirements. Many of strains that are phylogenetically distinct from *Synechococcus* have been important models for physiological and biochemical studies. Unfortunately, early attempts at classification were unsuccessful at validly describing these interesting organisms. This manuscript validates these taxa using the names by which they are known in the literature and recognizes earlier authors as the authors of the genera.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Review of the main described and revised *Synechococcus*–like cyanobacteria, selected from the literature.

Table S2. Taxonomic position of sequenced synechococcoid strains.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)

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