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Research Article

RESEARCH ARTICLE

Diversity of cyanobacteria at the Alaska North Slope with description of two new genera: *Gibliniella* and *Shackletoniella*

Otakar Strunecky[1,*,†], Lenka Raabova[2], Alexandra Bernardova[3], Anna Pavlovna Ivanova[1], Alexandra Semanova[2], Joel Crossley[3] and David Kaftan[3]

[1]University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, CENAKVA, Institute of Aquaculture and Protection of Waters, Na Sádkách 1780, 370 05 České Budějovice, Czech Republic, [2]Department of Biology, University of Ss. Cyril and Methodius, J. Herdu 2, 917 01 Trnava, Slovak Republic and [3]Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic

*Corresponding author: University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, CENAKVA, Institute of Aquaculture and Protection of Waters, Na Sádkách 1780, 370 05 České Budějovice, Czech Republic. Tel: +420 387776230; E-mail: otakar.strunecky@gmail.com

One sentence summary: Novel cyanobacteria from the Alaskan North Slope.

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†Otakar Strunecky, http://orcid.org/0000-0001-9735-4662

ABSTRACT

The diversity of cyanobacteria along the Alaskan North Slope was investigated. We isolated and cultivated 57 strains of cyanobacteria and sequenced a section of their rRNA operon containing a fragment of the 16S rRNA gene. Here, we describe 17 found species belonging mainly to families Coleofasciculaceae, Microcoleaceae, Oculatellaceae, Leptolyngbyaceae and to the order Synechococcales. In pursuing a conservative polyphasic approach, we utilized suggested thresholds in 16S rRNA gene differences in parallel with morphological differences between new and already described taxa for the description of new species and genera. Based on a combination of morphological, molecular and ecological analysis of collected and cultured strains we describe two genera *Gibliniella* and *Shackletoniella* as well as six cyanobacterial species; *Cephalothrix alaskaensis, Tildeniella alaskaensis, Pseudophormidium americanum, Leptodesmis alaskaensis, Albertania alaskaensis* and *Nodosilinea alaskaensis*. Here, a polyphasic approach was used to identify eight novel and nine established cyanobacterial taxa from a previously non–investigated region that uncovered a high degree of biodiversity in extreme polar environments.

Keywords: Alaska; cyanobacteria; *Gibliniella*; morphology; new taxa; *Shackletoniella*

INTRODUCTION

Due to high ecological valence cyanobacteria thrive in many extreme habitats (Whitton 2012). Cyanobacteria are well adapted to freezing and desiccation while being capable of restoring an active metabolism in minutes following their unfreezing or rehydration (Rajeev *et al.* 2013). Cyanobacteria are often regarded as dominant primary producers in polar regions. This is especially true in polar deserts (Vincent 2002) and glaciers

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(Anesio *et al.* 2017). Cyanobacteria play an important role in carbon and nitrogen economy in polar soils (Vincent 2002) and they are the main nitrogen fixers both as free-living cells or as a mutualistic part of lichen or in moss–cyanobacterial associations (Chapin and Bledsoe 1992).

The diversity of cyanobacteria is still highly underestimated as can be deduced from recent studies in previously less-explored environments (Powell *et al.* 2005; Sciuto, Moschin and Moro 2017; Mai *et al.* 2018). More recently, next generation sequencing (NGS) has been used extensively with the aim to accurately describe cyanobacterial diversity in particular ecosystems. NGS is undoubtedly an essential tool to uncover the biological diversity, history and processes of natural habitats (e.g. Monchamp *et al.* 2016). However, NGS results often provide a high number of abstract organizational taxonomic units (OTUs), higher taxonomical groups or a pure description of alpha diversity (e.g. Staley *et al.* 2013). A combination of NGS methods with classic phytological determination approaches (including, but not limited only to, detailed light microscopy observations of various life stages) results in the most accurate estimation of real numbers of species residing on site (Strunecky *et al.* 2019). This approach however requires employing extensive and lengthy observations and often does not provide answers about metabolomic properties of the studied taxa.

Classic phycology approaches based on microscopic evaluation of microalgal morphologies and cultivation of strains that are complemented by their basal sequencing hold strong potential for bringing new knowledge about community structure and diversity (Bohunická *et al.* 2015; Strunecky *et al.* 2017). New discoveries of species diversity is important for many previously non-investigated environments, particularly in habitats where climatic changes pose a risk to future sustainability. Here, we isolated and cultivated eight novel and nine previously identified cyanobacterial species from the Alaskan North Slope. This research provides a foundation for further detailed ecological, biogeographical and genomic studies.

MATERIAL AND METHODS

Sampling

A total of 111 samples were taken at the Alaska North Slope. The sampling area was limited by the Atigun Pass to the south and Prudhoe Bay to the north spanning 240 km and was ∼10 km eastwards or westwards away from Dalton Highway (Fig. 1). Samples were taken from freshwater environments (i.e. rivers, streams, lakes, pools), soil and bare rock. The geographic position and biotopes of cultivated strains are indicated in Supplementary Table S1, see online supplementary material. Cyanobacterial mats were sampled to Nalgene 1.8 mL cryogenic tubes in duplicates. For several hours after sampling one tube was put on ice and kept there at low light until cultivation. A second tube was frozen at –20°C for transportation and storage. The majority of samples were determined from first subsamples on site using a Zeiss Axioskop Upright Fluorescence Microscope at the Toolik Field Station (Institute of Arctic Biology, University of Alaska, Fairbanks, AK, USA, 68° 37' 39.11'' N, 149° 35' 51.93'' W).

Cultivation

A total of 66 samples with cyanobacteria previously confirmed by optical microscopy were cultivated on Petri dishes and in test tubes in Z medium (Zender in Staub 1961) solidified by 2% agar and in the mineral nutrient medium BG11 (Rippka *et al.* 1979) solidified by 1.5% agar. Cultures were maintained in a growth chamber with a 12:12 hour light:dark photoperiod provided by cool-white fluorescent illumination (4400 lx, PAR 12 Wm^{-2}, 48.6 μmol (photon) m^{-2} s^{-1}) at 15°C. Thirty-six samples yielded vitally growing cyanobacteria and altogether contained 57 individual cyanobacterial strains.

PCR amplification and sequencing

Total genomic DNA was isolated using NucleoSpin® Soil (Machery–Nagel) using a standard protocol. A section of the rRNA operon containing a fragment of the 16S rRNA gene and the 16S–23S internal transcribed spacer (ITS) was amplified using the primers 359F (5′–GGGGAATYTTCCGCAATGGG–3′) (Nübel, Garcia–Pichel and Muyzer 1997) and 23S30R (5′–CTTCGCCTCTGTGTGCCTAGGT–3′) (Taton *et al.* 2003) yielding a fragment of expected size 450 bp. The template DNA was mixed with 5 pmol of each primer in 50 μL of commercial PCR mix with Taq polymerase (Plain PP Master Mix, Top Bio, Czech Republic). Amplification was performed using the following settings: a starting denaturation step at 94°C for 5 min; 40 cycles of 30 s at 94°C, 30 s at 53°C and 3 min at 72°C; final extension for 7 min at 72°C and cooling to 4°C. The PCR was verified by running a sub-sample on a 1.5% agarose gel stained with ethidium bromide. Sequencing was performed at Macrogen (Amsterdam, The Netherlands) using the primers 23S30R (5'–CTTCGCCTC TGTGTGCCTAGGT–3'), 1492R (5'–TACGGYTACCTTGTTACGACTT–3') and 810R (5'–GTTATGGTCCAGCAAAGCGCCTTCGCCA–3') (Strunecky, Komarek and Smarda 2014).

Sequences were aligned in MAFFT (www.mafft.cbrc.jp) (Katoh and Toh 2010). A fragment of ∼1081 nt of the 16S rRNA gene was used for the phylogenetic analysis (starting at *Escherichia coli* ATCC 11 775 residue 302).

Phylogenetic analysis

The sequences of cyanobacterial strains outside of Alaskan clades were added as outgroup taxa for inferring congruent phylogeny as used elsewhere (Bohunická *et al.* 2015; Mareš *et al.* 2019b). The phylogenetic tree of the 16S rRNA gene was inferred in MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003). For the Bayesian analysis, four runs of four Markov chains were calculated for 30 million generations, each one with one cold and three heated chains, sampled every 2000 generations. Stop early if the convergence diagnostics falls below 0.01 was set to yes. An initial 25% of the generated trees were discarded as burn-in. The ML analysis in RAxML v. 8 (Stamatakis 2006) was via the CIPRES supercomputing facility (Miller *et al.* 2012). The most suitable substitution evolutionary model was identified using jModelTest 2 based on both the Akaike and Bayesian criteria employed. The general time-reversible + invariant + gamma (GTR+I+G) substitution model, with 1000 bootstrap pseudo–replications was used.

RESULTS

Phylogenetic results based on 16S rRNA phylogeny

Altogether 57 cultivated and sequenced cyanobacterial strains belonged to 20 OTUs (Figs 2, 3 and 4). Description of taxa follows according to their position in the phylogenetic tree inferred from the 16S rRNA gene (Figs 2, 3 and 4).

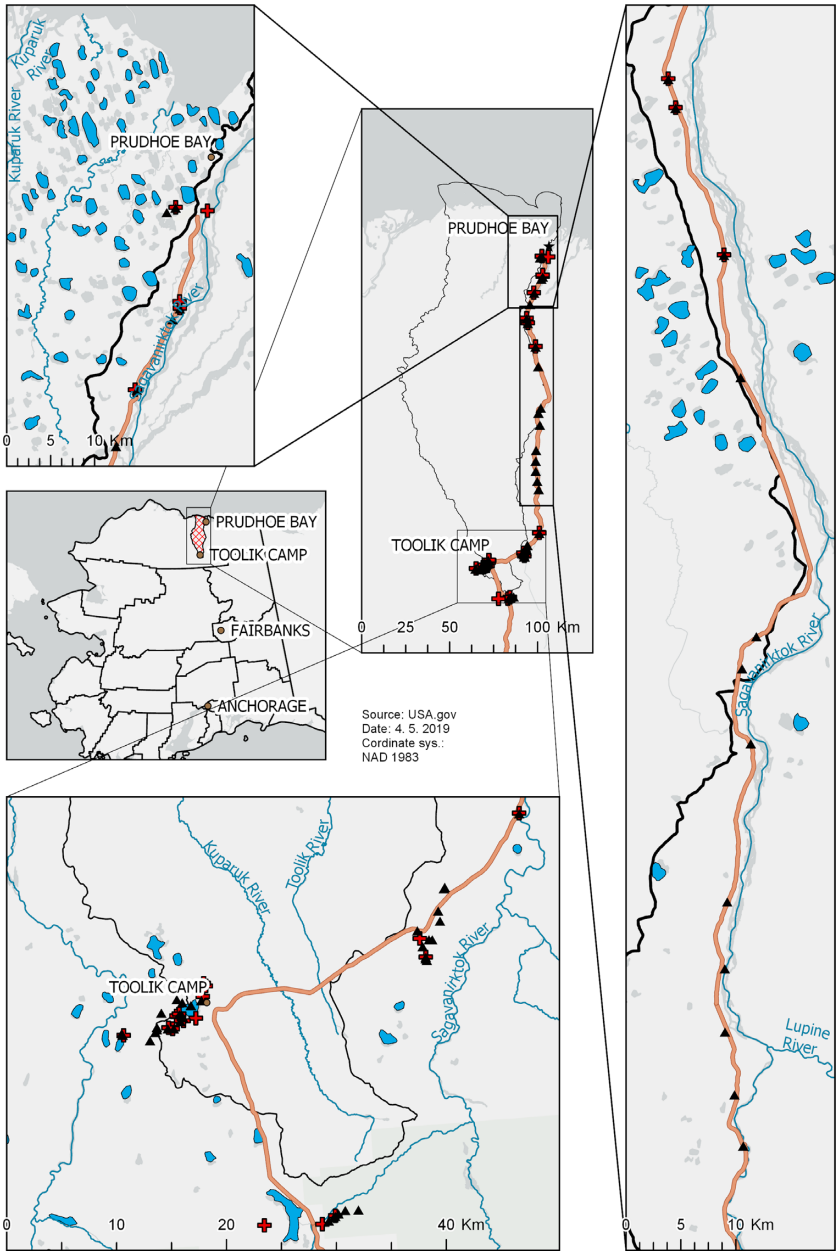

Figure 1. Location of sampling sites at Alaska, USA. Red crosses indicate sites with cultivated cyanobacteria. Triangles indicate the remaining sampling sites.

In Coleofasciculaceae, three strains belonged to *Cephalothrix* Malone (Fig. 2) that formed a distinct clade standing out among the other described species within this genus. Delimitation of a new species *Cephalothrix alaskaensis* described below is sufficiently supported by both their 2.4% nucleotide difference in the 16S rRNA gene and their distinct morphology that will be described later. Nucleotide sequence in the 16S rRNA gene of three strains placed into genus *Anagnostidinema,* Strunecky, Bohunicka, and Johansen et Komarek differed from the other sequences always less than 1.2%.

Three strains KT20, T28 and KL29 are placed within the Microcoleaceae (Fig. 2). Their 99.9% 16S rRNA gene similarity with the adjacent strains qualify them as representative members of the ubiquitous *Microcoleus vaginatus*. Similarly, the 16S rRNA gene sequence of the strains V9 and L24 (Fig. 2) corresponded well with the genus *Kamptonema* Strunecky, Komarek et Smarda with <1.1% difference in the 16S rRNA gene in comparison with a typical strain of the genus *K. animale* CCALA 139.

Four isolated strains belonged to order Chroococcales (Fig. 3). Three strains of *Gloeothece* KL21, L26 and L33 (Fig. 3) were isolated from a single locality. The sequence of their 16S rRNA gene reveals 98.3% similarity with *Gloeothece tepidariorum* CCALA 1112 (A. Braun in Rabenh.) Lagerheim 1883, considered as a valid member of this cyanobacterial lineage. A strain of *Geminocystis* sp. KT25 was isolated and sequenced but perished before thorough microscopic documentation.

The 16S rRNA gene of 12 isolated strains clustered into Oculatellaceae (Fig. 3). Six strains L1, L11, KL12, KV23, L27 and T27 formed a distinct clade. Their 16S rRNA gene nucleotide similarity was 97.36% with *Albertania skiophyla* Zammit. Three strains of Alaskan cyanobacteria T16, KL28, KL16 fell close to a genus *Tildeniella* Mai, Johansen et Bohunická with 16S rRNA gene nucleotide

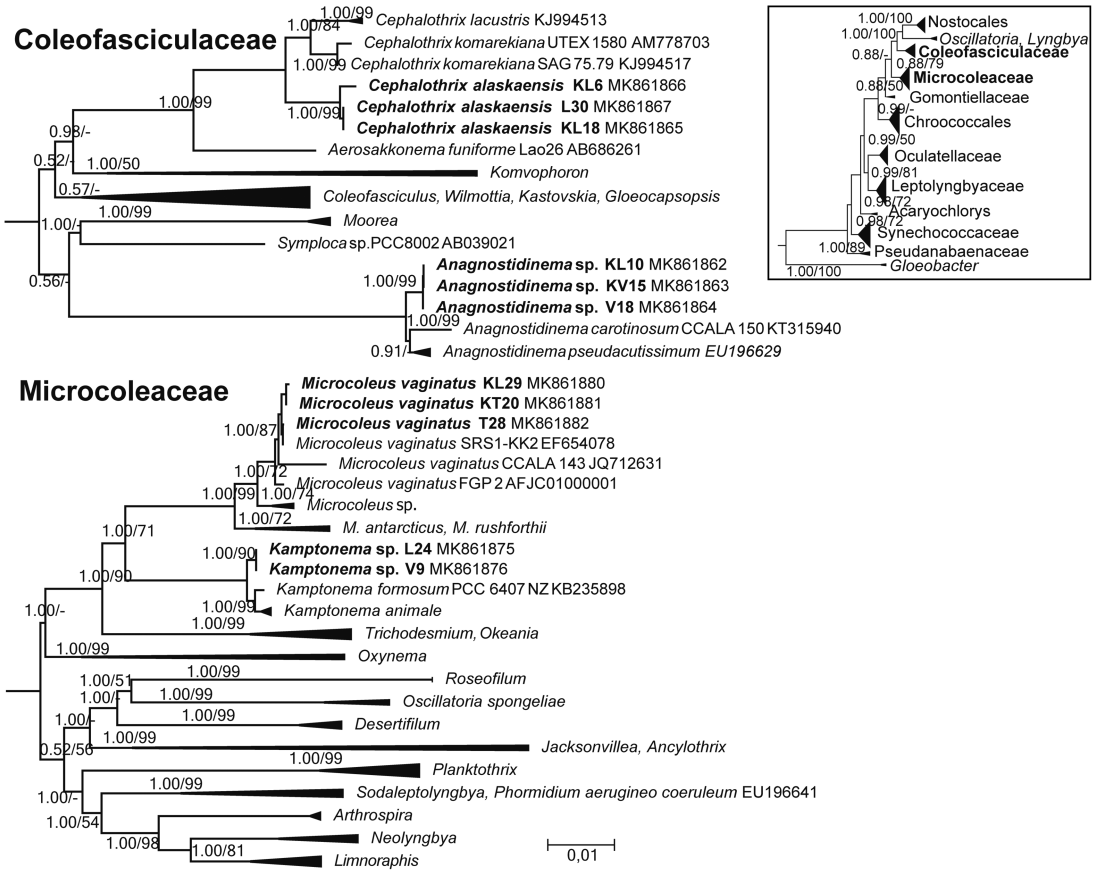

Figure 2. Phylogenetic relationships of the cultivated cyanobacteria from families of Coleofasciculaceae and Microcoleaceae from Alaska. The topology of the complete phylogenetic tree is shown in the box. This was done using Bayesian analysis in MrBayes 3.2.6 and ML in RAxML v. 8. Values indicate Bayesian posterior probabilities and bootstrap pseudo-replications; values <0.5/50% are not shown. A fragment of ~1081 nt was used for phylogenetic analysis of the 16S rRNA gene.

similarity between 98.1 and 95.1% with *Tildeniella torsiva* and only 91.3% identity with *Tildeniella nuda*. The other two cultivated strains KL8 and KL15 were identified as being phylogenetically closely related to *Leptolyngbya antarctica* (West and West 1911) Anagnostidis et Komárek.

A distinct part of the phylogenetic tree of Leptolyngbyaceae contained eight strains (Fig. 4). The strain L25 clustered with *Stenomitos* Miscoe *et al.* 2016 Johansen with 97.8% similarity. Strains KT1 and KT18 come together with the strains designated as *Pseudophormidium* sp. from Atacama Desert with high similarity of 98.4% in 16S rRNA gene sequence. The taxonomic designation of these strains however does not fully correspond with its current taxonomic designation to *Microcoleaceae*. Resolving this taxonomic issue lies outside the scope of this study due to the lack of the 16S rRNA gene sequence of the type of species *Pseudophormidium*, the *P. phormidioides*. Strain V20 was a second cultivated strain within the clade of *Leptodemis* Raabová, Kovacik et Strunecký with 97.1% 16S rRNA gene sequence similarity with strain *Leptodemis paradoxa* LK021. Strain KT5 collected in Sagavanirhtok river had 99.0% nucleotide identity in 16S rRNA gene with *Phormidesmis communis* Raabová, Kovacik, Elster et Strunecký.

A total of 23 strains were cultivated that belong to the core Synechococcales (Fig. 4). Two strains L5 and L31 formed a separate clade with maximal 92.6% 16S rRNA gene sequence identity to the closest taxa *Haloleptolyngbya alcalis*. Strains T21, T29 and L32 belong to *Nodosilinea* Perkerson *et al.* according to their 98.1% nucleotide similarity in 16S rRNA gene to the closest strain *N. nodulosa* UTEX B2910. The rest of *Nodosilinea* strains from Alaska were found within previously published strains of *Nodosilinea* with various species descriptions (Fig. 4).

All obtained sequences were submitted to Genbank under accession numbers MK861856-MK861912 (Supplementary Table S1, see online supplementary material).

Strain morphology

Light microscopy examination of the freshly collected samples at the Toolik Field Station led to the determination of several species that were not isolated later in the laboratory. They belonged mainly to the Chroococcales, Nostocales and Oscillatoriales and their morphological appearance is shown in Fig. 5A–W.

Two species were found in Coleofasciculaceae. The morphology of strains within *Cephalothrix* differs from *C. komarekiana* and *C. lacustris* from South America by the morphology of apical cells with absent conical calyptra (Figs 7A–G and 9A, Table 1). Trichomes are characteristically twisted unlike the straight trichomes of South American strains, additionally, the aerotopes were not found by optical microscopy. The morphology of *Anagnostidinema* strains KV15, KL10 and V18, including the presence of typically bent 'hockeystick'-like ends with rather thin trichome exhibiting distinctive rotational motility, corresponded well with typical traits of *A. pseudacutissimum* Strunecký, Bohunická, Johansen et Komárek.

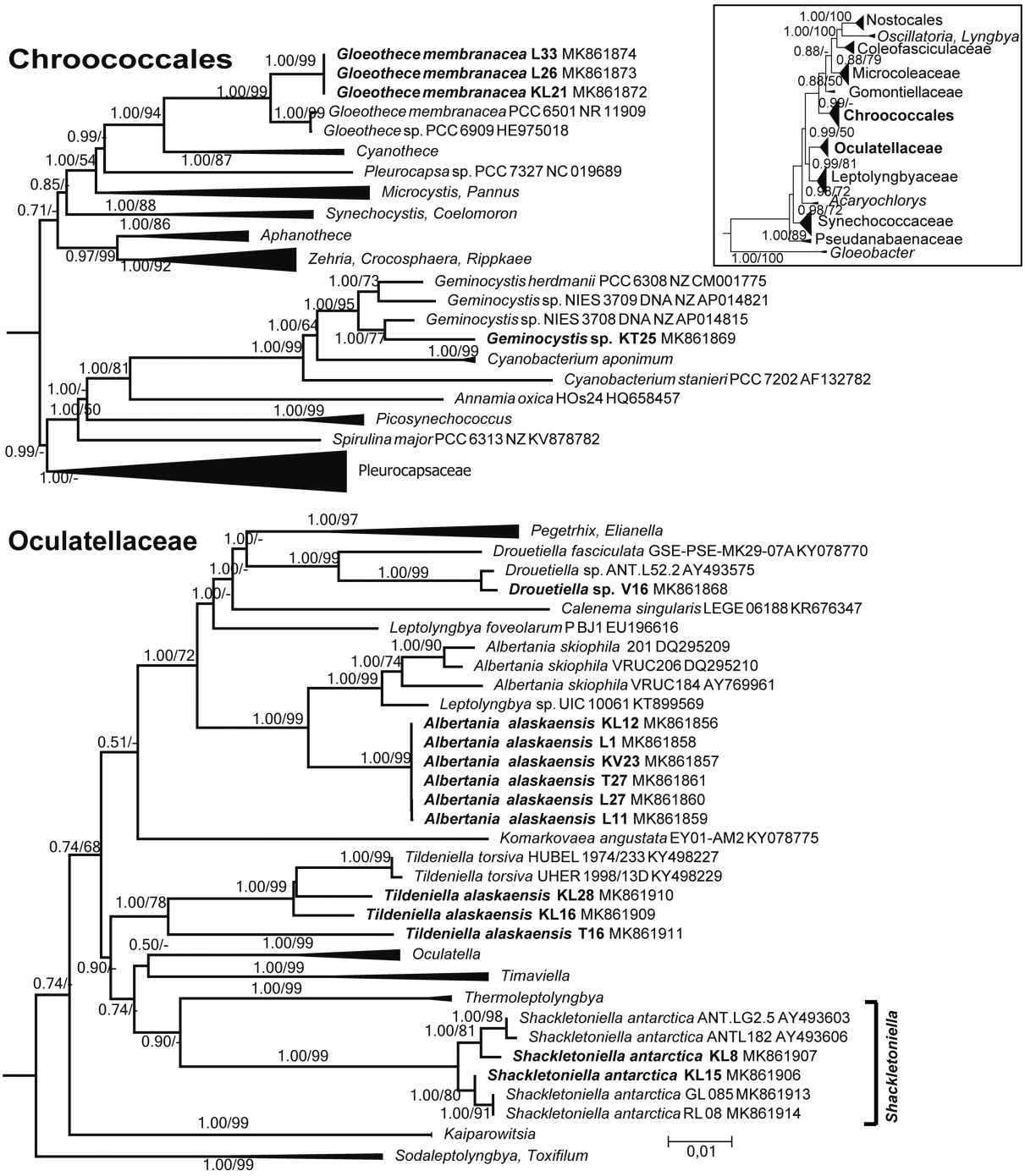

Figure 3. Phylogenetic relationships of the cultivated cyanobacteria from order of Chroococales and family Oculatellaceae from Alaska. The topology of the complete phylogenetic tree is shown in the box. This was done using Bayesian analysis in MrBayes 3.2.6 and ML in RAxML v. 8. Values indicate Bayesian posterior probabilities and bootstrap pseudo-replications; values <0.5/50% are not shown. A fragment of ~1081 nt was used for phylogenetic analysis of the 16S rRNA gene.

In Microcoleaceae the strains of *Microcoleus* possess typically attenuated trichomes with calyptra occasionally present (Fig 5K). The morphology of *Kamptonema* strains KV15, KL10 and V18, including the presence of a typically bent terminal cell and the trichome exhibiting a typical S bend (Fig. 5L) followed by rapid release of tension causing movement, corresponded well with typical traits of *Kamptonema*. The only observable difference found was the presence of a blunt terminal cell that was not as long and attenuated in natural populations as in a typical *K. animale*. Altogether, the morphology of strains collected at Alaska is very similar to known *Kamptonema* strains collected around the world.

The taxonomic designation of *Gloeothece membranacea* (Rabenhorst) Bornet 1886 was supported by morphological traits of studied strains, explicitly the formation of cell clusters joined by common mucilage into gelatinous or membranous

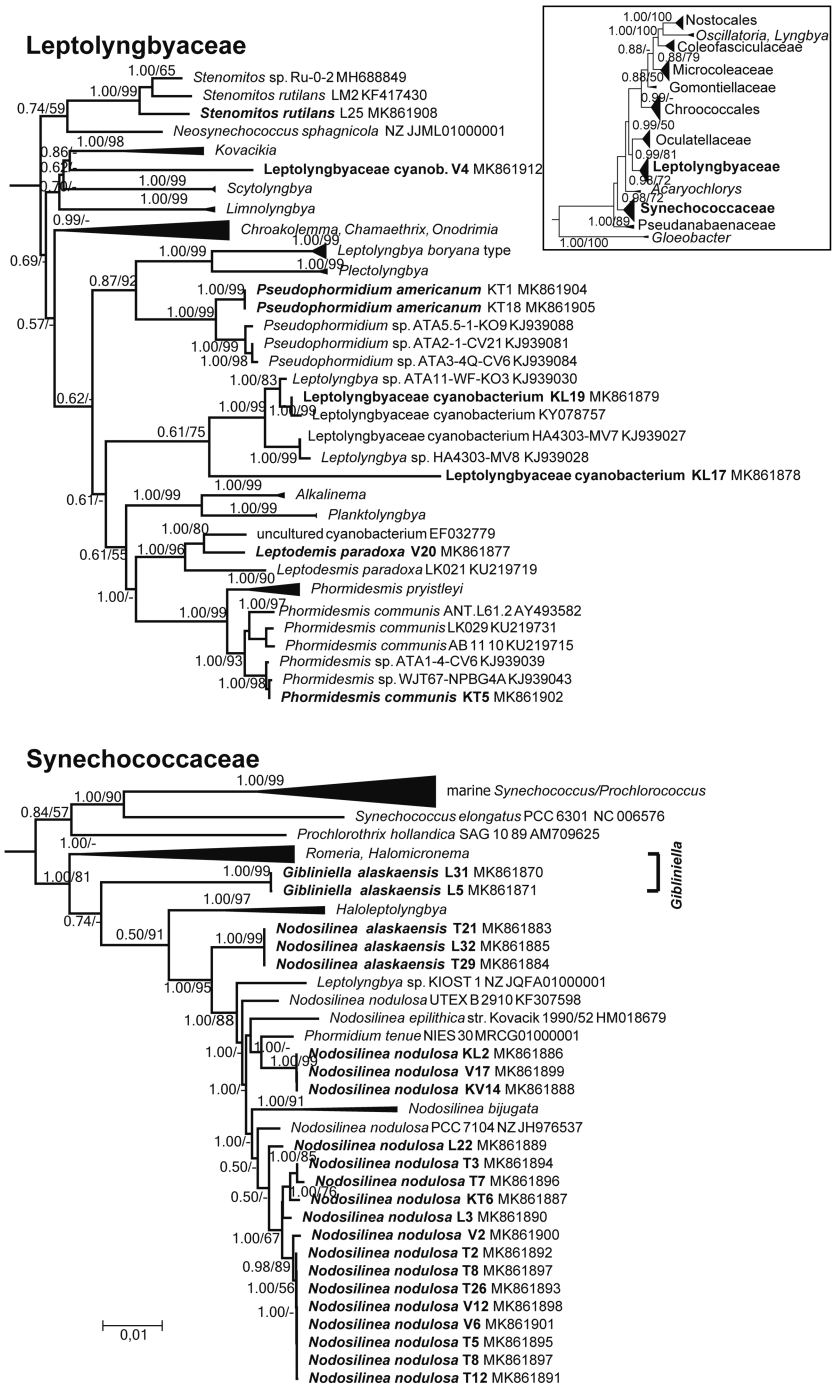

Figure 4. Phylogenetic relationships of the cultivated cyanobacteria from Leptolyngbyaceae and Synechococcales. The topology of the complete phylogenetic tree is shown in the box. This was done using Bayesian analysis in MrBayes 3.2.6 and ML in RAxML v. 8. Values indicate Bayesian posterior probabilities and bootstrap pseudo-replications; values <0.5/50% are not shown. A fragment of ~1081 nt was used for phylogenetic analysis of the 16S rRNA gene.

colonies, formation of colorless but lamellated envelopes surrounding the cells, cell size of 6–8 × 4–6 μm, along with their presence in a freshwater biotope (Fig. 5F).

Strain V16 with Oculatellaceae-like morphology was isolated from stream periphyton. It belonged to a clade with *Drouetiella* sp. ANT.L52, however, no specific morphologic features were observed in this strain. The solitary, non-fasciculated trichomes 3 μm wide are slightly constricted at septal cell walls with green, isodiametric or longer than wide cells with no distinct morphological features (Fig. 5M–O). The overall appearance resembled well the *Drouetiella lurida* (Gomont 1892) Mai, J.R. Johansen et Pietrasiak with the exception of a liver-brown filament coloration.

Six cultivated strains were grouped within Ocullateaceae showing typical morphology of the family with thin cells that are longer than they are wide. These strains were isolated from

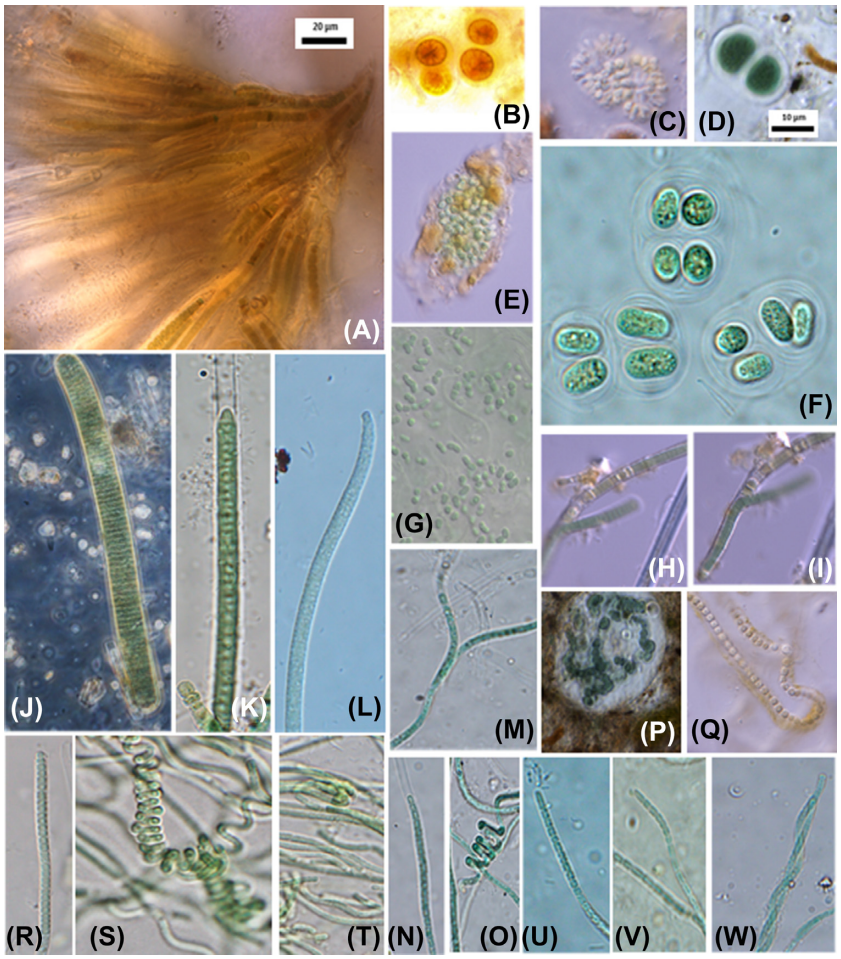

Figure 5. Morphological diversity of cyanobacteria found at site and within the cultivated strains. (**A**) *Rivularia* sp., (**B**) *Gloeocapsa* sp., (**C**) *Snowella* sp., (**D**) *Chroococcus* sp., (**E**) *Aphanothece* sp., (**F**) *Gloeothece membranacea* (strain L33), (**G**) *Aphanocapsa* sp., (**H, I**) *Scytonema* sp., (**J**) *Oscillatoria* sp., (**K**) *Microcoleus vaginatus* (strain T20), (**L**) *Kamptonema* sp. (strain L24), (**M–O**) *Drouetiella* sp. (strain V16), (**P, Q**) *Nostoc* sp., (**R, T**) *Nodosilinea nodulosa* strain L22, (**S**) *Nodosilinea nodulosa* strain V2, (**U–V**) *Stenomitos sp.* (strain L25), (**W**) *Phormidesmis communis* (strain KT5). Scale bar for microphotographs (A) and (J–l) is 20 μm in 1000X magnification and for (B–I) and (M–W) is 10 μm in 1000X magnification.

freshwater and one was found on a permanent snow field. During the preparation of the manuscript the description of the genus *Albertania* Zammit was validly published. Species of *Albertania skiophila* grew in aerophytic biofilms in Malta and were phylogenetically close to this group. The Alaskan strains exhibited a distinct morphology with coiled or undulating trichomes with occasionally present perpendicular cells (Fig. 6A–L). In addition, their distinct ecological traits provide further support for the formation of a new species *Albertania alaskaensis* (Figs 3, 6A–L and 9F, Table 1). Strains of *Tildeniella* (Figs 7M–P, 9C, Table 1) possessed morphological features similar to Oculatellaceae members with very thin (1–2.2 mm) cells that were partially longer than they were wide. Strains KL28 and KL16 differ morphologically from *Tildeniella torsiva* in their distinct arctuation both in nature and in cultures. We therefore included them in a new species, *T. alaskanesis* (Fig. 7M–P).

The last two cultivated strains in Oculatellaceae, KL8 and KL15, were identified as being closely related to previously described Antarctic species *Leptolyngbya antarctica* (West and West 1911) Anagnostidis et Komárek. They had very thin trichomes 1–1.5 (2) µm wide with predominately cells longer than they were wide up to 7 (10) μm. The formerly established species *L. antarctica* however did not comply either phylogenetically or morphologically with the genus *Leptolyngbya*. We therefore combined the strains of *L. antarctica* collected at the Antarctic and at Alaska and collectively described them in a newly formed genus *Shackletoniella*. (Figs 7U–Z, 9B, Table 1).

Unlike Oculatellaceae which had essentially cells longer than they were wide, the main diagnostic markers in the family Leptolyngbyaceae are very thin trichomes up to 4 µm combined generally with cells shorter than their length after division. Additionally, a visible circular structure of unknown function in the middle of the cell was recognisable by optical microscopy (Figs 6 and 7, Table 1). Strain L25 with short untapered trichomes, unconstricted at the crosswalls, occasionally coiled, 2 µm wide with cells growing to two times longer size before division, with parietal thylakoids and colorless sheaths, tightly adhering to trichomes, visible only when empty (Fig. 5U–V), corresponded well to the original description of *Stenomitos* Miscoe *et al.* 2016 Johansen. Strain V4 was lost before proper morphological analysis, but its micrographs obtained during cultivation showed morphological similarity with *Scytolyngbya* Song et Li except for missing false branching and incrustation of sheaths present in *Scytolyngbya timoleontis*. The width of filaments was ~3 µm, cells were isodiametric, up to 5 times longer than wide before division, with distinct, long and rounded-end cells.

Table 1. Comparison of morphological features of new species with morphological features of type species.

	Thallus	Filaments	False branching	Motility	Filament width (μm)	Sheaths	Color of trichomes
Cephalothrix alaskaensis	Fasciculate blue-green yellowish in 4–6 weeks older culture	Cylindrical, straight or twisted sometimes bent and coiled not attenuated	None	None	6.4–7.1 (7.8)	Hyaline, firm, sometimes loosening at the end of filaments	Colorless bright green to pale green in young cultures, yellowish-green in old cultures
Cephalothrix komarekiana	Fasciculate blue-green	Cylindrical, straight trichomes bent at the end slightly attenuated	None	None	Not available	Facultative hyaline, firm, narrow or wide	Not available
Albertania alaskaensis	Characteristic tufted, isolated colonies penetrating a few mm into agar, hardly removable bright green to yellow-brown when old	Solitary or fasciculate, narrow or coiled, wavy, heterogeneous filaments, width front of filament wider than end, with shorter cells, end markedly narrower with longer cells (1–12) forming hair	None in young culture, often in the old culture	None	0.8–1.3 end cells 1.8–3 cells	Lamellate, thin, firm, closed at the ends of the filament, sometimes widened colorless	Bright yellow-green to olive green
Albertania skiophila	Compact subaerophytic biofilms on solid substrata green, green-brown and black	Single trichome per sheath, trichomes occasionally slightly attenuated toward their ends, trichome fragmentation via random occurrence of necridic cells	Occasionally	None	Not available	Firm, thin, often open at the ends, colorless	Colorless
Tildeniella alaskaensis	Compact mats blue-green to dark green	Solitary, flexuous, irregularly curved and coiled, untampered, cylindrical, isopolar, uniseriate, one per sheath	None	None	1–2.2	Thin, firm, colorless	Bright green
Tildeniella torsiva	Colonies fasciculate, spreading irregularly, irregular clumps, filaments penetrating the agar bright blue-green, olive green (old)	Straight, flexuous or spirally coiled, often entangled	Rare false branching in older cultures	None	1.7–2.5	Thin, up to 0.7 μm wide, often not evident, colorless	
Shackletoniella antarctica	Compact biofilms greenish or reddish	Fine, short to long, arctuated, waved or intensely coiled, ensheathed and unbranched without necridic cells	None	None	1–1.5 (2)	Thin, firm, enveloping one trichome, usually firmly attached, colorless	Pale blue-green, brownish, olive green, yellowish or reddish

Table 1. Continued

	Thallus	Filaments	False branching	Motility	Filament width (μm)	Sheaths	Color of trichomes
Pseudophormidium americanum	Filamentous, joined into microscopic mats, bright green	Solitary, cylindrical, isopolar, uniseriate, irregularly curved an coiled, without necridia, not attenuated at the ends	Intensive and irregular	None	2.9–3.6 (4)	Thick, firm, tube like, colorless	Green
Pseudophormidium phormidioides	Thin, membranaceous, somewhat lubricous dark or blackish blue green, expanded	Rarely solitaire, densely coiled	Present	None	5.6–7	Narrow, colorless	Blue-green, greyish, blue-green, olive green, dirty brownish-violet
Leptodesmis alaskaensis	Entangled in mats intensely green surface layer	Straight, curved, flexuous or wavy, solitary, one filament per sheath pale-blue green to dark green	None	Motile	1.8–2.6	Thick, colorless	Pale blue-green to green
Leptodesmis paradoxa	Compact mats, filamentous green to dark green	Straight, curved, flexuous or wavy, solitary, one filament per sheath pale blue-green to dark green	None	None	2.5–3.5 (4)	Thick, colorless	Not available
Nodosilinea alaskaensis	Compact mats bright green	Single trichome, sometimes presenting circular growth, long or short, straight or bent	None	Motile in young cultures	2–2.5 (3)	Usually present, thin, soft, colorless	Green to pale green
Nodoillinea nodulosa	Not available	Filaments typically with a single trichome, but sometimes becoming multiseriate, with nodules forming under low light conditions	None	None	Not available	Usually present, thin, soft, inflated, colorless	Not available
Gibliniella alaskaensis	Thin yellow-green to green	Cylindrical filaments without necridic cells, solitary or densely entangled to mats	None	Intensely motile	1.2–1.7	Thin, firm clear	Yellow-green, green or yellow-brown
Cell width (μm)	**Crosswalls**	**Cell form**	**Apical cell**	**Cell length (μm)**	**Hormogonia**	**Special features**	**Ecology**
(4.7) 5–6.5 (7.1) narrow end (3) 4–5	Constricted	Always wider than long, sometimes longer than wide, without aerotopes prominent parietal thylakoid pattern with orange granules and visible stacked thylakoids no aerotopes	Rounded, not capitate, without calyptra	(1.4) 1.8–2 (2.9)	Formation by characteristic biconcave necridic cells	Not observed	Periphyton of the water soil shoreline

Table 1. Continued

	Thallus	Filaments	False branching	Motility	Filament width (μm)	Sheaths	Color of trichomes
4.8–7.3	Constricted or not constricted	Wider than long cell content with facultative aerotopes	Slightly or strongly capitate	2.0–3.5	Hormogonia formation by biconcave necridic cells	Not observed	Grows in an alkaline lake in the Brazilian Pantanal wetlands
2.1–3.2	Slightly constricted	Isodiametric or longer than wide before division, parietal thylakoids, end cells markedly narrowed, longer	Tapered, rounded, sometimes form mushroom like calyptra	1.4–4	Not observed	Characteristic 90 degrees turned cell within the trichome	Freshwater, periphytic on roots or in snow
2–3	Not constricted	Variable shape, mostly isodiametric, sometimes slightly shorter or longer than , cell content homogenous, occasionally grainy necridic cells sometimes present	Rounded	2–4	Straight, made up of 2–8 cells	Filaments grow preferentially in dimly lit areas	Subaerophytic biofilms attached to calcareous substrata
1.1–1.6	Not constricted	Isodiametric or slightly longer than wide	Rounded without calyptra	1.6–3	Not observed	Arctuation of majority of filaments, but they are never spirally coiled	Meltwater, periphyton on roots
1.4–1.9	Slightly constricted	Rarely isodiametric, mostly longer than wide, contents usually homogeneous, without granulation	Rounded	1.5–2.7	None	Cell division occurring throughout the trichome	Limestone wall
1.7–7 (10)	Constricted	Longer than wide, without aerotopes, prominent parietal thylakoid pattern	Rounded, long with prominent granules at the apex	1.7–7 (10)	Not observed	End cells without thickened cell walls or calyptras, cells divide by a symmetrical crosswise binary fission, cells grow longer before next division	Freshwater, flowing in the water, or form mats in pelagial
(1.9) 2.2–2.7 (2.9)	Constricted	Barrel shaped, isodiametric or slightly shorter than wide, no aerotopes	Conically-rounded without calyptra	(1) 1.2–2.5 (3.4)	Not observed	Cell division crosswise, perpendicular to the long axis of the trichome, daughter cells grow to the original size before the next division	Soils

Table 1. Continued

	Thallus	Filaments	False branching	Motility	Filament width (μm)	Sheaths	Color of trichomes
5.6–7	Constricted	1/2–1/3 longer than wide	Rounded	Not available	None	Cell division crosswise	Freshwater, stones in mountain clear stream
1–1.5	Slightly constricted	Barrel shape or shorter than wide, occasionally longer than wide, central granules, uniseriate, length of cells differs at opposite trichome ends	Rounded occasionally slightly conical without calyptra	1.4–4.3	Not observed	Two types of cells in filaments, beginning of filaments have shorter, wider cells, end of filaments have longer cells	Green mat in dry channels on side of lake
2.5–3.5 (4)	Slightly constricted	Barrel shaped or shorter than wide, occasionally longer than wide, central granules	Rounded without calyptra	1–2	Not observed	Filament morphology changes during the life cycle, young filaments similar to *Leptolyngbya corticola* or *L. boryana*, older ones similar to *Phormidesmis*	Mud of pool littoral
(1) 1.4–1.8	Not constricted	More or less longer than wide, lacking aerotopes, peripheral thylakoids	Rounded or conical	(1.5) 2–4.1 (4.8)	Not observed	Tolerates growth on nitrogen-free media	Crust on stones, freshwater
1–1.5	Distinct	Isodiametric, flattened	Rounded or bluntly rounded	1–2.4	Common	Capable of anaerobic N fixation	Marine phytoplankton euryhaline
0.7–1.2	Not constricted	Isodiametric, rarely longer than wide before division, parietal thylakoids	Rounded without calyptra	0.9–3.6	Not observed	Filaments coiled together like fishing-net filaments without necridia, intensely motile	Freshwater, crust on stones

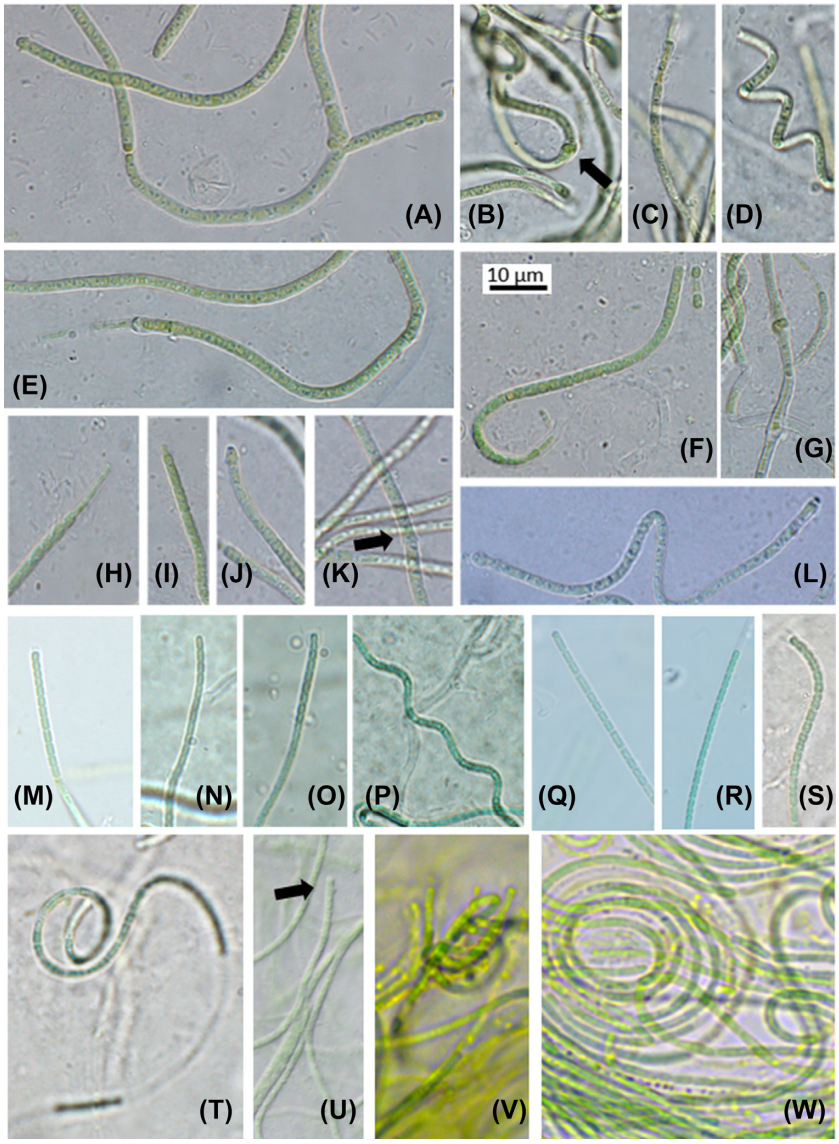

Figure 6. Morphological diversity of cultivated cyanobacteria. (**A–L**) *Albertania alaskaensis* [(A–D) strain KL12, (E–J) L1, (K–L) L11], (**M–P**) *Tildeniella alaskaensis* (strain KL16), (**Q–W**) *Nodosilinea alaskaensis* [strains (Q–S), (W) L32, (T–U) V2, (V) L22]. Scale bar for all microphotographs is 10 μm in 1000X magnification.

The morphology of strains KT1 and KT18 from Alaska (Figs 7H–M, 9G, Table 1) closely resembles *Pseudophormidium* (Hansgirg et Forti 1907) Anagnostidis et Komárek with its characteristic false branching that is a strikingly evident morphologic feature. These strains were thicker than the other species within Leptolyngbyaceae reaching 4 µm in width. The cells were however slightly thinner than typical cells of *P. phormidioides* that should be 5.6–7 µm. The other ubiquitous group of *Phormidesmis* included strain KT5 collected in Sagavanirhtok river. This strain was morphologically identical with *Phormidesmis communis* Raabová, Kovacik, Elster et Strunecký. It had thin filaments (1–3 µm), barrel-shaped isodiametric cells with prominent centroplasma with a central granule and parietal thylakoids (Fig. 5W). Morphologically similar strain V20 (Figs 7N–T and 9D) exhibited changes in its morphology throughout its life cycle. Namely, changing the cell ratios, from shorter to longer than their width throughout aging of its culture, suggested that it belonged to genus *Leptodemis* Raabová, Kovacik et Strunecký (Fig. 7N–T).

A total of 23 cultivated strains belonged to the core Synechococcales. Two strains were quite morphologically different from all others found in Alaska as they did not show any characteristic morphological features of their filaments due to their restricted size (Figs 8A–G and 9H, Table 1) and absence of heterocysts. Both strains exhibited sleek, yellow-green, intensely motile and exceptionally thin filaments with 0.7–1.2 µm wide cells, with thin and firm sheaths. The filaments were bundled altogether in one common sheath forming a characteristic fish-net like structure (Figs 8E–G, 9H). The morphological traits observed during microscopic examination, including the formation of string-like filaments, and altogether highly distinct habitus with respect to all other Syne-

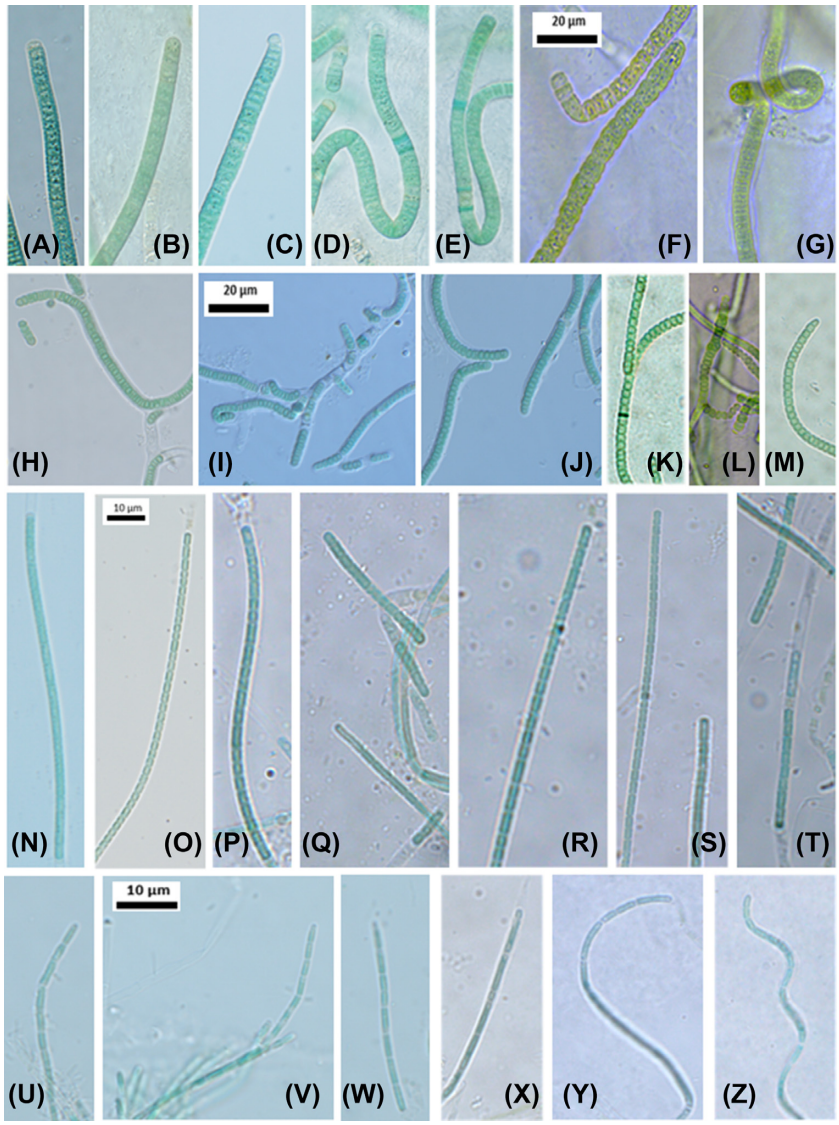

Figure 7. Morphological diversity of cultivated cyanobacteria. (**A–G**) *Cephalothrix alaskaensis* [(A) strain KL6, (B–E) KL18, (F–G) L30], (**H–M**) *Pseudophormidium americanum* [(H–K) KT1, (L–M) KT18], (**N–T**) *Leptodesmis alaskaensis* (strain V20), (**U–Z**) *Shackletoniella antarctica* (strain ULC037). Scale bar for microphotographs (A–M) is 20 μm in 1000X magnification and for (N–Z) 10 μm in 1000X magnification.

chococcales, provide support for the creation of a new genus *Gibliniella*.

The rest of the cultivated strains belonged to *Nodosilinea*. Strains T21, T29 and L32 showed similar knot-like traits (Figs 6Q–W, 9E) originating from cell division in more than one plane. They were motile during approximately the first month of cultivation in culture unlike previously reported strains of genus *Nodosilinea*. Barrel-shaped cells of slightly variable cell length with thicker sheaths retain morphologic features of straight filamentous forms similar to phylogenetically close *Halolephtolyngbya* Dadheech *et al.* 2012, contrasting with arctuated filaments with almost invisible sheaths in the strains of *Nodosilinea nodulosa* (Fig. 5R–S, Table 1). We find the differences sufficient to place these strains into a new species *N. alaskaensis* (Fig. 6Q–W). On the other hand, none of our strains designated as *Nodosilinea* species showed morphological features typical of the genus with cells dividing throughout the trichome forming nodular regions. Eleven of the other strains that we isolated and designated as *Nodosilinea nodulosa* (Fig. 5R–S) showed the other typical features, such as cells dividing perpendicularly to the trichome length that converge with the typical morphology of *Nodosilinea*. The last isolated strain found in the Pseudanabaneales perished before proper morphologic analysis.

TAXONOMIC TREATMENT

Cephalothrix alaskaensis sp. nov.

Description: Thallus fasciculated, blue-green or yellow-green when old. Cylindrical, straight or twisted trichomes, sometimes bent and coiled, not attenuated, constricted at the cross-walls with variable cell size within trichomes; with pairs of cells having conical trim outside of common cell wall, hyaline and firm sheath. End cells rounded, not calyptrate. Cells yellowish-green to pale green, sometimes with orange granules and visible stacked thylakoids, always wider than long, (1.4) 1.8–2 (2.9) µm

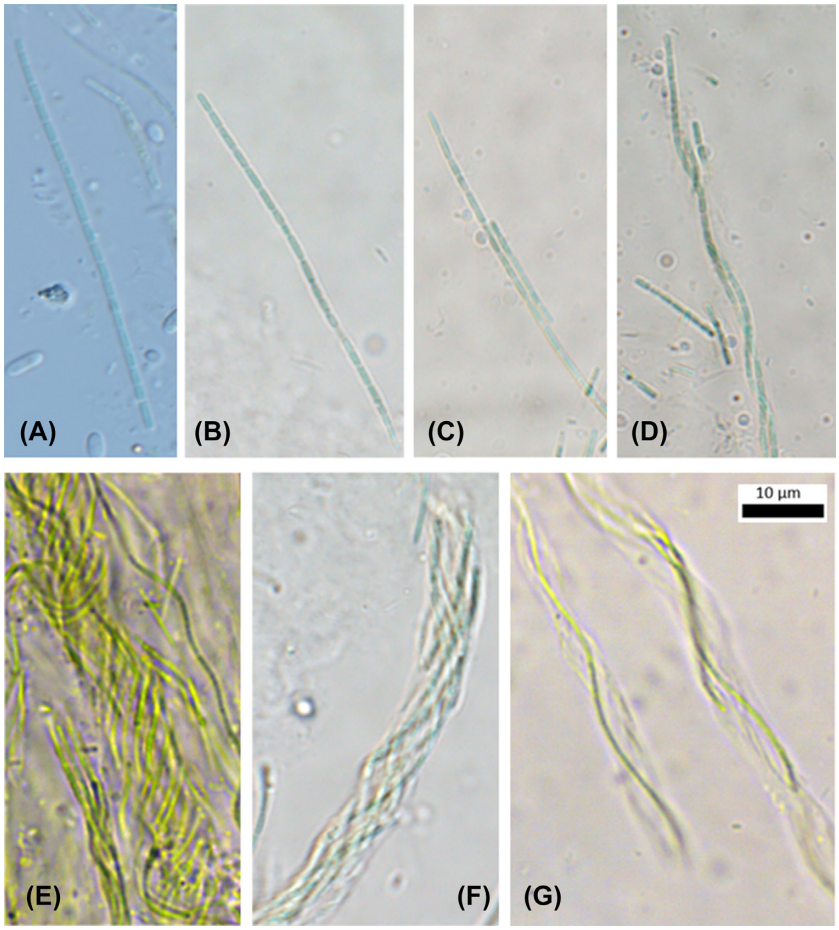

Figure 8. Morphological diversity of *Gibliniella* gen. nov. *Gibliniella alaskaensis*. (**A**) strain L5, (**B–G**) L31. Scale bar for all microphotographs is 10 μm in 1000X magnification.

long, (4.7) 5–6.5 (7.1) µm wide. Apical cell not capitate, cell content without aerotopes. Hormogonia formation by characteristic thin biconcave necridic cells. (Figs 7A–G, 9A)

Etymology: *alaskaensis* (L.): found at Alaska.

Holotype (here designated):–dried specimen of the strain L30 is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–108–1.

Reference strain: L30, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861867.

Type locality: Toollik lake area, Lake S15, periphyton on the water soil shoreline.

Taxonomic notes: *C. alaskaensis* differs from *C. komarekiana* and *C. lacustris* from South America by the morphology of apical cells, i.e. conical calypthra is not present. Trichomes are characteristically twisted and bent unlike the straight trichomes of South American strains. Also, the aerotopes were not found by optical microscopy. The 2.4% difference in 16S rRNA gene sequence and rather big geographical distance provide additional means for the validity of the new species.

Albertania alaskaensis sp. nov.

Description: Thallus bright green to yellow-brown when old with characteristic growth pattern on agar making well isolated spots . Trichomes solitary, not fasciculated, bright yellow-green to olive green, with characteristic 90 degrees turned cell within the trichome (Fig. 6B and G), occasionally 1–12 thin cells (hair) at the end of filament (0.8–1.3 µm wide, 1.4–3 µm long) (Fig. 6E, F and H), occasionally false branching (Fig. 6A), not constricted at cross-walls, necridia not observed. Cells mostly isodiametric, or longer than wide before division, with parietal thylakoids, 1.8–3 µm wide, 1.9–4 µm long. End cells tapered, rounded, making characteristic mushroom like calyptra. Sheath clear, thin, and firm, occasionally widened. Freshwater, periphytic on roots or in snow. (Fig. 6A–L, 9F)

Etymology: *alaskaensis* (L.): found at Alaska.

Holotype (here designated): dried specimen of the strain KL12 is deposited in the herbarium collection of the University of South Bohemia, under the number A–109–1.

Reference strain: KL12, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861856-MK861912.

Type locality: Meltwater brook close to Poplar lake, periphyton on willow roots.

Tildeniella alaskaensis sp. nov.

Description: Trichomes flexuous, irregularly curved and coiled, untapered, cylindrical, isopolar, uniseriate, not constricted

at the visible cross-walls, always with thin sheath, without necridia and hormogonia. Cells 1.1–1.6 µm wide, isodiametric or slightly longer 1.6–3 µm than wide. End terminal cells rounded. The main diacritical feature is the arctuation of the majority of

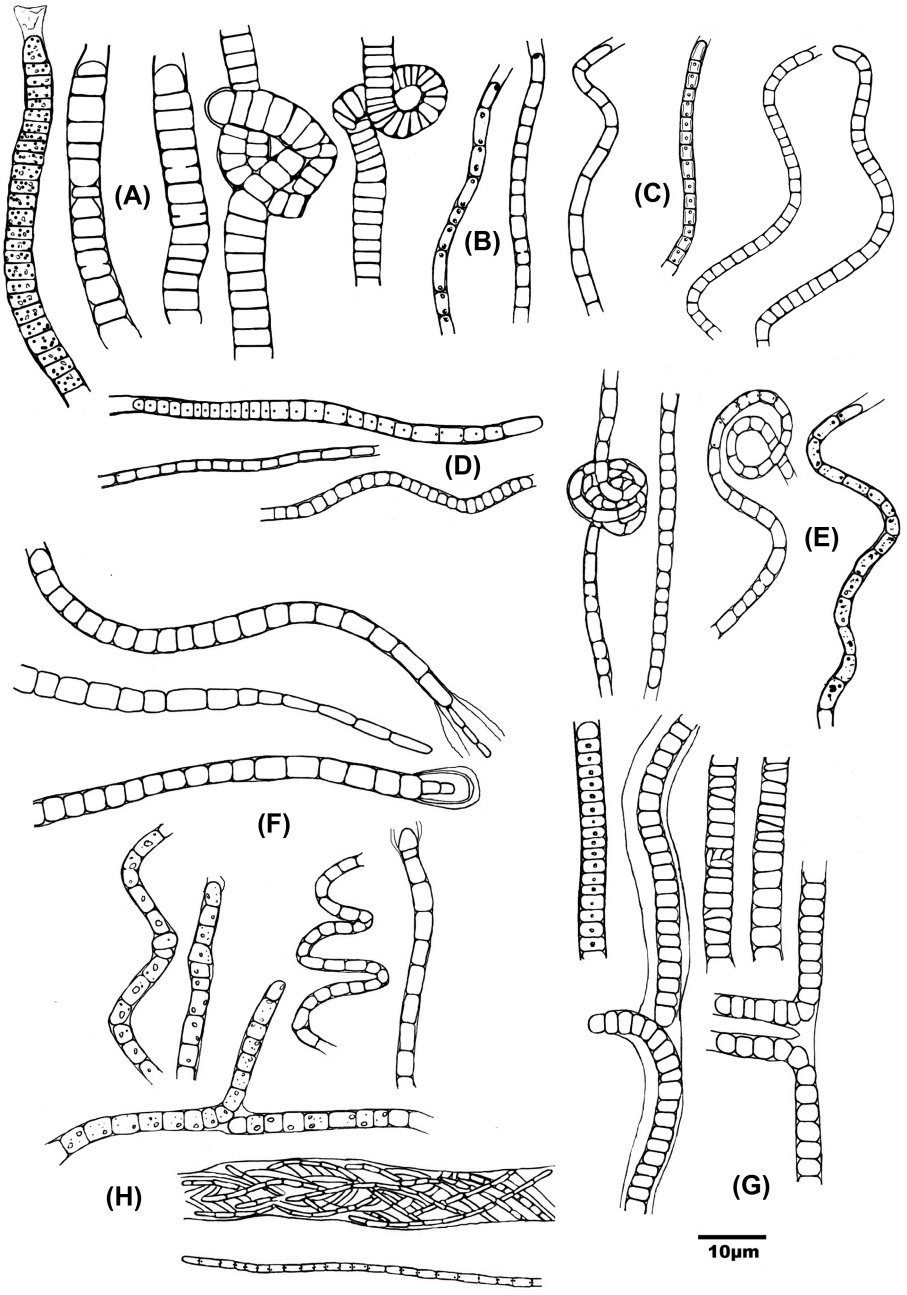

Figure 9. Illustrations of newly described taxa. (**A**) *Cephalothrix alaskaensis*, (**B**) *Shackletoniella antarctica*, (**C**) *Tildeniella alaskaensis*, (**D**) *Leptodesmis alaskaensis*, (**E**) *Nodosilinea alaskaensis*, (**F**) *Albertania alaskaensis*, (**G**) *Pseudophormidium americanum*, (**H**) *Gibliniella alaskaensis*. Scale bar for all illustrations is 10 μm.

filaments but they are never spirally coiled nor false branched. (Figs 6M–P, 9C Table 1)

Etymology: *alaskaensis* (L.): found at Alaska.

Holotype (here designated):–dried specimen of the strain KL16 is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–116–1.

Reference strain: KL16, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861909.

Type locality: Meltwater brook close to Poplar lake, periphyton on willow roots.

Taxonomic notes: Different from *T. torsiva* and *T. nuda* by the absence of constriction between cells in fillaments, in their arctuation, and in the occasional presence of false branching. Molecularly distinguished by from T. torsiva by et least 3%.

Pseudophormidium americanum sp. nov. Strunecky et Raabova

Description: Filamentous; filaments solitary, joined into microscopic mats, firm, tube-like, colorless multilayered sheaths, commonly and intensely irregularly falsely branched. Trichomes, cylindrical, isopolar, uniseriate, irregularly curved and coiled, (1.9)2.2–2.7(4) μm wide, composed of barrel-shaped cells constricted at cross-walls, not attenuated to the ends, non-motile. Cells isodiametric or slightly shorter than wide with cell

length (1)1.2–2.5(3.4) μm, without aerotopes, without necridia and hormogonia, green. End cells conically-rounded. Cell division crosswise, perpendicular to the long axis of a trichome, daughter cells grow to the original size before the next division. Isolated from soil. (Figs 7H–M, 9G, Table 1)

Etymology: *americanum* (L.): from the Americas, to date all sequenced strains were found at North or South America.

Holotype (here designated):–dried specimen of the strain KT1 is deposited in the herbarium collection of the University of South Bohemia, under the number A–115–1.

Reference strain: KT1, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861904.

Type locality: Dry soil at top of Jade Mountain.

Taxonomic notes: The original morphological designation of all described species within *Pseudophormidium* (Forti 1907) (Anagnostidis and Komarek 1988) with typical short non-constricted cells, clearly visible multilayered sheaths and intense false branching was identical to our strains designated as *Pseudophormidium*. Independent taxonomic designation of morphologically identical and phylogenetically closest strains from South America, designated as *Pseudophormidium* by Miscoe *et al.* 2016 supported our definition of new species according the rules of the botanical code as *Pseudophormidium americanum*.

Leptodesmis alaskaensis sp. nov.

Description: Thallus entangled in mats forming an intensely green surface layer. Filaments straight, curved, flexuose or wavy, solitary, one filament per sheath. Motile. Filaments pale blue-green to dark green, slightly constricted at cross-walls, may become slightly wider on the apical part of filament. Sheaths thick, colorless. Filaments 1–1.5 (1.6) μm wide, without necridic cells. Cell length 1.4–4.3 μm. Cells barrel-shaped longer than wide, isodiametric or shorter than wide, with central granules in cells. Uniseriate, length of cells differs at opposite trichome ends. Apical cell is rounded without calyptra. In young cultures cells are longer than wide whereas with central granules, in old cultures (∼4–6 weeks) cells isodiametric or shorter than wide. (Figs 7N–T and 9D, Table 1)

Etymology: *alaskaensis* (L.): found at Alaska.

Holotype (here designated): dried specimen of the strain V20 is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–111–1.

Reference strain: V20, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861877.

Type locality: Green mat in dry channels on east side of Lake NE2 in Toolik lake area.

Gibliniella gen. nov. Strunecky et Raabova

Thallus thin, yellow-green, green or yellow-brown. Cylindrical filaments without necridic cells solitary or densely entangled to mats. Trichomes long, wavy, typically spirally coiled, rarely straight, untapered, isopolar with rounded-end cells, 0.6–1 μm wide, intensely motile. Sheets thin and firmly attached to filaments. Cells not or slightly constricted at the translucent cross-walls. Phylogenetically distinct from all other genera in the Synechococcales.

Gibliniella alaskaensis sp. nov. Strunecky et Raabova

Description: Thallus yellow-green to green. Trichomes solitary or fasciculated, wavy, spirally coiled, rarely straight, bright yellow-green to bright green, without false branching, not constricted at cross-walls, necridia not observed, intensely motile. Cells mostly longer than wide, with parietal thylakoids, 0.7–1 μm wide. End cells rounded. Sheath clear, thin and firm. Freshwater in lake. (Figs 8A–G, 9H, Table 1)

Etymology: *Gibliniella* named in honor of Anne E. Giblin, a well–known North American ecologist who studies anthropogenic inputs to ecosystems.

Holotype (here designated): dried specimen of the strain L31 is deposited in the herbarium collection of the University of South Bohemia, under the number A–114–1.

Reference strain: L31, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861870.

Type locality: Research lake S7 in Toolik lake area, crust on stones.

Shackletoniella gen. nov. (West et G.S.West), Strunecky, Raabova et Bernardova

Filamentous cyanobacteria, forming greenish or reddish compact biofilms. Filaments fine, long, arctuated, waved or intensely coiled, ensheathed and unbranched without necridic cells. Sheaths colorless, thin, firm, enveloping one trichome and usually firmly attached. Trichomes fine 1–3 μm wide, cylindrical, slightly constricted at cross-walls. Up to date found in the Antarctic and north of North America.

Shackletoniella antarctica (West et G.S.West) Strunecky, Raabova et Bernardova

Description: Filaments short to long, curved, wavy or coiled, with colorless, thin sheaths. Filaments 1–1.5 (2) μm wide, slightly constricted on cross-walls, without necridic cells, cells longer than wide 1.7–7 (10) μm long, without aerotopes, with prominent parietal thylakoid pattern, pale blue-green, brownish, olive green, yellowish or reddish; end cells without thickened cell walls or calyptras. Cells divide by a symmetrical crosswise binary fission; cells grow long before next division. Heterocytes and akinetes absent. Apical cell rounded, long with prominent granules at the apex. Freshwater in flowing water or forming thick mats at lake bottom. (Figs 7U–Z, 9B, Table 1)

Etymology: the generic name '*Shackletoniella*' was derived from the name of Sir Ernest Shackleton, a polar explorer who led British expeditions to the Antarctic including scientific investigations.

Basionym: *Phormidium antarcticum* West and West 1911: 292, Syn.: *Leptolyngbya antarctica* (West and West 1911) Anagnostidis and Komárek 1988: 390

Type species: *Shackletoniella antarctica*

Holotype (here designated): dried specimen of the strain is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–155.

Reference strain: *Shackletoniella* ULC037 (ANT.LH18.2) kept at BCCM/ULC Cyanobacteria Collection at University of Liege, Belgium. Its 16S rRNA gene sequence has Genbank accession number AY493606.

Type locality: Prydz Bay at East Antarctica, Larsemann Hills, Lake 18, microbial mat.

Nodosilinea alaskaensis sp. nov.

Description: Thallus bright green. Filaments with a single trichome, sometimes presenting circular growth. Trichomes long or short, straight or bent, immotile or motile. Cells more or less longer than wide (1)1.4 × 1.8 wide, 2–4.1(8) μm long, lacking aerotopes, with peripheral thylakoids not constricted at the cross walls. Sheath usually present, thin, soft, colorless. Can grow on nitrogen free media. Freshwater. (Figs 6Q–W, 9E, Table 1)

Holotype (here designated): dried specimen of the strain L32 is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–113–1.

Reference strain: L32, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861885.

Type locality: Research lake S5 in Toolik lake area, crust on stones.

DISCUSSION

To date there have been limited studies into cyanobacterial diversity in Alaska, providing little understanding into the biodiversity of their life in extreme climates. Here, we present evidence that cyanobacteria exhibit high levels of species richness in a relatively small sample area. We found 17 unique cyanobacterial genera despite the limitations of our methodical approach being focused on cultivable species. As expected, all the cultivated strains belonged to faster growing species of cyanobacteria. Attempts to cultivate species belonging to Nostocales failed despite the fact they were frequently identified in our samples upon collection. These slower growing species were probably overgrown by the faster growing Oculalteaceae and Lepotolynbyaceae. A total of 36 strains were lost after their isolation from the initial sample to single species culture prompting speculation about the supposed need for a particular chemical compound(s) produced by a co–symbiont within the cyanobacterial consortium.

NGS is the most efficient method in describing natural diversity, however, this technique faces complications in the presence of a high amount of cyanobacterial extrapolysacharides such as is the case in biofilms at the majority of sampling sites in Alaska. These chemical agents disproportionally lower the yields of DNA isolated from environmental samples as well as primer mismatch during amplification processes in NGS (Strunecky *et al.* 2019). Following our previously successful approach we employed here a combination of methods comprising the cultivation of cyanobacteria, optical microscopy and 16S rRNA gene sequencing (Raabova *et al.* 2019). Observed cyanobacterial diversity supported by the sequencing efforts underestimate the true biodiversity at the sample collection sites. The results of our cultivation trials suggest that diversity at the North Slope is much higher. Our discovery of new species demonstrates that further research using NGS is needed to uncover the true biodiversity of the Alaskan region.

Our investigation resulted in identification of members of many ubiquitous cyanobacterial genera such as *Nodosilinea* (Fig. 5R–T and 6Q–W), *Phormidesmis* (Fig. 5W) and *Microcoleus* (Fig. 5K). We also found genera that were described only recently and their known species have few recorded incidences such as *Cephalothrix* (da Silva Malone *et al.* 2015), *Albertania* (Zammit 2018) and *Stenomitos* (Miscoe *et al.* 2016). Their exact phytogeography is expected to emerge in the near future due to the growing number of sequenced specimens.

We described here *Cephalothrix alaskaensis* gen. nov. belonging to Coleofasciculaceae according the original description by Malone *et al.* 2015 and our current phylogenetic analysis. Morphology of *Cephalothrix* resembles the phylogenetically close *Coleofasciculus* (Siegesmund *et al.* 2008) and *Wilmottia* (Strunecky *et al.* 2011). Their soft pale green constricted >5 μm wide trichomes and simple rounded-end cell helps identify this family. A meticulous observation under the microscope is imperative for distinguishing constrictions between the adjacent cells and the characteristic cell granulation.

Two ubiquitous species in Chroococcales were evaluated. Chroococcales in general have similar cellular morphologies, and typically lose their mucilage and colony characteristics in culture (Mareš *et al.* 2019a), often contributing to their misidentification. *Gloeothece membranacea* found in our study however showed its typical morphology as was described in recent revision by Mareš *et al.* (2019a) as well as *Geminocystis* (Korelusova, Kastovský and Komarek 2009). Both species currently have a small number of confirmed observations in polar environments, in particular the Alaskan region.

As the largest family in the Synechococcales the Leptolyngbyaceae have received significantly more attention in recent years (Mai *et al.* 2018). The species within the family have been repeatedly shown to be highly divergent in their 16S rRNA gene sequence, which led to the split into several families. The recently recognized daughter family of Oculateaceae (Mai *et al.* 2018) acquired many species during the latest taxonomical reevaluations. Species within this family have cells about 3 μm wide, generally longer than wide, with visibly parietal thylakoids. Cells are soft and it is easy to crush them during manipulation. Filaments often have a pigmented eye (oculus) in the terminal cell during their growing phase concomitant with the filament motility. Oculatellaceae contained four taxa in our set of cultivated cyanobacteria. Two of them were identified on the species level. *Albertania alaskaensis* exhibits several morphological peculiarities such as perpendicularly oriented cells within trichomes.

The last clade contained strains found to date only in the Arctic and Antarctic. These Alaskan strains clearly do not belong to Leptolyngbyaceae due to their long cells and the arctuated character of the trichomes. Similar strains were previously reported only from mats at the bottom of Antarctic lakes (Taton *et al.* 2006). Our samples were collected in the periphyton of the flowing Alaskan rivers. The members of our newly established genus *Shackletoniella* are strictly aquatic according to current knowledge. The long cells within the trichomes observed in optical microscopy display rounded structures at both ends of cells (Figs 7U–Z, 9B, Table 1). The origin of these structures is unknown, reflecting possibly a special thylakoid pattern (Mareš *et al.* 2019b). Due to recent recognition of many species within Oculatellaceae we expect even more species and genera described within this family that were previously determined as *Leptolyngbya* (Figs 6 and 7).

Unexpectedly, we cultivated a small amount of strains belonging to the Leptolyngbyaceae family. Surprisingly, three strains were not able to grow for an extended time under laboratory conditions. The members of the *Leptolyngbya* group are generally considered versatile, undemanding species growing under any conditions. Our research demonstrates that bacterial dark matter (Lloyd *et al.* 2018) may be more common among cyanobacteria than expected. On the other hand, we found several rarely found species within Leptolyngbyaceae. One of them was *Pseudophormidium* that has a clearly distinguishable overall appearance with typical short non-constricted cells, visi-

bly multilayered sheaths and intense false branching filaments. The phylogenetic placement of *Pseudophormidium* is still unclear according to Komárek *et al.* (2014) because all sequenced representatives are still assigned to Leptolyngbyaceae. According to the older morphology based studies *Pseudophormidium* was assigned to *Phormidium* (Komárek and Anagnostidis 2005) which was later transferred to *Microcoleus* (Komárek *et al.* 2014). Strains within the same cluster as Alaskan strains were previously recognized as *Pseudophormidium* sp. by Osorio-Santos *et al.* (2014) during their study of cyanobacteria from the Atacama Desert. All morphological features of *Pseudophormidium americanum* sp. nov. agree well with the original description of its type *Pseudophormidium phormidioides* (Hansgirg ex Forti 1907). To resolve the question about the proper taxonomic designation of *Pseudophormidium*, the type material shall be obtained, and more sequences compared in further studies. Until then, we follow the morphology based determination which is moreover in congruence with previously sequenced strains. Because the species designation was not figured out previously, we follow the geographic origin of strains found to date in both the South and North Americas and selected the name for this species as *Pseudophormidium americanum*.

Microscopic determination of *Leptodesmis alaskaensis* sp. nov. is highly problematic due to the changes in cell and trichome morphology. Originally, it was described as having long cells without constrictions in young trichomes while older trichomes exhibit short constricted barrel-shaped cells. We found *L. alaskaensis* to be motile. This characteristic was not observed in any other previously described species belonging to the Leptolyngbyaceae.

The largest group of strains that were cultivated belonged to Synechococcales. *Gibliniella alaskaensis* sp. nov. fell phylogenetically between th moderately halophilic *Halomicronema* (Abed, Garcia-Pichel and Hernandez-Marine 2002) and halophilic *Haloleptolyngbya* indicating possible halophily of this species (Fig. 4). Our strains were isolated from a lake undergoing a long-term nutrient enrichment experiment. We cannot rule out either the possibility of their transport to the site along with the fertilizer (Daniels, Kling and Giblin 2015) or their genuine preference for this type of environment. The *Gibliniella* species are extremely thin and intensely motile. Their morphological identification is possible under an optical microscope equipped with precise size measurement suitable for single filament width measurements or localization of characteristic fish-net like fascicles (Fig. 8E–G, 9H). The other cultivated strains in Synechococcales were classified as members of *Nodosilinea* genus despite lacking some of the morphological features typical of *Nodosilinea* such as formation of specific nodules in trichomes (Perkerson *et al.* 2011).

Description of cyanobacterial taxa is based on three pillars: morphology, ecology and similarity of a few selected genes. Morphological determination poses many challenges, especially in cyanobacteria exhibiting simple morphology such as thin trichomes or small coccoid cells. Strains that prosper under laboratory conditions may lose features such as mucilage layers, oculus or end cell shape, rendering morphology based identification difficult or close to impossible. On the other hand, cells exposed to stress or suboptimal conditions can form peculiar structures in nature as we witnessed many times in the samples from Alaska. Correct determination to family or generic level therefore requires inspection of the studied natural material as well as cultured strains.

Ecology and biogeography play a very important role in modern classification of cyanobacteria (Komárek and Anagnostidis 2005; Strunecky, Komarek and Elster 2012). Future discussions will shape the judgement of how much weight should be given to ecological factors in making taxonomic decisions. The assumption that two organisms cannot be the same species if they colonize significantly different habitats may seem intuitive, but increasingly available molecular data indicates that this is not always the case. Examples of this are evident in the fact that several ubiquitous species of Leptolyngbyaceae have been found in hot deserts as well as in freshwater of cold regions (Raabova *et al.* 2019).

The most used gene in bacterial taxonomy is still the 16S rRNA gene. Many of the current bacterial species with validly published names do not respect minimal 95% (for genus) and 98.7% (for species) sequence similarity thresholds that are currently recommended to classify bacterial isolates (Rossi-Tamisier *et al.* 2015). A similar situation can be found in the description of cyanobacterial taxa during the last few years, making recent cyanobacterial classification even more vague, although no detailed analysis has been published.

In conclusion, we followed an established approach to investigate the cyanobacterial diversity of a previously unexplored region of the Alaskan North Slope. This methodology led us to discover two new genera and six new species of cyanobacteria, suggesting the existence of high biodiversity in extreme polar environments. This research provides a foundation for further detailed ecological, biogeographical and genomic studies.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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