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New cyanobacterium *Aliterella vladivostokensis sp. nov.* (Aliterellaceae, Chroococcidiopsidales), isolated from temperate monsoon climate zone (Vladivostok, Russia)

SHAMIL R. ABDULLIN[1,2*], ARTHUR YU. NIKULIN[1,3], VERONIKA B. BAGMET[1,4], VYACHESLAV YU. NIKULIN[1,5] & ANDREY A. GONTCHAROV[1,6]

[1]*Laboratory of Botany, Federal Scientific Center of East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, 690022 Vladivostok, Russia*
[2] *crplant@mail.ru; https://orcid.org/0000-0002-6946-2321*
[3] *artyrozz@mail.ru; https://orcid.org/0000-0001-6113-2136*
[4] *chara1989@yandex.ru; https://orcid.org/0000-0002-1193-7689*
[5] *nikulinvyacheslav@gmail.com; https://orcid.org/0000-0002-6643-4325*
[6] *gontcharov@biosoil.ru; https://orcid.org/0000-0003-2918-730X*
**Author for correspondence: crplant@mail.ru*

Abstract

A new coccoid cyanobacterium *Aliterella vladivostokensis sp. nov.* was described from an urban aerophytic habitat in a temperate monsoon climate (Vladivostok, Russia) using a polyphasic approach. Phylogenetic analyses based on the 16S rRNA gene sequences confirmed that our isolate was a member of the *Aliterella* genus clade. *Aliterella* species are hardly distinguishable from each other morphologically and were described from highly contrasting natural and artificial environments with only a few records from several continents. Despite high similarity of morphometric data for *A. vladivostokensis* and *A. antarctica* cells and a compensatory base change in the D1–D1′ helix shared by these species; high percent of dissimilarity (11.6±1.3) between their 16S–23S internal transcribed spacer sequences with at least 5 autapomorphic mutations in the D1–D1′ and Box-B helices, and distinct folding patterns of the Box-B helix allowed us to erect a new species.

Keywords: *Aliterella*, coccoid cyanobacteria, geography, morphology, secondary structures, taxonomy, 16S rRNA, 16S–23S ITS rRNA

Introduction

Cyanobacteria adapted to a wide range of environmental conditions, and some of these bacteria form specific aerophytic communities on rock walls and stone surfaces that are either natural or artificial. The impact of abiotic factors, particularly temperature, solar radiation and water availability is significantly pronounced and frequent, rapid changes to extreme ranges of these factors are stressful for the organisms living in those habitats (Hauer *et al.* 2015, Pinheiro *et al.* 2019, Villa & Cappitelli 2019). Air pollution can also adversely affect aerophytic organisms, especially those inhabiting urban ecosystems (Ortega-Morales *et al.* 2019). Cyanobacteria damage building surface materials and cause their biodeterioration, reducing life span and reliability. This problem is particularly acute for buildings with cultural and historical value (Hauer *et al.* 2015, Ortega-Morales *et al.* 2019, Pinheiro *et al.* 2019, Coelho *et al.* 2021, Duan *et al.* 2021). Thus, the study of cyanobacterial diversity in such habitats is of great economic importance. Cyanobacterial communities on rock walls and stone surfaces have been studied in various regions of the world (Abdullin 2011, Komárek *et al.* 2012, Mühlsteinová & Hauer 2013, Hauer *et al.* 2015, González-Gómez *et al.* 2018, Czerwik-Marcinkowska & Massalski 2018, Ortega-Morales *et al.* 2019, Pinheiro *et al.* 2019, Abdullin *et al.* 2021). However, the taxonomic composition of aerophytic cyanobacteria inhabiting anthropogenic substrates in temperate monsoon climate is under-researched (Tripathi *et al.* 2007, Lee *et al.* 2020).

During our assessment of cyanobacterial biodiversity in aerophytic habitats of Vladivostok, Russia, a strain of coccoid cyanobacterium was isolated and studied using a polyphasic approach. Phenotypic features of the strain

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fitted the diagnosis of the genus *Aliterella* J.Rigonato & *al.* (2016: 2859) (solitary or irregular mucilaginous colonies; spherical, oval, irregular or elongated cells; cell division by binary fission in multiple planes; Rigonato *et al.* 2016), which is a member of the family Aliterellaceae (Chroococcidiopsidales). To date this genus accounts for four species. Two of these are marine species:—*A. atlantica* J.Rigonato & al. (2016: 2859) and *A. antarctica* J.Rigonato & al. (2016: 2860); one is a freshwater species, *A. shaanxiensis* Q. Zhang et L.R. Song (2018: 213), and *A. chasmolithica* P. Jung, Schermer, Mikhailyuk et Büdel (2020: 1222) was described as chasmoendolithic in granite boulders (Atacama Desert, Chile). In this study we describe a new *Aliterella* species, *Aliterella vladivostokensis sp. nov.*, using morphological and molecular characterization techniques.

Materials and Methods

Strain isolation and culture:—A sample was collected in Vladivostok, Russia (43°10'21.4"N, 131°56'10.9"E) on August 7, 2018. The coccoid cyanobacterial strain was isolated from the green spot on the concrete fence, and cultivated in liquid Waris-H nutrient medium (McFadden & Melkonian 1986) at 20–22°C with an irradiance of light intensity of 17.9–21.4 μmol photons·m^{-2} s^{-1} under a 16:8 h light:dark regime. The strain was maintained in the culture collection of the Laboratory of Botany in the Federal Scientific Center of East Asia Terrestrial Biodiversity, Russia (strain number VCA-43 (Vl15-3)) and the specimen was deposited to the Herbarium of the Federal Scientific Center of East Asia Terrestrial Biodiversity, Russia (exsiccatum number VLA-CA-1212).

Morphological examination:—General morphological observations, measurements and microphotographs were performed using an Olympus BX 53 light microscope (Olympus, Tokyo, Japan) equipped with Nomarski DIC optics, an Olympus DP27 digital camera (Olympus, Tokyo, Japan) and Olympus software cellSens Standard (v. 1.14).

DNA extraction and PCR amplification:—For DNA analysis, the unialgal culture was harvested during the exponential growth phase and concentrated by centrifugation. Total genomic DNA was extracted according to Echt *et al.* (1992) and Kiselev *et al.* (2015) with some modifications. The specimen of homogenized cells (ground to a fine powder with a pestle and mortar after being frozen in liquid nitrogen) was mixed with 800 μl of the buffer containing 0.2% mercaptoethanol, 100 mM Tris (pH 7.5–8.0), 0.7 M NaCl, 40 mM EDTA (pH 7.5–8.0), and 1% cetyltrimethylammonium bromide (CTAB). The mixture was incubated at +60 °C for 1 h under stirring. The sample was then mixed with 300 μl of chloroform with constant gentle agitation for 5 min and centrifuged at 13–14,000 rpm for 5 min. 300–400 μl of the aqueous phase was precipitated with 2.5V 96% ethanol (at −20 °C for 20 min) and pelleted by spinning in a microcentrifuge at 13–14,000 rpm for 5 min. The DNA sample was dried at +37 °C and dissolved in 100 μl of distilled water. The amplification of the 16S rRNA gene and 16S–23S ITS region was performed using universal cyanobacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3'; Lane 1991) and 340 (5'-CTCTGTGTGCCTAGGTATCC-3'; Iteman *et al.* 2000) that amplify 16S and complete ITS region of the ribosomal operon. Polymerase chain reaction (PCR) cycling profile included an initial step of 5 min at 95 °C, followed by 35 cycles of 20 s at 95 °C, 20 s at 51 °C, 2 min at 72 °C, and terminating with a final hold of 5 min at 72 °C using the Encyclo Plus PCR kit (Evrogen, Moscow, Russia), a T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, California, USA). The PCR products were visualized on 1% agarose gel then purified by ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, Santa Clara, California, USA) and sequenced in both directions using an ABI 3500 genetic analyzer (Applied Biosystems, Foster City. California, USA) with a BigDye terminator v3.1 sequencing kit (Life Technologies Corporation, Austin, Texas, USA). The sequencing was carried out from one PCR product with primers 27F, 1492R (5'-GGTTACCTTGTTACGACTT-3'; Turner *et al.* 1999), and 340. Sequences were assembled with the Staden Package v1.4 (Bonfield *et al.* 1995). The sequence comprising 16S rRNA gene and 16S–23S ITS region (1800 bp) was deposited in GenBank under accession number MW646373.

Phylogenetic analyses:—A dataset was assembled following Zhang *et al.* (2018), Wang *et al.* (2019), Jung *et al.* (2020), and Komárek *et al.* (2020). 16S rRNA gene sequences retrieved from the NCBI were aligned in the SeaView program (Galtier *et al.* 1996) with manual corrections. For the phylogenetic inferences ambiguous regions were removed that resulted in the alignment with 1533 aligned base positions. *Gloeobacter violaceus* Rippka, J.B.Waterbury & Cohen-Bazire (1974: 436) PCC 7421 (NR_074282) and *Gloeobacter kilaueensis* J.W. Saw *et al.* (2014: 3) JS1 (NR_121745) were used as an outgroup. The best evolutionary model GTR+I+G was determined using jModelTest 2.1.1 (Darriba *et al.* 2012). Phylogenetic trees were constructed using maximum likelihood (ML) in RAxML (v.7.2.6) (http://embnet.vital-it.ch/raxml-bb/; Kozlov *et al.* 2019) and Bayesian inference (BI) in MrBayes (v.3.1.2) (Huelsenbeck & Ronquist 2001). In BI, four runs of four Markov chains were executed for 2 million generations, sampling every 100 generations

for a total of 20,000 samples. Convergence of the two chains was assessed, and stationarity was determined according to the 'sump' plot with the first 5000 samples (25%) discarded as burn-in; posterior probabilities were calculated from trees sampled during stationary phase. The robustness of the ML trees was estimated by bootstrap percentages (BP; Stamatakis *et al.* 2008) and posterior probabilities (PP) in BI. BP < 50% and PP < 0.95 were not considered. The Mfold web server (http://www.unafold.org/mfold/applications/rna-folding-form.php; Zuker 2003) was used with the default settings to elucidate the folding pattern of D1–D1′, Box-B helices (16S–23S ribosomal RNA intergenic spacer) secondary structure and visualized in the program VARNA v.3.93 (Darty *et al.* 2009). The boundaries of the helices are determined based on the studies of Rigonato *et al.* (2016), Zhang *et al.* (2018) and Jung *et al.* (2020). MEGA v.7.0.26 (Kumar *et al.* 2016) was used to estimate intrageneric pairwise distances (uncorrected *p*-distances) representing the percentages of sequence similarities or dissimilarities and mean distances in clades.

Results

Aliterella vladivostokensis Sh.R. Abdullin, A.Yu. Nikulin, V.B. Bagmet et V.Yu. Nikulin *sp. nov.* (Fig. 1)

Description:—Cells solitary, more commonly irregular or in rounded colonies with many cells (up to 32–64 or more), usually aggregated irregularly, extended (Fig. 1 A–C). Mucilage unstratified, colorless and firm, surrounding cells and colonies. Cells cylindrical 1.6–6.87 μm long, 1.17–5.85 μm wide, 1 to 1.75 × longer than wide (mean, 1.28 ×) (Fig. 1 C–D). Cells easily squeezing from colonies with pressure (Fig. 1 C). The chromatoplasm and centroplasm usually recognizable with light microscopy (Fig. 1 C–D). Cell contents blue-green, slightly granulated, or sometimes homogeneous. Reproduction by simple binary cell division in three or more planes.

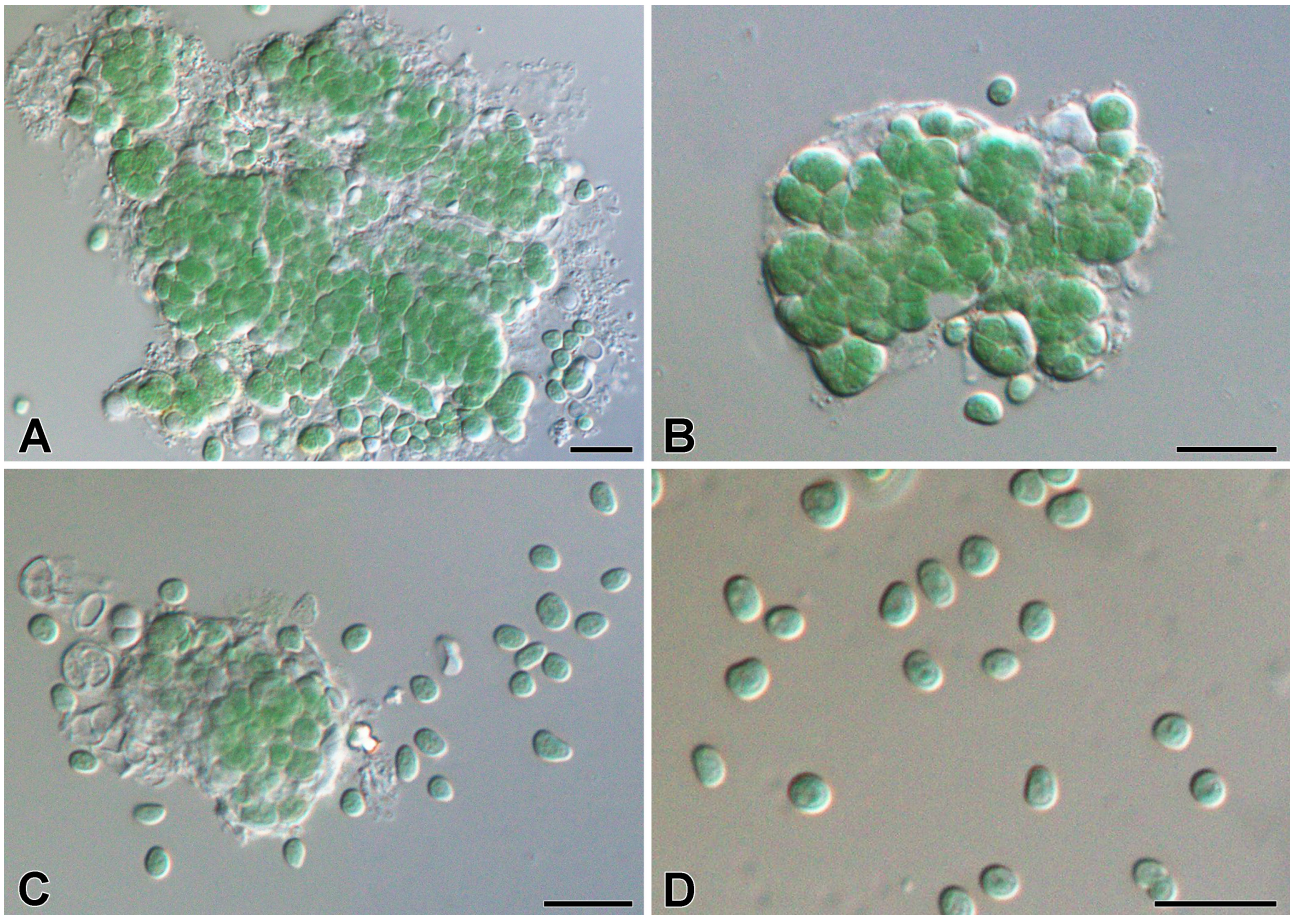

FIGURE 1. Light micrographs of *Aliterella vladivostokensis*. A–C. Compact and irregular thallus composed of numerous irregular or rounded colonies or solitary cells with colorless and firm mucilaginous envelopes. D. Cylindrical or irregular cells. Scale bars: 10 μm.

Holotype:—The dried biomass of authentic strain was deposited to the Herbarium of the Federal Scientific Center of East Asia Terrestrial Biodiversity, Russia (exsiccatum number VLA-CA-1212) as a holotype.

Type locality:—RUSSIA. Primorsky Territory, Vladivostok, 43°10'21.4" N, 131°56'10.9" E, collected by *Shamil R. Abdullin* on August 7, 2018. The authentic strain VCA-43 (Vl15-3) is available in the culture collection of the Laboratory of Botany, Federal Scientific Center of East Asia Terrestrial Biodiversity, Russia.

Habitat:—This cyanobacteria occurred in aerophytic habitat (on the concrete fence).

Etymology:—The species epithet '*vladivostokensis*' is derived from the type locality Vladivostok City.

Molecular phylogeny and sequence analyses:—Results of the BLAST searches showed that the sequence of the 16S rRNA gene and internal transcribed spacer (ITS) region (1800 bp) in our strain was highly similar to those in other species of *Aliterella,* several uncultured bacterial clones, and *Synechocystis* sp. PCC 7509 (> 94.5%). When only the ITS region was compared, the similarity to *Aliterella* spp. was just above 85%. Such relatively low similarity percentages suggested that we were likely dealing with a new species.

Phylogenetic analyses (ML and BI) clearly indicated that *A. vladivostokensis* was a member of the strongly supported (99/1.00; BP/PP; Fig. 2) *Aliterella* generic clade. Branching pattern between the clade members remained largely unresolved (Fig. 2).

We observed the highest 16S rRNA sequence similarities based on *p*-distance analysis between our isolate and an uncultured bacterial clone (DQ532167) isolated from clean spacecraft assembly rooms where spacecrafts are assembled (99.5%; Table 1). *A. antarctica* (KU291459) and *A. shaanxiensis* (MH023997) were the most similar to each other among described *Aliterella* species, (99.1%). Since no ITS sequences were available for the uncultured bacterial clones resolved as members of the *Aliterella* clade (5 accessions), we only compared the percentage of sequence dissimilarity between aligned 16S–23S ITS regions for the described taxa and *Synechocystis* sp. PCC 7509 (6 accessions). The dissimilarity varied from 6.4% to 17.5% (11.6%–17.5% between *A. vladivostokensis* and the other species; Table 2).

TABLE 1. 16S rRNA gene sequence similarities (100 x uncorrected *p*-distance) among four *Aliterella* strains and their relatives.

	1.	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**
1. *A. vladivostokensis sp. nov.* MW646373										
2. *A. chasmolithica* MN243145	98.8% (±0.3)									
3. *A. antarctica* KU291459	99.1% (±0.3)	96.5% (±0.4)								
4. *A. atlantica* NZ_JYON01000056	98.5% (±0.3)	93.7% (±0.4)	97.4% (±0.4)							
5. *A. shaanxiensis* MH023997	99.1% (±0.3)	97.2% (±0.3)	98.2% (±0.4)	98.3% (±0.3)						
6. Uncultured bacterium KF037769	98.5% (±0.3)	97.6% (±0.4)	98.2% (±0.4)	97.9% (±0.4)	99.0% (±0.3)					
7. Uncultured bacterium DQ532167	99.5% (±0.2)	98.7% (±0.3)	98.3% (±0.3)	98.0% (±0.4)	98.5% (±0.3)	98.3% (±0.4)				
8. Uncultured bacterium KF037911	99.2% (±0.2)	98.8% (±0.3)	98.3% (±0.3)	97.9% (±0.4)	98.5% (±0.3)	98.3% (±0.3)	99.8% (±0.2)			
9. Uncultured bacterium AB696285	99.4% (±0.2)	98.5% (±0.3)	98.2% (±0.4)	97.7% (±0.4)	98.3% (±0.3)	98.2% (±0.3)	99.7% (±0.1)	99.7% (±0.2)		
10. Uncultured bacterium AB696383	99.3% (±0.2)	98.7% (±0.3)	97.7% (±0.4)	97.4% (±0.4)	98.4% (±0.3)	98.0% (±0.4)	99.6% (±0.2)	99.6% (±0.2)	99.9% (±0.1)	
11. *Synechocystis* sp. PCC 7509	97.2% (±0.4)	96.5% (±0.4)	96.3% (±0.4)	96.2% (±0.5)	96.4% (±0.5)	96.0% (±0.4)	97.0% (±0.4)	96.8% (±0.4)	97.0% (±0.4)	96.8% (±0.4)

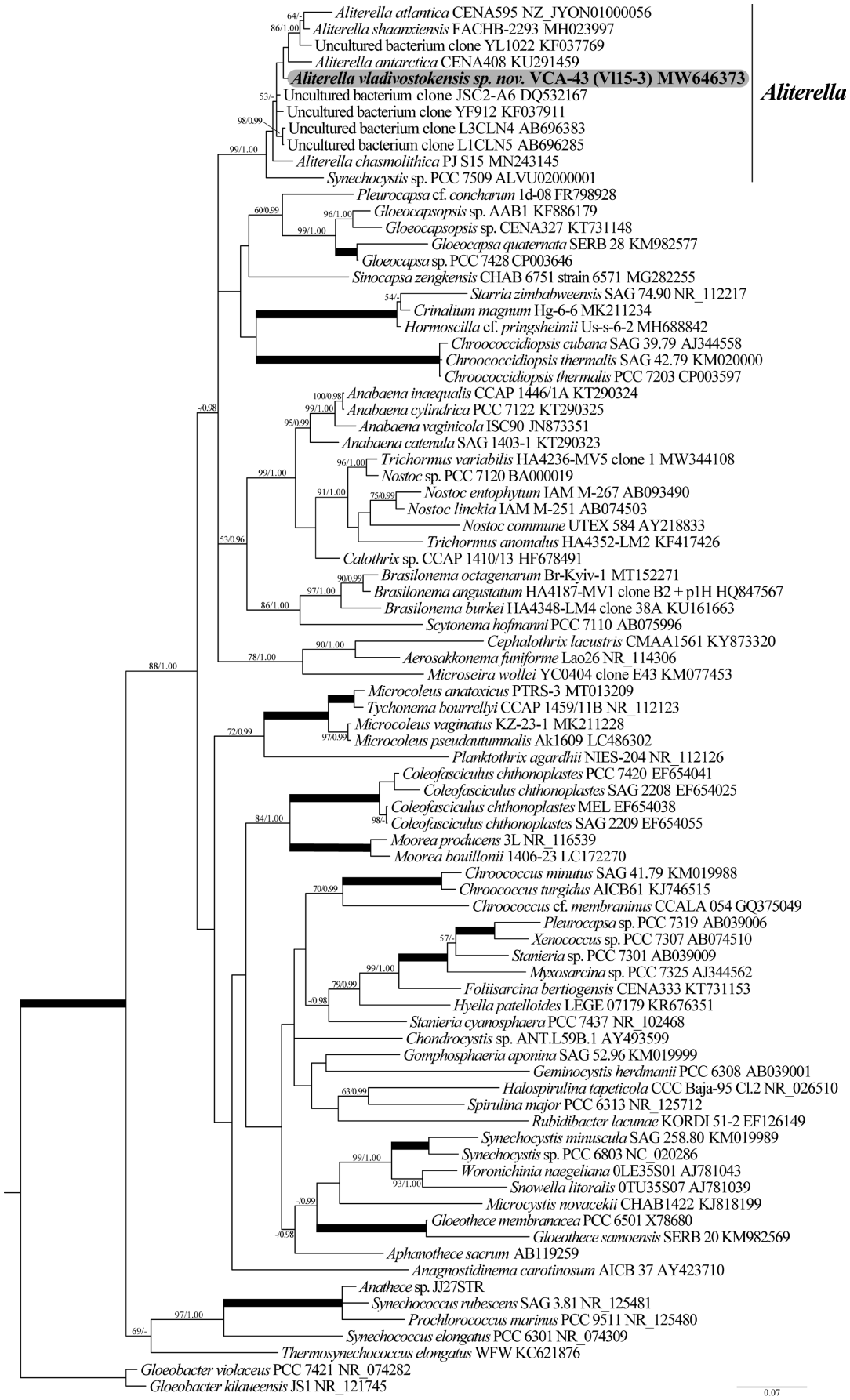

FIGURE 2. ML tree showing phylogenetic position of the new species *A. vladivostokensis* based on 16S rRNA gene sequence data (GTR+I+G model). Support [ML/BI, (BP) ≥ 50% and (PP) ≥ 0.95] are given above/below the branches. Branches with 100% BP, 1.00 PP and sequences obtained for this study are shown in boldface. Scale bar – substitutions per nucleotide position.

TABLE 2. Percent dissimilarity (100 x uncorrected *p*-distance) among aligned 16S–23S ITS regions of *Aliterella* species.

	1.	2.	3.	4.	5
1. *A. vladivostokensis sp. nov.*					
2. *A. chasmolithica* PJ S15	13.0 (±1.5)				
3. *A. antarctica* CENA 408	11.6 (±1.3)	14.5 (±1.3)			
4. *A. atlantica* CENA 595	17.0 (±1.6)	14.3 (±1.3)	14.3 (±1.4)		
5. *A. shaanxiensis* FACHB-2293	13.8 (±1.6)	12.8 (±1.4)	15.4 (±1.6)	6.4 (±0.1)	
6. *Synechocystis* sp. PCC 7509	17.5 (±1.8)	13.5 (±1.5)	14.9 (±1.5)	14.0 (±1.5)	15.0 (±1.7)

Comparison of ITS secondary structures of the D1–D1′ and Box-B helices showed that they have similar patterns of bulges and terminal loops (Fig. 3, 4). The D1–D1′ helix was almost invariant in length (65–67 bp) and structure with 42 conserved sites among *Aliterella* species (Fig. 3). In *Synechocystis* sp. PCC 7509 the helix was shorter (57 bp) due to the deletion in the terminal loop (Fig. 3). The basal part of the D1–D1′ helix consisted of a conservative 4-bp double-stranded region followed by the internal (bilateral) loop (positions 5–6 and from 55–57 to 61–63 in different *Aliterella* species; 5–6 and 47–53 in *Synechocystis* sp. PCC 7509) and side loop with a single unpaired base (position 51–52, position 43 for *Synechocystis* sp. PCC 7509). Substitutions in the bilateral loop (base change U → C at position 57) and in the side loop (A → C at position 51) differentiated *A. vladivostokensis*. The terminal loop of the helix consisted of five bp and also harbored a unique marker mutation for the new species (A → C at position 29). Most species had two internal loops in the central part of D1–D1′ helix with the exception of *A. shaanxiensis*, which had three loops and *Synechocystis* sp. PCC 7509 with the one loop. We found one compensatory base change (CBC) and five hemi-compensatory base changes (hCBCs) in our secondary structure models. While CBC (A-U → G-C) was shared between *A. vladivostokensis* and *A. antarctica* (at positions 19–42 in both cases), the hCBCs were attributed to *A. atlantica* (C-G → U-G, at position 22), *A. antarctica* (two substitutions C-G → U-G at positions 20 and 27), *A. chasmolithica* (G-C → G-U, at position 33; Fig. 3), and *Synechocystis* sp. PCC 7509 (C-G → U-G at position 13).

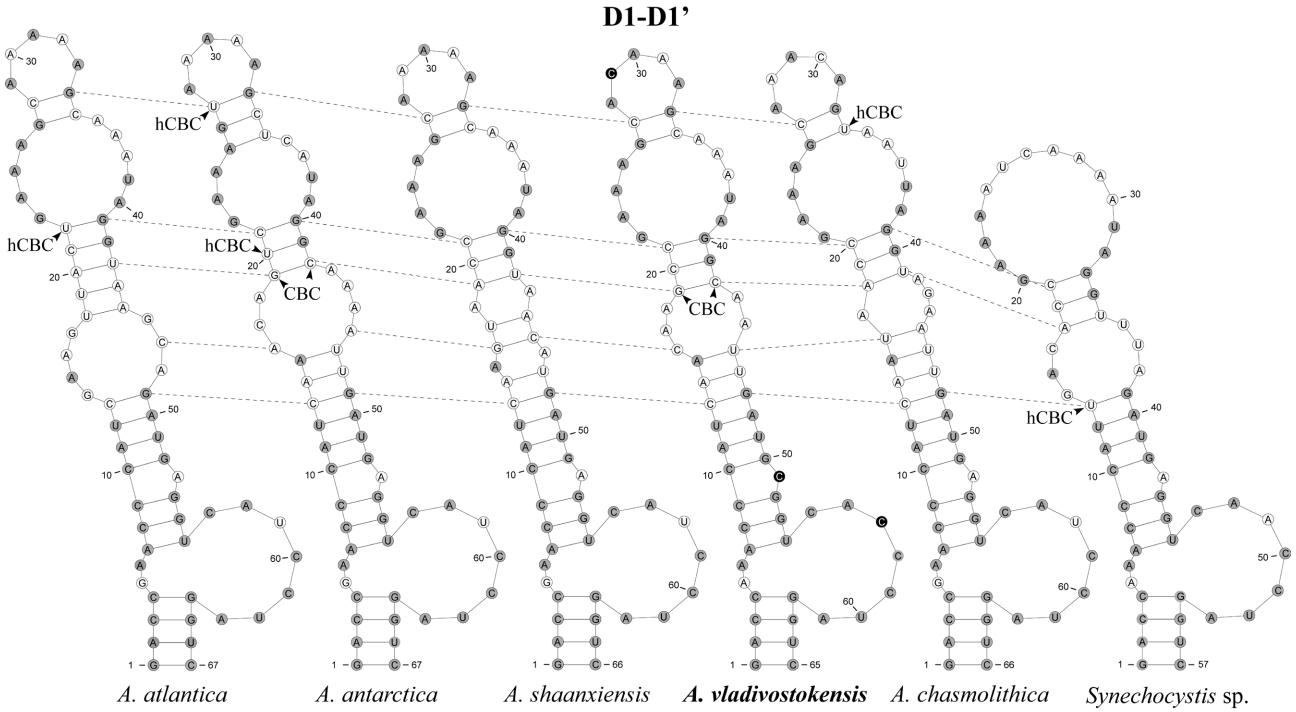

FIGURE 3. Secondary structures for the D1–D1′ helices in the ITS regions for five *Aliterella* species and putative genus member *Synechocystis* sp. PCC 7509. Conservative nucleotides are grey colored. The unique marker mutations for the new species *A. vladivostokensis* are black colored. Arrowheads show compensatory (CBCs) and hemi-compensatory base changes (hCBCs). Homological base pairs among different species are indicated by dotted lines.

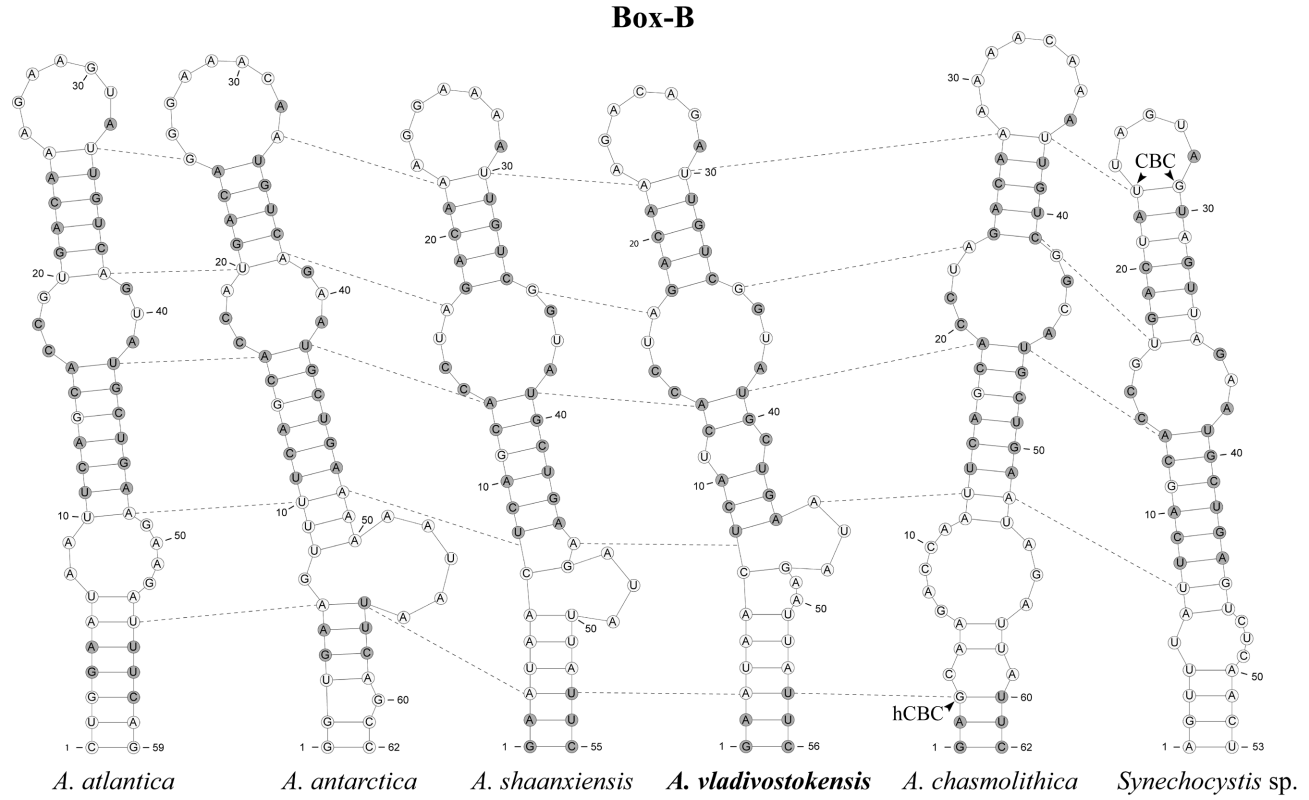

FIGURE 4. Secondary structures for the Box-B helices in the ITS regions for five *Aliterella* species and putative genus member *Synechocystis* sp. PCC 7509. Conservative nucleotides are grey colored. Arrowheads show compensatory (CBCs) and hemi-compensatory base changes (hCBCs). Homological base pairs among different species are indicated by dotted lines.

The Box-B helix was more variable in both primary and secondary structure (53–62 bp, 29 conservative sites); therefore, homologous nucleotides were difficult to identify in some cases (Fig. 4). The middle part of the Box-B helix had two (*A. atlantica*) to four (*A. vladivostokensis*) mostly bilateral internal loops. Point mutations, mostly found in single stranded domains, and indels frequent in the basal part of the Box-B altered the bulges location between species. A base substitution G → U at position 11 in the Box-B disrupted nucleotides pairing that led to an additional internal loop formation in *A. vladivostokensis* sequence (Fig. 4). The Box-B helix in *Synechocystis* sp. PCC 7509 differed from those in other species in lacking conservative motive at the base of the helix and shorter terminal loop (5 vs. 7–9 bases; Fig. 4). We found one CBC in *Synechocystis* sp. PCC 7509 (A-U → U-G, at positions 3 and 29) and one hCBC in *A. chasmolithica* (A-U → G-U, at position 3).

Discussion

Using molecular characteristics (sequences of 16S–23S rRNA ITS region, secondary structure data, and phylogenetic reconstructions), we found evidence to describe our cyanobacterial strain as a new species *Aliterella vladivostokensis*. Comparisons of 16S rRNA gene sequences confidently revealed that *A. vladivostokensis* was a member of the distinct *Aliterella* clade with high sequence similarity typical for its members. Percent similarity values based on *p*-distance between the new species and the rest of the clade were < 99% (the upper limit of the most optimistic threshold) in three instances only (Table 1). Lack of sufficient variation in the 16S rRNA gene in some cyanobacterial groups prompts students to rely on sequence data from 16S–23S ITS region along with 16S rRNA gene (Erwin & Thacker 2008, Perkerson *et al.* 2011, Johansen *et al.* 2014, Osorio-Santos *et al.* 2014, Pietrasiak *et al.* 2014, Abdullin *et al.* 2021). High divergence of the 16S–23S ITS sequences (6.4%–17.5%; Table 2) reflects significant genetic differentiation between the species and the isolates in *Aliterella*. From morphological point of view these entities could be regarded as cryptic species due to poor, mostly qualitative differences in cell dimensions (Table 3). The new species *A. vladivostokensis* is distinct from other *Aliterella* members (including *A. antarctica* that is highly similar in morphometric data and shares

a compensatory base change in the D1–D1′ helix (see Table 3 and Fig. 3)) in the high percent of dissimilarity ≥11.6 (±1.3) of the 16S–23S ITS sequences (Table 2), with at least 5 unique (autapomorphic) mutations in the secondary structures of the D1–D1′ and Box-B helices, and a distinct folding pattern of 4 loops of the Box-B helix (Fig. 3, 4). This divergence is also supported by a marked difference in the habitats the species were found in, great distances between the findings and their rarity (Table 4). Occurrence in marine, freshwater, aerophytic, chasmoendolithic, and soil environments on different continents and climate zones suggests a presence of specific adaptations that are not reflected by morphology and 16S rRNA gene sequences in case of *Aliterella*. Similar cryptic diversity was uncovered in some other cyanobacterial lineages e.g. *Synechococcus* C.Nägeli (1849: 56) (Synechococcales; Erwin & Thacker 2008); *Desertifilum* P.K.Dadheech & L.Krienitz (2012: 263) (Oscillatoriales; Cellamare *et al.* 2018) *Oculatella* Zammit, Billi & Albertano (2012: 351) (Synechococcales; Osorio-Santos *et al.* 2014, Becerra–Absalón *et al.* 2020), *Roholtiella* Bohunická, Pietrasiak & J.R.Johansen (2015: 88) (Nostocales; Abdullin *et al.* 2021), etc. with a number of species described based on ITS data evidence only.

TABLE 3. *Aliterella* species phenotypic characteristics.

	A. atlantica	*A. antarctica*	*A. shaanxiensis*	*A. chasmolithica*	*A. vladivostokensis sp. nov.*
Thallus	Extended, compact, irregularly shaped				
	composed of numerous colonies or isolated ensheathed cells			composed of numerous colonies or rarely isolated ensheathed cells	
Colony shape	Mostly irregular, sometimes rounded				
Number of cells in colony	Variable (up to 32–64 cells or more)				
Mucilage	Firm surrounding cells and colonies				
	colorless	colorless to brownish	colorless	colorless to brownish	colorless
Cell size	3.0–4.8 µm long (mean = 3.7 µm), 2.0–3.3 µm wide (mean = 2.7 µm)	3.4–5.8 µm long (mean = 4.5 µm), 2.5–4.6 µm wide (mean = 3.5 µm)	2.8–3.7 µm long (mean = 3.3 ± 0.3 µm, n = 40), 2.0–2.7 µm wide (mean = 2.2 ± 0.2 µm, n = 40)	1.5–3.2 µm long (mean = 2.6 ± 0.4 µm), 1.5–2.4 µm wide (mean = 1.9 ± 0.2 µm)	1.6–6.87 µm long (mean = 4.41 ± 0.16 µm, n = 40), 1.17–5.85 µm wide (mean = 3.45 ± 0.12 µm, n = 40)
Length/ Wide	1.1–2 (mean = 1.4)	1.0–1.7 (mean = 1.3)	1.3–1.7 (mean = 1.5)	0.7–2.0 (mean = 1.3 ± 0.2)	1.00–1.75 (mean = 1.28)
Cell shape	Cylindrical with rounded ends				
	sometimes irregular	less frequently near spherical or irregular		less frequently near spherical or irregular	
Cell content	Granulated, sometimes homogeneous		Slightly granulated, sometimes homogeneous	Granulated, sometimes homogeneous	
Color	Blue-green				
Reproduction	Simple binary cell division in three or more planes				
References	Rigonato *et al.* (2016)		Zhang *et al.* (2018)	Jung *et al.* (2020)	this study

Composition and the structure of the *Aliterella* clade (Fig. 2) coupled with genetic distance data (Tables 1 and 2) suggests presence of an undescribed species in the genus (uncultured bacterial clones and *Synechocystis* sp. PCC 7509). It is likely that the "bacterial" accession KF037769 (97.6%–99% of 16S rRNA gene sequence similarities; Table 1) represents a distinct species that is a sister of *A. atlantica/A. shaanxiensis* clade (Fig. 2). In addition to that, four sequences (DQ532167, KF037911, AB696285, and AB696383) characterized by relatively low genetic divergence (0.4% of 16S rRNA sequence dissimilarity) could be a single soil-inhabiting species. Its occurrence in the artificial strictly controlled environment of a clean spacecraft-associated room (Houston, USA; Moissl *et al.* 2007) could be a result of long-distance dispersal by air. Unfortunately, no morphological and ITS data, which could allow

the species characterization and delimitation, are available on these putative species. Molecular data strongly suggest that *Synechocystis* sp. PCC 7509 also belongs to the genus *Aliterella* where it likely occupy basal position (Wang *et al.* 2019; present study) and distinct in secondary structures of the D1–D1′ and Box-B helices (Figs. 3, 4).

Jung *et al.* (2020) hypothesized a possible split in the genus between aquatic (*A. shannxiensis*, *A. antarctica*, and *A. atlantica*) and terrestrial/lithophilous species (*A. chasmolithica* and uncultured bacterial clones DQ532167, KF037911, AB696285, and AB696383). This assumption is not supported by the results of our phylogenetic analyses which placed uncultured bacterial clone KF037769 (from the surface of a carbonate rock) in the "aquatic" clade. Aerophytic *A. vladivostokensis* also showed affinity to this lineage (Fig. 2).

There is more data on the geographic distribution of the bloom-forming and invasive toxic species due to their ecological, sanitary, and economic importance (Cirés & Ballot 2016, Harke *et al.* 2016), whereas quite limited biogeographic information is available for taxa inhabiting arduous geographic areas and habitats (Ribeiro *et al.* 2018). The genus *Aliterella* apparently has a wide distribution with its members found in the Asia (including *A. vladivostokensis*), North and South America, Europe (*Synechocystis* sp. PCC 7509 – the northernmost finding to date) and the Antarctic islands (Fig. 5; Table 4). There is no genus record from Africa, continental Antarctica, and Australia yet, although in Antarctica the diversity of cyanobacteria has been explored quite extensively using metagenomic approach (e.g. Wilkins *et al.* 2013, Alvarenga *et al.* 2017, Li *et al.* 2019, Panwar *et al.* 2020). An interesting fact is that nearly each finding of *Aliterella* represents a distinct species. Only in one instance likely two undescribed species were recorded in one locality (KF037769 and KF037911; Yunnan, China).

TABLE 4. Comparison of climate conditions and habitat of *Aliterella* genus clade members.

	Climate conditions (Köppen 1936)	Habitat
A. atlantica	Tropical savanna climate (Aw).	Marine plankton, Atlantic Ocean, at the level coinciding with the deep chlorophyll maximum layer (ca. 107 m) continental shelf of south-eastern Brazil
A. antarctica	Tundra climate (ET).	On a green turf alga growing together with decomposed *Deschampsia* grass on a ornithogenic soil (*Pygoscelis adeliae*), Admiralty Bay, King George Island and South Shetlands Archipelago, Antarctica
A. shaanxiensis	Humid subtropical climate (Cwa).	Freshwater plankton, Hongsi Lake, Hanzhong, Shaanxi Province, China
A. chasmolithica	Hot desert (arid) climate (BWh).	Chasmoendolithic in granite, National Park Pan de Azúcar, Atacama Desert, Chile
A. vladivostokensis sp. nov.	Humid continental climate (Dwb)	On the concrete fence, Vladivostok, Primorsky Territory, Russia
Uncultured bacteria DQ532167	Humid subtropical climate (Cfa).	Spacecraft-associated clean room, Houston, Texas, USA
Uncultured bacteria AB696285 and AB696383	Semi-arid climate (BSh).	Soil, Loess Plateau, China
Uncultured bacterium KF037769 and KF037911	Subtropical highland variety of oceanic climate (Cwb).	On the carbonate rock, Yunnan, China
Synechocystis sp. ALVU02000001	Subarctic climate (Dfc)	Rock, Switzerland, Europe

High level taxonomic affinity of *Aliterella* remains questionable. The genus *Aliterella* composes the family Aliterellaceae that is a part of the order Chroococcidiopsidales along with the family Chroococcidiopsidaceae (Rigonato *et al.* 2016). Phylogeny based on 16S rRNA gene sequences representing main cyanobacterial groups suggested non-monophyly of the order and revealed unrecognized earlier putatively related lineages distinct in their morphological, cytological and ecological features (Wang *et al.* 2019). In contrast to that, extensive analyses based on 260 housekeeping genes confidently resolved the order clade and confirmed its sistership to the order Nostocales (Komarek *et al.* 2020).

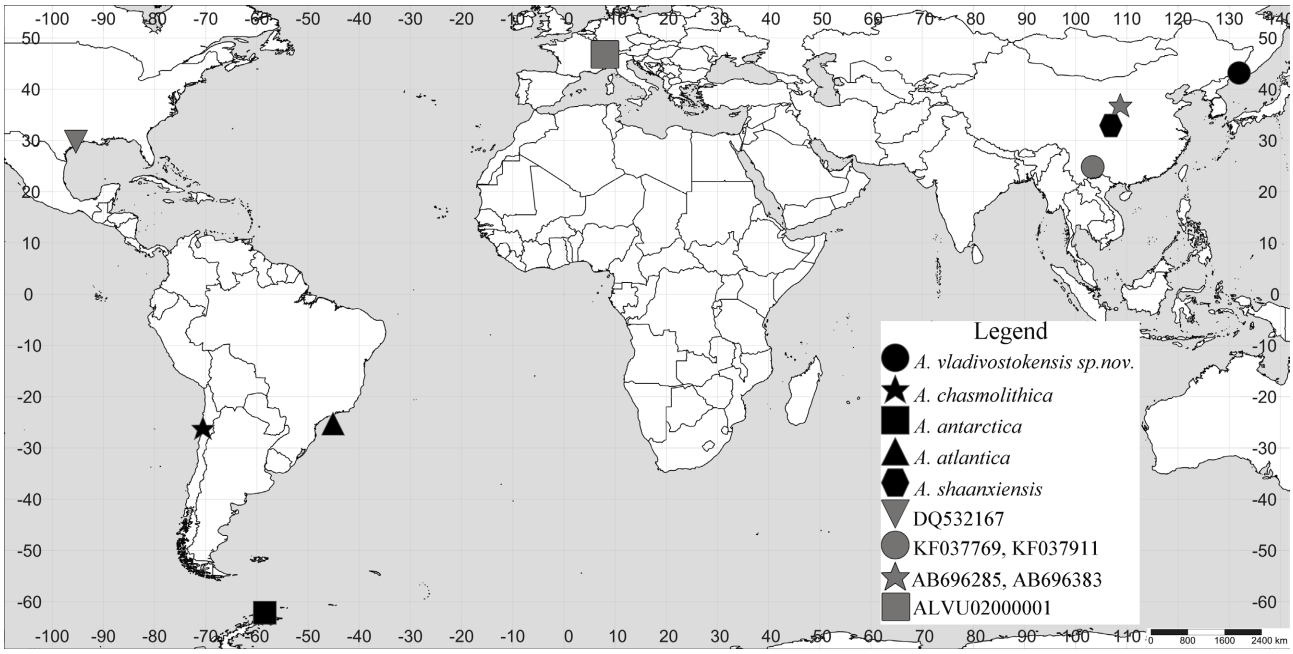

FIGURE 5. Map showing distribution of sampling locations of *Aliterella* genus members according of Rigonato *et al.* (2016), Zhang *et al.* (2018), Jung *et al.* (2020), present study and GenBank data (created with https://www.simplemappr.net, CC 1.0; Shorthouse 2010). For the uncultured bacterial clones and *Synechocystis* sp. PCC 7509 GenBank accessions are given (see the legend).

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