FEMS Microbiology Ecology, 97, 2021, fiab104

https://doi.org/10.1093/femsec/fiab104
Advance Access Publication Date: 13 July 2021
Research Article

RESEARCH ARTICLE

Ecology and biogeography of the 'marine *Geitlerinema*' cluster and a description of *Sodalinema orleanskyi* sp. nov., *Sodalinema gerasimenkoae* sp. nov., *Sodalinema stali* sp. nov. and *Baaleninema simplex* gen. et sp. nov. (Oscillatoriales, Cyanobacteria)

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One sentence summary: Phylogenetic relationships among widespread filamentous cyanobacteria from 'marine *Geitlerinema*' cluster are systemized; three new *Sodalinema* species and a new genus *Baaleninema* are described with the emphasis on their ecological diversity and biogeography.

Editor: Max Haggblom

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ABSTRACT

Filamentous cyanobacteria belonging to the 'marine *Geitlerinema*' cluster are spread worldwide in saline environments and considered to play an important ecological role. However, the taxonomy of this group remains unclear. Here, we analyzed the phylogeny, ecology and biogeography of the 'marine *Geitlerinema*' cluster representatives and revealed two subclusters: (1) an 'oceanic' subcluster containing PCC7105 clade and black band disease (BBD) clade with free-living and pathogenic strains distributed in Atlantic, Indian and Pacific Ocean-related localities, and (2) a *Sodalinema* subcluster containing free-living strains from marine, hypersaline, saline-alkaline and soda lake habitats from the Eurasian and African continents. Polyphasic analysis using genetic and phenotypic criteria demonstrated that these two groups represent separate genera. Representatives of *Sodalinema* subcluster were phylogenetically attributed to the genus *Sodalinema*. Our data expand the ecological and geographical distribution of this genus. We emended the description of the genus *Sodalinema* and proposed three new species differing in phylogenetic, geographic and ecological criteria: *Sodalinema*

Received: 22 January 2021; **Accepted:** 11 July 2021

orleanskyi sp. nov., *Sodalinema gerasimenkoae* sp. nov. and *Sodalinema stali* sp. nov. Additionally, a new genus and species *Baaleninema simplex* gen. et sp. nov. was discribed within the PCC7105 clade. By this, we put in order the current confusion of the 'marine *Geitlerinema*' group and highlight its ecological diversity.

Keywords: 'marine *Geitlerinema*'; *Sodalinema*; *Baaleninema*; taxonomy; biogeography; cyanobacteria; polyphasic approach

INTRODUCTION

The taxonomy of cyanobacteria is still in its infancy. Advances in molecular phylogeny have led to the revision of many traditional taxa and the description of new taxa. However, for many cyanobacteria, there is considerable confusion over taxonomic names. Sometimes the erroneous identification of newly isolated strains based only on morphological criteria leads to the subsequent incorrect placement of their genotypes in the GenBank database. Simultaneously, using molecular criteria without correlation with phenotypic features and the absence of molecular data for the type species also lead to incorrect taxonomic identification of strains (Komárek 2020). The assignment of many objectively different organisms to the genus *Geitlerinema* has become a consequence of such confusion.

The genus *Geitlerinema* (Anagnostidis et Komárek; Anagnostidis 1989) *sensu stricto* was separated from the genus *Phormidium* (Anagnostidis 1989). According to the original description, *G. splendidum* was established as a type species, but the genus included several other strains distributed worldwide that differ in morphological features and inhabit ecologically various biotopes. Phylogenetic studies revealed that *Geitlerinema sensu lato* represents a highly heterogeneous and polyphyletic group in its present state (Margheri *et al.* 2003; Do Carmo Bittencourt-Oliveira *et al.* 2009; Perkerson, Perkerson and Casamatta 2010; Hašler *et al.* 2012). In 'Bergey's Manual of Systematics of Archaea and Bacteria', *Geitlerinema* was defined as a form-genus that requires careful taxonomic revision due to the diverse ecology and phylogeny of its representatives (Castenholz, Rippka and Herdman 2015).

Molecular-biological justification of the genus *Geitlerinema* with the type species *G. splendidum* (strains CCALA 1004^T, P014 and P017) was recently made using a polyphasic approach (Strunecký *et al.* 2017). It was shown that some strains defined as various *Geitlerinema* species should belong to other genera; and therefore, a new genus *Anagnostidinema* Strunecký, Bohunická, Johansen et Komárek was separated from *Geitlerinema sensu lato*. Representatives of *Geitlerinema sensu stricto* and *Anagnostidinema* are usually found in freshwater habitats and soils in the form of mats or biofilms on different substrates. Strains of *Geitlerinema sensu lato* inhabiting saline environments are grouped in a phylogenetic cluster distant from both *Geitlerinema sensu stricto* and *Anagnostidinema*. This cluster was designated 'group IV' (Perkerson, Perkerson and Casamatta 2010) or 'marine *Geitlerinema*' (Strunecký *et al.* 2017). It included strain PCC7105 and a group of black band disease (BBD) strains. *Geitlerinema* sp. PCC7105 was recognized as one of the reference strains for the form-genus *Geitlerinema* according to Bergey's Manual (Rippka *et al.* 1979; Castenholz, Rippka and Herdman 2015). BBD strains are known to be pathogenic and strongly associated with black band disease of corals (Myers, Sekar and Richardson 2007). They contribute to disease pathobiology by producing the cyanotoxin microcystin and by giving energy and nutrients to the BBD microbial consortium in a sulphide-rich environment (Richardson *et al.* 2009; Den Uyl *et al.* 2016). Recently, Cellamare *et al.* (2018) described a new genus and species, *Sodalinema komarekii* Cellamare, Duval, Touibi, Djediat and Bernard, which was phylogenetically related to BBD strains. However, its phylogenetic position in the 'marine *Geitlerinema*' cluster was not established. It was isolated from a saline-alkaline crater lake where it floated in the water column with *Arthrospira*-dominated blooms and attached to stromatolites. We have previously found that representatives of the 'marine *Geitlerinema*' cluster were widespread in soda and saline-alkaline lakes in southern Siberia in Russia (Namsaraev *et al.* 2018a; Samylina *et al.* 2014, 2019) and considered them to play an important role in the precipitation of carbonate minerals through the functioning of extracellular carbonic anhydrase (Kupriyanova *et al.* 2007) and the release of exopolysaccharides (Samylina, Zaytseva and Sinetova 2016). Thus, a great number of representatives of 'marine *Geitlerinema*' have been described to date, but the phylogenetic relationships between them remain unresolved.

In this work, six novel haloalkaliphilic strains isolated from soda and saline-alkaline lakes belonging to the 'marine *Geitlerinema*' cluster were characterized by their morphology, ultrastructure, ecology and phylogeny. In addition, we used two haloalkaliphilic and one marine strain from official culture collections, including the type strain of *S. komarekii*. Furthermore, we report here the revision of the 'marine *Geitlerinema*' phylogenetic cluster based on a polyphasic approach and provide a description of *Sodalinema orleanskyi* sp. nov., *Sodalinema gerasimenkoae* sp. nov., *Sodalinema stali* sp. nov. and *Baaleninema simplex* gen. et sp. nov. By this, we put in order the current confusion of the 'marine *Geitlerinema*' group and highlight the ecological diversity of its representatives.

MATERIALS AND METHODS

Strains, their origin and growth media

Cyanobacterial strains Z-D0802, P-1104, T6-1124, T3-1112, G3-1101 and Ey-1201 were isolated previously (Namsaraev *et al.* 2018b; Samylina *et al.* 2014) or in this work from phototrophic biomass collected from soda lakes located in Russia and Tanzania (Tables 1 and 2) using enrichment cultures as described in Namsaraev *et al.* (2018b). Cultures of these strains were deposited in the Culture Collection of Microalgae IPPAS at the K.A. Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences, Moscow (the collection numbers of strains are listed in Table S1, Supporting Information). Strain IPPAS B-353 was obtained from the Culture Collection of Microalgae IPPAS (Russia), strain PMC 869.14 was obtained from the Paris Museum Collection of Cyanobacteria at the National Museum of Natural History (France) in 2018, and strain CCY9619 was obtained from the Culture Collection Yerseke (The Netherlands) in 2013 (this strain is now available at the German Collection of Microorganisms and Cell Cultures GmbH DSMZ at the Leibniz Institute (DSM 101427) and at the Culture Collection of Algae at Göttingen University (SAG 31.92)).

Strains were grown photoautotrophically in M, S and ASN-III media depending on their ecology. The type of medium used for each strain is specified in Table 1. Medium M has the following composition (g/L): Na_2CO_3 – 79.5, $NaHCO_3$ – 21.0, KCl – 2.0, $K_2HPO_4{\cdot}3H_2O$ – 0.5, KNO_3 – 2.0, Na_2SO_4 – 1.4, $FeCl_3$ – 0.0003,

Table 1. Strains used in this work, their growth media and accession numbers of 16S rRNA, *nifH, rpoB, gyrB* genes and 16S-23S internal transcribed spacer (ITS) regions.

Strain	Original definition	Isolator (Reference)	Growth medium	GenBank ID[1]					Definition in this study[2]
				16S rRNA	16S-23S ITS	*nifH*	*rpoB*	*gyrB*	
PMC 869.14	*Sodalinema komarekii*	Cellamare *et al.* (2018)	S	MG772676	MT067569, MT067570	MT093248	MZ230656	MZ230663	*Sodalinema komarekii*
IPPAS B-353	*Microcoleus chthonoplastes*	Gerasimenko L.M. (Gerasimenko, Mityushina and Namsaraev (2003))	S, M	KU375124	MT067571, MT067575	MT093245	ML776472: 4019298–4022627	ML776472: 3274525–3276468	*Sodalinema gerasimenkoae*
Z-D0802	*Geitlerinema* sp.	Samylina O.S. (p.i.)	S, M	MT067568	MT067577	MT093246	MZ230652	MZ230659	*Sodalinema gerasimenkoae*
P-1104	*Geitlerinema* sp. group IV *sensu* Perkerson, Perkerson and Casamatta (2010)	Samylina O.S., Samylina *et al.* (2014)	S, M	MT081568	MT067578	MT093241	SMDP01000048: c12102-8773	SMDP01000010.1: c18012-16069	*Sodalinema* sp.
T6-1124	*Geitlerinema* sp.	Samylina O.S. (p.i.)	S, M	MG197457	MT067579	MG197461	MZ230658	MZ230665	*Sodalinema* sp.
T3-1112	*Geitlerinema* sp.	Samylina O.S. (p.i.)	S, M	MT081569	MT067580	MT093242	MZ230657	MZ230664	*Sodalinema* sp.
G3-1101	*Geitlerinema* sp.	Samylina O.S. (p.i.)	S, M	MT081570	MT067581	MT093243	MZ230655	MZ230662	*Sodalinema* sp.
Ey-1201	*Geitlerinema* sp.	Samylina O.S. (p.i.)	S, M	MT081571	MT067582,MT067572	MT093244	MZ230654	MZ230661	*Sodalinema orleanskyi*
CCY9619	*Microcoleus chthonoplastes* strain 11	Stal and Krumbein (1985)	ASN-III, S	MT067567	MT067573	MT093247	MZ230653	MZ230660	*Sodalinema stali*

[1]Sequences with accession numbers starting with MT and MZ were obtained in this work;
[2]*Sodalinema* sp. stands for unresolved species. *Abbreviations*: p.i.—preliminary identification.

Table 2. Geographic locations and hydrochemical characteristics of habitats from which the studied *Sodalinema* strains were isolated.

					Selected hydrochemical parameters (ranges of values for specified years)				
Strain	Locality	Lake	Coordinates	Type of habitat	Years	Total mineralization (g/L)	Total soluble carbonates (g/L)	pH	References
S. komarekii PMC 869.14	Mayotte Island, Western Indian Ocean	Dziani Dzaha	12°46'13.3'S, 45°17'17.8'E	Saline-alkaline lake	2009–2011	51.8–65.7	10.6–14.5	9.1–9.4	Leboulanger *et al.* (2017)
S. gerasimenkoae IPPAS B-353	Transbaikalia, Russia	Khilganta	50°42'53.5'N, 115°06'08.6'E	Saline-alkaline lake	1995, 2007–2008, 2013–2014	6.5–260 (until completely dry)	0.1–1.5	8.2–9.8	Namsaraev *et al.* (2010); Zamana and Borzenko (2010); Borzenko and Shvartsev (2019)
S. gerasimenkoae Z-D0802	Transbaikalia, Russia	Doroninskoe	51°14'10.0'N, 112°14'05.9'E	Soda lake	1974, 2004–2007, 2015, 2017	16.5–46.9	7.4–25.7	9.6–9.9	Borzenko *et al.* (2007); Borzenko and Zamana (2008); Borzenko (2009); Gorlenko *et al.* (2010); Borzenko and Shvartsev (2019)
Sodalinema sp. T6-1124	Kulunda Steppe, Russia	Tanatar VI	51°37'08.4'N, 79°48'53.0'E	Soda lake	2011–2020	12–250	7.3–207.4	9.8–10.1	Own data
Sodalinema sp. T3-1112	Kulunda Steppe, Russia	Tanatar III	51°39'15.7'N, 79°46'40.3'E	Soda lake	2007, 2011–2020	55–234	27.4–97.6	9.9–10.2	Own data
Sodalinema sp. P-1104	Kulunda Steppe, Russia	Petukhovskoe	52°6′20.52″N, 79°9′22.19″E	Soda lake	2011–2020	29–200	19.5–145.2	9.6–10.0	Own data
Sodalinema sp. G3-1101	Kulunda Steppe, Russia	Bitter-3	51°40'00.4'N, 79°54'43.9'E	Soda lake	2011–2020	25–200	18.3–164.7	9.5–10.5	Own data
S. orleanskyi Ey-1201	Tanzania	Eyasi	3°36'48.5'S, 35°04'24.4'E	Saline-alkaline lake	1960, 1999	11.5–23.5	2.3–7.1	9.5–9.6	Hecky and Kilham (1973); Deocampo (2005)
S. stali CCY9619	Mellum Island, Germany	-	53°43'19.5'N, 8°08'55.4'E	Marine tidal flats	1982	23–34	n.d.	n.d.	Stal, van Gemerden and Krumbein (1985)

EDTA – 0.0005, modified trace element solution (TES) A5+Co – 1 mL/L and pH 10.0. Medium S was less extreme (g/L): $NaHCO_3$ – 16.8, NaCl – 30.0, KCl – 1.0, $K_2HPO_4{\cdot}3H_2O$ – 0.5, $NaNO_3$ – 2.5, K_2SO_4 – 1.0, $MgSO_4{\cdot}7H_2O$ – 0.1, $CaCl_2$ – 0.04, $FeSO_4$ – 0.01, EDTA – 0.08, modified TES A5+Co – 1 mL/L and pH 9.0–9.5. The composition of the modified TES A5+Co was the following (g/L): H_3BO_3 – 2.86, $MgCl_2{\cdot}4H_2O$ – 1.81, $ZnSO_4{\cdot}7H_2O$ – 0.222, $Na_2MoO_4{\cdot}2H_2O$ – 0.39, $CuSO_4{\cdot}5H_2O$ – 0.079, $Co(NO_3)_2{\cdot}6H_2O$ – 0.0494 and pH 10. Medium ASN-III was prepared as described in Rippka *et al.* (1979). Unless indicated otherwise, cells were grown in batch cultures at 31°C under continuous illumination at 50 μmol/m^2/s from cool white fluorescent lamps.

Hydrochemical characteristics of the studied habitats and observations in nature

Kulunda Steppe soda lakes (Petukhovskoe, Tanatars III and VI, Bitter-3) were investigated for several years (Table 2). The total mineralization (salinity) and pH of the brines were measured in the field using a WTW field potentiometer-conductometer (Germany) as described in Samylina *et al.* (2019). The soluble carbonate alkalinity was determined by a two-step titration with 1 M HCl. Samples of benthic phototrophic biomass (*Ctenocladus* communities and cyanobacterial biofilms) were collected from the littoral area and shores of the lakes. The presence of *Sodalinema* in environmental samples of different years was established by morphological criteria using a Jenaval light microscope (Germany).

The hydrochemical characteristics of Transbaikalian lakes Khilganta and Doroninskoe are given according to Borzenko (2009), Borzenko, Zamana and Bukaty (2007), Borzenko and Shvartsev (2019), Borzenko and Zamana (2008), Gorlenko *et al.* (2010), Namsaraev *et al.* (2010) and Zamana and Borzenko (2010). Data for Dziani Dzaha crater lake are given according to Leboulanger *et al.* (2017); data for Lake Eyasi are given according to Deocampo (2005) and Hecky and Kilham (1973) and data for the Aral Sea are given according to Friedrich and Oberhänsli (2004), Sapozhnikov, Kalinina and Nikitin (2017) and Andrulionis and Zavyalov (2019). Data for the coastal tidal flats of Mellum Island (North Sea) are given according to Stal, van Gemerden and Krumbein (1985) and correspond to the period of isolation of strain CCY9619.

The ranges of values of total mineralization, total soluble carbonate concentration and pH are given in Table 2. Detailed data, including the composition of major ions, are given in Table S2 (Supporting Information).

Growth experiments

Growth experiments were performed for the strains IPPAS B-353, T6-1124, G3-1101, PMC 869.14 and CCY9619. The three above-mentioned media (ASN-III, S and M) were used for each strain. The comparable amount of biomass of each strain was transferred into 50-mL Falcon tubes with 30 mL of 0.5 M NaCl solution, precipitated by centrifugation, washed three times and resuspended in 5–10 mL of the same NaCl solution. For inoculation, 1 mL of the obtained suspension was added into the flask with 30 mL of corresponding media and 2 mL of this suspension was used to estimate the initial biomass (dry weight). The cultures were incubated under continuous illumination of 50 μmol/m^2/s from cool white fluorescent lamps either at 32°C (IPPAS B-353, T6-1124, G3-1101 and PMC 869.14) or at 27°C (CCY9619) for 7–8 days. Then, the biomass from the flasks was collected by centrifugation, washed three times with distilled water and used to estimate the final dry weight. To estimate the biomass productivity of the strains in each media, the difference between final and initial values of dry weights was calculated.

Morphological characterization of strains

The morphology of strains was examined in wet mounts at a magnification of ×1000 under a Jenaval microscope equipped with a Zeiss Bundle Canon PS G9 digital camera (Germany). The images were obtained using the AxioVision Rel. 4.7 software package. For the morphological studies, all cultures were incubated at room temperature and natural light-dark cycles for 2 weeks. The parameters analyzed included cell width and length, the morphology of cells and filaments and motility. Assessment of sheath presence and type of granulation at cross-walls was made using older cultures if necessary. In this case, data are indicated by asterisks in Table 3.

Ultrastructure

The ultrastructure of cyanobacterial cells was analyzed by transmission electron microscopy as described in Samylina, Zaytseva and Sinetova (2016). Briefly, the cyanobacterial filaments were fixed with 4% paraformaldehyde solution in 0.2 M sodium phosphate buffered saline (pH 7.2), postfixed in 1% OsO_4, dehydrated and embedded in Epon resin (Sigma-Aldrich, St. Louis, MO). Ultrathin sections prepared with a LKB microtome (Sweden) were sequentially contrasted with uranyl acetate and lead citrate according to Reynolds (1963) and analyzed with a TEM Libra-120 (Carl Zeiss, Germany) and JEM-1400 (JEOL, Japan). To visualize exopolysaccharides, samples were stained with a 1% solution of Alcian blue in 1% acetic acid (Nermut and Svobodová 1965) before postfixation in OsO_4.

Amplification, cloning and sequencing

Standard molecular biology protocols were used for all DNA manipulations. All synthetic oligonucleotides were synthesized by Evrogen (Moscow, Russia). PCR was performed using high fidelity Tersus polymerase (Evrogen). Unless otherwise indicated, amplified fragments were gel-purified, extracted from agarose with a GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced with a capillary Sanger-type automated DNA sequencer (Evrogen). Alternatively, purified DNA fragments were ligated into the pTZ57R/T vector (Thermo Fisher Scientific). The recombinant plasmids were cloned in *Escherichia coli* cell strain XL1-Blue (Agilent Technologies, Santa Clara, CA, USA). The nucleotide sequences of cloned DNA fragments were also determined by Sanger sequencing. If necessary, the sequences were assembled using the SeqMan Pro module of the Lasergene v. 12.3.1 software package (DNAStar Inc., Madison, WI, USA) and edited using BioEdit (Hall 2011) or Lasergene EditSeq. Primary comparison of the *de novo* determined sequences with those available from the GenBank database was performed using the BLASTN algorithm (http://www.ncbi.nlm.nih.gov/blast). All nucleotide sequences determined in this work were deposited in GenBank with the accession numbers indicated in Table 1.

16S rRNA gene and 16S-23S rRNA internal transcribed spacer (ITS)
For the strains P-1104 and T6-1124, the nucleotide sequences of the 16S rRNA gene were determined previously (Namsaraev *et al.* 2018b; Samylina *et al.* 2014). In other cases, genomic DNA was isolated with the hot-phenol method as described

Table 3. Morphological features of the *Sodalinema* strains used in this study.

Species	Strain	Cell dimensions (μm)		Cell shape	Apical cell	Polar gas vacuole	Granulation at cross walls		Arrangement of thylakoids	Motility
		Length	Width				C	Y		
S. komarekii	PMC 869.14	(3)3.9–5.7	2.6–3.0	E	R	+	+	n/o	PCF	+
S. orleanskyi	Ey-1201	2.9–5.0	2.1–2.7	E	R	+	+*	+	PCF	+
S. gerasimenkoae	IPPAS B-353	3.1–5.0	2.8–3.5	I or E	R	+	+	+	PCF	+
S. gerasimenkoae	Z-D0802	2.4–3.2	2.3–2.8	I or E*	R	+	+	+	PCF	+
Sodalinema sp.	T6-1124	2.9–3.4	2.3–3.0	I	R	+	n/o	+*	PCF	+
Sodalinema sp.	T3-1112	3.0–3.7	2.8–3.5	I	R	+	+	+*	PCF	+
Sodalinema sp.	G3-1101	3.3–3.8	3.1–3.5	I or E*	R	+	+	+	PCF	+
Sodalinema sp.	P-1104	3.3–4.6	3.2–4.6	I or E*	R	+	+	+	PCF	+
S. stali	CCY9619	2.6–4.8(5.5)[1]	2.6–2.9 (3.5)[1]	I or E*	R or Ex	+	+	n/o	PCF	+

[1]Dimensions in brackets denote the maximal length/width according to the initial description of the strain CCY9619 by Stal and Krumbein (1985); *Abbreviations*: E – elongated, I – isodiametric, PCF – parietal with a central fascicle, R – rounded, Ex – with extrusions; Y – yellowish granulations with homogeneous or finely granular content, C – a refractive cyanophycin granule(s) on either or both sides of cross-walls; '+' – feature is present, 'n/o' – feature was not observed. *Asterisk* marks data obtained for old cultures or natural samples.

in Mironov *et al.* (2016). Amplification of the 16S rRNA gene was performed with two universal bacterial-specific primers: 8F (5′-AGAGTTTGATCCTGGCTCAG) and 1492R (5′-AAGGAGG TGATCCAGCCACA; Lane 1991). For amplification of the ITS region, the forward primer 5′-TGTACACACCGCCCGTC and the reverse primer 5′-CTCTGTGTGCCTAGGTATCC were used (Iteman *et al.* 2000). *Escherichia coli* recombinant colonies containing cyanobacterial fragments of the 16S rRNA gene were selected by PCR using specific CYA106F (5′-CGGACGGGTGAGTAACGCGTGA) and CYA781R (5′-GACTACWGGGGTATCTAATCCCWTT) primers (Nübel, Garcia-Pichel and Muyzer 1997).

nifH gene

Genomic DNA was isolated from the biomass as described previously (Bulygina *et al.* 2002). Universal bacterial primers nifH-F (5′-AAAGGYGGWATCGGYAARTCCACCAC-3′) and nifH-R (5′-T TGTTSGCSGCRTACATSGCCATCAT-3′; Rösch, Mergel and Bothe 2002) were used for amplification of the *nifH* gene. The PCR products were purified in a 0.7% agarose gel using the Wizard SV Gel and PCR Clean-Up System kit (Promega, Madison, WI, USA). Sequencing of the PCR products was conducted on an ABI 3730 automatic sequencer (Applied Biosystems, Waltham, MA, USA) using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's recommendations.

rpoB gene

Genomic DNA was extracted using an InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA, USA). A total of two overlapping fragments of *rpoB* (1492 and 1068 bp), which included the most variable gene regions, were amplified using the corresponding specific primer pairs: (1) F1405 (5'-TACATGACCGCCGACGAAG) and R2896 (5'-CGACCAAGTGAACCAGTTTGAG) and (2) F1997 (5'-TCGTGGCCTATATGCCCTGG) and R3064 (5'-CGGATTTGACGGTGAGCAGTTC). The primers corresponded to the highly conserved regions of *rpoB* gene sequences extracted from whole-genome shotgun contigs of 'marine *Geitlerinema*' representatives: IPPAS B-353 (GenBank, ATDS01000001.1), P-1104 (GenBank, SMDP01000048.1), PCC7105 (GenBank, ANFQ01000107.1), HE10JO (GenBank, FSSI02000012.1), FCII (GenBank, NRIU01002520.1) and BBD 1991-9 (GenBank, LWRO01000075.1). The primers were numbered on the basis of the IPPAS B-353 *rpoB* gene sequence.

gyrB gene

Genomic DNA was extracted using an InstaGene Matrix kit (Bio-Rad). Almost complete (approximately 1915 bp) *gyrB* gene sequences were amplified using primers gyrB-F (5'-ATGACTAGTAGCTACAGTGCCGATCAAATCC) and gyrB-R1 (5'-GGNCCTTCTGTTTCGATGAATTCKCGG). The primers corresponded to the highly conserved regions of *gyrB* gene sequences extracted from whole-genome shotgun contigs of the strains IPPAS B-353 (GenBank, ATDS01000001.1) and P-1104 (GenBank, SMDP01000010.1). The first round of DNA fragments sequencing was performed with the same forward and reverse primers that were used in the amplification. Then, the additional primers were designed to determine the nucleotide sequence of the middle part of the *gyrB* fragments: gyrB-R2 (5'-CTTCGCCCTTAATCCCTAACC) for CCY9619 and gyrB-R3 (5'-GTCTTCCCCTTTAATCCCTAATCC) for the strains PMC 869.14, Z-D0802, T6-1124, T3-1112, G3-1101 and Ey-1201. Full-length or partial nucleotide sequences of the *gyrB* gene for other studied strains were retrieved from their whole-genome shotgun contigs at GenBank with the following accession numbers: ATDS01000001.1 (for IPPAS B-353), SMDP01000010.1 (for P-1104), ANFQ01000049.1 (for PCC7105), FSSI02000059.1 (for HE10JO), NRIU01001594.1 (for FCII) and LWRO01000045.1 (for BBD 1991-9).

Phylogenetic analysis and analysis of the secondary structures of 16S-23S rRNA ITS regions

In order to determine the phylogenetic position of 'marine *Geitlerinema*' representatives among other cyanobacteria, we conducted a comparative analysis of their 16S rRNA gene sequences with those of type strains of the validly described species of the genera belonging to families *Coleofasciculaceae* and *Microcoleaceae* within order *Oscillatoriales* according to AlgaeBase (Guiry and Guiry 2021). We used only genera supported by molecular phylogeny, including a 16S rRNA gene sequence of the type species. Nucleotide sequences of the 'marine *Geitlerinema*' strains were obtained in this study or retrieved from the GenBank database at the NCBI. Only strains of cyanobacteria belonging to the 'marine *Geitlerinema*' cluster with available 16S rRNA and 16S-23S rRNA spacer region nucleotide sequences were used for the phylogenetic analysis (including analysis of the *nifH*, *rpoB* and *gyrB* genes sequences). The exception was strains from the BBD clade (BBD

HS217, BBD HS223 and BBD P2b-1), which were not used in 16S-23S ITS region analysis because the relevant sequences were not available from any databases.

Multiple alignments of nucleotide sequences were performed with CLUSTAL OMEGA 1.2 (Sievers *et al.* 2011) or Lasergene MegAlign. Since the minimal sequence length of 16S rRNA genes retrieved from GenBank was 1160 bp, all sequences were truncated to this length after alignment. Maximum likelihood phylogenetic trees were constructed using the MEGA 7.0 (Kumar, Stecher and Tamura 2016) software. The statistical significance of the branching order was determined by bootstrap analysis of 1000 alternative trees and expressed as percentages.

Both conserved (D1, D1', D2, D3 and D4) and variable (V2 and V3) regions, tRNA genes and the antiterminators (Box-B and Box-A) of the 16S–23S rRNA ITS sequence were identified according to Iteman *et al.* (2000) or on the basis of information on respective region locations in 'marine *Geitlerinema*' group organisms, which was available from the NCBI database or research papers. Secondary structures of ITS regions were obtained by the Sfold software version 2.2 (Ding, Chan and Lawrence 2004) using default settings. Structures with minimal Gibbs energy were chosen for the analysis. The locations of variable stems (V2 and V3) were also verified by folding of the potential nucleotide sequence region with the Sfold software. For several strains, the analysis of secondary structures of the entire nucleotide sequence located between two tRNAs ($rRNA^{Ile}$ and $rRNA^{Ala}$) showed that this area contains stem-loop structure V2 with free nucleotide 'tails' on its ends. If the sequence corresponding only to the V2 helix was reanalyzed with the Sfold tool, the hairpin region shifted to release new free 'tails'. However, the Gibbs energy for these second structures was higher. Thus, it was assumed that the first structure with lower Gibbs energy reflects the actual position of the V2 helix.

Fatty acid analysis

For determination of the fatty acid (FA) composition of total lipids, the strains PMC 869.14, Ey-1201, IPPAS B-353 and CCY9619 were grown in batch cultures for 7–10 days as described above. Next, 30mL samples were precipitated by centrifugation, washed with deionized water, transferred to 2-mL microtubes and again precipitated by centrifugation. Then, the precipitates were resuspended in 400 μL of 1 M KOH in 80% ethanol and incubated at 75°C for 1 h. The obtained extracts were washed with 400 μL of *n*-hexane to remove unsaponified components (e.g. carotenoids, sterols and so on) and then neutralized by the addition of 100 μL of 20% aqueous H_2SO_4. Furthermore, free FAs were extracted with 300 μL of *n*-hexane, transferred to new microtubes, dried by argon flow, and subsequently converted into their methyl esters by incubation in 1% H_2SO_4 methanolic solution at 55°C for 1 h.

The obtained fatty acid methyl esters (FAMEs) were extracted with 200 μL of *n*-hexane and analyzed by a GC-MS Agilent 7890A gas–liquid chromatography system with an Agilent 5975S mass spectrometric detector (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-88 capillary column (60 m × 0.25 mm, 0.2 μm; (88% cyanopropyl)-arylpolysiloxane; Agilent Technologies). Other separation conditions for FAMEs were as follows: the helium pressure in the injector was 245 kPa, the flow rate was 1.2 mL/min, the volume of the sample solution was 1 µL (ca 10 μg FAMEs), the split ratio was 10:1 and the evaporation temperature was 250°C. The program of the column gradient temperature was as following: from 120 to 175°C at 10°C/min (10 min hold), from 175 to 210°C at 5°C/min (5 min hold) and from 215 to 230°C at 5°C/min (20 min hold). The operating temperature of the mass spectrometric detector and ionization energy were 240°C and 70 eV, respectively.

RESULTS

Analysis of 16S rRNA gene sequence data

A comparative analysis of the partial sequences of 16S rRNA genes (1160 bp) of the strains in the focus of this study, type species and strains from genera belonging to families *Coleofasciculaceae* and *Microcoleaceae* within order *Oscillatoriales* showed a clear separation of the 'marine *Geitlerinema*' cluster (Fig. 1). The phylogenetic relationships between the selected representatives of 'marine *Geitlerinema*' are shown in the subtree in Fig. 1. These strains were divided into two groups: (1) an 'oceanic' subcluster containing the PCC7105 clade and BBD clade with free-living and pathogenic strains distributed in Atlantic, Indian and Pacific Ocean-related localities (Fig. 2) and (2) a *Sodalinema* subcluster that included the 16S rRNA gene sequence of the type strain of the recently described species *S. komarekii* PMC 869.14 (Cellamare *et al.* 2018) and sequences of free-living strains isolated from different geographical localities on the Eurasian and African continents (Fig. 2).

The nucleotide identity of the partial 16S rRNA sequences between the strains belonging to *Sodalinema* and 'oceanic' subclusters was approximately 95% (Table S3, Supporting Information), suggesting that these groups can represent two closely related but separate genera. The nucleotide identity of the partial 16S rRNA gene sequences within the *Sodalinema* subcluster was 99–100% (Table S3, Supporting Information). This implies their affiliation with the genus *Sodalinema*; however, delineation of species requires further analysis. Simultaneously, 16S rRNA gene sequences within the 'oceanic' subcluster demonstrate approximately 97–100% nucleotide identity, which also indicates that the group can include different species.

Analysis of nucleotide sequences and secondary structures of 16S–23S ITS regions

A phylogenetic tree based on the analysis of 16S–23S ITS nucleotide sequences of strains belonging to the 'marine *Geitlerinema*' cluster revealed that they are clearly divided into two separate groups (Fig. 3), identical to those previously identified by 16S rRNA gene sequence analysis (Fig. 1): (1) an 'oceanic' subcluster containing the PCC7105 clade, and (2) a *Sodalinema* subcluster. Within each subcluster, ITS sequences demonstrated high similarity (Figure S1, Supporting Information).

Furthermore, the 'oceanic' subcluster was divided into three clades (subgroups) corresponding to different geographic locations: (i) a USA clade with PCC7105 and Flo1; (ii) a Brazilian clade with CENA552 and CENA556 and (iii) an Indian clade, including only one strain, FC II. The strains from the *Sodalinema* subcluster were also divided into geographic subgroups (Fig. 3): (i) a Transbaikalian subgroup (IPPAS B-353 and Z-D0802), (ii) a Kulunda subgroup (P-1104, T3-1112 and G3-1101), (iii) an East African subgroup (PMC 869.14 and Ey-1201), (iv) a North Sea subgroup (CCY9619, SAG 31.92, 'Schier', OL 75, OL 'sphere', OL S6, OL S12 and HE10JO) and (v) an Aral Sea subgroup (FO-SIORAS-13 and SK-SIORAS-13). Strain T6-1124 isolated from Kulunda Steppe soda lake was the only problematic strain that was closer to the Transbaikalian subgroup than to the Kulunda subgroup.

All the ITS sequences possessed conserved domains (D1, D1', D2, D3 and D4), two transfer RNA genes ($tRNA^{Ile}$ and $tRNA^{Ala}$),

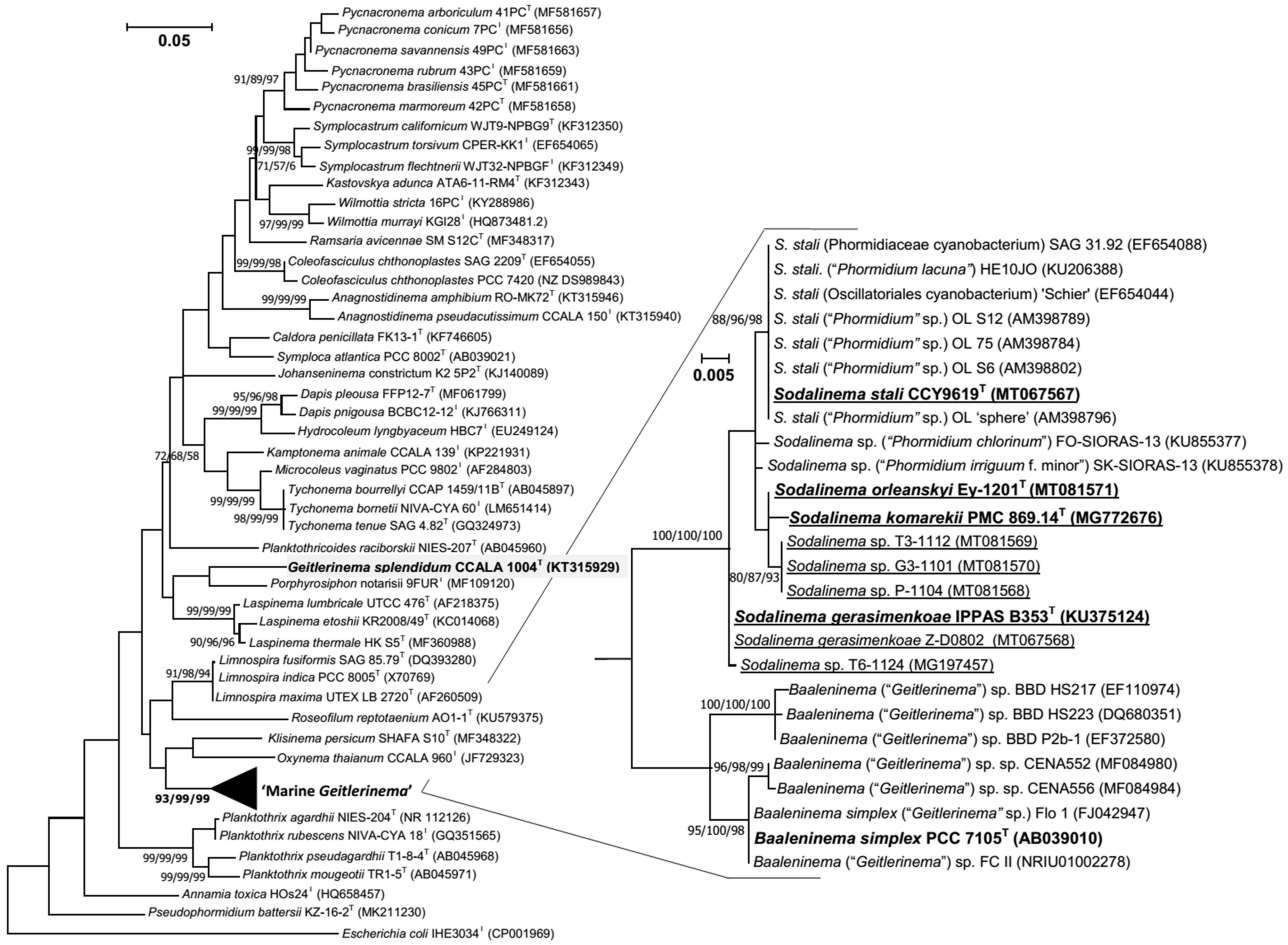

Figure 1. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences (1160 bp) showing the positions of the studied strains among type strains and species of genera belonging to families *Coleofasciculaceae* and *Microcoleaceae* within order *Oscillatoriales* and detailed view of the 'marine *Geitlerinema*' cluster showing phylogenetic relationships within it. The strains used in this work are underlined. The type strains of *Sodalinema* and *Baaleninema* species are shown in bold. The type strain and species of the genus *Geitlerinema sensu stricto* (*G. splendidum* CCALA 1004^T) is in the grey box. The numbers above branches indicate statistical significance of the branching order as determined by bootstrap analysis of 1000 alternative trees (only values higher than 70% are shown). The levels of bootstrap support are given for the ML/NJ/ME algorithms. The scale bar shows evolutionary distance corresponding to five substitutions per 100 (left panel) or 1000 (right panel) nucleotides.

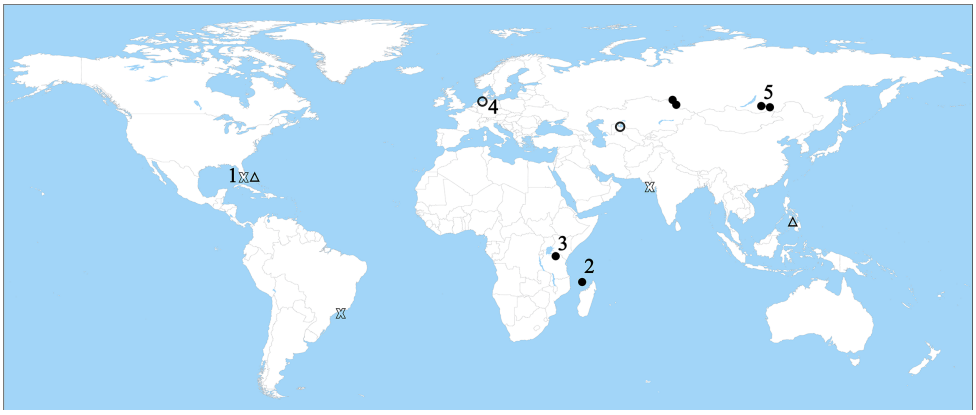

Figure 2. Worldwide distribution of the 'marine *Geitlerinema*' strains used in this study. Signs: *crosses* – strains from the PCC7105 clade, *triangles* – strains from the BBD clade, *circles* – strains from the *Sodalinema* subcluster, hollow signs – saline habitats (marine and inland) and filled signs – saline-alkaline and soda habitats. (1) *B. simplex* (strain Flo1), (2) *S. komarekii*, (3) *S. orleanskyi*, (4) *S. stali* and (5) *S. gerasimenkoae*. For detailed information on the localities, see Table 2 and Table S2 (Supporting Information).

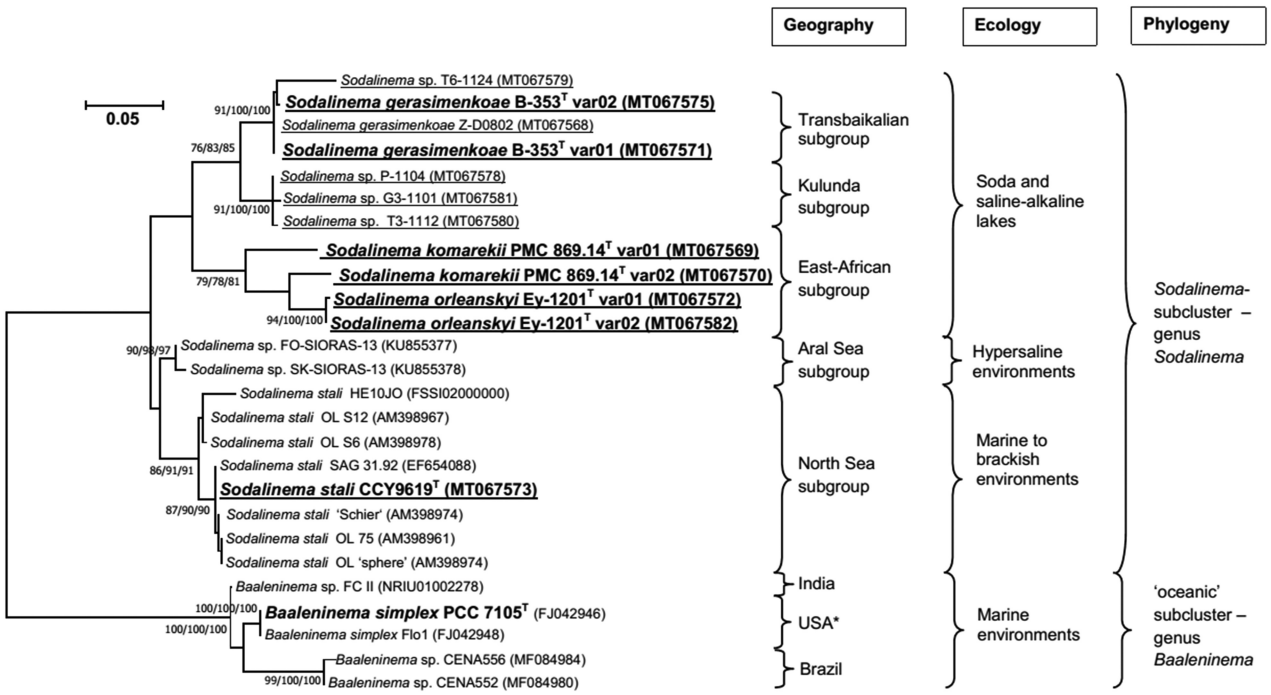

Figure 3. Maximum likelihood phylogenetic tree based on 16S–23S ITS region sequences showing the position of the studied strains among representatives of the 'marine *Geitlerinema*' cluster. The strains used in our work are underlined. The type strains of the *Sodalinema* and *Baaleninema* species are shown in bold. The numbers above branches indicate statistical significance of the branching order as determined by bootstrap analysis of 1000 alternative trees (only values higher than 70% are shown). The levels of bootstrap support are given for the ML/NJ/ME algorithms. The scale bar shows the evolutionary distance corresponding to five substitutions per 100 nucleotides. *Asterisk* – the exact original habitat for strain PCC7105 is unknown.

two variable regions (V2 and V3) and two antiterminator sites (box B and box A; Figure S1, Supporting Information). In the case of strains IPPAS B-353, Ey-1201 and PMC 869.14, amplification and subsequent cloning of the ITS region led to the identification of two variants of ITS. This is probably due to the presence of two rRNA operons in the genomes of these organisms. This fact has been confirmed for strain IPPAS B-353 with the genome available in GenBank (ac. no. PRJNA203668). In strains IPPAS B-353 and Ey-1201, two variants of the ITS region differed by only one or two nucleotides. In Ey-1201, the nucleotide substitution was not a part of any region with a potential secondary structure. In IPPAS B-353, one of the nucleotide replacements was a part of the Box-B helix sequence. In the case of *S. komarekii* PMC 869.14, two variants of the ITS region varied significantly and had only 92% identity. Nucleotide replacements were found in the D1-D1', V2 and Box-B regions in this strain, but they did not change the secondary structures of the corresponding helices (Fig. 4 and Figure S1, Supporting Information). Thus, these substitutions were considered but did not affect the final conclusions regarding the taxonomic affiliation of the strains.

Secondary structure analysis was performed for the D1–D1', V2 and Box-B regions (Fig. 4). Strains belonging to the *Sodalinema* subcluster notably differed from representatives of the 'oceanic' subcluster in the predicted secondary structures of the D1–D1' and V2 helices (Fig. 4A and B). The V2 helix was the most variable, and its length was 27–56 nucleotides for the *Sodalinema* group or 72–80 nucleotides for the 'oceanic' strains (Fig. 4B and Figure S1, Supporting Information). The secondary structures of the Box-B motif (Fig. 4C) were more uniform than those of the D1–D1' and V2 regions, but the nucleotide sequences of Box-B in strains belonging to the *Sodalinema* subcluster were one nucleotide longer and had several substitutions compared to those of the 'oceanic' subcluster.

Based on the secondary structures of the D1–D1' and V2 regions, each of the two groups forming the 'marine *Geitlerinema*' cluster could be split into several separate subgroups (Fig. 4A and B). Thus, 'oceanic' strains were subdivided into three subgroups identical to those identified by ITS sequence analysis (Fig. 3): (i) a USA subgroup with PCC7105 and Flo1, (ii) a Brazilian subgroup with CENA552 and CENA556 and (iii) an Indian subgroup with FC II. Joint analysis of the secondary structure of D1–D1' and V2 helices within *Sodalinema* subcluster representatives revealed five subgroups: (i) strain PMC 869.14, (ii) strain Ey-1201 (i and ii both from the East African region), (iii) a joint Transbaikalian and Kulunda subgroup (IPPAS B-353, Z-D0802, P-1104, T3-1112, T6-1124 and G3-1101), (iv) a North Sea subgroup (CCY9619, SAG 31.92, 'Schier', OL 75, OL 'sphere', OL S6, OL S12 and HE10JO) and (v) an Aral Sea subgroup (FO-SIORAS-13 and SK-SIORAS-13). All these subgroups were generally similar to those identified by ITS sequence analysis except that Transbaikalian and Kulunda strains fell into one subgroup; likewise, East African strains were divided into two subgroups. Although strains PMC 869.14 and Ey-1201 were geographically and phylogenetically close (Figs 2 and 3), their V2 regions differed both in sequence length and secondary structure (Fig. 4B).

Phylogenetic analysis of *rpoB* and *gyrB* genes sequence data

To determine whether all of these subgroups from the 'oceanic' and *Sodalinema* subclusters belong to different species or represent geographic populations and/or ecotypes, we additionally

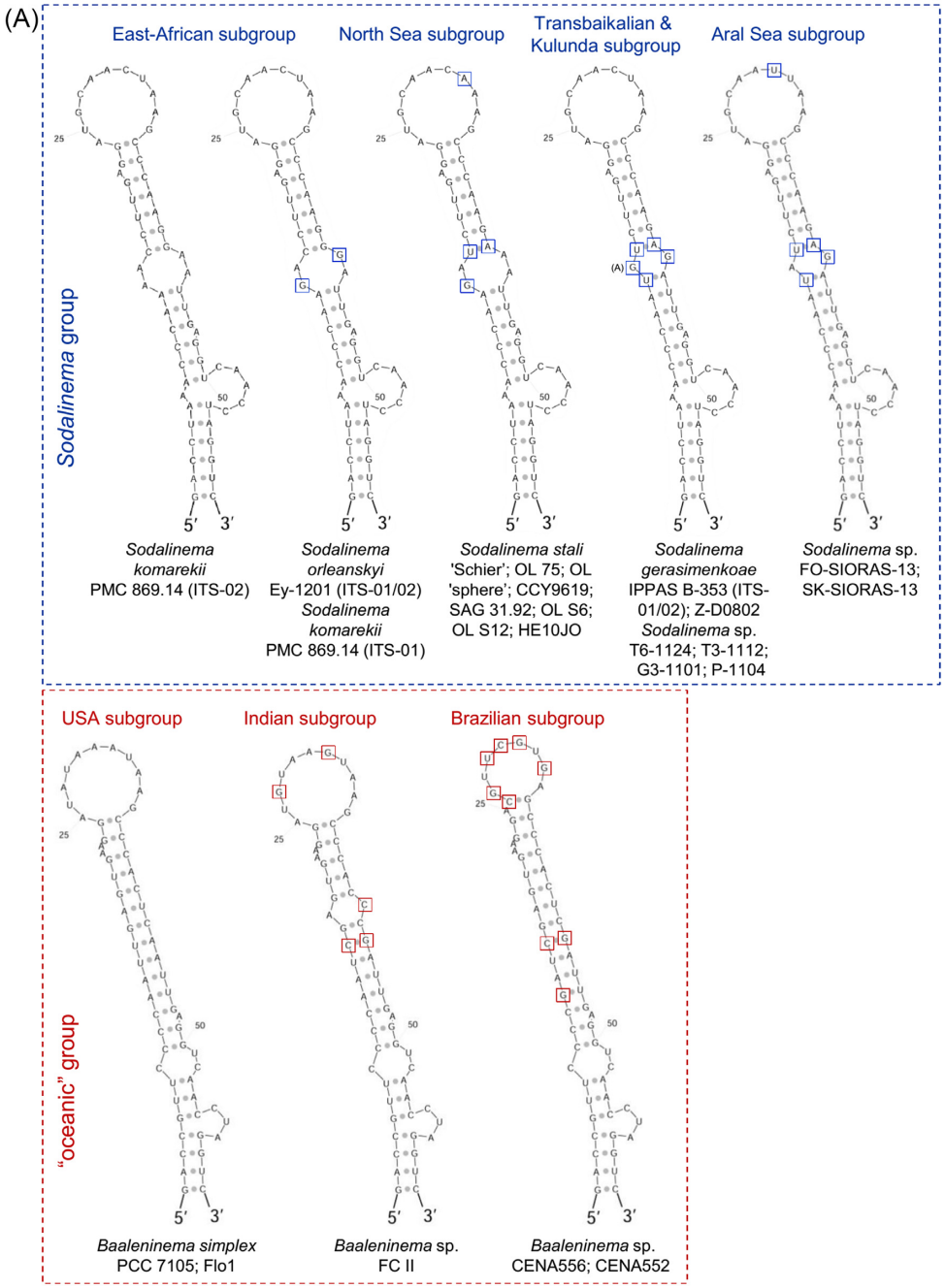

Figure 4. Putative secondary structures of the 16S–23S rRNA ITS regions of the strains belonging to the 'marine *Geitlerinema*' cluster: **(A)** D1–D1' helix. Nucleotides different from those in the consensus (D1–D1' region of the ITS-02 sequence of PMC 869.14 for the *Sodalinema* group or that of the ITS sequence of PCC7105 for the 'oceanic' group) are outlined with squares. **(B)** V2 helix. Nucleotides of the V2 region that are different in two variants of PMC 869.14 ITS are indicated. **(C)** Box-B helix. Variable nucleotides within the Box-B region secondary structures of all studied strains of *Sodalinema* and *Baaleninema* are indicated by blue squares. Nucleotides in the Box-B helix of the genus *Baaleninema* that are different from those of *Sodalinema* are outlined with arrows. For the strains with two sequenced variants of ITS, both types of D1–D1', V2 and Box-B helix structures are shown if there are differences.

analyzed the sequences of the housekeeping genes encoding the beta subunits of RNA polymerase (*rpoB*) and DNA gyrase (*gyrB*).

A phylogenetic tree based on the analysis of the combined *rpoB*+*gyrB* genes sequences (Fig. 5) generally confirmed the clustering of strains on the 16S rRNA tree (Fig. 1). The 'oceanic' subcluster (strains PCC7105, FC II and BBD 1991-9) clearly separated from the *Sodalinema* subcluster at the genus level. The nucleotide identity of partial (1550 bp) *rpoB* sequences between the strains belonging to these subclusters was approximately 80–84% (Table S4, Supporting Information), which correlates with membership of different genera according to Adékambi *et al.* (2008). Further clustering of *Sodalinema* strains revealed several clades: (i) North Sea strains (CCY9619 and HE10JO), (ii) strain PMC 869.14, (iii) strain Ey-1201 combined with Kulunda strains P-1104 and G3-1101 and (iv) joint Transbaikalian (IPPAS B-353 and Z-D0802) and Kulunda (T3-1112 and T6-1124) strains. Thus, clustering based on the analysis of housekeeping genes did not directly reflect the geography of the strains as it did for 16S–23S ITS sequences

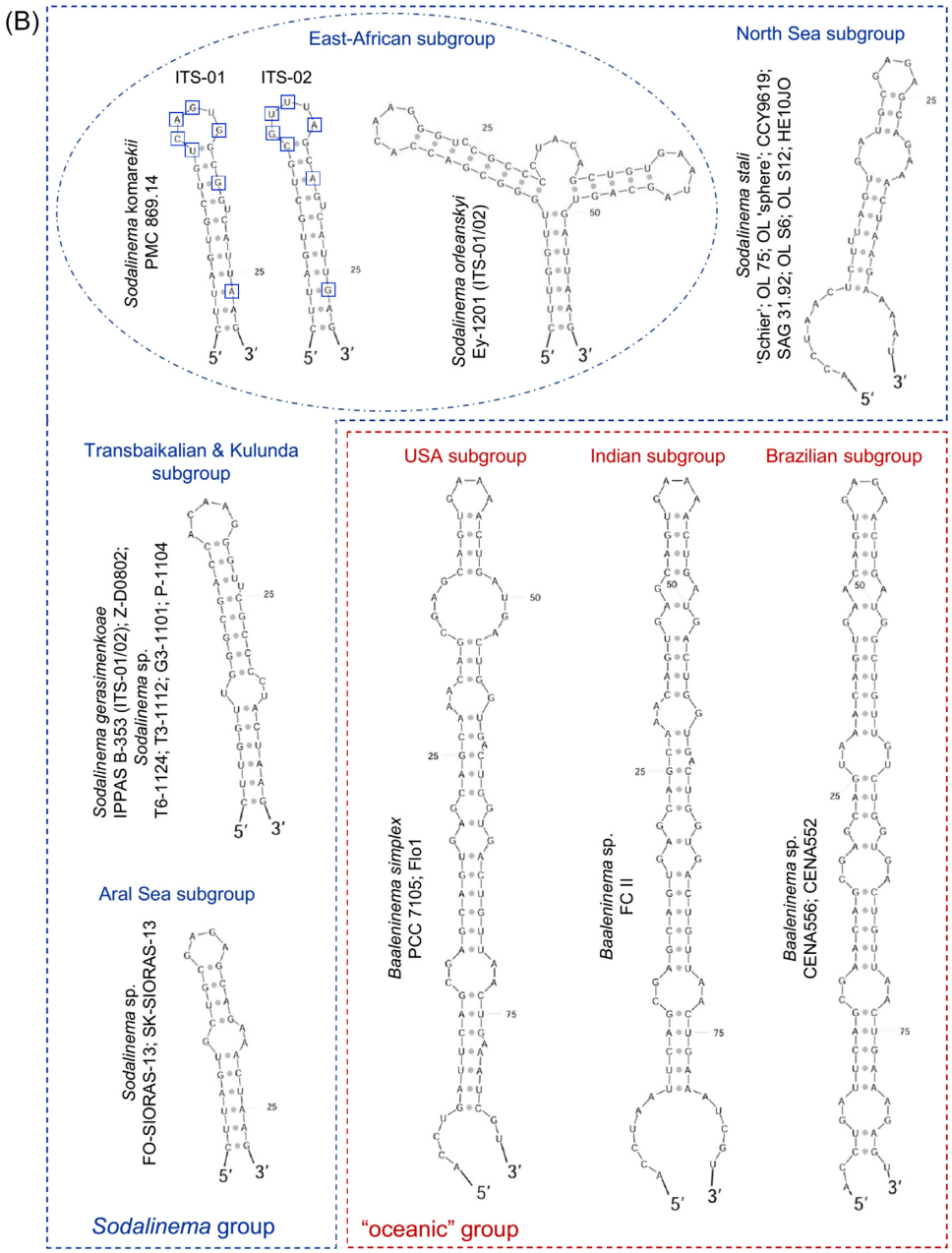

Figure 4. *Continued.*

(Fig. 3). According to the 93–97.5% nucleotide identities of partial *rpoB* sequences between these clades (Table S4, Supporting Information), they represent different species (Adékambi *et al.* 2008). Nevertheless, the species affiliation of the couples of strains T6-1124 + T3-1112 and P-1104 + G3-1101 requires further discussion.

Phylogenetic analysis of *nifH* gene sequence data

We established earlier that representatives of the 'marine *Geitlerinema*' from soda lakes of southwestern Siberia in Russia possess *nifH* genes (Namsaraev *et al.* 2018b) and considered them to be phototrophic diazotrophs potentially active in up to 100 g/L of total salts (Samylina *et al.* 2019). To determine whether other representatives of 'marine *Geitlerinema*' also possess *nifH* genes, we conducted additional phylogenetic analysis.

Amplification products with universal bacterial primers for *nifH* genes were obtained for all studied *Sodalinema* strains (Fig. 6, underlined). In a phylogenetic tree based on a comparison of amino acid sequences translated from *nifH* genes, these strains formed a single group with a high level of identity (99.7–100%). This group was a part of an extensive cluster formed by *nifH* gene sequences of the representatives of *Deltaproteobacteria/Chlorobi* phyla. The cyanobacterial clade also included *nifH* genes belonging to *Coleofasciculus chthonoplastes* PCC7420 and *Roseofilum reptotaenium* AO1-A. It was suggested for *C. chthonoplastes* PCC7420 that its *nif* operon (*nifHDKENB*) was most likely obtained during horizontal gene transfer from

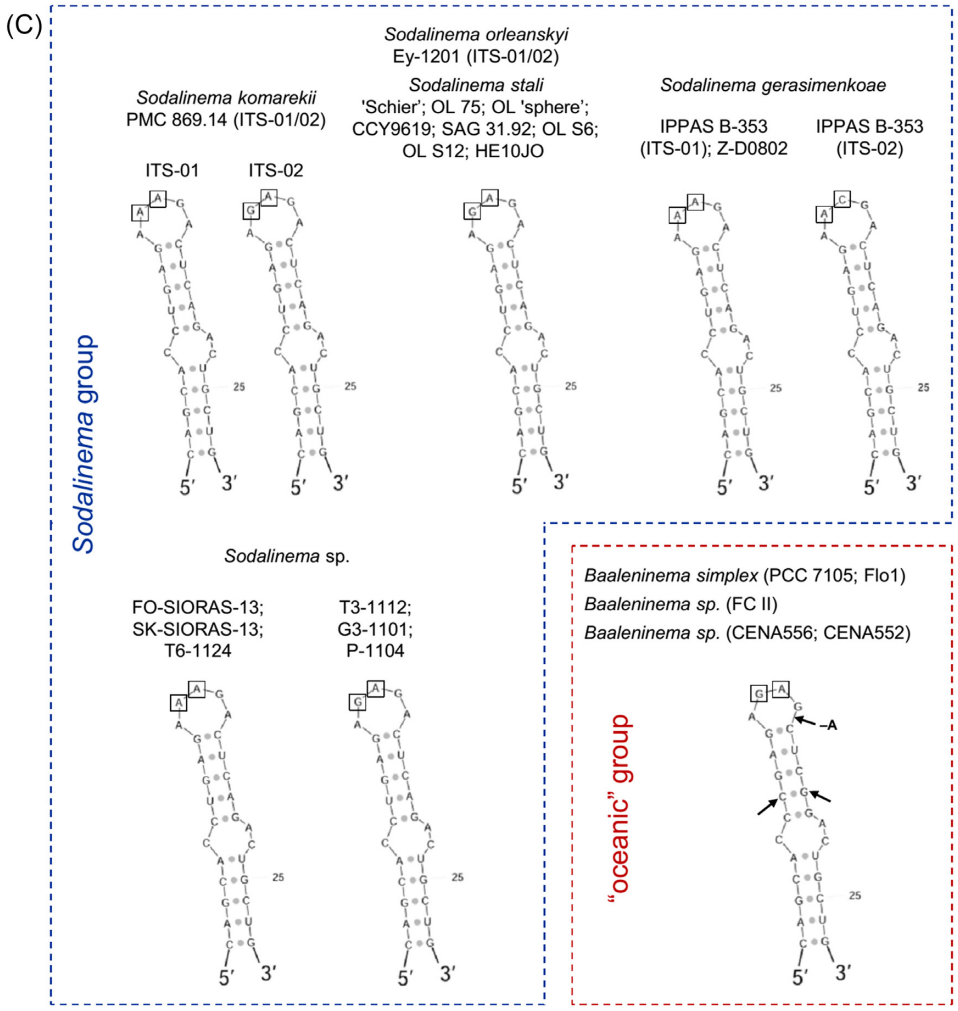

Figure 4. *Continued.*

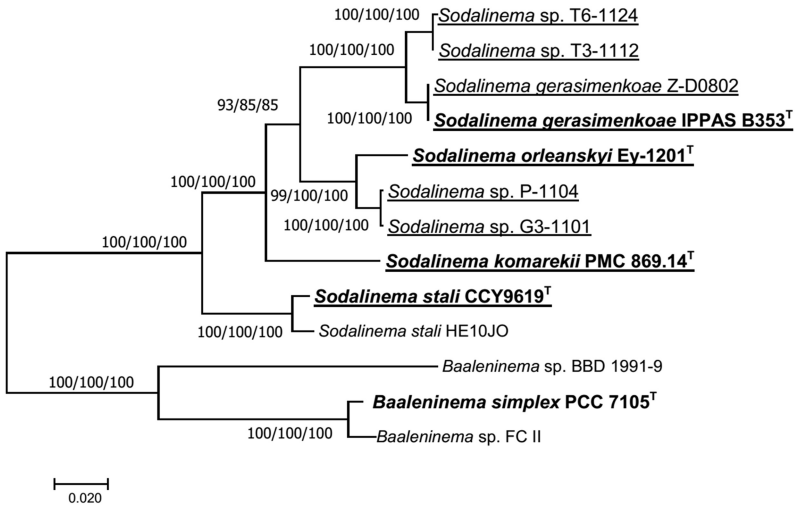

Figure 5. Maximum likelihood phylogenetic tree based on the combined *rpoB+gyrB* genes sequences showing the relationships of the *Sodalinema* and *Baaleninema* strains. The strains used in this work are underlined. The type strains of *Sodalinema* and *Baaleninema* species are shown in bold. The numbers above branches indicate statistical significance of the branching order as determined by bootstrap analysis of 1000 alternative trees (only values higher than 70% are shown). The levels of bootstrap support are given for the ML/NJ/ME algorithms. The scale bar shows the evolutionary distance corresponding to five substitutions per 100 nucleotides.

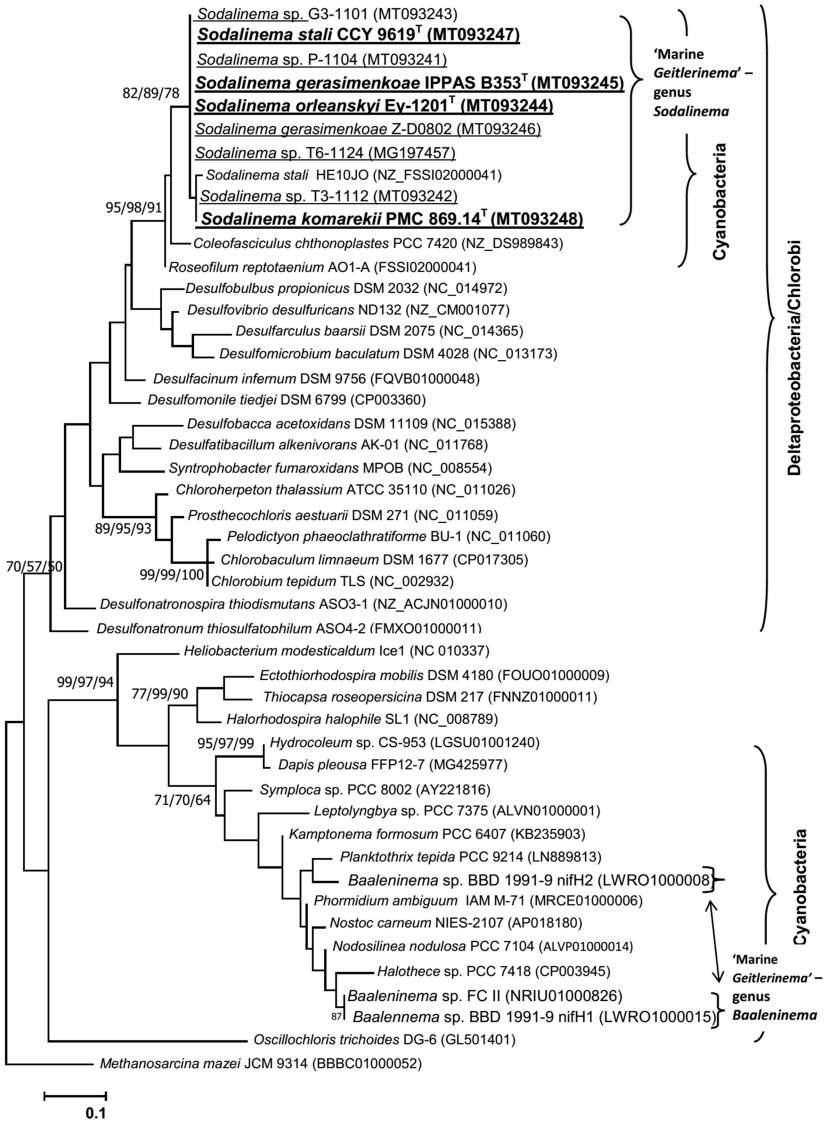

Figure 6. Maximum likelihood phylogenetic tree based on NifH amino acid sequences showing the position of the *Sodalinema* strains among diazotrophic bacteria. The strains used in our work are underlined. The type strains of *Sodalinema* species are shown in bold. The numbers above branches indicate statistical significance of the branching order as determined by bootstrap analysis of 1000 alternative trees (only values higher than 70% are shown). The levels of bootstrap support are given for the ML/NJ/ME algorithms. The scale bar shows the evolutionary distance corresponding to five substitutions per 100 amino acids.

sulphate-reducing bacteria (Bolhuis *et al.* 2010). Therefore, we designated this type of *nifH* gene the '*desulfo*' type.

Analysis of the available genomes of three strains belonging to the PCC7105 clade revealed that *Geitlerinema* sp. PCC7105 lacks *nif* genes and *Geitlerinema* sp. FC II and *Geitlerinema* sp. BBD 1991-9 have typical cyanobacterial *nifH* genes (Fig. 6). Thus, the presence of '*desulfo*' type *nifH* genes can be a distinctive feature of the genus *Sodalinema* within the 'marine *Geitlerinema*' cluster.

Morphology and ultrastructure of *Sodalinema* strains

In liquid culture, all the studied strains existed either as macroscopic stellate aggregates or as thin pellicles on the surface of the liquid and on the glass walls. Under stress conditions, ball-like formations were often observed. Old cultures became highly viscous due to large amounts of mucilage released into the surrounding medium. On agar plates, all the strains were able to form many small spirals.

The results of microscopic observations are summarized in Table 3. Light microscopy revealed that all strains appeared morphologically similar (Fig. 7): nonheterocystous, bright blue-green trichomes unbranched and slightly attenuated towards ends, with gliding motility, with an average cell width of 2.1–4.6 μm and length of 2.3–5.7 μm. The cell shape usually ranged from quadratic (isodiametric) to elongated. 'Young' cells (shortly after division) could be even wider than long. Thus, cell shape depended on the stage of the life cycle. Refractive granules on either or both sides of cross-walls and/or yellowish granulations with homogeneous or finely granular content were typical. Refractive granules were probably cyanophycin (Fig. 8E, G and H; Figures S2 B–E and I, Supporting Information). The nature of yellowish granulations was unclear. Apical cells of all strains were rounded, often with polar gas vesicles, but marine strain CCY9619 could also possess apical cells with extrusions (Fig. 7P–S). For comparison, apical cells were never bent and capitated, as in type species of the genus *Geitlerinema sensu*

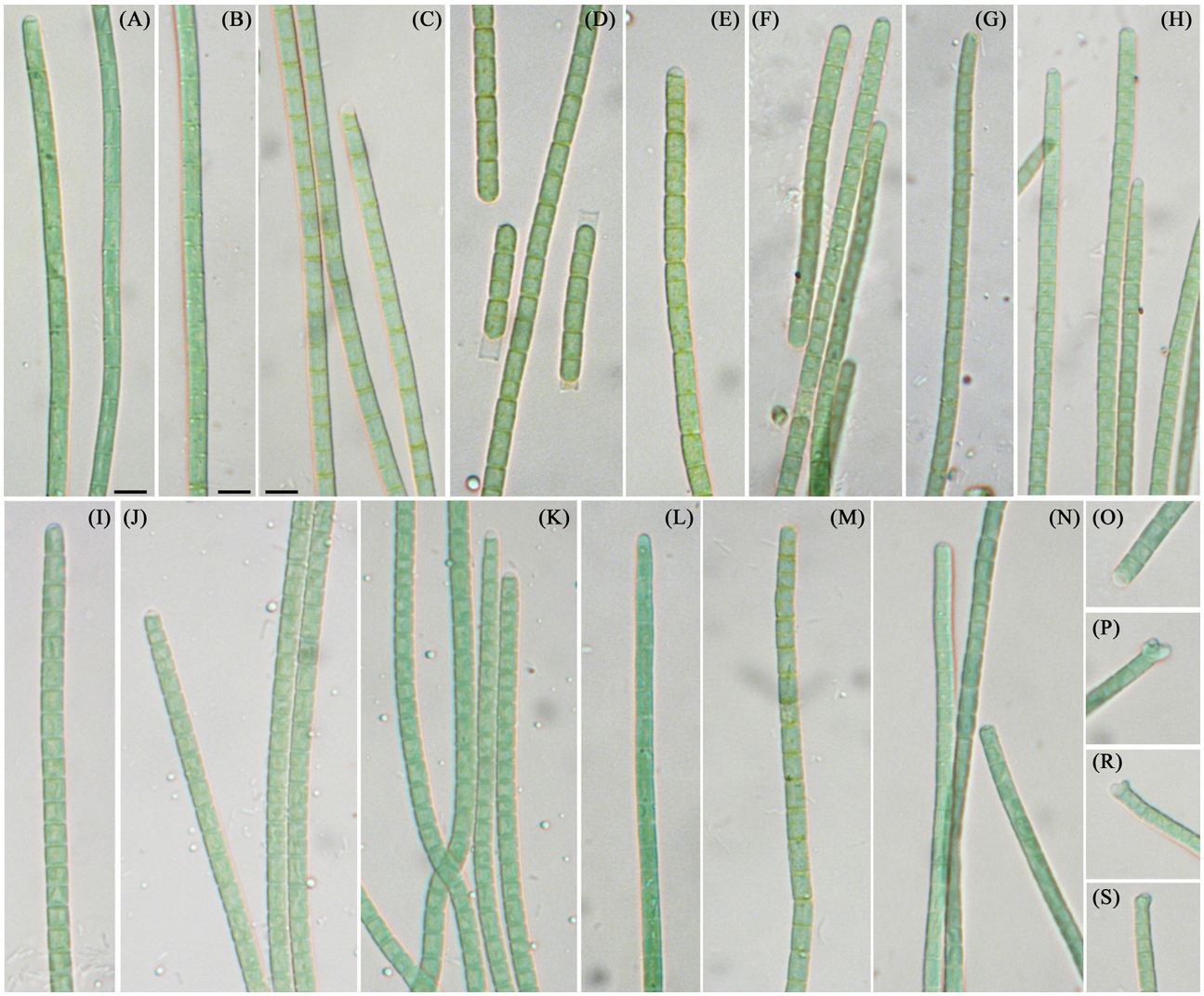

Figure 7. Morphology of the studied strains of the genus *Sodalinema* (light microscopy): **(A and B)** *S. komarekii* PMC 869.14, **(C)** *S. orleanskyi* Ey-1201, **(D and E)** *S. gerasimenkoae* IPPAS B-353, **(F and G)** *S. gerasimenkoae* Z-D0802, **(H)** *S. gerasimenkoae* T6-1124, **(I and J)** *S. gerasimenkoae* G3-1101, **(K)** *S. gerasimenkoae* T3-1112, **(L and M)** *S. gerasimenkoae* P-1104 and **(N–S)** *S. stali* CCY9619. The scale bars are 5 μm.

stricto (*G. splendidum*). Sheaths were absent or thin and colorless, containing only one trichome, visible where it extended beyond the terminal cell of the trichome (Fig. 7D). Bundles of trichomes could be surrounded by amorphous, colorless slime stainable with Alcian blue. Reproduction occurred through the formation of motile hormogonia and by fragmentation of whole trichomes without necridic cells.

TEM revealed that all studied strains have parietal thylakoids running parallel to the cytoplasmic membrane (Fig. 8 and Figure S2, Supporting Information). Solitary thylakoids (Fig. 8I) or their small fascicles (Fig. 8B–D, F and G and Figure S2 D–I, Supporting Information) penetrated the inner part of cells, and sometimes inner thylakoid bundles were observed as spherical formations (Fig. 8D and F and Figure. S2 D, H and I, Supporting Information). One to several carboxysomes were usually present in each section of the cell (Fig. 8 and Figure S2, Supporting Information). Besides carboxysomes, other electron-dense solitary inclusions, supposedly containing cyanophycin and polyphosphates, were observed. Constrictions between adjacent cells were absent or very shallow. Cell division was completed before new cross-wall formation began (Fig. 8A, D and G). The polysaccharides excreted by the cells formed an amorphous fibrous matrix stainable with Alcian blue (Fig. 8F, G and I and Figure S2 A, C, E, H and I, Supporting Information).

Analysis of fatty acid composition of *Sodalinema* strains

Analysis of the FA composition of total lipids is a useful tool that, in addition to other approaches of polyphasic analysis, may be used to clarify the taxonomic positions of cyanobacterial strains (Los and Mironov 2015). The studied strains were characterized by similar and rather simple FA compositions (Table 4): the main FAs were palmitic (16:0), hypogeic (16:1Δ^7), palmitoleic (16:1Δ^9) and 7,10-hexadecadienoic (16:2$\Delta^{7,10}$) acids; C_{14} and C_{18} FAs were present in low amounts–3–10% of total FAs. Saturated FAs comprised 43–55% of the total FAs, and unsaturation indexes (UIs) were in the range of 0.50–0.67. Strains Ey-1201 and IPPAS B-353 had two times higher amounts of 16:2$\Delta^{7,10}$ than the two other strains, PMC 869.14 and CCY9619. Strain Ey-1201 was characterized by the highest proportion of C_{18} FAs, and strain CCY9619 had the lowest UI.

Table 4. Fatty acid composition of total lipids of the type strains of the species of the genus *Sodalinema*.

	Content of fatty acids (mean[a] ± SD, mass %)			
Fatty acids	*S. komarekii* PMC 869.14	*S. orleanskyi* Ey-1201	*S. gerasimenkoae* IPPAS B-353	*S. stali* CCY9619
14:0	1.6 ± 0.2	2.5 ± 0.1	0.8 ± 0.2	1.3 ± 0.0
14:1Δ^9	1.1 ± 0.2	-	-	-
16:0	41.5 ± 0.4	40.2 ± 0.7	40.6 ± 1.5	52.4 ± 0.5
16:1Δ^7	13.4 ± 0.0	4.6 ± 0.1	7.5 ± 0.1	9.9 ± 0.2
16:1Δ^9	35.4 ± 0.2	35.9 ± 0.3	37.5 ± 1.2	30.9 ± 0.6
16:2 $\Delta^{7,10}$	4.2 ± 0.1	8.1 ± 0.3	8.9 ± 0.6	3.9 ± 0.2
16:2 $\Delta^{9,12}$	0.6 ± 0.0	1.2 ± 0.1	1.3 ± 0.1	-
18:0	1.0 ± 0.0	3.0 ± 0.2	1.7 ± 0.2	0.8 ± 0.4
18:1 Δ^9	0.4 ± 0.1	1.3 ± 0.7	1.8 ± 0.1	0.3 ± 0.0
18:1 Δ^{11}	0.5 ± 0.1	2.3 ± 0.1	-	0.2 ± 0.0
18:2 $\Delta^{9,12}$	0.4 ± 0.0	0.9 ± 0.2	-	0.2 ± 0.0
UI	0.611	0.644	0.671	0.497

[a]Mean percentage from three technical replicates. SD: standard deviation of the replicates. UI: unsaturation index (relative units).

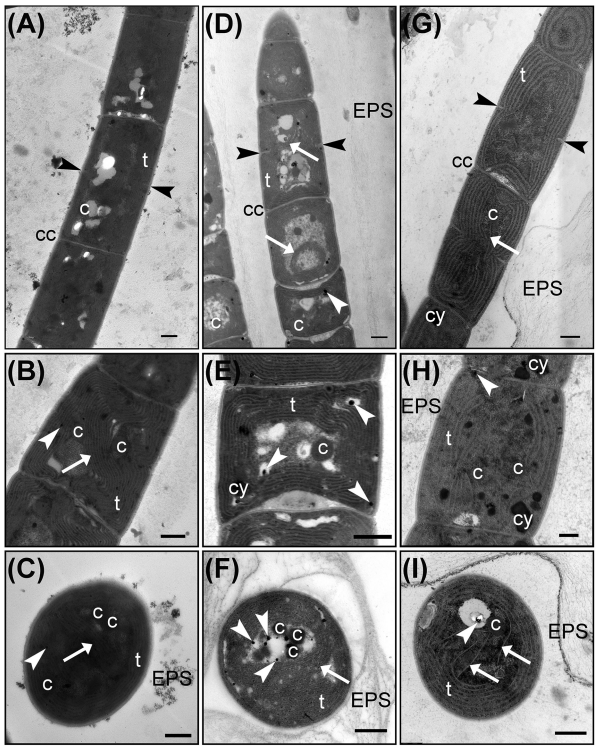

Figure 8. Ultrastructure of the type strains of the newly described species of the genus *Sodalinema*. **(A–C)** *S. orleanskyi* Ey-1201, **(D–F)** *S. gerasimenkoae* IPPAS B-353 and **(G–I)** *S. stali* CCY9619. (A, D and G) General view of filament parts, longitudinal sections; (B, E and H) magnified longitudinal sections; and (C, F and I) cross sections of filaments. All micrographs except for D and E were obtained on samples stained with 1% Alcian blue. c – carboxysomes; cc – cross wall constriction; cy – electron-dense inclusions, supposedly cyanophycin; EPS – exopolysaccharides; t – thylakoids; *white arrowhead* – electron-dense granules, supposedly polyphosphates; *black arrowheads* – new cross-wall formation and *arrows* – thylakoids crossing internal part of the cell and sometimes forming spherical formation. The scale bars are 0.5 μm.

Distribution of *Sodalinema* strains in the environmental habitats and growth experiments

According to the total mineralization (TM), all the *Sodalinema* habitats could be divided into the following: (i) marine to brackish with TM up to 35 g/L (Mellum Island coastal tidal flats), (ii) mixosaline with TM up to 30 g/L (Lake Eyasi and Lakes Khilganta, Dorоninskoe and Tanatar VI in some periods) and (iii) eusaline to hypersaline with TM generally higher than 30 g/L and up to 260 g/L (Large Aral Sea, Dziani Dzaha, Transbaikalian and Kulunda Steppe lakes; Fig. 9 and Table 2). According to the concentration of total soluble carbonates (TSC) and pH, three types of habitats can be distinguished: (i) soda lakes, (ii) saline-alkaline lakes and (iii) saline environments. Soda lakes are characterized by the dominance of HCO_3^-+CO_3^{2-} anions and a stable alkaline pH of approximately 10. The following studied lakes belong to this type: Tanatars III and VI, Petukhovskoe Lake, Bitter-3 and Dorоninskoe Lake. Saline-alkaline lakes are characterized by alkaline pH 9.0–9.5 and elevated concentrations of HCO_3^-+CO_3^{2-} but dominance of other major anions in the chemical composition (Cl^- or SO_4^{2-}). Lakes Eyasi, Dziani Dzaha and Khilganta are examples of this hydrochemical type. Saline environments are represented by the North Sea and Aral Sea habitats and characterized by a slightly alkaline pH of approximately 8 and low soluble carbonate content (Fig. 9, Table 2 and Table S2, Supporting Information).

Most of these habitats have fluctuating hydrological regimes that are expressed in a wide range of changes in hydrochemical parameters. The detailed descriptions of our observations on the Kulunda Steppe soda lakes have been published in part earlier (Samylina *et al.* 2014, 2019; Samylina, Zaytseva and Sinetova 2016; Samylina and Namsaraev 2020). Here, we briefly summarize all currently available data (including previously unpublished). According to our long-term (2007 and 2011–2020) observations, representatives of the *Sodalinema* subcluster were morphologically detected in the ranges of TM from 25 to 200 g/L and TSC from 18.3 to 164.7 g/L in the lakes Tanatars III and VI, Petukhovskoe and Bitter-3 (Fig. 9). *Sodalinema* morphotypes were widespread in the benthic cyanobacterial biofilms and phototrophic communities with chlorophyte *Ctenocladus circinnatus* Borzì in the lake brines, and in the biological soil crusts developed on the moist soil between thickets of *Salicornia altaica* Lomon. near the lakes. Less often, they were detected in plankton during water blooms. In Petukhovskoe Lake, *Sodalinema* was found on stromatolites.

Using morphological identification, we can't unambiguously state that the annually observed morphotypes were the same organisms with wide hydrochemical limits, and not genetically

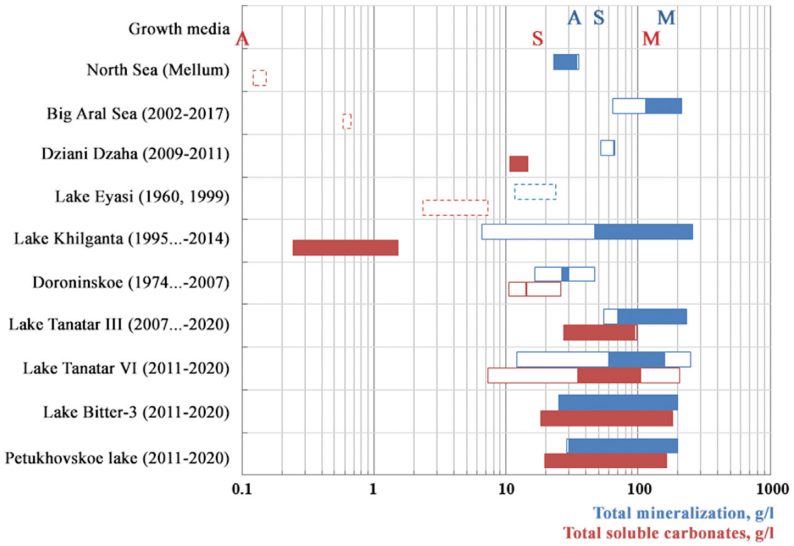

Figure 9. Ranges of development of *Sodalinema* in natural habitats. *Horizontal columns* – ranges of total mineralization (*blue*) and total soluble carbonate concentration (*red*) recorded during specified years (see Table S2, Supporting Information, for details) with *filled areas* that indicate the ranges with the documented development of *Sodalinema*. Growth media parameters are given in comparison to natural environments (A stands for medium ASNIII, S and M – for media S and M, respectively). *The dotted line* was used for horizontal columns when information on the development of *Sodalinema* was absent.

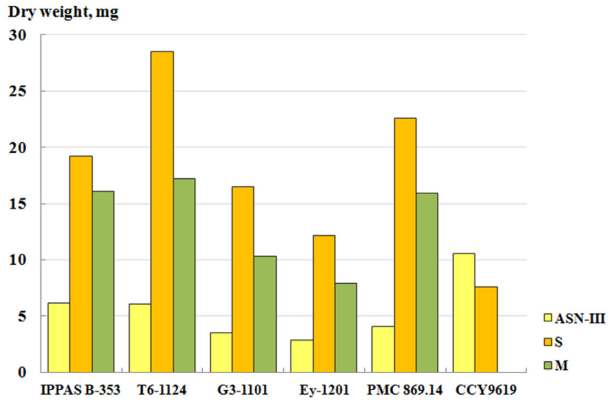

Figure 10. Growth of the selected haloalkaliphilic and marine *Sodalinema* strains in the media simulating marine (ASN-III), mixosaline to eusaline alkaline (S) and hypersaline soda (M) conditions. The columns represent the biomass yield of each strain after 7 days of incubation.

different ecotypes adapted to narrower ranges and replacing each other. To solve this, we set up laboratory experiments with the strains IPPAS B-353, T6-1124, G3-1101, PMC 869.14, Ey-1201 and CCY9619. We carried out a comparative cultivation of these strains in three media simulating marine (ASN-III), mixosaline to eusaline alkaline (S) and hypersaline soda (M) conditions (Fig. 9). This experiment pursued two goals: (1) to reveal whether there is a difference in hydrochemical requirements between the studied strains; and (2) to determine if the strains can grow in a wide range of TM and TSC. As a result, it was revealed that after the first passage from the medium S, the haloalkaliphilic strains IPPAS B-353, G3-1101, T6-1124, Ey-1201 and PMC 869.14 were able to grow in all three media (Fig. 10). They preferred medium S (maximum biomass yield and dark blue-green color of the cultures). The biomass yield was lower in medium M, but the cultures retained their dark blue–green color and formed dense biofilms. The lowest yield associated with the yellow–green color of the cultures was obtained on ASN-III medium. In the laboratory collection, these strains are stably maintained in media M and S and can successfully be transferred from one medium to another. The marine strain CCY9619 yielded maximum biomass in marine ASN-III medium, but surprisingly, it grew well in the saline-alkaline S medium. There was no growth in the most extreme medium M. (Fig. 10). Its growth was also stimulated by the addition of 0.2 M (16.8 g/L) $NaHCO_3$ and totally inhibited by the addition of 0.2 M (21.2 g/L) Na_2CO_3 to the ASN-III medium (data not shown) meaning this strain is adopted to hydrochemical fluctuations within the ranges that may be typical for marine tidal flats but not adopted to soda lake environments. These results suggest that representatives of genus *Sodalinema* are organisms with wide hydrochemical limits which generally correspond to their natural habitats.

DISCUSSION

Genera justification

According to genetic, morphological and ecological criteria, the representatives of the 'marine *Geitlerinema*' cluster are clearly separated from the type species of genus *Geitlerinema sensu stricto* (*G. splendidum* CCALA 1004) and should be named differently.

A comparative analysis of partial sequences of 16S rRNA genes revealed two genus level subclusters within the 'marine *Geitlerinema*' cluster (Fig. 1). According to the analyzed data, the tight *Sodalinema* subcluster contains free-living strains from marine, hypersaline, saline-alkaline and soda lake habitats located on the Eurasian and African continents. The other ('oceanic') subcluster divides into two clades (PCC7105 clade and BBD clade) and contains marine free-living and pathogenic strains probably distributed worldwide (North and South Americas, India and the Philippines; Fig. 2 and Table S5, Supporting Information). Comparative analysis of the nucleotide sequences of 16S–23S ITS (Fig. 3), secondary structures of its D1–D1', V2 and Box-B regions (Fig. 4) and analysis of housekeeping genes *rpoB* and *gyrB* (Fig. 4 and Table S4, Supporting Information) confirmed the difference of representatives of *Sodalinema* and 'oceanic' subclusters at the genus level.

Additionally, analysis of *nifH* genes also confirmed the genus level separation of the *Sodalinema* and 'oceanic' subclusters of 'marine *Geitlerinema*'. It was revealed that the representatives of the *Sodalinema* subcluster possess *nifH* genes of the '*desulfo*' type regardless of ecology and geographical distribution whereas representatives of the PCC7105 and BBD clades from the 'oceanic' subcluster either lack *nifH* genes or possess typical cyanobacterial *nifH* genes (Fig. 6).

Finally, we analyzed the morphological features of strains belonging to the *Sodalinema* subcluster and compared them with those of the PCC7105 clade, available from the literature. According to Rippka *et al.* (1979), Wilmotte (1991), Schrübbers (2007, 2011), Gonzalez-Esquer *et al.* (2016) and Batchu *et al.* (2019), the strains belonging to the PCC7105 clade are characterized by the following morphological features: highly motile filaments with isopolar straight or slightly waved trichomes 1.2–4.0 μm wide with cylindrical cells (up to 6.9 μm in length) and cyanophycin granules at the cross-walls (Table S5, Supporting Information). Apical cells are almost spherical without polar gas vacuoles. They produce little or no sheath material. Filaments are usually attached to substrates and form bundles, biofilms or membranaceous mats. The morphological features of the *Sodalinema* strains used in this study are listed in the Results section and Table 3. Thus, the comparison of morphological characteristics showed that the strains belonging to both the *Sodalinema* subcluster and PCC7105 clade are simple, highly motile filamentous cyanobacteria with similar diameters of trichomes (2.1–4.6 vs. 1.2–4.0 μm, respectively), which produce fine diffluent mucilage and little or no sheaths. However, morphologically, the representatives of *Sodalinema* subcluster differ from the representatives of the PCC7105 clade in the shape of trichomes (slightly attenuated towards ends vs. not attenuated, respectively), the presence of yellowish granulations at cross walls (usually present *vs.* absent), the presence of polar gas vesicles (present *vs.* absent) and thylakoid arrangement (parietal with a central fascicle vs. simple parietal).

Eventually, according to genetic and morphological features, *Sodalinema* and 'oceanic' subclusters represent two separate genera (Table 5). *Sodalinema* subcluster refers to the previously described genus *Sodalinema* (Cellamare *et al.* 2018) with an amended description given below. A new genus name should be proposed for the representatives of the 'oceanic' subcluster, and PCC7105 should be established as the type strain of the new genus: it was deposited in the Pasteur Culture Collection of Cyanobacteria (PCC) and recognized as the reference strain for marine strains of the form-genus *Geitlerinema* according to Bergey's Manual (Castenholz, Rippka and Herdman 2015). Recently, a new genome-based species name proposal was made for the strain PCC7105 – *Geitlerinema catellasis* (Walter *et al.* 2017). The problem of the approach used in that study is that any taxa that have been validly described earlier but lacked full genome sequences were not considered. The taxonomic name *Geitlerinema catellasis* could not be accepted for strain PCC7105 as it does not belong to the validly described and phylogenetically confirmed genus *Geitlerinema* (Anagnostidis and Komárek; Anagnostidis 1989; Strunecký *et al.* 2017). Thus, we propose a new genus name for the representatives of the 'oceanic' subcluster – *Baaleninema* gen. nov. It refers to the name of Chase van Baalen (1925–1986), an American microbiologist who isolated and pioneered studies of the strain PCC7105 as *Microcoleus tenerrimus* (Van Baalen 1962) or *Oscillatoria williamsii* (Van Baalen and Marler 1963) and deposited it to the Pasteur Culture Collection of Cyanobacteria (⟨?Undefined?⟩https://catalogue-crbip.pasteur.fr/fiche_catalogue.xhtml?crbip=PCC%207105). The description of *B. simplex* gen. et sp. nov. with PCC7105 as a type strain is given below (Table 6).

As an additional criterion that can be useful in substantiating new taxa within the 'marine *Geitlerinema*' cluster, we analyzed the FA composition of the studied strains. It was revealed that all studied *Sodalinema* strains have highly similar FA compositions and produce only saturated, mono- and dienoic FAs, of which 90–97% are C_{16} FAs (Table 4). As reported by Parker, Van Baalen and Maurer (1967), strain PCC7105 (referred to there as *Oscillatoria williamsii*) has the same main FAs, and the only significant difference is that the proportion of C_{16} FAs was less – 74% of the total FAs. Thus, we did not reveal any clear differences in FA composition between representatives of the genera *Sodalinema* and *Baaleninema*, and this criterion apparently cannot be used as a taxonomic criterion in this situation. However, the obtained data may have important ecological significance. Therefore, according to the updated system of FA-based classification of cyanobacteria (Los and Mironov 2015), all studied *Sodalinema* strains and *B. simplex* PCC7105 belong to Group 2 that produces only mono- and dienoic FAs. This group also includes marine strains of *Prochlorococcus* and *Synechococcus* (Los and Mironov 2015), freshwater thermophilic *Desertifilum* sp. IPPAS B-1220 (Sinetova *et al.* 2017), halophilic *Euhalothece* sp. PCC7418 (Oren *et al.* 1985) and haloalkaliphilic *Spirulina* strain 'pantelleria' (Romano *et al.* 2000). However, the distinctive feature of the 'marine *Geitlerinema*' strains is the high proportion of C_{16} FAs and the production of hypogeic ($16{:}1\Delta^{7}$) and 7,10-hexadecadienoic ($16{:}2\Delta^{7,10}$) acids. Interestingly, $16{:}2\Delta^{7,10}$ FA was considered a biomarker of *Euhalothece/Halothece* cyanobacteria from the upper layer of gypsum crusts from saltern ponds (Oren 2010; Jahnke *et al.* 2014). Considering that *Sodalinema* and *Euhalothece* co-occur in the composition of phototrophic communities in a wide range of salinities in soda and saline-alkaline lakes (Samylina *et al.* 2019), it is possible to suppose that such FA composition reflects some adaptation to high-salt conditions, but further studies are needed to prove this.

Species justification

In the framework of the polyphasic approach, the following definition of a species in cyanobacteria is used: 'group of populations (+ strains), which belong to one and the same genotype (genus), which are characterized by a stabilized phenotype with characteristic features (definable and recognizable with distinct limits of variation) and by the same ecological demands; they should occur repeatedly (in time) in various localities with the same ecological conditions' (Komárek 2016). The particular problem for taxonomy is the existence of cryptotaxa, morphotaxa and ecotaxa in cyanobacteria (Dvořák *et al.* 2015; Komárek 2016). Therefore, we used a set of criteria including genetic data, morphology, ecology, geography and secondary structures of corresponding helices of 16S-23S ITS regions in order to determine species in the genera *Baaleninema* and *Sodalinema*.

According to our results, the genus *Baaleninema*, in addition to PCC7105, also includes strains Flo1, FCII, CENA552 and CENA556. Strain Flo1 belongs to the proposed species *B. simplex* according to the identity of nucleotide sequences and secondary structures of corresponding helices of the 16S–23S ITS region (Figs 3 and 4) and cell dimensions (Table S5, Supporting Information). Strains FCII, CENA552 and CENA556 can represent other closely related species in the genus *Baaleninema* because of differences in the secondary structures of D1–D1' and V2 helices of the 16S–23S ITS region, as well as in the geographic distribution (Fig. 4 and Table S5, Supporting Information). Finally, BBD strains

Table 5. Morphological and molecular characteristics of genera *Sodalinema* Cellamare, Duval, Touibi, Djediat and Bernard and *Baaleninema* gen. nov.

Genus		*Sodalinema* Cellamare, Duval, Touibi, Djediat and Bernard	*Baaleninema* gen. nov.
Type species		*Sodalinema komarekii* Cellamare, Duval, Touibi, Djediat and Bernard	*Baaleninema simplex* sp. nov.
Other species		*Sodalinema orleanskyi* sp. nov., *Sodalinema gerasimenkoae* sp. nov., *Sodalinema stali* sp. nov.	Unresolved
16S–23S ITS (nt):	D1-D1'	61	62
	tRNA	Ile, Ala	Ile, Ala
	V2	27–56	72–80
	Box-B	28	27
nifH genes		'*Desulfo*'-type	Absent or typical cyanobacterial gene
Thallus in culture		Filaments solitary, free-floating or attached to substrates and forming biofilms	Filaments attached to substrates and forming biofilms
Trichomes		Nearly straight, flexuous, slightly attenuated towards the ends and slightly or very slightly constricted at cross-walls	Nearly straight, flexuous, not attenuated towards the ends and very slightly constricted at cross-walls
Cell shape		Cylindrical, elongated up to 2× or isodiametric after division	Cylindrical, isodiametric or elongated up to 4× times
Cell dimensions, length × width (μm)		(2.3)2.6–5.7 × 2.1–4.6	(1.6)1.9–4.8(6.9) × 1.2–2.5(2.8)
Granulation at cross walls		1–2 cyanophycin granules on either or both sides and/or yellowish granulations with homogeneous or finely granular content	Mostly two cyanophycin granules on either or both sides
Apical cell		Rounded without calyptra, sometimes with extrusions	Rounded without calyptra
Polar gas vacuole at apical cell		Present	Absent
Polarity		Isopolar	Isopolar
Branching		Absent	Absent
Exopolysaccharides	Sheath	Absent or thin and colorless, contain only one trichome	Absent or thin and colorless, contain only one trichome
	Mucilage	Present	n/d
Motility		Motile	Motile
Thylakoids arrangement		Parietal with a central fascicle	Parietal
Ecology		Haloalkaliphilic, halophilic, marine to brackish	Marine
Occurrence		Planktonic and benthic phototrophic communities, stromatolites in soda and saline-alkaline lakes and biological soil crusts near its shores; marine microbial mats	Unknown for the type strain (PCC7105); mangrove wood; black band disease of corals

surely represent a separate species in the genus *Baaleninema*, but they cannot be validly described due to a lack of cultures and necessary data at present.

Considering all the criteria used, we propose three new species within the genus *Sodalinema* (Table 6) that differ in phylogeny, ecology and geographical distribution (with minimal morphological differences): (1) *S. orleanskyi* sp. nov. for strain Ey-1201 (type); (2) *S. gerasimenkoae* sp. nov. for strains IPPAS B-353 (type) and Z-D0802 and (3) *S. stali* sp. nov. for strains CCY9619 (type), SAG 31.92, 'Schier', OL 75, OL 'sphere', OL S6, OL S12 and HE10JO. Strains T6-1124, T3-1112, G3-1101 and P-1104 remain problematic (see below).

Sodalinema orleanskyi is the closest relative of *S. komarekii*. The strains *S. orleanskyi* Ey-1201 and *S. komarekii* PMC 869.14 are similar in morphology (Tables 3 and 6) and ecology (haloalkaliphilic) and relatively close in geography (Tanzania and Mayotte Island, i.e. East Africa), but the V2 helix of *S. orleanskyi* Ey-1201 has a characteristic configuration due to the presence of 28 additional nucleotides in both ribosomal operons (Fig. 4B and Figure S1, Supporting Information). In addition, a phylogenetic tree based on the combined *rpoB+gyrB* genes sequences unambiguously separates these strains into different species (Fig. 5). Thus, *S. orleanskyi* and *S. komarekii* represent an example of cryptic species (Dvořák *et al.* 2015; Komárek 2016).

Sodalinema gerasimenkoae includes strains IPPAS B-353 and Z-D0802 isolated from saline–alkaline and soda lakes in Transbaikalia. According to 16S rRNA and 16S-23S ITS phylogenetic trees, strain T6-1124 isolated from the Kulunda Steppe soda lake

Table 6. Distinctive features of *Sodalinema* and *Baaleninema* species.

Species	*Sodalinema komarekii* Cellamare, Duval, Touibi, Djediat and Bernard	*Sodalinema orleanskyi* sp. nov.	*Sodalinema gerasimenkoae* sp. nov.	*Sodalinema stali* sp. nov.	*Baaleninema simplex* gen. et sp. nov.
Type strain	PMC 869.14	Ey-1201	IPPAS B-353 (= CCALA 1011)	CCY9619 (= SAG 31.92; = DSM 101427; = IPPAS B-2050)	PCC7105
Other strains	PMC strain numbers: 741.11–746.11; 861.14–865.14; 886.15–889.15; 898.15; 899.15; 907.15	Unknown	Z-D0802 (= IPPAS B-2033)	'Schier', OL 75, OL 'sphere', OL S6, OL S12, HE10JO	Flo1
Length of V2 region in 16S–23S ITS (nt):	28	56	37	30	77
Cell dimensions, length × width (μm)	(3)3.9–5.7 × 2.6–3.0	2.9–5.0 × 2.1–2.7	2.4–5.0 × 2.3–3.5	2.6–4.8(5.5) × 2.0–3.5	(1.6)1.9-4.8(6.9) × 1.2–2.5(2.8)
Ecology	Haloalkaliphilic	Haloalkaliphilic	Haloalkaliphilic	Marine to brackish	Marine
Geography	Western Indian Ocean, Mayotte Island	Africa, Tanzaniya	Asia, Russia, southeastern Siberia, Transbaikalia	Europe, Germany/Netherlands, North Sea	Unknown for the type strain (PCC7105); North America, USA, Atlantic coast (Flo1)
References	This study; Cellamare *et al.* (2018)	This study	This study	This study; Stal and Krumbein (1985); Garcia-Pichel, Prufert-Bebout and Muyzer (1996); Marquardt and Palinska (2007); Nies *et al.* (2017)	Rippka *et al.* (1979); Wilmotte (1991); Schrübbers (2007); Schrübbers *et al.* (2008); Schrübbers (2011); Gonzalez-Esquer *et al.* (2016)

should also belong to this species while other Kulunda strains (T3-1112, G3-1101 and P-1104) could probably be separated into one more species (Figs 1 and 3). However, the analysis of the combined *rpoB*+*gyrB* genes sequences revealed slightly different clustering (Fig. 5): the couple of strains P-1104 + G3-1101 appeared close to *S. orleanskyi* Ey-1201, and the couple of strains T6-1124 + T3-1112 appeared close to *S. gerasimenkoae* IPPAS B-353. However, the values of nucleotide identity of partial *rpoB* sequences between the problematic couples and closest type strains (approximately 96%) testify in favor of classifying them as separate species according to Adékambi *et al*. (2008; Table S4, Supporting Information). Thus, we consider it premature to classify these couples of strains as the indicated species; and the Kulunda Steppe strains T6-1124, T3-1112, G3-1101 and P-1104 remain unresolved.

Sodalinema stali differs from other *Sodalinema* species by ecology (marine), morphological peculiarities (apical cells with extrusions), secondary structure of the V2 helices and geographical distribution (North Sea, i.e. Europe; Figs 2–4 and 7). Thus, *S. stali* is currently the only described marine species belonging to the genus *Sodalinema*, but other marine and halophilic species certainly exist and can be widely distributed in marine and saline (not alkaline) environments, at least on the Eurasian continent. Apparently, strains *Sodalinema* sp. FO-SIORAS-13 and SK-SIORAS-13 may represent halophilic species inhabiting the hypersaline Aral Sea (Figs 2–4). Unfortunately, these cultures were lost (Dr Sapozhnikov F.V. and Dr Kalinina O.Y., isolators of the strains; personal communication) and therefore cannot be validly described yet. It is likely that morphospecies *Phormidium anissimovae* (Elenk.) O.N. Vynogr. distributed in mineral lakes, estuaries and salt marshes in the steppe zone of Ukraine (Vinogradova 2011) may also appear to belong to the genus *Sodalinema*. It was first described as *Oscillatoria tambi* f. *anissimovae* Elenk., and its ecology and morphology correspond well to the genus *Sodalinema* described in the current study: trichomes slightly attenuated towards the ends; and the cells are mostly quadratic, 2.5–4.2 μm long and 3.5–3.8 μm wide, apical cells often with a polar gas vacuole (Elenkin 1949). However, genetic data are not available for this species, and its taxonomic affiliation cannot be reliably established yet.

Thus, *Sodalinema* represents a widely distributed but still understudied genus.

Ecological and biogeographic criteria in relation to the taxonomy of *Sodalinema*

Although the 16S rRNA phylogenetic tree (Fig. 1) did not show clear clustering reflecting ecological differences, the phylogenetic tree based on the analysis of 16S–23S ITS sequences (Fig. 3) revealed two main groups associated with the concentration of TSC and pH rather than TM: strains from soda and saline–alkaline lakes and strains from saline environments (including athalassic hypersaline lakes and marine tidal flats). This division was also confirmed by the growth experiments (Fig. 10) and some morphological peculiarities (apical cells with extrusions in marine strain, Fig. 6). These groups, probably, should differ not only phylogenetically, but also physiologically due to the chemistry of their habitats, in particular the content of soluble carbonates. According to the concept of 'natronophily' the different electrolytic properties of the sodium carbonates and sodium chloride may be the reason of the fundamental difference between life in these two solutions and may require special adaptations (Sorokin, Banciu and Muyzer 2015). For cyanobacteria, the content of dissolved inorganic carbon outside the cells is important also from the point of view of their autotrophy and functioning of the CO_2-concentrating mechanism (Kupriyanova and Samylina 2015). Not surprisingly, the analysis of *rpoB* and *gyrB* genes sequences of these groups confirmed interspecies differences between marine and haloalkaliphilic strains (Fig. 5 and Table S4, Supporting Information). TM seems to be not important for the phylogenetic clustering of the haloalkaliphilic strains, probably, because this parameter is not stable in natural environments. It fluctuates from mixosaline to hypersaline status of the studied soda and saline-alkaline lakes (Fig. 9, Table 2 and Table S2, Supporting Information) and the strains are capable of stable growth on mixosaline to hypersaline media (Fig. 10). To date, too few marine and halophilic strains of *Sodalinema* are known to make any conclusions about the interrelation of TM of saline habitats with the distribution of various species or ecotypes.

Finer clustering of the ecological groups in the 16S–23S ITS phylogenetic tree reflects the biogeography of the studied *Sodalinema* strains (Fig. 3). For most strains, this clustering corresponds to the geographic distribution of the described species, i.e. separate species (and not geographic populations of the same species) inhabit different geographic areas (Fig. 2). But some of the analyzed strains suggest that the situation may be more complicated. Thus, four haloalkaliphilic strains from the Kulunda Steppe soda lakes appeared to be not united according the phylogenetic trees based on 16S rRNA, *rpoB*+*gyrB* genes and 16S-23S ITS region sequences. We suppose that strains P-1104 and G3-1101 can be designated as one more new species in future, and it will match with all the phylogenetic trees. But its relationships with the strains T6-1124 and T3-1112 from the same geographic location remain unclear. The strain T6-1124 is likely to be a representative of distant geographic population of *S. gerasimenkoae* according to the comparative view of the phylogenetic trees (Figs 1, 3 and 5). On the other hand, the strains T6-1124 and T3-1112 may represent one more species according to the nucleotide identity of *rpoB* genes (Table S4, Supporting Information) and *rpoB*+*gyrB* phylogenetic tree (Fig. 5). But anyway, the phylogenetic position of the strain T3-1112 creates major confusion and prevents us from making clear conclusions. Moreover, we did not analyze all the diversity of cultured and uncultured representatives of 'marine *Geitlerinema*' since we limited our study to only those strains for which the 16S rRNA and 16S–23S ITS sequences were available. More strains and deeper genetic and ecophysiological research is needed to understand this.

Thus, detailed taxonomic studies of genus *Sodalinema* show that the analysis of 16S–23S ITS sequences and its correct taxonomic interpretation should be carried out within the framework of a comprehensive geographical–ecological–taxonomic work.

Formal description of genus *Baaleninema* gen. nov. and species *B. simplex* sp. nov.

Baaleninema Samylina, Sinetova, Kupriyanova and Tourova

Diagnosis

Thallus blue–green, fine, smooth and diffluent, forming biofilms. Filaments solitary, straight or slightly waved, flexuous. Trichomes motile, cylindrical, very slightly constricted at the cross walls, not attenuated towards the ends, sometimes with thin

and colorless sheath, containing only one trichome. Cells cylindrical, elongated up to 4 times (rarely isodiametric), (1.6)3.2–5.0(6.9) μm long and 1.2–2.8(4.0) μm wide and cyanophycin granules at the cross-walls. Apical cell rounded, without calyptra, polar gas vacuoles are absent. Thylakoid arrangement is parietal.

Habitat
The taxon occurs in marine environments, free-living and pathogenic on corals.

Iconotype
Baaleninema simplex Samylina, Sinetova, Kupriyanova & Tourova, based on strain PCC7105, Journal of General Microbiology, 111: 1–61, 1979: Fig. 27.

Holotype
A cryopreserved sample of the PCC7105 strain stored in the Pasteur Culture Collection of Cyanobacteria (https://catalogue-crbip.pasteur.fr; Paris, France).

Etymology
Baaleninema = The name of the genus refers to the name of Professor Dr Chase van Baalen (1925–1986), an American microbiologist who was the first who studied strain PCC7105 and deposited it to the Pasteur Culture Collection and to the filamentous morphology (Greek word νμα, or níma means 'filament').

Baaleninema simplex Samylina, Sinetova, Kupriyanova and Tourova

Diagnosis
Thallus attached to substrates and forming biofilms. Trichomes straight, very slightly constricted at the cross walls, not attenuated towards the ends, sheath absent or thin and colorless, contain only one trichome. Highly motile. Cells cylindrical, elongated, (1.6)1.9–4.8(6.9) μm long and 1.2–2.8 μm wide and cyanophycin granules at the cross-walls. Apical cell rounded, without calyptra, polar gas vacuoles are absent. Thylakoid arrangement is parietal.

Holotype
A cryopreserved sample of the PCC7105 strain stored in the Pasteur Culture Collection of Cyanobacteria (https://catalogue-crbip.pasteur.fr; Paris, France).

Type strain
A living strain PCC7105 deposited at PCC under no. 7105, Assembly: GCA_000332355.1, WGS: ANFQ01000001/ANFQ01000266.

Habitat
Unknown for the type strain.

Type locality
Unknown for the type strain.

Etymology
The specific epithet (*simplex*) refers to the simple morphology (Latin word 'simplex' means 'simple').

Amended description of genus *Sodalinema*

Sodalinema Cellamare, Duval, Touibi, Djediat and Bernard emend. Samylina, Sinetova, Kupriyanova and Tourova

Diagnosis
Thallus emerald green, diffluent, forming thin mats or free-floating filaments. Filaments solitary or parallel in bundles, nearly straight, flexuous, blue–green, pale blue–green or yellow–green. Trichomes motile, cylindrical, slightly constricted at the cross walls, slightly attenuated towards the ends, sheaths are absent or thin and colorless, containing only one trichome. Bundles of trichomes can be surrounded by amorphous, colorless slime. Cells cylindrical, isodiametric or elongated, (2.3)2.6–5.7 μm long and 2.0–4.6 μm wide. Cross-walls with 1–2 cyanophycin granules on either or both sides and/or with yellowish granulations with homogeneous or finely granular content. Apical cells rounded, hemispherical, sometimes with extrusions, often with a large polar gas vacuole. Thylakoid arrangement is parietal with a central fascicle, sometimes with spherical formations. Possess *nifH* genes of '*desulfo*' type.

Habitat
The taxon occurs in saline–alkaline and soda lakes and marine and mixosaline to hypersaline inland environments.

Iconotype
Sodalinema komarekii Cellamare, Duval, Touibi, Djediat and Bernard 2018, based on *S. komarekii* PMC 869.14, FEMS Microbiology Ecology, 94: fiy108, 2018: Figs 10 and 11.

Holotype
A cryopreserved and formaldehyde-fixed sample of *S. komarekii* PMC 869.14 deposited at PMC, Paris, France.

Etymology
Sodalinema = The name of the genus refers to the *soda* lake habitat where the holotype species was found and to the simple morphology (Greek word νμα, or níma means 'filament').

Formal description of the new *Sodalinema* species:

Sodalinema orleanskyi Samylina, Sinetova, Kupriyanova and Tourova, sp. nov.

Description
Thallus blue–green, diffluent, forming biofilms or free-floating solitary filaments. Trichomes blue–green or pale blue–green, motile, cylindrical nearly straight, flexuous, slightly attenuated towards the ends and very slightly constricted at cross-walls. Sheath thin and colorless, contain only one trichome. Cells cylindrical, elongated (2.9–5.0 × 2.1–2.7 μm). Granulations at cross-walls are yellowish with homogeneous or finely granular content. Apical cell rounded, hemispherical, often with a large polar gas vacuole. Thylakoid arrangement is parietal with a central fascicle. Differs significantly from other *Sodalinema* species in the length and structure of the V2 helix of the 16S–23S ITS region and geography (Africa).

Holotype
A cryopreserved sample of the type strain Ey-1201 deposited at IPPAS, Moscow, Russia.

Type strain
A living strain Ey-1201 deposited at IPPAS under the no. B-2037, GenBank IDs: MT081571 (16S rRNA), MT067582 and MT067572 (16S–23S ITS) and MT093244 (*nifH*).

Habitat
Biofilms in saline-alkaline lakes.

Type locality
A shallow soda lake Eyasi, Tanzania.

Etymology
The specific epithet (*orleanskyi*) named in honor of Dr Vladimir K. Orleansky, a Russian algologist who worked in the Winogradsky Institute of Microbiology of Russian Academy of Sciences.

Sodalinema gerasimenkoae Samylina, Sinetova, Kupriyanova and Tourova, sp. nov.

Description
Thallus blue–green, diffluent, forming biofilms or free-floating solitary filaments. Trichomes motile, cylindrical nearly straight, flexuous, slightly attenuated towards the ends and very slightly constricted at cross-walls. Sheath thin and colorless, contain only one trichome. Cells cylindrical, isodiametric or elongated ((2.3)3.0–4.6(5.0) × (2.2)3.0–4.6 μm). Cross-walls with a refractive granule on either or both sides coupled with minor yellowish granulations with homogeneous or finely granular content. Apical cell rounded, hemispherical, often with a large polar gas vacuole. Thylakoid arrangement is parietal with a central fascicle. Differs significantly from other *Sodalinema* species in the structure of the V2 helix of the 16S–23S ITS region, geography (Asia) and ecology.

Holotype
A cryopreserved sample of the type strain B-353 deposited at IPPAS, Moscow, Russia.

Type strain
A living strain B-353 deposited at IPPAS (Moscow, Russia) under the number B-353 and at CCALA (Třeboň, Czech Republic) under the number 1011, GenBank IDs: KU375124 (16S rRNA), MT067571 and MT067575 (16S-23S ITS) and MT093245 (*nifH*).

Habitat
Cyanobacterial mats, biofilms and *Ctenocladus* communities in saline-alkaline and soda lakes.

Type locality
Saline–alkaline lake Khilganta, Transbaikalia, Russia.

Etymology
The specific epithet (*gerasimenkoae*) is named in memory of the prominent microbiologist and cyanobacterial specialist Dr Lyudmila M. Gerasimenko who worked in the Winogradsky Institute of Microbiology of Russian Academy of Sciences and isolated the type strain of the species.

Sodalinema stali Samylina, Sinetova, Kupriyanova and Tourova, sp. nov.

Description
Thallus blue–green, diffluent, forming biofilms or free-floating solitary filaments. Trichomes motile, cylindrical nearly straight, flexuous, slightly attenuated towards the ends and very slightly constricted at cross-walls. Sheath thin and colorless, contain only one trichome. Cells cylindrical, elongated (2.6–4.8(5.5) × 2.0–3.3(3.5) μm). A refractive granule on either or both sides of the cross walls can be present. Apical cell rounded, sometimes with extrusions, often with a large polar gas vacuole. Thylakoid arrangement is parietal with a central fascicle. Differs significantly from other *Sodalinema* species in the structure of the V2 helix of the 16S–23S ITS region, geography (Europe) and ecology (halophilic).

Holotype
A cryopreserved sample of the type strain CCY9619 deposited at IPPAS, Moscow, Russia.

Type strain
A living strain CCY9619 deposited at DSMZ (Braunschweig, Germany) under the number DSM 101427, at SAG (Göttingen, Germany) under the number 31.92 and at IPPAS (Moscow, Russia) under the number B-2050, GenBank IDs: MT067567 (16S rRNA), MT067573 (16S-23S ITS) and MT093247 (*nifH*).

Habitat
Marine cyanobacterial mats.

Type locality
Mellum Island, North Sea, Germany.

Etymology
The specific epithet (*stali*) named in honor of Professor Dr. Lucas J. Stal, an outstanding microbiologist known for his long-term research in the field of cyanobacterial ecology and who isolated the type strain of the species.

CONCLUSION

We analyzed the phylogeny, ecology, morphology, ultrastructure and biogeography of the 'marine *Geitlerinema*' cluster representatives and described three new species, *S. orleanskyi* sp. nov., *S. gerasimenkoae* sp. nov. and *S. stali* sp. nov.; and a new genus and species *B. simplex* gen. et sp. nov. Both genera are spread worldwide and play an important ecological role. The genus *Baaleninema* contains free-living and pathogenic strains inhabiting ocean-related environments. The genus *Sodalinema* contains free-living cyanobacteria inhabiting marine, mixosaline to hypersaline inland environments, saline–alkaline and soda lakes. We also showed that in contrast to other studied 'marine *Geitlerinema*' strains, representatives of the genus *Sodalinema* possess '*desulfo*' type *nifH* genes, which are believed to have been obtained during horizontal gene transfer from sulphate-reducing bacteria. The biogeography of the genera *Sodalinema* and *Baaleninema* is still understudied. Many strains belonging to the 'marine *Geitlerinema*' cluster and distributed worldwide were not considered in this work due to the lack of necessary information. However, this study emphasizes the importance of using ecology and biogeography in the description of new cyanobacterial taxa.

ACKNOWLEDGMENTS

This study was performed using the scientific equipment of the Core Research Facility 'Bioengineering' (Research Center of Biotechnology RAS) and large-scale research facilities of the Collection of microalgae and cyanobacteria IPPAS (Institute of Plant Physiology RAS, Moscow, Russia). We thank Mr Michele Grego (Culture Collection Yerseke, the Netherlands), Ms Charlotte Duval and Mr Claude Yéprémian (Paris Museum Collection of Cyanobacteria at the National Museum of Natural History, France) for their generous help in obtaining the strains from

their Collections. We also thank Dr Muriel Gugger (Pasteur Institute) and Dr Henk Bolhuis (NIOZ Royal Netherlands Institute for Sea Research and Utrecht University) for the valuable information on the strains PCC7105 and CCY9619, respectively. Finally, we are grateful to Professor Aharon Oren (The Hebrew University of Jerusalem) for his kind help and valuable suggestions on the correct spelling of the new taxa names proposed in this work.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

FUNDING

This work was supported by grant number 19–04–00377 from Russian Foundation for Basic Research and, in part, by The Ministry of Science and Higher Education of the Russian Federation (Projects numbers № AAAA-A19–119020590109–3, 121041200194-7 and 121033000137-1).

Conflicts of interests. None declared.

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