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Microbiomes of Antarctic pearlwort (*Colobanthus quitensis*) of the maritime Antarctic: distinct diversity and core microbes in rhizosphere and endosphere compartments of the plant

Abstract. Plant microbiome plays a crucial role in the plants' performance and fitness to the environment. The latter is especially significant for the plants withstanding the unfavorable conditions of the Antarctic. The study aimed to evaluate the microbiome of Antarctic pearlwort *Colobanthus quitensis* (Kunth) Bartl. growing in the wide range from the South Shetland Islands in the North to Marguerite Bay in the South (63°S – 68°S) in the maritime Antarctic. The composition of *C. quitensis* microbiome (rhizosphere and endophytes of the plant's aerial part) was studied by 16S rRNA amplicon metagenomic sequencing on Illumina Novaseq 6000. The number of operational taxonomic units and diversity indices (Shannon, Simpson, Faith PD) of the endosphere microbiomes were lower ($p < 0.05$) than in the rhizosphere microbiomes, and the ANOSIM test revealed a difference ($R = 0.9$, $p = 0.0001$) in the microbiomes' taxonomic structure. The diversity of the barren's microbiome was lower compared to the rhizospheres'. *Proteobacteria*, *Acidobacteria, Actinobacteria, Bacteroidota, Chloroflexi*, and *Verrucomicrobia* were dominant in the rhizosphere. Similar phyla were found in the barren, yet the ratio of *Actinobacteria* was higher. *Proteobacteria* dominated in the endosphere, followed by *Firmicutes*, *Actinobacteria*, and *Bacteroidota*. *Alphaproteobacteria*, *Actinobacteria*, and *Acidobacteria* represented a large proportion of the core microbiota of *C. quitensis* rhizosphere. The endophyte microbiome's core was mainly composed of *Alphaproteobacteria*, *Gammaproteobacteria*, and *Firmicutes*. On the family taxonomic level, *Rhodobacteraceae*, *Microbacteriaceae*, *Rhizobiaceae*, *Xanthobacteraceae*, *Sphingomonadaceae*, *Comamonadaceae*, *Pseudomonadaceae*, and *Oxalobacteraceae* were determined as the core for rhizosphere and endosphere. The correlation was low ($R = 0.22$, $p = 0.04$) between the rhizosphere microbiome composition and the latitude. Nevertheless, differential abundance of some bacterial taxa in the rhizosphere was attributed to the region of the plant's growth: Northern, Central, or Southern part of the maritime Antarctic. The shift in the composition of microbial communities can be associated with the changing of the climatic conditions southwards along the Western coast of the Antarctic Peninsula.

Keywords: 16S rRNA gene, Antarctic pearlwort, endophytes, rhizosphere

1 Introduction

Plants host diverse microbial communities in the root surroundings, inner part of the tissues, leaf surface, and other compartments. They are considered the plant microbiome (Hardoim et al., 2015). Production of phytohormones and 1-aminocyclopropane-1-carboxylic acid (Glick, 2005; Singh & Jha, 2017), mobilization of insoluble nutrients (Chauhan et al., 2017) and N_2 fixation (Baldani et al., 2014) are known mechanisms of bacterial plants' growth promotion. Plant-associated microorganisms play a crucial function in the plants' fitness to the environment since they affect plant physiology and mitigate the negative effect of stresses (Berendsen et al., 2012). The composition of the plant-associated microbial community is affected by environmental factors (Taketani et al., 2017) and the hosts' metabolic inputs (Williams & de Vries, 2020).

Antarctica is one of the world's most severe environments, with its low temperatures, freeze-thaw cycles, intense UV radiation during the austral season, and desiccation (Convey et al., 2011; 2014). Adverse environmental conditions significantly impact the terrestrial biota in Antarctica, which is well illustrated by its extremely low diversity. In particular, the native vascular plants of Antarctica are presented only by two species: Antarctic hairgrass *Deschampsia antarctica* È. Desv. and Antarctic pearlwort *Colobanthus quitensis* (Kunth) Bartl. These species can thrive in unfavorable conditions, evidenced by their distribution along the Western coast of the Antarctic Peninsula from c. 61°S in the North to 69°S in the South (Komárková et al., 1990; Convey et al., 2011).

The exclusivity of Antarctic flora spurs the interest in these species, including the plant-associated microbiota. The studies of the cultured members of the endo-, phyllo-, and rhizosphere found an abundance of plant growth-promoting bacteria in the microbiome of Antarctic plants. Bacterial strains able to solubilize phosphorus (Barrientos-Díaz et al., 2008; Berríos et al., 2013; Peixoto et al., 2016) or enhance plants' salt stress tolerance (Gallardo-Cerda et al., 2018) were isolated from the rhizosphere of *D. antarctica*. Endophyte *Pseudomonas* sp. promoted tuft growth of *D. antarctica in vitro* (Podolich et al., 2021). Isolates from the phyllosphere of *D. antarctica* showed ice recrystallization inhibition activity (Cid et al., 2017), which contributes to the enhancement of the plant's cold tolerance. Fungal endophytes promoted the growth of *C. quitensis* under water deficiency (Hereme et al., 2020) and UVB (Ramos et al., 2018).

Besides the culture-based techniques, high-throughput DNA sequencing was used to study the composition, diversity, and predicted functions of the Antarctic plants' microbiomes (Teixeira et al., 2010; Molina-Montenegro et al., 2019; Zhang et al., 2020; Znój et al., 2022; Prekrasna et al., 2022). Teixeira et al. (2010) revealed similar patterns of bacterial diversity between *D. antarctica* and *C. quitensis*. On the contrary, Molina-Montenegro et al. (2019) showed a notable difference. The difference in the rhizosphere microbiomes of *D. antarctica* and *C. quitensis* growing on Galindez Island was shown by Prekrasna et al. (2022). However, this variability is likely connected to the microclimatic conditions of the sites.

The data on the microbiomes of Antarctic vascular plants are few, with the hairgrass studied more frequently than the pearlwort. The latter is much less abundant in the maritime Antarctic, often growing in hardly accessible sites, complicating the research. Moreover, the data come from a limited geographic range. Most of the available studies were conducted on the northernmost part of the Antarctic Peninsula (South Shetland Islands – 61°S), while the range of Antarctic plants expands more southwardly. The microbiome of *C. quitensis* inhabiting Galindez Island in the central part of maritime Antarctica was studied by Prekrasna et al. (2022). However, the research involved only one location of this plant, mainly focusing on *D. antarctica*. Habitat conditions tend to grow ever harsher with latitude in Antarctica and can affect the plants' performance and their microbiome composition. For these reasons, our study aims to evaluate the microbiome (rhizosphere and endophytes) of *C. quitensis* growing in a wide range from the Shetland Islands in the north to Marguerite Bay in the south (61°S – 68°S) in the maritime Antarctic.The study's objectives were as follows: i) estimate the diversity and taxonomic composition of endophyte and

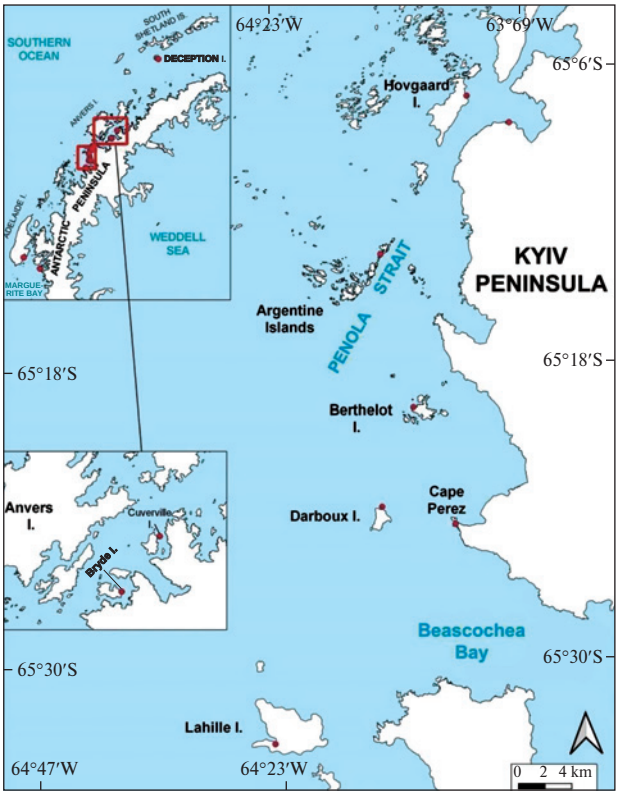


Figure 1. Location of the *Colobanthus quitensis* sampling sites

rhizosphere microbiomes of the Antarctic pearlwort; ii) estimate the core microbiota; iii) evaluate the latitudinal variation of the pearlworts' microbiome. In Section 2, methods applied in the study are briefly described. Main results on diversity and taxonomic composition of pearlwort-associated microbiota are considered in Section 3. A discussion is given in Section 4 and Section 5 provides a summary and conclusions.

2 Materials and methods

2.1 Sampling

Rhizosphere soil (11 samples) and plants' stems and leaves (5 samples) were collected along the Western coast of the Antarctic Peninsula in 2020 (Fig. 1, Table 1).

Non-rhizosphere soil is often used for comparison with the rhizosphere microbiome. Islands and coastlines suitable for the plants' colonization on the Antarctic Peninsula are composed of rocks and can be covered with ice/snow. Soil is rare and found sporadically in rock cracks and depressions, and usually associated with vegetation (vascular plants or bryophytes). To compare the rhizosphere soil microbiome, we sampled a weathering rock substrate recently exposed after the glacier had retreated on the Moot Point. The recent retreat of glacier on this spot occured, and new vegetation sites presented mainly by lichens, bryophytes and hairgrass began to appear on weathering rock substrates. For this reason, the bare weathering rock substrates were assumed as non-rhizosphere substrates before the plants' colonization. Substrate (1 sample) that was not colonized by vegetation was collected aseptically after removal of the top layer into sterile 15 ml tubes and stored at –80 °C.

For the rhizosphere analysis, only the small (1–2 mm) soil particles attached to the roots were aseptically collected, while the rest of the soil was discarded. Rhizosphere soil was collected in sterile 15 ml tubes and stored at –80 °C. Samples of soil and substrate were transported on dry ice (–30 °C).

Plant material (leaves and stems of alive plants) was collected aseptically with forceps and scissors into sterile 50 ml tubes and transported to the Ukrainian Antarctic Akademik Vernadsky station (hereinafter – Vernadsky station). Plant samples were surface-sterilized within 24–48 hours after collection, and DNA was extracted immediately in the laboratory.

Plants and rhizosphere samples were collected from 26.01.2020 to 08.03.2020. Plants were in the similar growth stage.

2.2 Surface sterilization of plants

Surface sterilization of plants was performed according to Barra et al. (2016) with modifications (Prekrasna et al., 2021). In brief, plants were successively washed in sterile distilled H_2O, ethanol (70%), and sodium hypochlorite (5.6%). Sterilized plant material was finally washed in sterile distilled H_2O, and this water (0.1 ml) was inoculated on CASO medium (Merck) for sterility control.

2.3 DNA extraction

DNeasy PowerSoil and DNeasy Plant mini (Qiagen, Germany) were used for DNA extraction from soil

and plants, respectively. Extraction was performed in two replicates for each rhizosphere sample, and for the plant material, it was performed in one replicate due to material scarcity. DNA concentration and the 260/280 ratio were determined on the Denovix DS-11 FX (Denovix, USA) spectrophotometer. Only samples with a sufficient concentration of DNA were included (the DNA concentration ranged from 11.5 to 90.9 ng/μl, and the ratio was ~1.8).

2.4 PCR and DNA sequencing

The V3-V4 sites of the 16S rRNA gene were amplified using 515F-806R barcode universal primers (Walters et al., 2016). All PCR reactions were performed using Phusion High-Fidelity PCR Master Mix (New England Biolabs). Thermal cycling was started at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s, and final elongation within 72 °C for 5 min. PCR products were tested in a 2% agarose gel. Samples with an available PCR product length of 400–450 bp were selected for further analysis. PCR products (repeated PCRs for one sample) were mixed in an equal ratio and purified using Qiagen Gel Extraction Kit (Qiagen, Germany).

Libraries for sequencing were prepared using the NEBNext Ultra DNA Library Pre Kit for Illumina according to the manufacturer's instructions. Library quality was determined using a Qubit 2.0 fluorometer (Thermo Scientific, USA) and an Agilent Bioanalyzer 2100 system. Libraries were infiltrated on the Illumina platform Novaseq, and 250 bp reads were generated.

Sequences were uploaded to the National Center for Biotechnology Information (NCBI) database under accession number PRJNA876844.

2.5 Bioinformatics and statistical analysis

The resulting nucleotide sequences were sorted into individual samples according to barcode sequences. The barcode and primer sequences were then removed.

Table 1. Description and coordinates of the sampling locations

Sample	Location	Type of sample	Latitude	Longitude	Region of sampling
R.C.1	Deception Island	Rhizosphere	–62.982010	–60.520370	North
R.C.2	Hovgaard Island	Rhizosphere	–65.120110	–64.067830	Central
R.C.4	Bryde Island	Rhizosphere	–64.875540	–62.927760	North
R.C.5	Girard Bay	Rhizosphere	–65.138450	–64.001100	Central
R.C.6	Irizar Island	Rhizosphere	–65.219067	–64.200167	Central
R.C.7	Darboux Island	Rhizosphere	–65.395217	–64.214917	Central
R.C.8	Cape Pérez	Rhizosphere	–65.407730	–64.097250	Central
R.C.9	Léonie Island	Rhizosphere	–67.593330	–68.338360	South
R.C.10	Deception Island	Rhizosphere	–62.982010	–60.520370	North
R.C.12	Eight Island	Rhizosphere	–65.225717	–64.209817	Central
R.C.13	Lagotellerie Island	Rhizosphere	–67.884860	–67.387650	South
S.6.1	Lahille Island	Aerial part of the plant	–65.553580	–64.394883	Central
S.11.1	Deception Island	Aerial part of the plant	–62.982120	–60.520600	North
S.14.1	Ukraine Island (the biggest of the Berthelot Islands)	Aerial part of the plant	–65.329040	–64.161650	Central
S.19.1	Cape Pérez	Aerial part of the plant	–65.407730	–64.097250	Central
S.20.1	Darboux Island	Aerial part of the plant	–65.395217	–64.214917	Central
R.K.5	Moot Point	Bare substrate	–65.203826	–64.075375	Central

Paired ends were pooled in the FLASH V1.2.7 program. Reads' quality assessment and removal of poor-quality data were done in the QIIME V1.7.0 program. The filtered data were compared with the Gold database using the UCHIME algorithm and the detection and removal of quirky reads. The filtered data were clustered into operational taxonomic units (OTUs) at 97% similarity in Uparse v7.0.1001. Taxonomic annotation was done for a representative sequence of each OTU by comparison with the GreenGene database using Ribosomal Database Project (RDP) classifier. OTU representative sequences were classified taxonomically by a QIIME-based wrapper of the RDP (Version 2.2) (Wang et al., 2007) naive Bayesian classifier retrained on the Greengenes 16S rRNA gene database (DeSantis et al., 2006).

The obtained data was analyzed in QIIME V1.7.0 and RStudio (version 4.0.4, packages: *vegan*, *microbiome*, *phyloseq*, *Complex Heatmap*, *geosphere*, *ggplot2, edgeR*). Alpha diversity indices (the number of OTUs, Shannon index, Simpson index and Faith PD) were determined in QIIME V1.7.0. OTUs number and diversity indices were compared between the groups (endosphere and rhizosphere microbiomes) with the *t-test* (p-value < 0.05).

ANOSIM test with Bray–Curtis dissimilarity matrix and 9999 permutations were performed in the *vegan* package to test for the differences in taxonomic composition in endosphere and rhizosphere microbial communities, which was visualized by principal coordinate analysis. Rhizosphere and endosphere microbiomes' parameters (diversity indices, Bray-Curtis distances) were compared by the Wilcoxon rank sum test (p-value < 0.05).

The core microbiota of rhizosphere microbial communities was estimated in *phyloseq* and *microbiome* packages with the threshold detection = 0.0002 of the OTU ratio and threshold occurrence = 80%. Core microbiota of endosphere microbial communities with the threshold detection = 0.00001 of the OTU ratio and threshold occurrence = 80%, and OTU belonging to chloroplast and mitochondria were filtered out afterward. The core microbiome of the bare substrate was defined with the threshold detection = 0.0002 of the OTU ratio.

A general linear model with a negative binomial distribution (*edgeR* package) was used to identify whether bacterial families are significantly associated with rhizosphere or endosphere. P-values were corrected to account for multiple comparisons using the false discovery rate (q-value) method (Benjamini & Hochberg, 1995). Results with a q-value < 0.05 were considered to be significant.

A Mantel test with 9999 permutations was performed to infer whether the variance in taxa abundance is explained by geographical distance, using the abundance matrix (based on Bray–Curtis measure) and the geographical distance matrix (based on Haversine distance). ANOSIM test with Bray–Curtis dissimilarity matrix and 9999 permutations were performed to test for the differences in the rhizosphere microbime composition according to the region of sampling: North (61°S), Central (65°S) and South (68°S). A general linear model with a negative binomial distribution was used to identify the differentially abundant taxa within these three groups.

3 Results

3.1 Sequencing output and diversity of the community

Illumina Novaseq 6000 high-throughput sequencing was used to analyze the diversity and composition of prokaryotic communities inhabiting the rhizosphere

Table 2. Estimated OTU richness and diversity indices for 16S rRNA libraries of rhizosphere, endophyte microbial communities of *Colobanthus quitensis*, and microbial community of bare substrate from the maritime Antarctic

Microbiome	Reads number	OTU number	Shannon	Simpson	Faith PD
Rhizosphere	143.437 ± 11.052	3061 ± 451	8.8 ± 0.6	1.0 ± 0.008	302.1 ± 49
Endosphere	127.480 ± 28.005	444 ± 116	2.1 ± 0.3	0.5 ± 0.05	65.5 ± 14
Bare substrate	17.539	2143	8.1	0.9	215

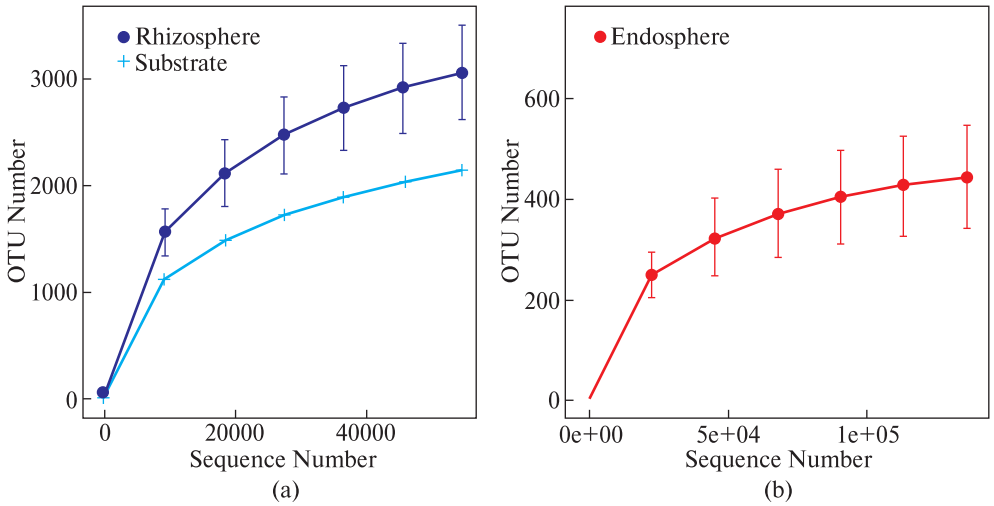


Figure 2. Rarefaction curves of partial sequences of bacterial 16S rRNA genes from the rhizosphere of *Colobanthus quitensis* and bare substrate (a) and endophytes of *Colobanthus quitensis* (b) from the maritime Antarctic

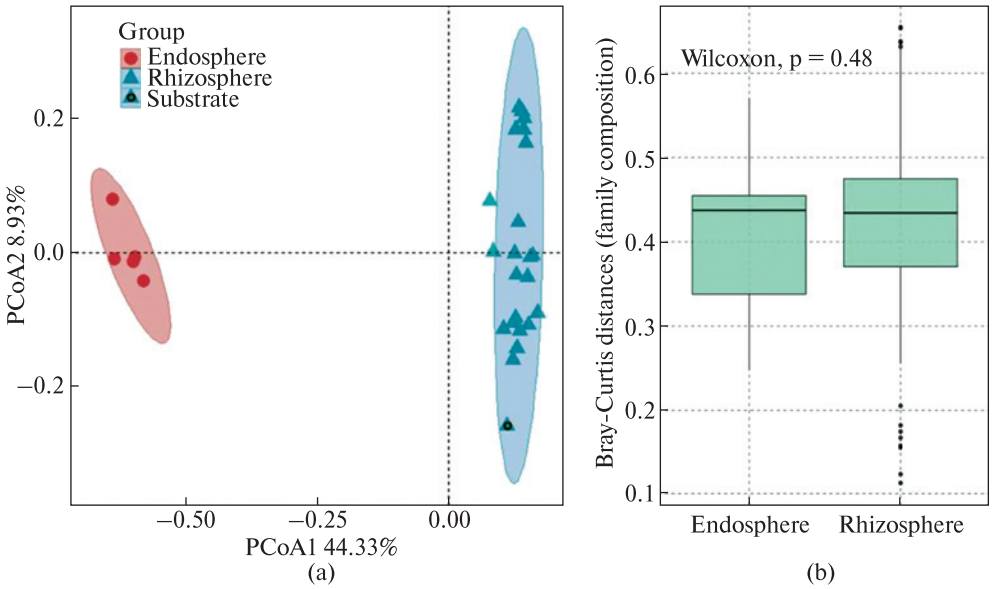


Figure 3. Principal coordinate analysis based on the Bray-Curtis distance matrix (a) and Bray-Curtis distances variations within the endosphere and rhizosphere microbial communities (b) of *Colobanthus quitensis* microbial community from the maritime Antarctic

and endosphere of *C. quitensis*. The number of partial 16S rRNA gene reads are presented in Table 2. The average length of the read was 415 bp. Rarefaction curves outlined from the samples indicated that deeper sequencing would not have resulted in significantly higher estimates in rhizospheres', substrate's and endospheres' microbiomes (Fig. 2).

The number of OTUs in the microbial community of the endosphere was significantly lower than in the rhizosphere (Table 2), as confirmed by the t-test (p-

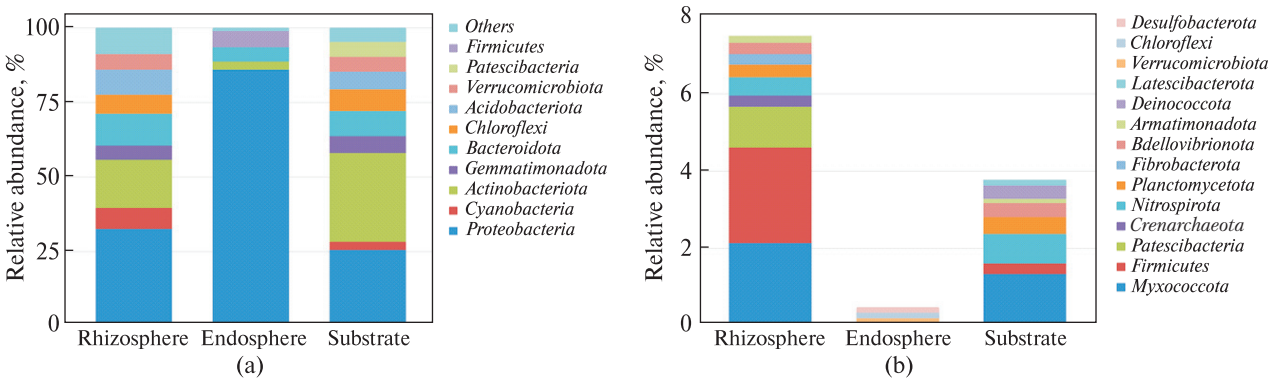


Figure 4. Major (a) and minor (b) bacterial phyla in the microbial communities of *Colobanthus quitensis* rhizosphere, endosphere, and the sample without vegetation (substrate). The threshold for major phyla was 2.5%, phyla numbering from ≥0.1% to ≤2.5% were considered minor. "Others" in Figure 4a refers to minor and unidentified phyla

value = 2.2×10^{-16}). Predictably, Shannon (p-value = $= 5.5 \times 10^{-12}$) and Simpson (p-value = 2.2×10^{-16}) indices, and phylogenetic diversity index Faith PD (p-value = 2.1×10^{-15}) were significantly higher in the rhizosphere microbial communities.

ANOSIM test based on the Bray–Curtis distance matrix revealed a significant difference (R = 0.993, p = = 0.0001) in the taxonomic structure of microbial communities inhabiting the rhizosphere and endosphere of *C. quitensis,* which is illustrated by the principal coordinate analysis (Fig. 3).

3.2 Taxonomic composition and core microbiome of rhizosphere and endosphere microbial communities of *Colobanthus quitensis*

Among the phyla detected, ten accounted for about 85% of the rhizosphere microbial communities. *Proteobacteria* (32.6 ± 9.1%), *Actinobacteriota* (16.2 ± ± 5.8%), *Bacteroidota* (10.6 ± 2.8%), *Acidobacteriota* (8.3 ± 3.3%), *Cyanobacteria* (6.9 ± 2.9%), *Chloroflexi* (6.5 ± 2.9%) and *Verrucomicrobia* (5.1 ± 3.1%) and *Gemmatimonadota* (4.6 ± 2.2%) were dominant phyla in the rhizosphere of *C. quitensis* (Fig. 4). *Gammaproteobacteria* (11.1 ± 10.8%) and *Alphaproteobacteria* (13.4 ± 4.3%) were the dominant classes within the phylum of *Proteobacteria*. *Myxococcota*, *Firmicutes*, *Patescibacteria* were among minor (0.1–2.5%) representatives of microbial communities (Fig. 4). *Crenarchaeota* (0.3 ± 0.05%) was found among the minority of the microbial communities as well. Moreover, 23 phyla accounting for <0.1% of the communities were found in the rhizosphere of *C. quitensis*. About 0.7% of the microbial communities remained unassigned to the phylum level.

The microbial community of the bare substrate was composed of similar phyla as in the rhizosphere, yet their proportion was different. The proportion of *Proteobacteria* (25.3%), *Bacteroidota* (8.5%), *Acidobacteriota* (5.9%), *Cyanobacteria* (2.8%), and *Verrucomicrobiota* (4.8%) in the community was lower than in the rhizosphere microbiome. On the other hand, the ratio of *Actinobacteriota* (29.7%) and *Chloroflexi* (7.2%) was higher in the bare substrate than in the rhizosphere.

The taxonomic composition of the endosphere microbial community significantly differed compared to the rhizosphere. *Proteobacteria* was a dominant phylum amounting to 85.9 ± 5.8%. *Actinobacteriota* (2.6 ± 1.3%), *Bacteroidota* (4.7 ± 3.4%), *Firmicutes* (5.4 ± 3.1%) were also found. An archaeal phylum, *Nanoarchaeota*, was also detected. About 0.3% of the endosphere microbial communities remained unidentified.

OTUs were assigned to 263 families in the endosphere microbiomes and 465 families in the rhizosphere microbiomes. 260 families were identified in the bare substrate's microbial community. The percentage of the microbial communities identified at the family level comprised 96.5–98.8%, 79.6–96.0%, and 92.0% of each group, respectively.

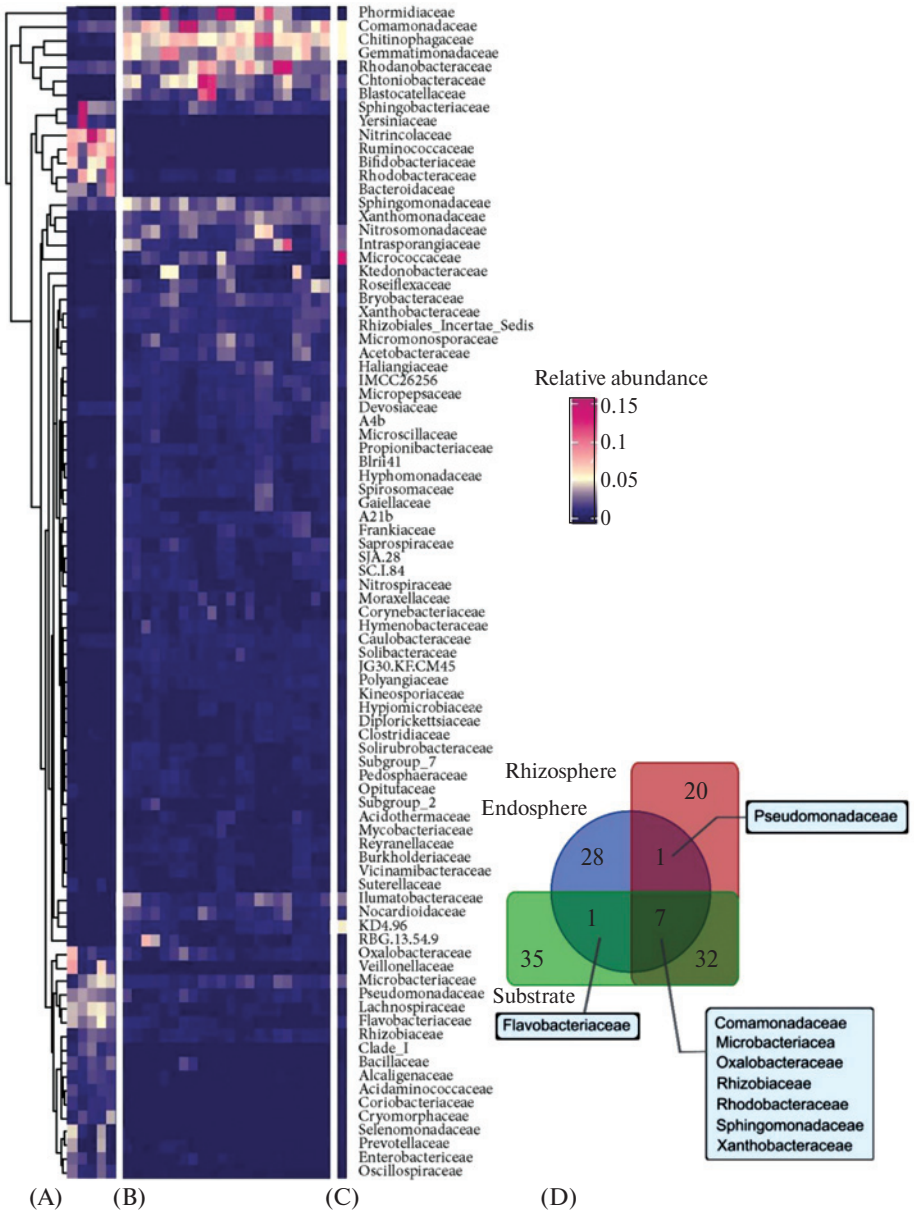
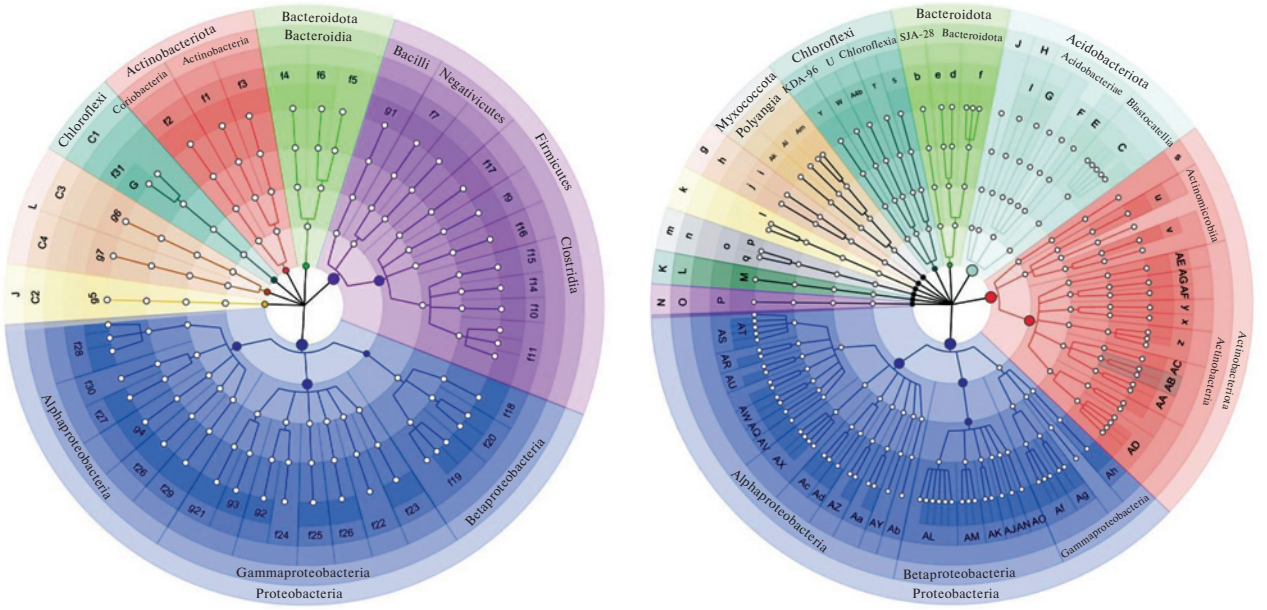


Figure 5. Relative abundance of the most distributed families (threshold = 0.02%) in the endosphere (A), rhizosphere (B) of *Colobanthus quitensis* and bare substrate (C); (D) – Shared families belonging to the endosphere, rhizosphere, and bare substrate core microbiome

General linear analysis with a negative binomial distribution revealed that 272 families had significantly different abundance (q-value <0.05) in *C. quitensis*' rhizosphere and endosphere. The list of the families with the corresponding p- and q-values is presented in Appendix Table 1.

Thirty families with an average abundance of 1–9% comprised 76.3–89.0% of the microbiome inhabiting the internal part of *C. quitensis's* leaves and stems (Fig. 5). The minor families found in the endosphere microbiomes constituted not more than a fourth (9–22%) of the communities. The 66 unique endo-



C1:Anaerolineae
C2:Defferrisomatia
C3:Desulfovibrionia
C4:Desulfomonadotia
G:Anaerolinea
J:Deferrisomatota
L:Desulfobacterota
f10:Oscillospiraceae
f11:Ruminococcaceae
f14:Butyricicoccaceae
f15:Eubacterium_copro-
stanoligenes_group
f16:Monoglobaceae
f17:Peptostreptococcaceae
f18:Alcaligenaceae
f19:Comamonadaceae
f1:Bifidobacteriaceae
f20:Oxalobacteraceae
f21:Enterobacteriaceae
f22:Nitrincolaceae
f23:Porticoccaceae
f24:Moraxellaceae
f25:Thioglobaceae
f26:Clade
f26:SAR86_clade
f27:Rhizobiaceae
f28:Rhodobacteraceae
f29:SAR116_clade
f2:Coriobacteriaceae
f30:Xanthobacteraceae
f31:Anaerolineaceae
f3:Microbacteriaceae
f4:Bacteroidaceae
f5:Cryomorphaceae
f6:Flavobacteriaceae
f7:Acidaminococcaceae
f8:Veillonellaceae
f9:Lachnospiraceae
g1:Bacillus
g2:Pseudomonas
g3:Serratia
g4:Sphingomonas
g5:Deferrisoma
g6:Bilophila
g7:Geobacter

(a)

AA:Intrasporangiaceae
AB:Micrococcaceae
AC:Microbacteriaceae
AD:Nocardioidaceae
AE:Kineosporiaceae
AF:Nocardiaceae
AG:Mycobacteriaceae
AJ:A21b
AK:Oxalobacteraceae
AL:Comamonadaceae
AM:Nitrosomonadaceae
AN:Burkholderiaceae
AO:TRA3-20
AQ:A0839
AR:Devosiaceae
AS:Xanthobacteraceae
AT:Rhodopseudomonas
AU:Rhizobiaceae
AV:Rhodomicrobiaceae
AW:Hyphomicrobiaceae
AX:Sphingomonadaceae
AY:Rhodobacteraceae
AZ:Acetobacteraceae
Aa:Reyranellaceae
Ab:Micropepsaceae
Ac:Caulobacteraceae
Ad:Hyphomonadaceae
Af:Xanthomonadaceae
Ag:Rhodanobacteraceae
Ah:Pseudomonadaceae
Ak:Haliangiaceae
Al:Blrii41
Am:Polyangiaceae
C:Blastocatellaceae
E:Solibacteraceae
F:Bryobacteraceae
G:Subgroup_2
H:Holophagae
I:Subgroup_7
J:Vicinamibacteria
K:Nitrospirota
L:Nitrospiria
M:Nitrospira
N:Firmicutes
O:Clostridia
P:Clostridiaceae
S:JG30-KF-CM45
T:Roseiflexaceae
U:Anaerolineae
W:RBG-13-54-9
Y:KD4-96
b:SJA-28
d:Spirosomaceae
e:Microscillaceae
f:Chitinophagaceae
g:Cyanobacteria
h:Cyanobacteriia
i:Coleofasciculaceae
j:Phormidiaceae
k:Gemmatimonadota
l:Gemmatimonas
m:Verrucomicrobiota
n:Verrucomicrobiae
o:Chthoniobacteraceae
p:Candidatus_Udaeo-
bacter
q:Chthoniobacter
s:Thermoleophilia
u:IMCC26256
v:Ilumatobacteraceae
x:Nakamurellaceae
y:Frankiaceae
z:Micromonosporaceae

(b)

Figure 6. The core microbiome of the endosphere (a) and rhizosphere (b) microbial communities of *Colobanthus quitensis*

phyte families (found only in one group of samples in the study) are shown in Appendix Figure and Appendix Table 1. *Nitrincolaceae* (9.0 ± 1.7%), *Clade I* (1.5 ± 0.4%), and *Coriobacteriaceae* (1.0 ± 0.2%) had the highest shares in the communities, while other unique taxa occurred in low quantities.

The 25 families with an average share >1.0% (up to 6.4%) in the rhizosphere microbiome can be considered as major (Fig. 5). The major families comprised 45.4–72.0% of the rhizosphere communities in total. About 30% of the communities were complemented by minor families, accounting for 0.2–0.9% on average. Rhizosphere microbiomes hosted 193 families that were unique compared to endosphere and substrate microbiomes (Appendix Figure).

Core microbiome analysis defined 201, 64, and 76 OTUs as core in rhizosphere, endophyte, and bare substrate microbial communities, respectively. On the class level, the core microbiome of the rhizosphere communities of *C. quitensis* was presented mainly by *Alphaproteobacteria*, *Actinobacteria*, and *Acidobacteria* (Fig. 6). Mainly members of *Alphaproteobacteria*, *Gammaproteobacteria*, and *Firmicutes* composed the core of endosphere microbiome of *C. quitensis* (Fig. 6). A comparison of the core microorganisms on the family level between groups revealed that the majori-

ty of them did not overlap (Fig. 5B). A narrow list of the shared core families is presented in Figure 5b. Nevertheless, all of them, besides *Oxalobacteraceae* (q = = 0.94) and *Rhodobacteraceae* (q = 0.4), had differential abundance in the groups of samples. *Pseudomonadaceae* ($q = 7.8 \times 10^{-7}$) was the core family for both endosphere and rhizosphere microbial communities (Fig. 6).

The 20 families were defined as core microbes only in the rhizosphere microbial communities (Fig. 5B). Among them, only *Subgroup 2*, RBG-13-54-9, SJA-28 were unique for the rhizosphere microbiomes of *C. quitensis*. The other families unique for pearlwort's rhizosphere, such as *Saprospiraceae* (0.6 ± 0.11%), *Fibrobacteracea* (0.3 ± 0.09%), *Corynebacteriaceae* (0.4 ± ± 0.12), *Pedospearaceae* (0.5 ± 0.06%) etc. (Appendix Table 1) were not evenly distributed in the samples.

The 28 families formed the core specification of the *C. quitensis'* endophyte microbial communities (Fig. 5B). Vast portions of these families were found in the rhizosphere microbial communities as well (Appendix Table 1), which may indicate the source of these bacteria in the internal parts of the plant. However, these families had a significantly higher ratio in the endosphere microbiomes (q-value < 0.05). *Nitrincolaceae*, *Coriobacteriaceae*, SAR86_clade, SAR116_ clade, *Porticoccaceae*, *Defferrisomataceae*, *Thioglobaceae*, and *Clade_I* were found only in the endosphere communities.

3.3 Association of variance in diversity and taxa abundance with the geographical distance

Samples of pearlworts' rhizosphere were collected along the Western coast of the Antarctic Peninsula (the maritime Antarctic) from 62.9°S to 68.3°S. That corresponds to the latitudinal range of about 630 km.

The Mantel test did not reveal the correlation between diversity indices (Shannon, Faith PD), family abundance in the rhizosphere microbiomes of *C. quitensis*, and latitudinal extent. On the other hand, ANOSIM statistics based on the Bray–Curtis measure revealed a low correlation (R = 0.22, p-value = = 0.04) between the composition of the rhizosphere microbial communities and the region of sampling (North, Central, South). General linear analysis with a negative binomial distribution revealed that 90 families had differential abundance depending on the region of sampling (Appendix Table 2). Most of these families had higher relative abundance in the rhizosphere of the plants sampled in the Northern region and, in total, comprised ~15% of the rhizosphere communities sampled in the North in this region (Fig. 7a). Families that were more numerous in the Southern region amounted to ~4% of the Southern communities in total, which is higher compared to their amount (>1%) in the communities sampled on the Northern sites (Fig. 7b, c).

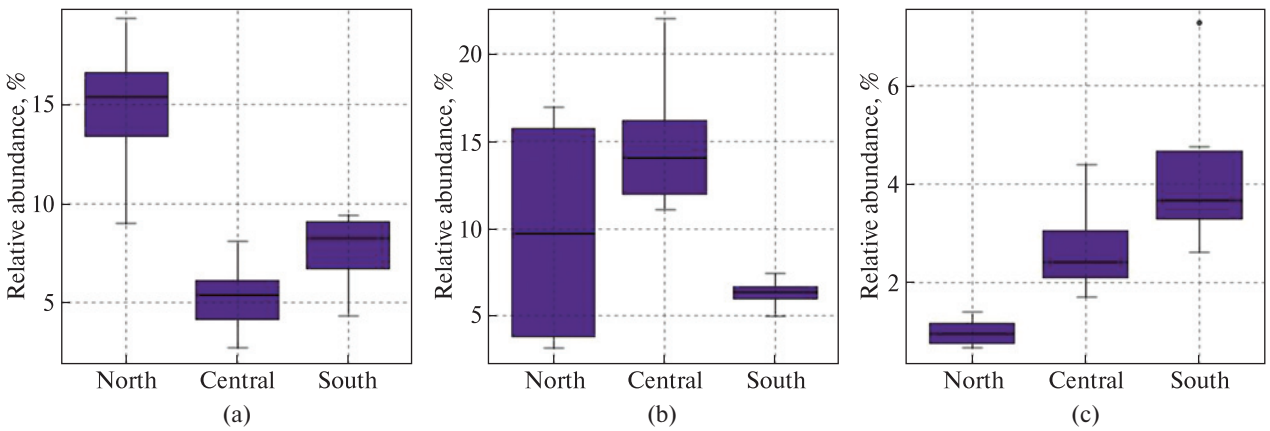


Figure 7. Total amount (%) of three groups of bacterial families that were abundant in the rhizosphere of *Colobanthus quitensis*, but were significantly higher in number in the Northern (a), Central (b), and Southern (c) parts of the Western coast of the Antarctic Peninsula

4 Discussion

Microorganisms associated with plants can improve fitness in harsh environmental conditions. The present study focuses on the diversity and composition of the microbial communities inhabiting the rhizosphere and the endosphere of *C. quitensis* growing along the Western coast of the Antarctic Peninsula.

The OTU number and diversity indices had significantly higher estimates in the rhizosphere of Antarctic pearlwort compared to the endosphere. Shannon, Simpson, and Faith PD indices indicated that the rhizosphere microbiome of *C. quitensis* had high diversity without a pronounced dominant component and was composed of phylogenetically diverse microorganisms. On the contrary, endosphere microbial communities had notably lower diversity and had dominant representatives, and a considerable part of the communities were presented by phylogenetically close bacteria. Our results are consistent with previous findings shown by Zhang et al. (2020) for *C. quitensis* or by Sarria-Guzmán et al. (2016) for *Anthurium andraeanum* Linden ex André. Rhizosphere enhances microbial diversity due to the production of additional nutrients (Li et al., 2021). On the contrary, the endosphere is considered a specific niche restricted by plant-bacteria interactions. In particular, plants exploit defence mechanisms (i.e., induced systemic resistance) to reduce the entrance of microorganisms into the internal part of the plant (Romera et al., 2019). Moreover, we consider that microbiome of the aerial part of *C. quitensis* faces more intense adverse factors of the Antarctic environment compared to the relatively protected rhizosphere microbiome or even root endophytes.

Based on our results, *Proteobacteria* were dominant among the endophytes of *C. quitensis*, which is typical for this niche (Hardoim et al., 2015; Santoyo et al., 2016; Zhang et al., 2019; 2020), and had high estimates of *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*. Members of *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, *Acidobacteriota*, *Cyanobacteria, Chloroflexi*, and *Verrucomicrobia* were the most abundant in the rhizosphere of *C. quitensis*, which is in line with the previous studies of this plant species from the South Shetland Islands (Molina-Montenegro et al., 2019; Zhang et al., 2020; Znój et al., 2022). On the contrary, the rhizosphere microbiome of *C. quitensis* growing on Galindez Island was outnumbered by *Actinobacteria*, which was connected to the lower temperature of the vegetation site (Prekrasna et al., 2022). Similar bacterial phyla were abundant in the rhizosphere of the other Antarctic vascular plant *D. antarctica* inhabiting the Northern (Zhang et al., 2020; Znój et al., 2022) and Central parts of the maritime Antarctic (Prekrasna et al., 2022). Rhizosphere microbiomes of other non-Antarctic plants are composed of similar bacterial phyla (e.g., Sarria-Guzmán et al., 2016; Zuo et al., 2022), and they are usually found in a wide variety of soils (Yergeau et al., 2007; Delgado-Baquerizo et al., 2018). The ratio of *Actinobacteriota* and *Chloroflexi* was lower in the rhizosphere compared to the bare substrate, while other major phyla had higher numbers in the rhizosphere. The rhizosphere is generally enriched in low-weight and high-weight carbon nutrients, so a decrease in the oligotrophic bacteria compared to the bare substrate was expected. *Actinobacteria* are typically associated with higher-carbon content soils (Fierer et al., 2007). However, its members are also found in oligotrophic environments (Arocha-Garza et al., 2017) and can be enriched in such environments as bare substrate. Moreover, *Actinobacteria* are typically highly abundant in cold desert soils (Yergeau et al., 2012; Staebe et al., 2019).

Members of *Chitinophagaceae*, *Gemmatimonadaceae*, *Comamonadaceae* families represented the majority of the microbial communities found in the rhizosphere and bare substrate. These bacteria belong to phyla typically found in soil (Delgado-Baquerizo et al., 2018) can degrade macromolecules (Chung et al., 2012). The dominance of these families in the rhizosphere of *C. quitensis* and *D. antarctica* was shown earlier for plants growing on King George Island (Zhang et al., 2020), Galindez, and Anvers Islands (Prekrasna et al., 2022). A number of minor families that did not occur in the barren substrate were found in the rhizosphere of *C. quitensis*. We suggest that *C. quitensis*'s root system affects the microbiome of the initial substrate by enriching the minor taxa that constitute about 30% of the community. Additional sources of

nutrients, such as root exudates and rhizodeposition, positively affect the diversity of the microbial assembly in the close vicinity to the roots (Haichar et al., 2008; Li et al., 2021). The difference in the composition of the rhizosphere and bare substrate microbiomes underlines the plants' effect on the development of the terrestrial microbiomes in the Antarctic.

Much fewer bacterial families succeeded to proliferate in the endosphere of the *C. quitensis'* leaves and stems compared to the rhizosphere, consistent with other studies on the comparison of plants' microbiomes from different compartments (Sarria-Guzmán et al., 2016; Shi et al., 2021). The endosphere's niche cohesion to the plant's rhizosphere is inevitable since bacteria get into the plant through root hairs (Hardoim et al., 2015). Several families were expectedly found in both the rhizosphere and endosphere of *C. quitensis*. Relative abundance of some families like *Pseudomonadaceae, Rhodobacteriaceae, Flavobacteriaceae,* which are often found among the endophytes (Bredow et al., 2015; Sarria-Guzmán et al., 2016; Tamošiūnė et al., 2018; Tarquinio et al., 2021; Yue et al., 2022), was higher inside the pearlwort. Similarly, *Pseudomonadaceae* was more numerous in the endosphere of the pearlwort compared to the rhizosphere according to Zhang et al. (2020). It is worth noting that some cultured *Pseudomonas* sp. have profound growth plant-promoting effects (Devi et al., 2017; Lally et al., 2017; Tamošiūnė et al., 2018). Endosphere *Pseudomonas* sp. IMBG305 promoted an increase in the leaf number of the Antarctic hairgrass *in vitro* (Podolich et al., 2021). On the contrary, families with the highest abundance in the bare soil and rhizosphere (*Chitinophagaceae*, *Gemmatimonadaceae*, *Comamonadaceae*, etc.) were occasionally found or absent in the internal part of the Antarctic pearlwort. Other colonization routes through leaves' or flowers' surface, stomata, or vertical transmission with seeds (Hardoim et al., 2015) destine the presence of families exclusively abundant among endophytes of *C. quitensis*. Most of the unique families were in a minor quantity and unequally distributed among samples showing their situational colonization of the plant.

The core microbiome is commonly determined as taxa shared among the microbial communities in a particular niche and can be assumed as the most ecologically and functionally important part of the community (Neu et al., 2021). Endophyte and rhizosphere microbial communities of the Antarctic pearlwort had quite distinct core microbiomes. This underlines the different functional activity of the microbiomes of these two niches, which should be studied with specific functional assays. Bacteria mainly of *Alphaproteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacterioidetes* were found to be crucial in the rhizosphere microbial community of the *C. quitensis*. Whereas among the endophytes, *Alphaproteobacteria*, *Gammaproteobacteria*, and *Firmicutes* were mainly defined as core bacteria. A narrow list of bacteria was defined as a core for both the rhizosphere and endosphere. The list includes *Rhodobacteraceae*, *Microbacteriaceae*, *Rhizobiaceae*, *Xanthobacteraceae*, *Sphingomonadaceae*, *Comamonadaceae*, *Pseudomonadaceae*, *Oxalobacteraceae*. We suppose that the ecological and functional role of these taxa's members is crucial in both niches of *C. quitensis*; functional activity assays should be applied to estimate the bacteria's role in the pearlwort's growth.

Bacteria belonging to *Microbacteriaceae*, *Pseudomonadaceae*, *Lactobacillaceae*, and *Corynebacteriaceae* were defined as keystone taxa of the endosphere of Antarctic plants on the South Shetland Islands (Zhang et al., 2020). Members of *Rhodospirillaceae* were major keystone bacteria observed in the rhizosphere (Zhang et al., 2020). Except for *Microbacteriaceae* and *Pseudomonadaceae*, the above-mentioned families were not defined as core microbiota in the current study, which can be connected to applying different computation techniques. Our data differ from the findings for root endophytes of *C. quitensis* from different sites on King George Island (Znój et al., 2022). *Chitinophagaceae*, *Sphingobacteriaceae* were among the root core microbes of *C. quitensis* (Znój et al., 2022), while they were in low quantity inside the aerial part of the plant, according to our data. On the contrary, the *Pseudomonadaceae* and *Rhizobiaceae* were not defined as core bacteria among root endophytes of *C. quitensis* (Znój et al., 2022).

The notable part of the core endosphere bacteria was presented by the anaerobic taxa (e.g., *Clostridium*: *Lachnospiraceae, Veillonellaceae*, *Oscillospiraceae*, *Mo-*

noglobaceae, etc.). To our knowledge, this is the first study reporting anaerobic bacteria in the endosphere of *C. quitensis*. However, the presence of anaerobes in the endosphere of the plants was reported earlier (Miyamoto et al., 2004; Saito et al., 2008; Sarria-Guzmán et al., 2016). Clostridial cells were found in a large proportion among the diazotrophs inside the leaves, stems, and roots of *Miscanthus sinensis* Andersson (Miyamoto et al., 2004) and in non-leguminous plants, including their aerial parts (Minamisawa et al., 2004). *Clostridiaceae* and *Peptostreptococcaceae* were found inside the roots and flowers of the *A. andraeanum*, and *Lachnospiraceae* was found in all tissues of this plant (Sarria-Guzmán et al., 2016). Clostridia and other anaerobes conventionally inhabit anaerobic niches such as soil particles, sediments, rumen, etc. So the abundance of anaerobic bacteria in the aerial part of a plant uprises a discussion. Minamisawa et al. (2004) suppose that endosphere clostridia can proliferate in anoxic microzones mediated by other endophytes or plant respiration; the ability to form spores enables their survival under high concentrations of O_2.

Environmental conditions in Antarctica are rather extreme for terrestrial life, trending to change southward. Yergeau et al. (2007) showed a negative relationship between bacterial diversity and latitude for bare soils in the transect from Falkland Islands (51°S) to Ellsworth Mountains (78°S). On the contrary, no such pattern exists for moss-covered soil. The plant vegetation in Antarctica is considered to provide an advantageous habitat for bacteria shielding the rhizosphere soil from unfavorable conditions (Harris & Tibbles, 1997; Yergeau et al., 2007). Nevertheless, abiotic stresses are recognized to affect bacterial communities of the rhizosphere (Naylor et al., 2017). For this reason, we supposed that changes of climate severity could affect the rhizosphere microbiome of Antarctic plants, though the effect will be much less profound compared to the fell fields. The low correlation between rhizosphere microbiome composition and region of sampling underlines that the latitudinal gradient is not the main driving force of the rhizosphere microbiomes' variation, and microscale effects can exhibit a profound effect on the community structure (Prekrasna et al., 2022). Nevertheless, a substantial part of the bacterial taxa in our study dropped in abundance in the Central or Southern sites compared to the Northern sites. The other taxa, e.g., *Burkholderiaceae*, *Ktedonobacteraceae*, *Acetobacteraceae*, increased in the Central and Southern sites. According to our data, despite the vegetation shield, a variation is observed in the rhizosphere communities' composition from plants collected in the Northern, Central or Southern parts of the maritime Antarctic.

5 Conclusions

Microbial communities that inhabit the rhizosphere and internal part of *C. quitensis* are distinct in their diversity and taxonomic composition. Most rhizosphere bacteria belong to the phyla found in the barren soil in this study. However, the rhizosphere microbial community was enriched in taxa compared to the bare substrate. On the other hand, the endosphere microbial community was a narrow niche with low diversity and was represented mainly by *Proteobacteria*. Taxonomic data show that the rhizosphere was a source of some bacteria that colonize the internal part of the plant, yet other sources of the endosphere bacteria were also available. Core microbiotas of rhizosphere and endophyte communities were distinct, with a short list of overlapping taxa. *C. quitensis* tend to inhabit comparatively protected niches; hence comparison of the microbiomes of the plants growing in different regions of the maritime Antarctic did not reveal a drastic difference in the diversity or composition. Nevertheless, a shift was visible in the abundance of some bacterial taxa in the rhizosphere of plants growing in the Northern, Central, and Southern parts of the maritime Antarctic. Variations in microbial communities' composition can indicate the effect of the climatic conditions' harshening southwards along the Western coast of the Antarctic Peninsula on the rhizosphere microbiota.

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Мікробіоми перлинниці антарктичної (*Colobanthus quitensis*) морської Антарктики: різниця у різноманітті та ключових мікроорганізмах ризосфери та ендосфери рослини

Реферат. Мікробіом рослини відіграє важливу роль у розвитку рослини та її адаптації до середовища. Останнє є особливо суттєвим для рослин, що витримують несприятливі умови Антарктики. Метою роботи була оцінка мікробіому *Colobanthus quitensis* (Kunth) Bartl., що росте в географічному діапазоні від Південних Шетландських островів на півночі до затоки Маргарити на півдні (63°S – 68°S) в морській Антарктиці. Склад мікробіому *C. quitensis* (мікроорганізмів ризосфери та ендосфери наземної частини рослини) вивчали за допомогою метагеномного сиквенування ампліконів 16S рРНК на базі Illumina Novaseq 6000. Кількість операційних таксономічних одиниць та індекси біорізноманіття (Шеннона, Сімпсона, філогенетичного різноманіття Фейта) були нижчими ($p < 0.05$) у ендофітних мікробних угруповань порівняно з ризосферними, а ANOSIM виявив різницю ($R = 0.9$, $p = 0.0001$) у таксономічних структурах мікробіомів. Різноманіття мікробіому субстрату без вегетації було нижчим порівняно з ризосферою. *Proteobacteria*, *Acidobacteria, Actinobacteria, Bacteroidota, Chloroflexi* та *Verrucomicrobia* виявились домінантними типами в ризосфері. Ці типи бактерій домінували у мікробіомі субстрату, проте частка *Actinobacteria* була вищою. *Proteobacteria* домінувала в ендосфері рослини, а *Firmicutes*, *Actinobacteria* та *Bacteroidota* мали нижчу кількість. Представники *Alphaproteobacteria*, *Actinobacteria* та *Acidobacteria* становили значну частку ключового мікробіому ризосфери *C. quitensis.* Ключова частина ендофітного мікробіому переважно складалась із представників *Alphaproteobacteria*, *Gammaproteobacteria* та *Firmicutes*. На таксономічному рівні родин бактерії, що належать до *Rhodobacteraceae*, *Microbacteriaceae*, *Rhizobiaceae*, *Xanthobacteraceae*, *Sphingomonadaceae*, *Comamonadacea*, *Pseudomonadaceae* та *Oxalobacteraceae* входили до ключової мікробіоти як ризосферних, так і ендосферних мікробних угруповань. Кореляція між складом мікробного угруповання і регіоном росту рослини була низькою ($R = 0.22$, $p = 0.04$). Однак, деякі таксони в ризосфері рослини мали різну кількість залежно від регіону: північної, центральної чи південної частини морської Антарктики. Зміна у складі мікробних угруповань може бути пов'язана з погіршенням кліматичних умов в південному напрямку вздовж західного узбережжя Антарктичного півострова.

Ключові слова: 16S рРНК, ендофіти, перлинниця антарктична, ризосфера

APPENDIX

Figure. Shared families among endosphere, rhizosphere of *Colobanthus quitensis* and bare substrate

Table 1. Differential abundance of bacteria identified on a family-rank taxonomic level in rhizosphere, endopshere and bare substrate; unique, common and core microorganisms in these compartments. E – endosphere, R – rhizosphere, S – substrate; grey colour indicates unique families, light grey colour indicates families common for two groups of samples, black colour indicates families common for three groups of samples; dark grey colour indicates core families

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
Nitrincolaceae	2.0E-80	9.7E-78										
Clade_I	1.2E-53	2.0E-51										
Coriobacteriaceae	1.3E-53	2.0E-51										
Porticoccaceae	5.5E-53	6.5E-51										
Thioglobaceae	1.1E-42	6.3E-41										
Defferrisomataceae	2.6E-39	1.2E-37										
SAR86_clade	7.4E-34	2.5E-32										
Magnetospiraceae	2.4E-32	7.1E-31										
SAR116_clade	5.8E-32	1.6E-30										
Thermincolaceae	3.8E-24	9.4E-23										
Kangiellaceae	7.5E-24	1.8E-22										
Colwelliaceae	2.4E-20	4.5E-19										
Akkermansiaceae	1.1E-18	1.9E-17										
OPB41	7.6E-18	1.2E-16										
Barnesiellaceae	3.9E-17	6.0E-16										

Continuation of Table 1

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
Bacteroidetes_vadinHA17	5.7E-16	7.9E-15										
Marinifilaceae	4.5E-14	4.9E-13										
SJA.15.1	2.3E-13	2.3E-12										
Clade_IV	2.7E-13	2.5E-12										
SR.FBR.L83	3.0E-13	2.8E-12										
Methylophagaceae	5.0E-12	4.0E-11										
Pseudoalteromonadaceae	1.2E-10	8.5E-10										
AKIW659	2.3E-10	1.5E-09										
OM182_clade	3.8E-09	2.1E-08										
Spongiibacteraceae	4.4E-08	2.1E-07										
PHOS.HE36	1.3E-07	5.8E-07										
D8A.2	1.5E-07	6.6E-07										
Caldicoprobacteraceae	4.3E-07	1.7E-06										
Methylomonadaceae	5.4E-07	2.1E-06										
Nisaeaceae	5.6E-07	2.2E-06										
Limnochordaceae	6.3E-07	2.4E-06										
Sulfurimonadaceae	3.4E-06	1.2E-05										
Pleomorphomonadaceae	7.8E-03	1.5E-02										
Roseiflexaceae	5.1E-17	7.6E-16										
Pedosphaeraceae	4.2E-15	5.3E-14										
Saprospiraceae	1.3E-12	1.1E-11										
SJA.28	2.5E-12	2.2E-11										
Vicinamibacteraceae	1.4E-09	8.4E-09										
RBG.13.54.9	7.0E-09	3.8E-08										
Thermoanaerobaculaceae	8.4E-09	4.5E-08										
S085	2.1E-08	1.1E-07										
Subgroup_17	5.7E-08	2.7E-07										
Rhodomicrobiaceae	9.4E-08	4.2E-07										
SM2D12	1.8E-07	7.8E-07										
Sandaracinaceae	1.9E-07	8.1E-07										
Phaselicystidaceae	3.6E-07	1.5E-06										
WD2101_soil_group	4.7E-07	1.8E-06										
WPS.2	7.3E-07	2.8E-06										
Pyrinomonadaceae	1.8E-06	6.8E-06										
WD260	3.9E-06	1.3E-05										
Fibrobacteraceae	6.2E-06	2.0E-05										
X01D2Z36	8.6E-06	2.7E-05										

Continuation of Table 1

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
Corynebacteriaceae	3.4E-05	1.0E-04										
Sumerlaeaceae	4.3E-05	1.2E-04										
X0319.7L14	1.8E-04	4.6E-04										
Pirellulaceae	2.1E-04	5.3E-04										
S0134_terrestrial_group	3.0E-04	7.6E-04										
Sporichthyaceae	3.1E-04	7.8E-04										
Parcubacteria	7.4E-04	1.7E-03										
Phycisphaeraceae	7.7E-04	1.8E-03										
Paracaedibacteraceae	1.1E-03	2.5E-03										
Tepidisphaeraceae	1.4E-03	3.2E-03										
Sericytochromatia	1.5E-03	3.3E-03										
Dermabacteraceae	2.3E-03	5.1E-03										
Anaerolineae	2.4E-03	5.3E-03										
F082	3.0E-03	6.4E-03										
Phormidesmiaceae	3.2E-03	6.8E-03										
Longimicrobiaceae	3.3E-03	6.9E-03										
Oligoflexales	3.6E-03	7.5E-03										
Brevibacteriaceae	3.7E-03	7.5E-03										
PB19	3.8E-03	7.8E-03										
Rhodospirillaceae	3.8E-03	7.8E-03										
Aeromonadaceae	4.2E-03	8.5E-03										
OM190	4.5E-03	9.0E-03										
Parachlamydiaceae	4.6E-03	9.2E-03										
Brevibacillaceae	5.3E-03	1.0E-02										
Holophagaceae	7.0E-03	1.3E-02										
KF.JG30.C25	7.3E-03	1.4E-02										
Eubacteriaceae	8.1E-03	1.5E-02										
Silvanigrellaceae	8.5E-03	1.6E-02										
Rickettsiales	1.2E-02	2.1E-02										
Subgroup_22	1.3E-02	2.4E-02										
Paenibacillaceae	1.6E-02	2.8E-02										
Ga0077536	1.7E-02	3.1E-02										
Crocinitomicaceae	1.8E-02	3.2E-02										
Subgroup_5	2.2E-02	3.8E-02										
Subgroup_2	5.4E-07	2.1E-06										
Dietziaceae	2.8E-02	4.8E-02										
Bacteroidaceae	2.4E-49	1.9E-47										

Continuation of Table 1

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
Cryomorphaceae	1.7E-40	8.8E-39										
Monoglobaceae	8.3E-36	3.6E-34										
Yersiniaceae	6.7E-35	2.6E-33										
Veillonellaceae	2.4E-34	8.6E-33										
Bifidobacteriaceae	2.0E-33	6.2E-32										
Tannerellaceae	4.9E-30	1.3E-28										
Butyricicoccaceae	2.8E-23	6.3E-22										
Erysipelotrichaceae	5.5E-23	1.2E-21										
Eggerthellaceae	2.3E-22	4.8E-21										
Opitutaceae.1	2.3E-20	4.5E-19										
Sphingomonadaceae	8.6E-20	1.6E-18										
Desulfovibrionaceae	3.9E-17	6.0E-16										
Eubacterium_ coprostanoligen	2.4E-16	3.4E-15										
Rhodobacteraceae	9.3E-16	1.3E-14										
Geobacteraceae	3.4E-15	4.3E-14										
Acidaminococcaceae	7.0E-15	8.3E-14										
Reyranellaceae	2.6E-13	2.5E-12										
Ruminococcaceae	4.0E-12	3.3E-11										
PeM15	8.1E-12	6.3E-11										
Saccharimonadales	3.1E-11	2.3E-10										
Erysipelatoclostridiaceae	3.5E-11	2.5E-10										
Sphingobacteriaceae	4.7E-10	2.9E-09										
Solibacteraceae	4.9E-10	2.9E-09										
Selenomonadaceae	3.0E-09	1.7E-08										
Oscillospiraceae	1.1E-08	6.0E-08										
Pseudonocardiaceae	1.3E-08	6.8E-08										
Polyangiaceae	1.7E-08	8.7E-08										
Xanthomonadaceae	2.4E-08	1.2E-07										
Puniceicoccaceae	2.8E-08	1.4E-07										
Subgroup_7	3.6E-08	1.8E-07										
Solirubrobacteraceae	7.5E-08	3.5E-07										
Rhizobiaceae	7.7E-08	3.5E-07										
Pseudomonadaceae	1.8E-07	7.8E-07										
Rhizobiales_Incertae_Sedis	1.9E-07	8.2E-07										
SC.I.84	2.4E-07	9.8E-07										
Alteromonadaceae	2.4E-07	1.0E-06										

Continuation of Table 1

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
Xanthobacteraceae	1.8E-06	6.7E-06										
Propionibacteriaceae	2.2E-06	8.0E-06										
TRA3.20	2.7E-06	9.4E-06										
Lentimicrobiaceae	3.2E-06	1.1E-05										
Parvibaculaceae	3.3E-06	1.2E-05										
Verrucomicrobiaceae	3.6E-06	1.2E-05										
Peptostreptococcaceae	5.2E-06	1.7E-05										
Atopobiaceae	1.9E-05	5.8E-05										
Clostridia_UCG.014	6.0E-05	1.7E-04										
Fusobacteriaceae	6.1E-05	1.7E-04										
R7C24	7.3E-05	2.1E-04										
Anaerovoracaceae	7.5E-05	2.1E-04										
Weeksellaceae	7.9E-05	2.2E-04										
Phormidiaceae	1.8E-04	4.6E-04										
Williamwhitmaniaceae	2.9E-04	7.5E-04										
Staphylococcaceae	3.0E-04	7.6E-04										
Pseudohongiellaceae	4.6E-04	1.1E-03										
Actinomycetaceae	4.9E-04	1.2E-03										
Spirosomaceae	1.7E-03	3.7E-03										
Sporomusaceae	2.0E-03	4.4E-03										
Azospirillaceae	3.1E-03	6.6E-03										
Enterobacteriaceae	3.3E-03	7.0E-03										
Steroidobacteraceae	4.5E-03	9.0E-03										
Rikenellaceae	6.6E-03	1.3E-02										
JG30.KF.AS9	6.9E-03	1.3E-02										
Xiphinematobacteraceae	8.8E-03	1.6E-02										
Prevotellaceae	1.5E-02	2.6E-02										
Synergistaceae	0.03	0.05										
C0119	1.4E-13	1.5E-12										
Hymenobacteraceae	2.9E-12	2.4E-11										
Iamiaceae	5.3E-11	3.7E-10										
Hyphomonadaceae	6.5E-11	4.5E-10										
A0839	1.3E-10	8.5E-10										
Frankiaceae	2.6E-10	1.7E-09										
OLB14	3.9E-08	1.9E-07										
AKYH767	5.0E-08	2.4E-07										

Continuation of Table 1

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
JG30.KF.CM66	6.8E-08	3.2E-07										
Lineage_IIa	4.1E-07	1.7E-06										
Dermacoccaceae	2.2E-06	7.8E-06										
MBNT15	2.5E-06	8.7E-06										
Coleofasciculaceae	4.0E-06	1.4E-05										
Gemmataceae	4.8E-06	1.6E-05										
Leptolyngbyaceae	4.8E-06	1.6E-05										
Armatimonadales	6.3E-06	2.0E-05										
AKYG1722	9.0E-06	2.8E-05										
Chloroflexaceae	1.1E-05	3.3E-05										
Caldilineaceae	1.5E-05	4.6E-05										
LWQ8	1.3E-06	4.8E-06										
Geminicoccaceae	2.6E-05	8.0E-05										
Obscuribacteraceae	3.3E-05	9.7E-05										
Herpetosiphonaceae	3.9E-05	1.2E-04										
Latescibacterota	6.7E-05	1.9E-04										
Frankiales	2.7E-05	8.2E-05										
Blfdi19	1.0E-04	2.7E-04										
Dongiaceae	1.2E-04	3.2E-04										
Koribacteraceae	1.7E-04	4.5E-04										
Nostocaceae	1.7E-04	4.5E-04										
Gallionellaceae	2.9E-04	7.5E-04										
Lineage_IIb	3.6E-04	9.0E-04										
Chthonomonadaceae	3.6E-04	9.1E-04										
Corynebacteriales_ Incertae_S	3.8E-04	9.4E-04										
Nitrososphaeraceae	4.1E-04	1.0E-03										
Abditibacteriaceae	4.7E-04	1.1E-03										
DS.100	5.3E-04	1.3E-03										
Candidatus_Levybacteria	6.9E-04	1.6E-03										
Candidatus_Pacebacteria	1.1E-03	2.5E-03										
Amb.16S.1323	1.2E-03	2.7E-03										
Inquilinaceae	1.4E-03	3.2E-03										
Elev.16S.1166	1.8E-03	3.9E-03										
Candidatus_Nomurabacteria	3.5E-03	7.2E-03										
Candidatus_ Yanofskybacteria	4.3E-03	8.7E-03										
KF.JG30.B3	1.7E-05	5.3E-05										

Continuation of Table 1

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
NB1.j	4.5E-03	9.0E-03										
Geodermatophilaceae	5.7E-03	1.1E-02										
Labraceae	8.9E-03	1.7E-02										
KD3.93	1.2E-02	2.2E-02										
Candidatus_Peribacteria	1.3E-02	2.3E-02										
X37.13	1.3E-02	2.3E-02										
Candidatus_Adlerbacteria	2.4E-02	4.2E-02										
Fodinicurvataceae	0.03	0.05										
Halieaceae	1.1E-08	5.8E-08										
Methylophilaceae.1	3.2E-45	2.2E-43										
Gemmatimonadaceae	4.6E-19	8.1E-18										
Bryobacteraceae	1.9E-15	2.5E-14										
A21b	6.7E-15	8.1E-14										
IMCC26256	1.8E-14	2.1E-13										
Blastocatellaceae	2.7E-14	3.0E-13										
Haliangiaceae	5.1E-14	5.5E-13										
Anaerolineaceae	1.5E-13	1.6E-12										
JG30.KF.CM45	2.3E-13	2.3E-12										
Alcaligenaceae	4.4E-13	4.0E-12										
X67.14	8.2E-13	7.3E-12										
A4b	2.3E-12	2.0E-11										
Nitrosomonadaceae	7.6E-12	6.0E-11										
Kineosporiaceae	1.3E-11	1.0E-10										
Chitinophagaceae	4.2E-11	3.0E-10										
Micromonosporaceae	2.4E-10	1.5E-09										
Micropepsaceae	3.2E-10	2.0E-09										
Chthoniobacteraceae	4.4E-10	2.7E-09										
Intrasporangiaceae	4.8E-10	2.9E-09										
Lachnospiraceae	7.9E-10	4.7E-09										
Nocardioidaceae	1.8E-09	1.0E-08										
Opitutaceae	1.9E-09	1.1E-08										
Hyphomicrobiaceae	8.2E-09	4.4E-08										
Methyloligellaceae.1	4.2E-10	2.6E-09										
Myxococcaceae	5.1E-08	2.4E-07										
Ilumatobacteraceae	1.7E-07	7.3E-07										
Flavobacteriaceae	2.3E-07	9.4E-07										
Acetobacteraceae	3.7E-07	1.5E-06										

Continuation of Table 1

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
X0319.6G20	1.6E-06	5.8E-06										
Microscillaceae	2.0E-06	7.2E-06										
Nitrospiraceae	4.3E-06	1.4E-05										
KD4.96	5.3E-06	1.7E-05										
Diplorickettsiaceae	2.7E-05	8.2E-05										
Ktedonobacteraceae	3.3E-05	9.7E-05										
Gracilibacteraceae	4.2E-05	1.2E-04										
Microbacteriaceae	8.8E-05	2.4E-04										
Acidobacteriae	9.5E-05	2.6E-04										
Micrococcaceae	1.0E-04	2.8E-04										
Methylophilaceae	1.3E-04	3.4E-04										
Nakamurellaceae	1.4E-04	3.8E-04										
Isosphaeraceae	1.8E-04	4.6E-04										
Methyloligellaceae	4.3E-04	0.001										
mle1.27	4.6E-04	0.001										
Mycobacteriaceae	4.8E-04	0.001										
Fimbriimonadaceae	5.6E-04	0.001										
Acidothermaceae	0.001	0.001										
Deinococcaceae	0.001	0.003										
Cellvibrionaceae	0.001	0.003										
AKIW781	0.001	0.003										
Gaiellaceae	0.002	0.005										
Beijerinckiaceae	0.003	0.005										
Acidobacteriaceae_. Subgroup	0.003	0.005										
Burkholderiaceae	0.00	0.01										
Bdellovibrionaceae	0.01	0.01										
Holosporaceae	0.01	0.01										
Methylacidiphilaceae	0.01	0.01										
Bacteriovoracaceae	0.01	0.01										
Candidatus_Kaiserbacteria	0.01	0.02										
Comamonadaceae	0.01	0.02										
Microtrichaceae	0.01	0.02										
MB.A2.108	0.01	0.02										
Bacillaceae	0.01	0.02										
Legionellaceae	0.02	0.03										

End of Table 1

Family	p-value	q-value	Unique			Common				Core		
			E	R	S	R E	R S	E S	R E S	E	R	S
NS11.12_marine_group	0.03	0.05										
Trueperaceae	0.21	0.27										
Rhodanobacteraceae	0.42	0.48										
Rubritaleaceae	0.42	0.48										
Oxalobacteraceae	0.93	0.94										
Moraxellaceae	0.04	0.06										
Nannocystaceae	0.04	0.07										
BIrii41	0.05	0.09										
Morganellaceae	0.08	0.12										
Kapabacteriales	0.08	0.13										
Clostridiaceae	0.09	0.13										
Gitt.GS.136	0.09	0.13										
Cytophagaceae	0.09	0.14										
Hungateiclostridiaceae	0.12	0.17										
Caulobacteraceae	0.15	0.20										
Cellulomonadaceae	0.24	0.30										
bacteriap25	0.24	0.31										
Candidatus_Jorgensenbacteri	0.28	0.35										
Coxiellaceae	0.32	0.39										
NS9_marine_group	0.34	0.41										
Acidiferrobacteraceae	0.34	0.41										
Desulfitobacteriaceae	0.43	0.48										
Parvularculaceae	0.45	0.51										
Omnitrophales	0.47	0.52										
Anaeromyxobacteraceae	0.55	0.59										
Nocardiaceae	0.72	0.74										
Dojkabacteria	0.78	0.79										
Devosiaceae	0.85	0.87										
env.OPS_17	0.86	0.88										
Hydrogenophilaceae	0.94	0.95										

Table 2. Differential abundance of bacteria identified on a family-rank taxonomic level in the Northern (A), Central (B) and Southern (C) part of the Antarctic Peninsula

Family	p-value	q-value	Region, where taxa's amount was higher
Marine_Group_II	2,23E-14	1,25E-11	Northern
MB.A2.108	6,48E-13	1,81E-10	Northern
Intrasporangiaceae	2,85E-10	3,99E-08	Northern
Frankiales	1,92E-09	2,15E-07	Northern
Pyrinomonadaceae	3,62E-09	3,38E-07	Northern
Sporichthyaceae	6,98E-09	5,59E-07	Northern
R7C24	4,43E-07	3,10E-05	Northern
Sulfuricellaceae	1,30E-06	8,06E-05	Northern
Gitt.GS.136	2,41E-06	1,35E-04	Northern
Ilumatobacteraceae	2,95E-06	1,50E-04	Northern
Micromonosporaceae	4,19E-06	1,96E-04	Northern
Verrucomicrobiaceae	4,68E-06	2,01E-04	Northern
VHS.B3.70	3,01E-05	8,03E-04	Northern
Rickettsiales	3,65E-05	8,88E-04	Northern
Methyloligellaceae	5,40E-05	1,26E-03	Northern
Gaiellaceae	6,67E-05	1,44E-03	Northern
SC.I.84	2,14E-04	3,87E-03	Northern
S0134_terrestrial_group	2,27E-04	3,97E-03	Northern
AKYH767	2,50E-04	4,11E-03	Northern
Fimbriimonadales	2,72E-04	4,23E-03	Northern
AT.s3.28	4,54E-04	6,52E-03	Northern
KD4.96	5,03E-04	7,05E-03	Northern
Rubinisphaeraceae	6,56E-04	8,96E-03	Northern
Lineage_IV	8,01E-04	9,97E-03	Northern
UA11	1,20E-03	1,27E-02	Northern
Methylophilaceae	1,37E-03	1,42E-02	Northern
cvE6	2,14E-03	1,97E-02	Northern
Pseudanabaenaceae	2,29E-03	2,03E-02	Northern
S.BQ2.57_soil_group	4,85E-03	3,62E-02	Northern
Saccharimonadales	4,85E-03	3,62E-02	Northern
Subgroup_11	6,53E-03	4,45E-02	Northern
Subgroup_7	6,61E-03	4,45E-02	Northern
Lineage_IIc	6,71E-03	4,45E-02	Northern
X0319.7L14	7,54E-03	4,75E-02	Northern
Pseudonocardiaceae	6,33E-06	2,53E-04	Central
Cyclobacteriaceae	1,62E-05	5,33E-04	Central
Rhodomicrobiaceae	2,02E-05	6,28E-04	Central

Continuation of Table 2

Family	p-value	q-value	Region, where taxa's amount was higher
Anaerolineae	7,57E-05	1,57E-03	Central
JG30.KF.AS9	1,85E-04	3,57E-03	Central
Ktedonobacteraceae	2,14E-04	3,87E-03	Central
Acetobacteraceae	2,36E-04	4,01E-03	Central
Acidobacteriaceae_.Subgroup_1.	3,15E-04	4,77E-03	Central
Acidothermaceae	4,40E-04	6,48E-03	Central
Bacillaceae	6,87E-04	9,16E-03	Central
Subgroup_2	7,13E-04	9,29E-03	Central
Peptostreptococcaceae	7,85E-04	9,97E-03	Central
PAUC26f	8,45E-04	1,01E-02	Central
WD260	8,73E-04	1,02E-02	Central
Dermatophilaceae	1,54E-03	1,54E-02	Central
Koribacteraceae	1,72E-03	1,69E-02	Central
Burkholderiaceae	1,17E-03	1,26E-02	Central
Beijerinckiaceae	2,44E-03	2,13E-02	Central
Demequinaceae	2,77E-03	2,39E-02	Central
Absconditabacteriales_.SR1.	2,99E-03	2,53E-02	Central
Enterococcaceae	3,37E-03	2,70E-02	Central
Planococcaceae	3,83E-03	3,02E-02	Central
Rhodanobacteraceae	6,44E-03	4,45E-02	Central
Phormidesmiaceae	5,70E-03	4,20E-02	Central
Micrococcaceae	6,96E-03	4,53E-02	Central
type_III	1,91E-03	1,81E-02	Central
Elev.1554	2,12E-03	1,97E-02	Central
Cellvibrionaceae	2,38E-05	6,66E-04	Central and Southern
Hyphomonadaceae	9,98E-04	1,12E-02	Central and Southern
bacteriap25	1,04E-03	1,14E-02	Central and Southern
Inquilinaceae	2,69E-04	4,23E-03	Central and Southern
Micropepsaceae	1,52E-03	1,54E-02	Central and Southern
FFCH16263	3,29E-03	2,70E-02	Southern
NB1.j	3,36E-03	2,70E-02	Central and Southern
Reyranellaceae	3,37E-03	2,70E-02	Central and Southern
Chloroflexaceae	4,69E-03	3,59E-02	Central and Southern
Solibacteraceae	5,83E-03	4,24E-02	Central and Southern
Neisseriaceae	6,39E-03	4,45E-02	Central and Southern
Vampirovibrionales	6,39E-03	4,45E-02	Central and Southern
Amb.16S.1323	7,55E-03	4,75E-02	Central and Southern
Coleofasciculaceae	6,75E-03	4,45E-02	Central and Southern

End of Table 2

Family	p-value	q-value	Region, where taxa's amount was higher
Subgroup_22	3,56E-05	8,88E-04	Northern and Southern
P2.11E	6,19E-05	1,39E-03	Northern and Southern
Weeksellaceae	1,42E-04	2,84E-03	Northern and Southern
Fibrobacteraceae	7,47E-06	2,79E-04	Northern and Southern
Eubacteriaceae	1,15E-05	4,04E-04	Northern and Southern
Solimonadaceae	2,33E-05	6,66E-04	Northern and Southern
Pseudohongiellaceae	1,79E-03	1,72E-02	Northern and Southern
Subgroup_17	3,90E-03	3,03E-02	Northern and Southern
Acidobacteriae	6,01E-03	4,32E-02	Northern and Southern
WS2	7,46E-03	4,75E-02	Northern and Southern
Sandaracinaceae	2,28E-03	2,03E-02	Northern and Southern
Hydrogenophilaceae	9,57E-04	1,09E-02	Northern and Southern
Longimicrobiaceae	7,21E-11	1,35E-08	Northern and Southern