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Picocyanobacteria in Estuaries of Three Siberian Rivers and Adjacent Shelves of Russian Arctic Seas: Genetic Diversity and Distribution

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Abstract: Single-cell cyanobacteria, being an integral part of picoplankton in marine ecosystems, have been suggested to be important contributors to primary production and carbon cycles in the global ocean. The spatial distribution, abundance and diversity of natural communities of picocyanobacteria (PC) in estuaries of Khatanga, Indigirka and Kolyma rivers and adjacent shelves of the Laptev and East Siberian seas were studied in September 2017. The PC concentrations were higher in the estuaries than in the shelf stations of the seas. The abundance of PC was 1.25×10^6 cells/L, 0.42×10^6 cells/L and 1.58×10^6 cells/L in the surface layer of Khatanga, Indigirka and Kolyma estuaries, respectively. The contribution of PC to total autumn picophytoplankton abundance averaged 6% and 3% in the Khatanga and Indigirka estuaries and reached 5% in the Kolyma estuary. Phylogenetic analysis of the 16S rRNA gene and ITS region clone libraries revealed picocyanobacterial sequences related to marine *Synechococcus* subclusters 5.1-I, 5.2 and 5.3. Of the phylotypes from *Synechococcus* S5.1-I and S5.2 that were found, only several were discovered earlier, while the remaining clones were unique. Two groups of phylotypes (clades A and E) were found that were not closely similar to those previously described in both marine and freshwater habitats. It can be expected that a more detailed study of the phytoplankton of the Arctic seas will further expand our understanding of the diversity of these key components of the food chains of oceanic biocenoses.

Keywords: *Synechococcus*; cyanobacteria; picoplankton; 16S rRNA-ITS region; Laptev Sea; East Siberian Sea; Khatanga estuary; Kolyma estuary; Indigirka estuary

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1. Introduction

Unicellular cyanobacteria (cell size, 1–3 μm) are important contributors to the global primary production and carbon cycle [1,2]. In marine cold environments, only one genus, *Synechococcus*, is ubiquitous and inhabits the whole area from coastal waters to open ocean, including the Beaufort Sea [3], off Iceland [4], the Chukchi Sea [3,5], and an area neighboring the Norwegian, Greenland and Barents seas [6]. Recent research shows that *Synechococcus* is well adapted to the Arctic conditions and is indigenous in high-latitudinal ecosystems [7]. Several sources of PC in the Arctic marine environment are hypothesized: allochthonous inputs with river discharges or transport via advection from surrounding oceans [8–10], and an autochthonous origin of PC, which became adapted to cold environments [3,5].

The Laptev and East Siberian seas are characterized by the considerable river discharge of Siberian rivers and influenced by North Atlantic and Pacific water flows [11–14]. The Laptev Sea is second among the Arctic seas (after the Kara Sea) by volume of riverine discharge [15]. The western part of the sea is affected by the freshwater discharge of the Khatanga River (85 km^3/year), which flows through the long (>200 km) Khatanga Bay and is characterized by a rather late melt of seasonal sea ice. The East Siberian Sea is the widest of the Arctic Ocean shelf seas, with an area of ~900,000 km^2, but its mean depth is only

52 m [16]. The waters of the East Siberian Sea are heavily diluted first by the Lena River runoff and then by waters of the Indigirka and Kolyma rivers with annual water discharge of 61 km^3 for Indigirka and 132 km^3 for Kolyma [15].

Estuaries are characterized by pronounced gradients of physical and chemical components due to the mixing of fresh and marine waters [17]. These factors strongly influence the phytoplankton communities and lead to changes in their composition, structure and diversity along the resulting continuum. As the Arctic warms and the permafrost thaws, the total river discharge is increasing [18], resulting in the increased river export of nutrients, dissolved organic carbon matter [19,20]. All of these factors can lead to an increase in the allochthonous inputs of freshwater eukaryotic algae and cyanobacteria to marine ecosystems and to the growth of the autochthonous origin of PC in the Russian arctic seas.

In Russian Arctic ecosystems, mainly microbial eukaryotes, as part of the picophytoplankton, are studied through molecular techniques [21–23], while cyanobacterial communities in estuaries of Siberian rivers and adjacent areas of the Russian Arctic seas remain underexplored. PC diversity is most studied in the eastern part of the Fram Strait, in the coastal waters of the Chukchi Sea and the Beaufort Sea, in the Mackenzie Shelf and Franklin Bay areas [3,7,10,24]. PC are broadly classified into the genera *Prochlorococcus* and *Synechococcus*, which coexist in the open ocean, in polar regions; however, it is *Synechococcus* that has a wider geographical distribution [24]. *Synechococcus* is particularly difficult to identify and classify morphologically, and molecular methods are mostly used for its identification. Based on several genome-level analyses, Salazar et al. In 2020 proposed a new taxonomic framework that classified *Synechococcus* as polyphyletic at the order rank and suggested that it may refer to 15 genera, which are placed into five distinct orders within the phylum Cyanobacteria: Synechococcales, Cyanobacteriales, Leptococcales, Thermosynechococcales and Neosynechococcales [25]. Dufresne and coauthors (2008) analyzed the complete genomes of 11 marine *Synechococcus* isolates and classified them into three subclusters, 5.1, 5.2 and 5.3 [26]. Based on 16S rDNA and ITS sequence phylogenies, the *Synechococcus* isolates were clustered into 10 clades [27,28]. Other authors analyzing different markers proposed various nomenclatures of marine *Synechococcus* with up to 27 clades in subclusters 5.1–5.3 [5]. Ahlgren and Rocap, based on multiple gene loci, recognized more than 30 marine cultured *Synechococcus* clades in three subclusters [29].

The predicted increase in the PC role in the functioning of the Arctic ecosystems and the lack of data on their abundance in the Russian sector of the Arctic determined the goal of this research: to study natural communities of picocyanobacteria from estuaries of Khatanga, Indigirka and Kolyma rivers and adjacent shelves of the Laptev and East Siberian seas by constructing and sequencing clone libraries of internal transcribed spacer (ITS) sequences and 16S rDNA, and assess PC abundance, biomass and contributions to the total picophytoplankton abundance in these areas.

2. Materials and Methods

2.1. Water Sample Collection

Seawater samples were collected during the 69 cruise of the R.V. "Akademik Mstislav Keldysh" in September 2017. In total, twenty-three stations were sampled in the estuary of Khatanga river, the adjacent areas of Indigirka and Kolyma estuaries and adjacent shelves of the Laptev and East Siberian seas (Khatanga transect, Indigirka transect and Kolyma transect) (Figure 1). Seawater was collected directly from 5-L Niskin-type bottles mounted on a Rosette system equipped with a conductivity, temperature, depth profiler (CTD, Sea-Bird SBE-32, Sea-Bird Electronics Inc., Bellevue, DC, USA) and sensors for chlorophyll fluorescence (Seapoint Sensors Inc., Exeter, NH, USA) from the surface or chlorophyll (Chl a) maximum layers. Salinity was reported using the TEOS-10 Practical Salinity scale. The intensity of surface irradiance measured using a LI-190SA (LI-COR) sensor was used to estimate the depth of the euphotic zone (Zeu, 1% of surface irradiance). In the absence of underwater hydrooptical measurements, the diffuse attenuation coefficient for downwelling solar radiation in the visible spectrum was calculated according to Demidov et al. [30].

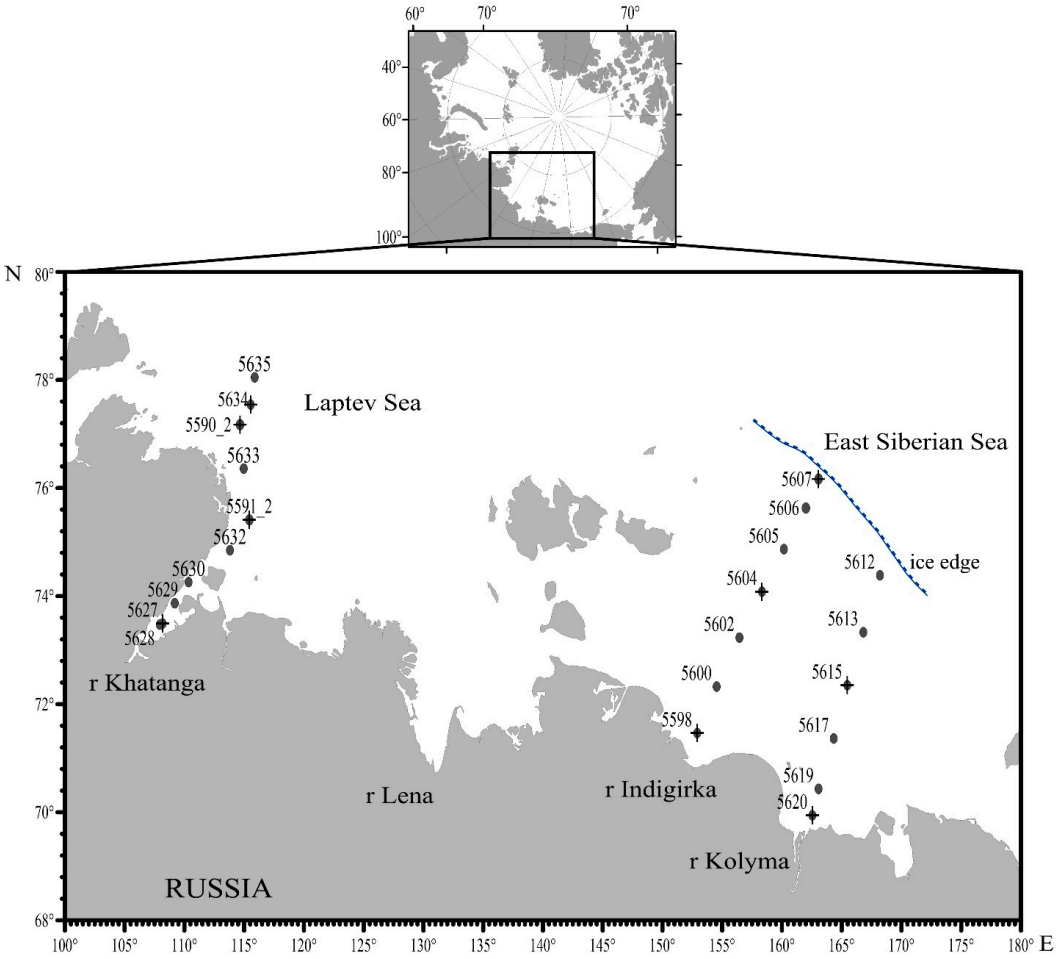

Figure 1. Map of the sampling sites. eDNA was collected from stations marked with crosses.

2.2. Nutrients and Chlorophyll a Concentration

Nitrate, nitrite, ammonium (DIN, the sum of NO_3 + NO_2 + NH_4), phosphate and silicate concentrations were measured calorimetrically within a few hours of collection on the ship board [31].

Water samples (500 mL) for the total chlorophyll a concentrations were filtered through Whatman GF/F filters (47 mm). For the pico-sized fractionated chlorophyll a concentrations, 1 L water samples were passed through a 3 µm-pore-size polycarbonate filter using a <50 mmHg vacuum. The filtrate (<3 µm) was then filtered through 0.7 µm Whatman GF/F filters (47 mm). The chlorophyll a concentrations were measured using a Turner Designs model Trilogy field fluorometer calibrated spectrophotometrically using a chemically pure Chl solution (Sigma) as the standard. The Chl and phaeophytine concentrations were calculated using the standard equations [32].

2.3. Picophytoplankton Abundance and Biomass

The abundance of cyanobacteria and picoeukaryotes was estimated (total, 23 stations) (Table S1) via flow cytometry (BD Accuri C6, Ann Arbor, MI, USA) and epifluorescence microscopy (Leica DM1000, St. Gallen, Switzerland) directly on board the research vessel. According to a previous study, the BD Accuri C6 gives adequate results in counting photosynthetic eukaryotes but not cyanobacteria [33]. Epifluorescence microscopy was used to count picocyanobacteria. The sub-samples (3.6 mL) were pre-filtered through a 20 µm nylon gas and immediately analyzed using a flow cytometer equipped with 488- and 640-nm laser sources. Forward angle light scatter, right angle light scatter, orange fluorescence from phycoerythrin (575 ± 20 nm) and red fluorescence from chlorophyll (675 ± 10 nm) were

measured. Microspheres (1 µm, Fluoresbrite plain YG, Polysciences, Warrington, PA, USA) were added to each sample as an internal standard. The average coefficient of variation for duplicate sample counting was 5.7%. For epifluorescence microscopy, the sub-samples (10 mL) were placed in a filtration funnel and incubated for 5–7 min after the saturated solution of primulin was added. Each sample was preserved with glutaraldehyde at a final concentration of 1%. Nuclear filters (0.12 µm-pore diameter, Dubna, Russia) prestained with Sudan black were used for filtration. The cells on the filter were counted under a Leica DM1000 epifluorescence microscope at ×100, ×10 and ×1.3 magnification. Depending on the cell concentration, 30 to 50 fields were examined and the cell size was measured. The "type" of fluorescence was also determined: spherical cells with a diameter $\leq$ 1.5 µm with orange fluorescence from phycoerythrin (575 $\pm$ 20 nm) were considered picocyanobacteria. Orange fluorescence under the blue excitation was also specific to Cryptophytes, but the latter can be easily identified by their asymmetric cell shape and were absent in our samples. The cell volume was converted to carbon using different conversion factors. For prokaryotes, for which cell sizes varied from 0.8 to 1.2 µm (average 1 µm), a conversion factor of 470 fg C/cell was used [34]. The carbon biomass of picoeukaryotes was estimated according to a conversion factor of logC = 0.941 logV –0.60 [35].

The depth of almost all stations did not exceed 63 m. The average PC abundance was calculated for the entire depth of the stations, with the exception of shelf station 5634 (186 m) and station 5635 (857 m) located at the continental slope where the average PC abundance was calculated for the 60 m depth.

2.4. DNA Extraction, PCR Amplification, Cloning and Sequencing

Nine stations were sampled for the investigation of PC diversity (Figure 1). Total DNA was extracted using a Nucleospin Plant II Kit (Macherey Nagel, Düren, Germany) according to the manufacturer's instructions. The ITS region of rDNA was amplified with the primers Picocya16S-F—Picocya23S-R flanking the spacer region (Table 1). The amplification conditions were as follows: initial denaturing at 95 °C for 3 min; 35 cycles: 94 °C for 30 s, annealing of the primers at 64 °C for 20 s, elongation at 72 °C for 1.0 min; final elongation for 5 min. For five clones, ITS sequences were extracted from partial sequences of rDNA (1163 bp of 16s rRNA gene and ITS). The primers used for those were Cya359F (forward) and Picocya23R (reverse), complementary to the 16S rRNA gene and the 5′ end of the 23S rRNA gene, respectively. The PCR conditions were as follows: initial denaturing at 95 °C for 3 min; 35 cycles: 94 °C for 30 s, annealing of the primers at 64 °C for 20 s, elongation at 72 °C for 2 min; final elongation for 10 min. Out of all of those clones, we selected five with differing spacer variants and sequenced them with the primers Cya359F, B1055 and Picocya23S-R. The amplification was carried out using an Encyclo Plus PCR Kit (Evrogen, Moscow, Russia) according to the manufacturer's instructions. The PCR products were separated from the primers through preparative electrophoresis in a 1% agarose gel and subsequently isolated from agarose with a Cleanup Mini kit (Evrogen, Russia). The purified amplicons were cloned into the pAL2-T vector using a Quick-TA (Evrogen, Russia) kit in accordance with the manufacturer's instructions. Clones were screened using M13F and M13R vector-specific primers. At least 10 clones were sequenced for each sample. A total of 81 ITS clones were sequenced. Partial 16S rRNA gene sequences were determined from five clones with differing ITS sequences. DNA sequencing was performed using the ABI PRISM® BigDye™ Terminator v. 3.1 kit, followed by an analysis of the reaction products on an Applied Biosystems 3730 DNA Analyzer in the Genome Center (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia).

Table 1. Primers used in this study.

Primer Name, Position	Sequence 5′-3′	Reference
Cya359F (359–378, 16S)	GGGGAATTTTCCGCAATGGG	[36]
B1055 (1055–1074, 16S, F)	ATGGCTGTCGTCAGCTCGT	[37]
Picocya16S-F	TGGATCACCTCCTAACAGGG	[38]
Picocya23S-R	CCTTCATCGCCTCTGTGTGCC	[38]

2.5. Phylogenetic and Statistical Analyses

The obtained sequences were then compared to GenBank entries using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/ (accessed on 1 August 2023)) in order to identify closely related sequences in the database. For the phylogenetic analysis, unique sequence variants (total 43) were selected from each sample. The tRNA-Ile gene sequences were identical in all investigated clones and removed from the alignment prior to the phylogenetic tree construction. ITS sequences were aligned using Muscle5 [39] with the default setting followed by a manual adjustment in BioEdit ver. 7.2.5 [40]. 16S rDNA sequences were aligned manually in BioEdit.

Phylogenetic trees were computed using the Minimum Evolution (ME) method [41] implemented in MEGA ver.11.0.13 [42] with 1000 bootstrap replicas. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). The differences in the composition bias among sequences were considered in evolutionary comparisons. *Prochlorococcus marinus* phylotypes were used as an outgroup.

ITS and partial 16S rRNA gene sequences obtained in this study were deposited in GenBank under access numbers OR395546, OR393610, OR451699–OR451701 and OR448688–OR448763.

We used Spearman's correlation assay to estimate the influence of environmental factors on PC abundance using PAST 3.10 [43].

3. Results

3.1. Environmental Parameters and Nutrients

In the Khatanga Bay and at stations located near Indigirka and Kolyma estuaries, the surface salinity was lower and the temperature was higher than those above the Laptev and East Siberian sea shelf (Table 2). In the Laptev Sea, the surface water temperature and salinity ranged from −1.3 to 3.6 °C and from 3.5 to 32.3, respectively, with the warmer temperatures and lowest salinity recorded at the southernmost station 5627. The environmental parameters at stations located at Indigirka and Kolyma transects were analyzed in detail by Demidov and Gagarin [44] and Sukhanova et al. [45]. Surface water temperature ranged from −1.4 to 6.7 °C, with the colder temperatures recorded at stations near the ice edge (<0 °C), and surface salinity varied from 15.2 to 30.3. The freshened waters were recorded near the estuaries of Siberian rivers Indigirka and Kolyma.

The DIN:PO_4 molar ratio at different depths ranged from 0.1 to 10 in Khatanga Bay and from 0.2 to 14.5 in the Laptev Sea, and the average was 1.28 ± 1.68 at Indigirka and Kolyma rivers and adjacent areas of the Laptev Sea. These values were lower than the 16:1 Redfield value [46], suggesting that dissolved inorganic nitrogen was the macronutrient in the lowest supply for phytoplankton growth throughout the studied areas. Nitrogen limitation is common in Arctic shelf seas, such as Baffin Bay [47–49] and Hudson Bay [50,51].

Table 2. Sampling sites, station depth (H, m), surface temperature (T_0) and salinity (S_0), surface PC abundance (N_0, $\times 10^6$ cells/L) and the average PC abundance in the 0–60 m layer (N, $\times 10^6$ cells/L), and contribution to total picophytoplankton abundance (%). Stations marked with an asterisk were sampled for DNA analysis, and the sampling horizon (in m) is indicated in brackets.

Region	Location	Station	H, m	T_0	S_0	$N_0 \times 10^6$ cells/L	$N_{0-60} \times 10^6$ cells/L	%
Laptev Sea	Khatanga estuary	5627 * (0)	15	3.6	3.5	1.16	0.72	6.7
Laptev Sea	Khatanga estuary	5628	12	3.6	3.5	1.25	0.93	4.8
Laptev Sea	Khatanga estuary	5629	21	3.3	11.2	0.22	0.13	6.5
Laptev Sea	Khatanga estuary	5630	26	2.3	17.2	0.31	0.11	7.1
Laptev Sea	shelf	5632	34	2.2	21.9	0.11	0.03	1.9
Laptev Sea	shelf	5591_2 * (0)	40	2.3	22.3	0	0	-
Laptev Sea	shelf	5633	33	1.5	27.9	0.02	0.03	1
Laptev Sea	shelf	5590_2 * (13)	63	0.7	31.6	0	0.004	0.1
Laptev Sea	shelf	5634 * (0)	186	−0.4	30.0	0	0.007	0.1
Laptev Sea	continental slope	5635	857	−1.3	32.3	0	0.008	0.6
East Siberian Sea	Indigirka estuary	5598 * (0)	12	6.2	15.2	0.04	0.07	0.8
East Siberian Sea	Indigirka estuary	5600	20	5.6	17.6	0.42	0.23	3.9
East Siberian Sea	shelf	5602	25	4.2	21.2	0.09	0.51	9.8
East Siberian Sea	shelf	5604 * (0)	22	2.9	25.7	0	0.03	1.8
East Siberian Sea	shelf	5605	43	1.1	29.1	0.02	0.08	5.3
East Siberian Sea	shelf	5606	44	0.7	30.1	0	0	-
East Siberian Sea	shelf	5607 * (0, 10)	56	−1.4	30.3	0	0	-
East Siberian Sea	Kolyma estuary	5620 * (0)	18	6.0	18.4	1.16	0.37	3.8
East Siberian Sea	Kolyma estuary	5619	16	6.7	19.0	1.58	0.98	6.1
East Siberian Sea	shelf	5617	22	6.1	23.3	0.4	0.16	3.4
East Siberian Sea	shelf	5615 * (0)	2	4.0	28.1	0	0.03	0.1
East Siberian Sea	shelf	5613	34	3.1	27.6	0	0.38	6.8
East Siberian Sea	shelf	5612	50	0.5	29.3	0.02	0.14	10.8

3.2. *Picocyanobacteria* Abundance and Role in Total Picophytoplankton

Synechococcus-like PC were found based on epifluorescence microscopy counts at 20 out of 23 studied stations. In the East Siberian Sea, the lowest PC abundance and biomass were detected in the central part of the sea, 0.03 $\times$ 10^6 cells/l and 0.014 µg C/L at stations 5615 and 5604. The PC concentration increased towards estuaries of the Siberian rivers Indigirka and Kolyma with the highest abundance and biomass found at station 5619, 0.98 $\times$ 10^6 cells/L and 0.46 µg C/L (Table 2; Figure 2). PC were absence at stations 5606 and 5607 located near the ice edge.

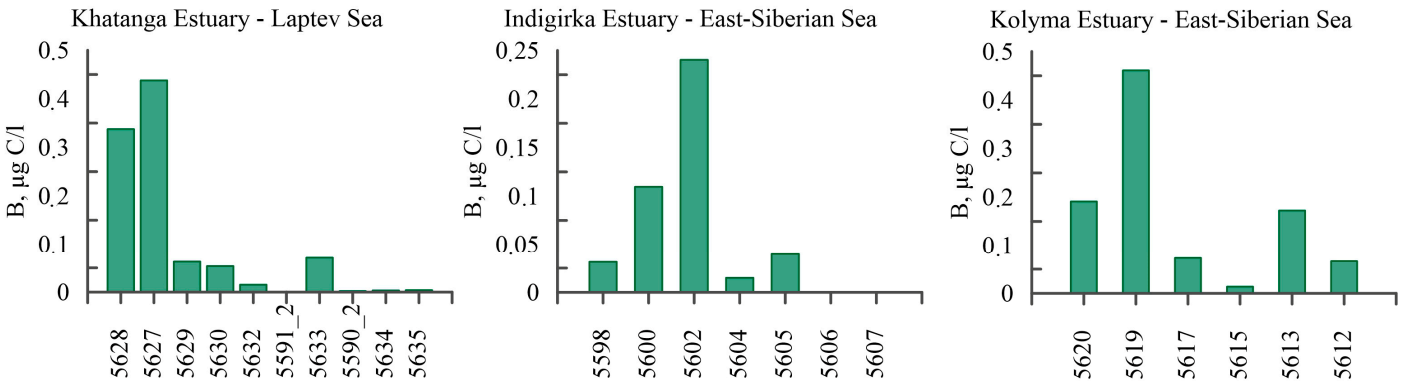

Figure 2. The average PC biomass (B, µg C/L) in the 0–60 m layer at studied estuaries and adjacent areas of the Laptev and east Siberian seas.

The spatial distribution of total picophytoplankton, including PC and picoeukaryotes, was described in detail for the Khatanga Bay and western part of the Laptev Sea in our previous study, but the phylogenetic diversity of PC has not been examined [52]. We consider it necessary to note some important points in the present study. In the Laptev Sea, the highest PC concentration was found in the estuary of the Khatanga River (0.93×10^6 cells/L and 0.43 µg C/L at station 5627) (Table 2, Figure 2). The lowest abundance (0.02×10^6 cells/L) was found at the western part of the Laptev Sea on the shelf and the continental slope (stations 5590_2, 5634 and 5635), where PC were detected only at one of the lower horizons (Table S1). PC were not found at station 5591_2.

All studied estuaries were characterized by high PC concentrations at the surface layer, whereas at the north stations of all transects, abundance was low and PC were found mostly at lower depths (Table 2).

Picocyanobacteria always represented a small percentage of the picophytoplankton cells. The contribution of PC to the total autumn picophytoplankton abundance averaged 6% and 3% in the Khatanga and Indigirka estuaries and reached 5% in the Kolyma estuary.

PC abundance was positively correlated with the water temperature, salinity and $Si(OH)_4$ concentrations and negatively correlated with the Z_{eu} depth (Table S2). There was no significant correlation with the other measured variables.

3.3. PC Molecular Diversity Using ITS and the 16S rRNA Gene

PC diversity in the samples was assessed by sequencing the libraries of ITS rDNA clones. The amplification of total DNA in each sample showed amplicons in only six stations. Samples from 5604, 5607 and 5615 stations did not contain a PCR product.

The ITS length among environmental clones varied widely, from 725 to 954 bp. The length of the alignment was 1564 bp. Phylogenetic analysis revealed eight highly supported clades (bootstap value (BV) = 82–100%), the relationship between which, due to high intergroup variability, remains uncertain (Figure 3, Table S3).

The largest clade A (BV = 100%) included most of the clones from stations 5627, 5620 and 5591_2, which shared 90.6–96.5% identity with uncultured cyanobacterium clone TH320–12-6 from the Baltic Sea (EF513348). It is a single entry from a Genbank BLAST search with an E value $<1 \times 10^{-50}$, relative to newly sequenced phylotypes. This group of clones is a sister to *Synechococcus* RCC 307, represented in the previously described marine subcluster 5.3 and was sufficiently ubiquitous to appear at three stations out of six (Figure 3). Clones from station 5620 near the Kolyma estuary (two clones out of ten are shown on the tree) were included in clade B (BV = 100%) and belonged to marine subcluster 5.1-I *Synechococcus* from the North Sea, California current and uncultured clones from Bering and Yellow Seas (identity >99%). One phylotype (total of three clones) from station 5627 (Khatanga estuary) with high sequence similarity (>98%) to freshwater cyanobacterium previously revealed in Lake Zhaling (China) and Fayetteville Green Lakes (USA) was combined in the maximally supported clade C. Four clones (three out of four are shown on the tree) from stations 5598 and 5620 (Indigirka and Kolyma estuaries) were related to *Synechococcus* sp. from the winter plankton of Chesapeake Bay (FJ547224) and from the Baltic Sea (EF513328), with similarity that did not exceed 89% (clade D, BV = 100%). Clade E with BV = 81% included phylotypes from six stations. A BLAST search did not reveal sequences in Genbank with E values $<1 \times 10^{-45}$ that could be included in this clade. Remaining clones exhibited low sequence similarity (87–94%) to sequences from the Baltic Sea, Atlantic estuaries and freshwater lakes of varying trophic statuses and a wide geographic distribution, including Turkey, China, Italy, the USA and Argentina. The phylogenetic analysis showed that these groups of clones are clustered with different known *Synechococcus* phylotypes related to marine subclusters 5.2 and 5.3. The highest diversity was found at the freshest station 5620 near Kolyma River where representatives of all three subclusters were revealed.

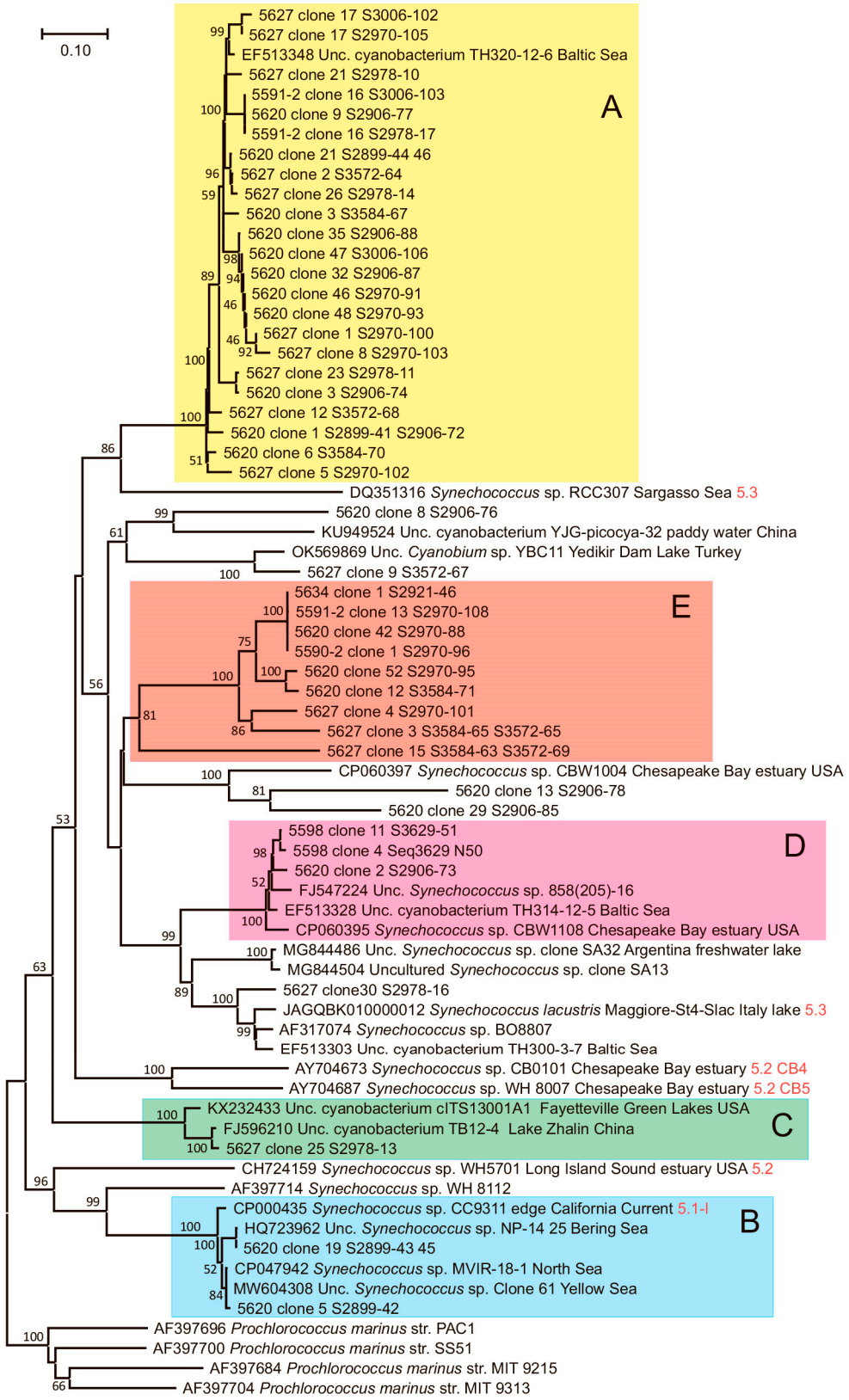

Figure 3. ME Phylogenetic tree of picocyanobacteria, based on ITS sequences. BVs > 50% are indicated. Subcluster numbers are highlighted in red. Unc.—uncultured. Clades A–E are marked by different colors. The scale bar is the number of base substitutions per site.

Partial 16S rRNA gene sequences were obtained for five divergent spacer variants, and their positions on the phylogenetic tree were determined (Figure 4). The length of the alignment was 1114 bp. All 16S rRNA gene sequences were obtained from stations 5627 and 5598 located near Khatanga and Indigirka estuaries, respectively. Phylogenetic analyses of clones from station 5627 revealed picocyanobacteria with varying degrees of relatedness to PCs from various freshwater lakes. The majority of Khatanga estuary cyanobacterial sequences were similar (>99%) to the genus *Synechococcus* from oligo-mesotrophic lakes located in Upper Austria (AY224198) and New Zealand (MZ648797). One clone was identical (>99%) to freshwater *Cyanobium* sp. (AM710378), a genus closely related to *Synechococcus*. Clones from stations 5598 and 5627 were included in a moderately supported clade (BV = 70), which also contained a *Synechococcus* strain isolated from the winter plankton of the Chesapeake Bay estuary, uncultured cyanobacterium clone from Baltic Sea, *Synechococcus* sp. belonging to subcluster 5.2 and three uncultured clones of freshwater origin.

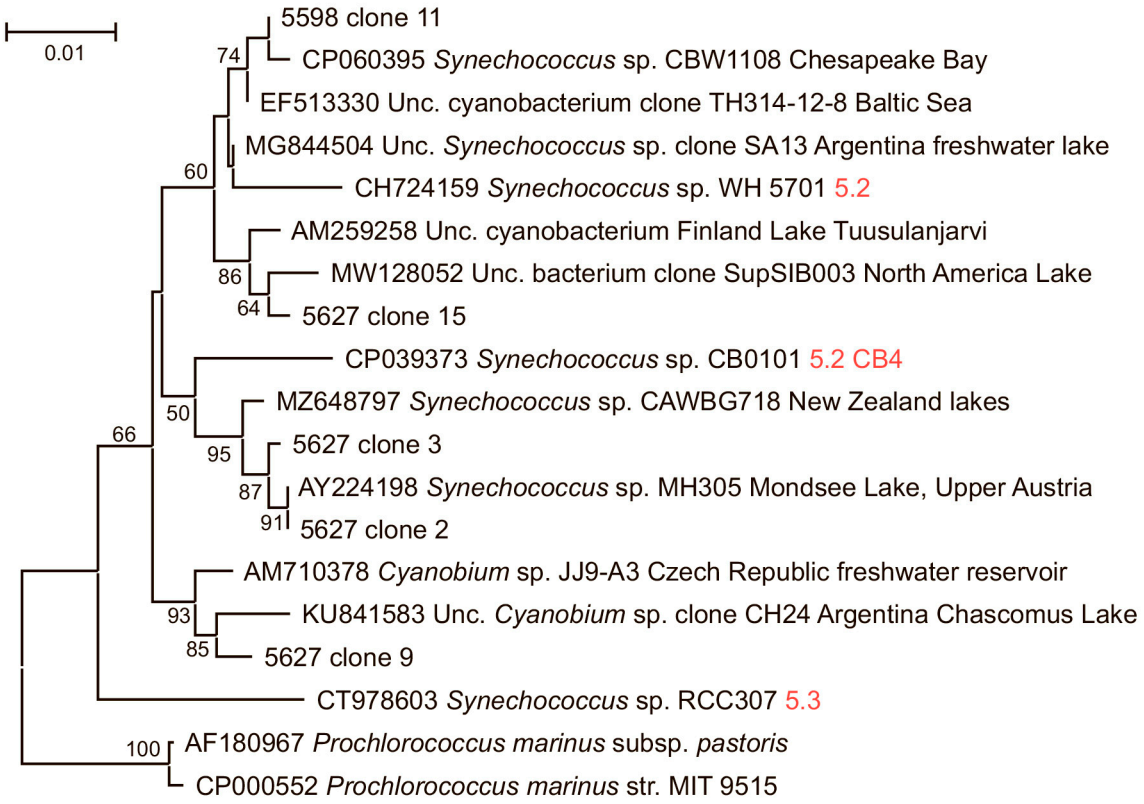

Figure 4. ME phylogenetic tree of picocyanobacteria, based on partial 16S rDNA sequences. BVs > 50% are indicated. Subcluster numbers are highlighted in red; Unc.—uncultured. The scale bar is the number of base substitutions per site.

4. Discussion

Estuaries of the Khatanga, Indigirka and Kolyma rivers and coastal waters of the Laptev and East Siberian seas are characterized by markedly changing environmental conditions from south to north. The decrease in the riverine water impact from the estuaries to the central part of the seas and the respective increases in salinity and decreases in temperature were the main factors that determined latitudinal changes in picocyanobacteria communities. PC abundance and biomass decreased northward in compliance with the decrease in the riverine water impact. Similar results were obtained in the Lena River delta [53], where PC abundance decreased with increasing salinity. Our estimates of PC abundance examined in the surface layers of Khatanga and Kolyma estuaries fall in a range similar to that measured in the estuary of the Mackenzie River in September 2002

(min–max range: 3500–6700 cells/mL) [10] and are an order of magnitude lower than in the Lena River delta (30,000 cells/mL) [53]. The greatest picophytoplankton abundance, with the predominance of *Synechococcus*, was revealed in the surface layer of the Lena River delta at a salinity of 3.17. The authors suggest that the high biomass was related to the supply of nutrients by the Lena River-enriched waters during the summer–autumn period [54]. We did not find any correlations between PC abundance and nutrients, except silicon. A relationship between total picophytoplankton (eukaryotes and cyanobacteria) abundance and the silicon concentration, as well as the absence of a correlation between nitrogen and phosphorus concentrations, and picophytoplankton abundance were revealed on the transect through Baffin Bay, the Northwest Passage and in the Beaufort Sea in the late summer [49]. Brzezinski et al. confirmed the accumulation of significant amounts of silicon by six clones of marine *Synechococcus* representing four clades (5.1.I-VII, 5.3) from a variety of marine habitats [55]. Both cellular Si levels and the rate of Si accumulation in *Synechococcus* increase in direct ratio with silicic acid concentrations in the external environment. The evolutionary, physiological or metabolic roles that Si may play in *Synechococcus* remain unclear.

The cyanobacteria abundance was lower in Indigirka estuary than in the Khatanga and Kolyma estuaries but similar to those reported by Parli et al. [24] in waters around Svalbard (80–830 cells/mL). The low PC abundance is probably due to the smaller volume of river runoff of the Indigirka, in contrast to the other two rivers. Moreover, hydrological and hydrophysical conditions of the East Siberian Sea are very complex, and two areas are identified: a Western area that is influenced strongly by the freshwater flux from the south and the water of Atlantic origin from the north, and an Eastern area that is under the influence of Pacific-derived waters. The average position of the border of these areas is situated roughly near 160 E, but from year to year, the longitude shift between Western and Eastern areas may reach 10 degrees and more [14]. We suppose that the difference in PC abundance between Indigirka (Western area) and Kolyma (Eastern area) transects is related to the origin of the waters at the studied areas.

Unlike the cold-adapted eukaryotic picophytoplankton, which was usually observed in the Arctic in high abundance [49,56–58], PC were considered to be in low abundance or absent in the Arctic Ocean [59]. The absence of PC in the surface layer of the shelf and continental slope of the Laptev Sea and in the northern stations of the Indigirka River transect near the ice edge at a temperature of 0.7–(−1.7 °C) and salinity of 30–31.6 is consistent with previous data obtained for the Chukchi Sea, Makarov basin [60] and Northern Baffin Bay [61].

The data revealed using the molecular approach usually confirmed the results obtained via luminescent microscopy: the PCR product was not obtained at the corresponding horizons of those stations where no PC were detected. It was only at the surface horizon of stations 5591_2 and 5634, where PC were not found via microscopy, that the molecular analysis revealed their presence. The detection of cyanobacteria sequences at these two stations can be caused by the sample collection method. The water for the environmental DNA analysis was collected on 0.2 μm filters that can accumulate not only dissolved live organisms but also the DNA of dead cells bound to particles in the marine sediment [62]. Dissolved extracellular DNA is ubiquitous in all marine environments [63] and can range from a few hundred to several thousand base pairs in length [64]. Thus, the revealed PC sequences may refer to the already dead cells.

The studied estuaries and adjacent areas of Siberian seas contain unique and diverse picocyanobacteria. Phylogenetic analyses of ITS region sequences revealed picocyanobacteria phylotypes related to marine *Synechococcus* subclusters 5.1-I, 5.2 and 5.3. Because only a few phylotypes previously classified as subclusters are represented on the tree, these subclusters seem to be polyphyletic in Figure 3. Only several earlier pre-discovered phylotypes from *Synechococcus* S5.1-I and S5.2 were found. The remaining clones were unique, and their similarity with sequences deposed in Genbank was under 97%. *Synechococcus* S5.1-I, largely confined to coastal and higher latitude regions (above 30°N or below 30°S

approximately) [6], exhibits great tolerance to cold temperatures and prefers high nutrient levels [65]. In our study, representatives of S5.1-I were found only in the Eastern part of the East Siberian Sea near the Kolyma estuary at a temperature of 6 °C, salinity of 19 and relatively low levels of nutrients. The absence of *Synechococcus* S5.1-I in an area near the Kolyma estuary and in the Khatanga estuary is probably related to the lowest DIN concentration and a reduced salinity ~15 in the first area and very low salinity (<4) in the second area. Such environmental conditions limited the distribution of *Synechococcus* S5.1-I. Earlier, *Synechococcus* S5.1-I was detected in two Arctic stations near the Norwegian coastline [6], but it was not detected in the Chukchi Sea [5] and the Beaufort Sea [10].

A total of 24 clones from two stations, 5627 and 5591_2 (Khatanga estuary), and station 5620 (near Kolyma estuary) fell within subcluster 5.3, which is globally abundant [66]. *Synechococcus* S5.3 is mainly present in the surface water layer, and in previous studies, was mostly detected in open-ocean habitats in the northwestern Atlantic and Pacific Ocean and in the Mediterranean Sea [5,6,29,67,68]. The closest known phylotype (<97% identity) was found in the Baltic Sea in an area with a salinity of 5–7 and a temperature >12 °C [69]. In our study, the salinity and temperature varied widely from 3.5 to 22.3 and from 3.6 °C to 6 °C, respectively. This is in contrast with the conclusion of the previous studies showing that 5.3 phylotypes are mainly present in warm, oligotrophic waters [65].

PC making up S5.2 were ubiquitous and found in all studied areas. This subcluster usually appears in the estuarine and freshwater environment [70] and includes both *Synechococcus* and *Cyanobium* [71,72], a genus closely related to *Synechococcus*. *Cyanobium* is known from freshwater and brackish environments [73,74], is composed of clusters that are distinguished by salt tolerance [75] and has similar physiological and ecological characteristics with *Synechococcus*.

The majority of cyanobacterial ITS sequences were unique, and their similarity with early known sequences from GenBank was low. The obtained results unveil the important novel genetic diversity of PC, which seems to be specific to Russian Arctic seas. In the Khatanga transect, several identical clones were revealed at stations 5591_2 and 5634 (Table S3), located on the shelf and continental slope of the Laptev Sea, respectively. Likewise, sequences of another phylotypes were found at stations 5627, 5598 and 5620, located in the inner part of the Khatanga estuary and near the Indigirka and Kolyma rivers, respectively. No one phylotype was found simultaneously in freshened, warm estuarine areas and in cold, saltier areas of adjacent seas.

Luminescent microscopy revealed PC on the shelf and continental slope of the Laptev Sea in layers deeper than 20 m. Samples for DNA analysis at these stations were collected only from surface horizons. We can assume that PC in the cold and salt waters of these areas were represented by autochthonous forms, and the possibility of PC advection with Atlantic waters to the north areas of the Laptev Sea cannot be excluded.

5. Conclusions

Our findings demonstrate that the spatial distribution of picocyanobacteria in the coastal waters of the Russian Arctic seas is influenced by the temperature and silicon concentration. Eurasian river discharge is one of the main factors determining PC abundance and distribution. Although the molecular data in this study are too sparse, we are confident that the waters of Siberian rivers are the main source of genetic biodiversity of the PC communities, considering the variability resulting from the mixing of marine and freshwater. The majority of revealed PC phylotypes were unique, and further research is needed. Global warming promotes cyanobacteria dominance in the Arctic region [76], and picocyanobacteria are one of the major sources of primary production for the upper trophic levels [77].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15101049/s1, Table S1: Sampling sites, date of sampling, coordinates of the station, sampling depth (D, m), concentration of DIN (NO_2+NO_3+NH_4), phosphate (PO_4), silicon (($Si(OH)_4$), total Chlorophyll a (Chl_{tot}), Chlorophyll a of picophytoplankton (Chl_{pico}),

picocyanobacteria abundance ($N_{PC} \times 10^6$ cells/L) at sampling depths and total picophytoplankton abundance in the 0–60 m layer (N, $\times 10^6$ cells/L). "-" no data. Table S2: Spearman correlation coefficients between PC abundance and environmental and biological factors at all stations and depths. * $p < 0.01$, ** $p < 0.001$, ns—not significant; Z_{eu}—euphotic depth. Table S3: The abundance of PC clone library phylotypes revealed at the studied stations of the Khatanga estuary, the Laptev Sea and areas of the East Siberian Sea adjacent to Kolyma and Indigirka river estuaries, and the closest strain/species/phylotype from NCBI matched for each clone.

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