MOLECULAR ECOLOGY RESOURCES

Supplemental Information for:

Opening a next-generation black box: ecological trends for hundreds of species-like taxa uncovered within a single bacterial >99% 16S rRNA operational taxonomic unit

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Supplementary Materials Text S1

Development of PCR primers targeting a protein-encoding gene

We aimed on development of a primer pair suitable for specific amplification of a protein-encoding gene present in all *Polynucleobacter* bacteria from environmental samples. Due to the high polymorphisms of protein-encoding genes in *Polynucleobacter* strains, we expected to find suitable primer binding sites in the more conserved intergeneric sequences. We screened 61 genomes of *Polynucleobacter* strains for single copy genes in the length range of 198 to 348 bp for primer binding sites in their up- and downstream spacers. This length range was chosen in order to ensure high quality sequences from paired-end sequencing by the Illumina MiSeq system (2x300 bp read length). This search resulted only in a single gene with a suitable phylogenetic resolution and rather conserved flanking primer binding sites. Unfortunately, it turned out that primers matching to the various sequences of all four *Polynucleobacter* subgroups are not possible if a high degree of primer degeneration should be avoided. Therefore, only a primer pair specific for subcluster PnecC was further developed. In order to reveal the sequence variability at the targeted primer binding sites, the priB gene including flaking regions of a large number of cultivated strains were sequenced. The finally available set of priB sequences used for checking the primer binding sites included 377 sequences (254 non-redundant priB sequences, Suppl. Mat. Table S2). The binding site of the developed forward primer priBinnFd starts in the spacer region upstream of the priB gene and covers the first 14 bp of the priB gene. The binding site of the reverse primer priBinnRd is located a few bp downstream of the priB gene. The forward primer and the reverse primer contain two and one degenerated positions, respectively. In total, 14 of the 351 priB sequences representing subcluster PnecC showed mismatches with the forward primer. These 14 sequences cluster in eight $OTU_{98\%}$ (see below), of which two represent endosymbionts. The reverse primer did not show any mismatch with sequences of strains affiliated with subcluster PnecC. The primer pair amplifies sequences of 285-288 bp length, whereas the length polymorphism originates from indels located immediately downstream the priB gene. In some cases, the priB genes of strains affiliated with other *Polynucleobacter* subclusters differ in length from the genes of PnecC strains.

Sequence similarity threshold for discrimination of species-like OTU

We aimed on sequencing of priB amplicons of PnecC bacteria in environmental samples representing ecological gradients. Furthermore, we aimed on defining a sequence similarity threshold for clustering priB sequences in OTU best reflecting species-like OTU. In order to reveal potential thresholds, 102 *Polynucleobacter* genome sequences were clustered in species-like taxa by using a genome-based species delineation threshold of 95% average nucleotide identity (ANI) [1, 2]. Next, we compared intra- and interspecific priB sequence similarity values (Fig. 1) and observed that the best priB threshold for species discrimination is > 4 bp sequence differences, which equals 98.3% sequence similarity. Two species showed for a few pairwise comparisons < 98% intraspecific priB sequence similarity, which accounted for 0.2% of all pairwise comparisons. This mainly involved comparisons between the amplus and simplex subgroups of *P. asymbioticus* [3]. On the other hand, some species delineated by ANI values < 95% showed priB sequence similarities > 98.3% (< 4 bp). This was the case in 2% of the pairwise comparisons of the 102 analyzed genomes. Such species were lumped into species complexes, of which seven were established. Based on a reference set of 254 priB sequences (non-redundant) and a threshold of 98% sequence similarity, 117 reference $OTU_{98\%}$ (refOTU) could be established (Suppl. Mat. Table S2). These 117 refOTU represent 108 species-like OTU, one species split into two refOTU and seven species complexes. The reference taxonomy list (Suppl. Mat. Table S2) was used for initial classification of $OTU_{98\%}$ obtained from environmental samples. All environmental $OTU_{98\%}$ not sharing with one of the reference sequences a sequence similarity of ≥ 98% were termed environmental OTU (eOTU; Suppl. Mat. Table S2).

Amplicon sequencing of environmental samples

Sequencing of the amplicons of 120 priB samples (117 environmental samples and three controls) resulted in 18.7×10^6 raw paired-end sequences. Quality filtering and denoising removed 5.7×10^6 sequences. Length filtering for sequences < 285 or > 288 bp, and exclusion of sequences encoding additional stop codons removed 2208 sequences. Most of the sequences with an additional stop codon within the priB gene were found among the sequences only present with copy numbers < 10. In order to remove other sequences potentially impacted by PCR or sequencing errors, all sequences, which appeared only in a single sample with copy numbers of < 10 were removed, which excluded another 900 sequences. Three samples were excluded from further analyses due to too low sequence numbers. The remaining 117 samples (114 environmental samples representing 99 habitats and three controls) were rarefied to 25 230 sequences per sample. A rarefaction analysis revealed that absolute sequence type (ASV) richness of all but one sample had reached an asymptote already for smaller sequence numbers (Supplementary Fig. S2). The remaining sequences represented 3210 ASV, which were clustered with a threshold of 98% sequence similarity in OTU, which were classified in a two-step process by using the reference sequence set. In the two controls with template DNA of one and four cultured strains, respectively, exclusively the expected OTU were detected. Another control consisted of a technical replicate of an OTU-rich environmental sample. The OTU specific read numbers of the two replicates correlated well (linear regression, R^2=0.98, p=2.2e-16).

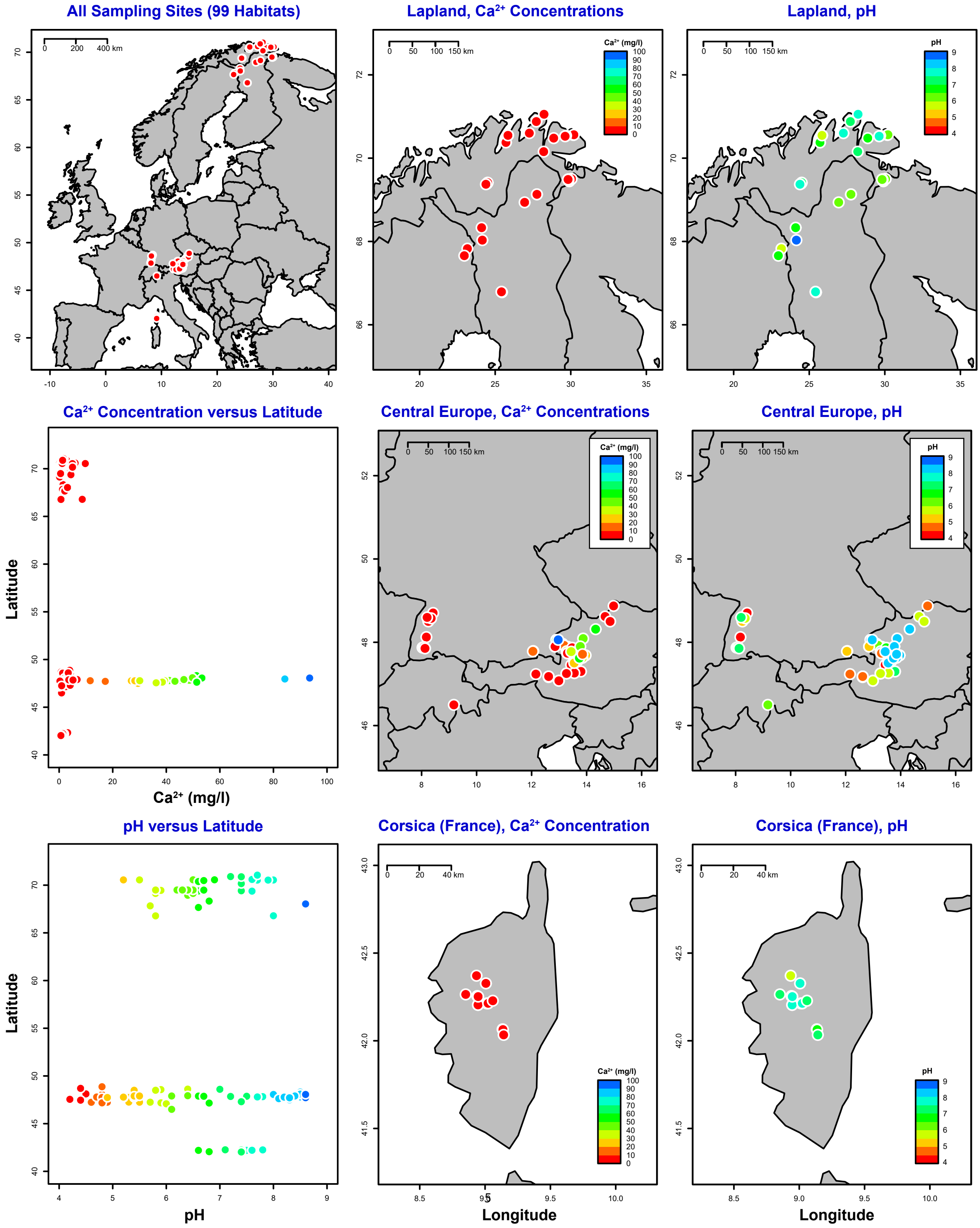
In order to test if all obtained environmental priB sequences represent PnecC sequences, a phylogenetic tree was calculated with all environmental and all reference sequences. This revealed that almost all sequences clustered within subcluster PnecC of the genus *Polynucleobacter*. However, despite slight primer mismatches, 41 of the 600 OTU clustered within subcluster PnecB1. However, these 41 PnecB1 eOTU represented only 0.14% of all reads obtained from the 114 environmental samples and none of those eOTU represented more than 0.05% of the total number of obtained reads. Interestingly, all those detections of PnecB1 eOTU originate from alkaline habitats characterized by higher Ca^{2+} concentrations, which fits to previous observations [4], however, due to the primer mismatches it is unlikely that the observed ratios of PnecC to PnecB1 reads reflect any real differences in relative abundances of the two groups.

References

1. Konstantinidis, K.T., A. Ramette, J.M. Tiedje, The bacterial species definition in the genomic era. Philosophical Transactions of the Royal Society B: Biological Sciences. 2006;361:1929-40.
2. Jain, C., L.M. Rodriguez-R, A.M. Phillippy, K.T. Konstantinidis, S. Aluru, High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nature Communications. 2018;9:5114.

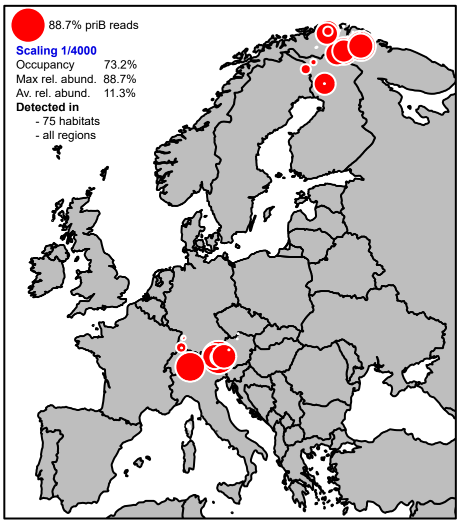
3. Hoetzinger, M., J. Schmidt, J. Jezberová, U. Koll, M.W. Hahn, Microdiversification of a pelagic *Polynucleobacter* species is mainly driven by acquisition of genomic islands from a partially interspecific gene pool. Appl. Environ. Microbiol. 2017;83:e02266-16.
4. Jezbera, J., J. Jezberova, U. Koll, K. Hornak, K. Simek, M.W. Hahn, Contrasting trends in distribution of four major planktonic betaproteobacterial groups along a pH gradient of epilimnia of 72 freshwater habitats. FEMS Microbiol. Ecol. 2012;81:467-79.

Suppl. Mat. Fig. S1. Maps of Europe depicting all sampling sites (top, left), Ca^{2+} concentrations (middle column) and pH of habitats (right column) in the regions Lapland, Central Europe and the Mediterranean island Corsica (France). **Next page,** geographic distribution of detection and pH-specific occupancy of some selected taxa. *Polynucleobacter paneuropaeus* and *P. sphagniphilus* represent common taxa with frequently high relative abundance and high occupancy. By contrast, *P. wuianus* and *P. meluiroseus* represent only locally abundant taxa with low occupancy values. Note the different scaling used for plotting the relative local abundance of eOTU0124.

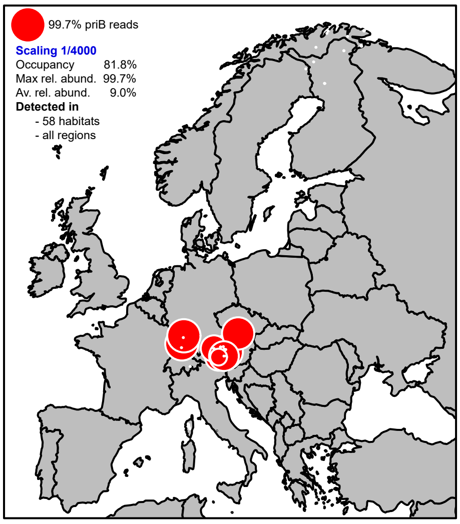


Suppl. Mat. Fig. S1 (continued).

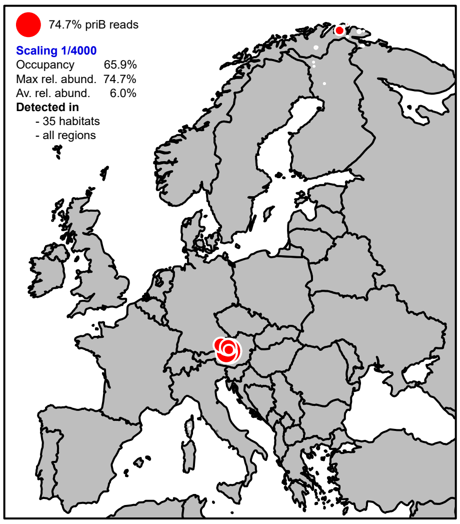
Polynucleobacter paneuropaeus



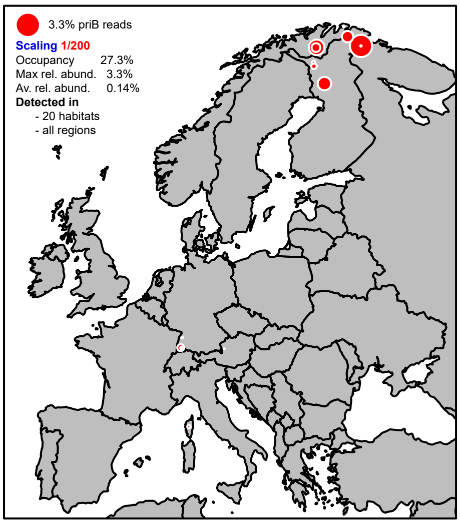
Polynucleobacter sphagniphilus



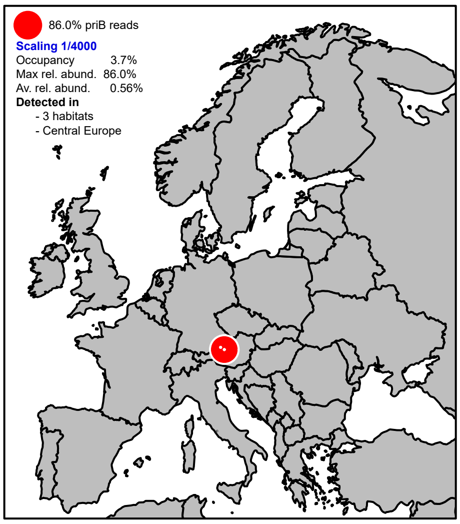
eOTU0002



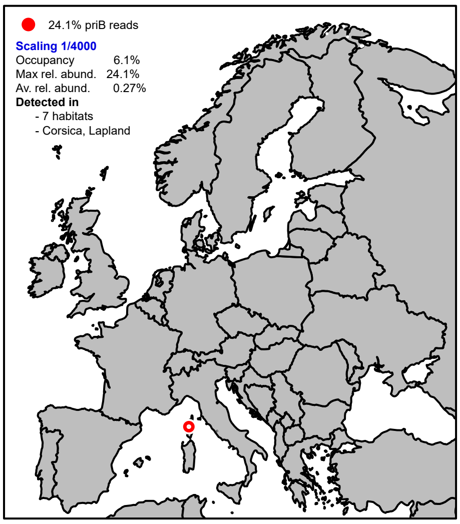
eOTU0124



Polynucleobacter wuianus

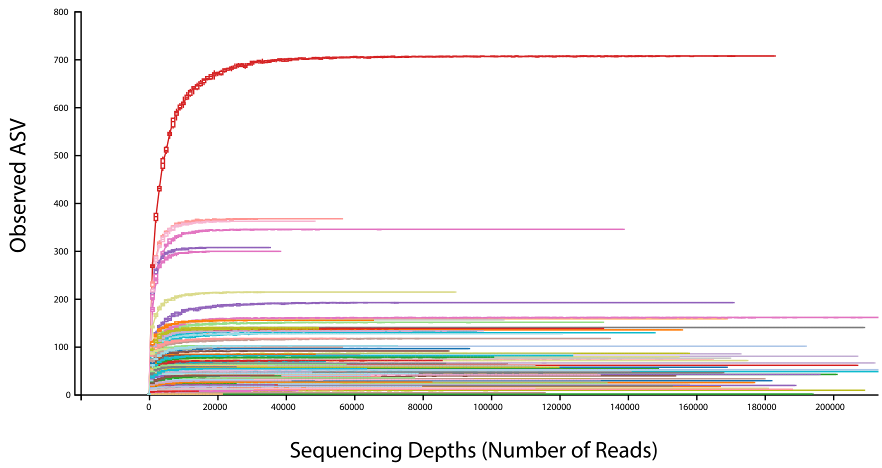


Polynucleobacter meluiroseus

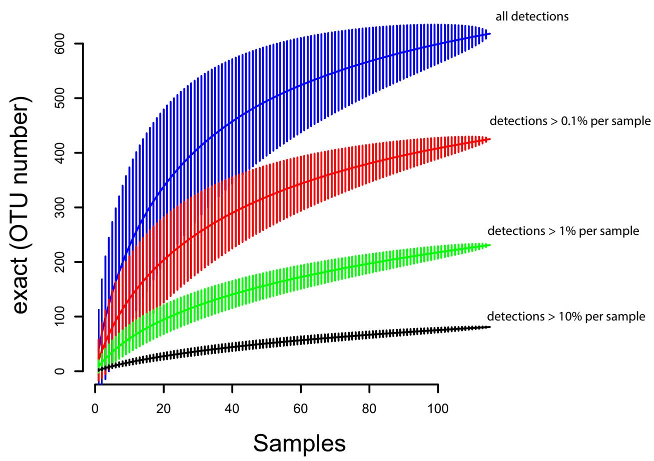


Suppl. Mat. Fig. S2. (A) Rarefaction analysis of number of amplicon sequence variants (ASV) observed in the different sequenced samples. Each curve represents an individual sample. The six samples with the highest estimated numbers represent the five investigated running water systems (including one technical replicate). In a subsequent step, the numbers of reads per sample were rarefied to 25230 reads. **(B)** Species accumulation curves determined by using a sample-based rarefaction method (Mao Tau estimates, R function specaccum, Vegan package). Richness of species-like OTU (> 98% sequence similarity threshold) was estimated for all detections and for detections above particular detection thresholds. The bars show unconditional standard deviations. **(C)** Log-normal rank-abundance curves of individual samples. The five samples with total taxon numbers > 100 represent the five running water samples.

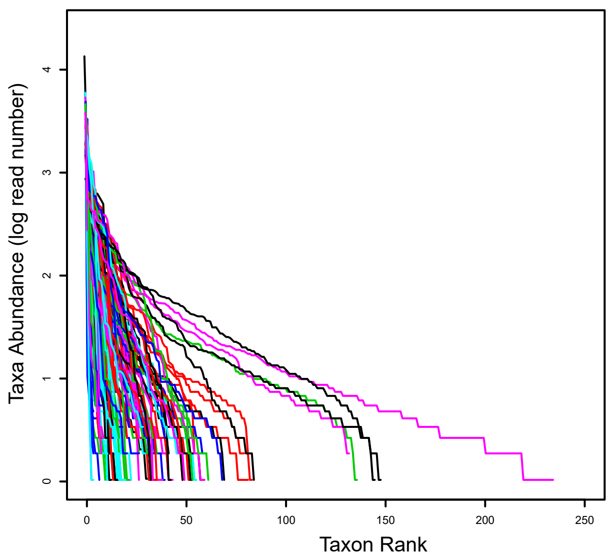
(A)



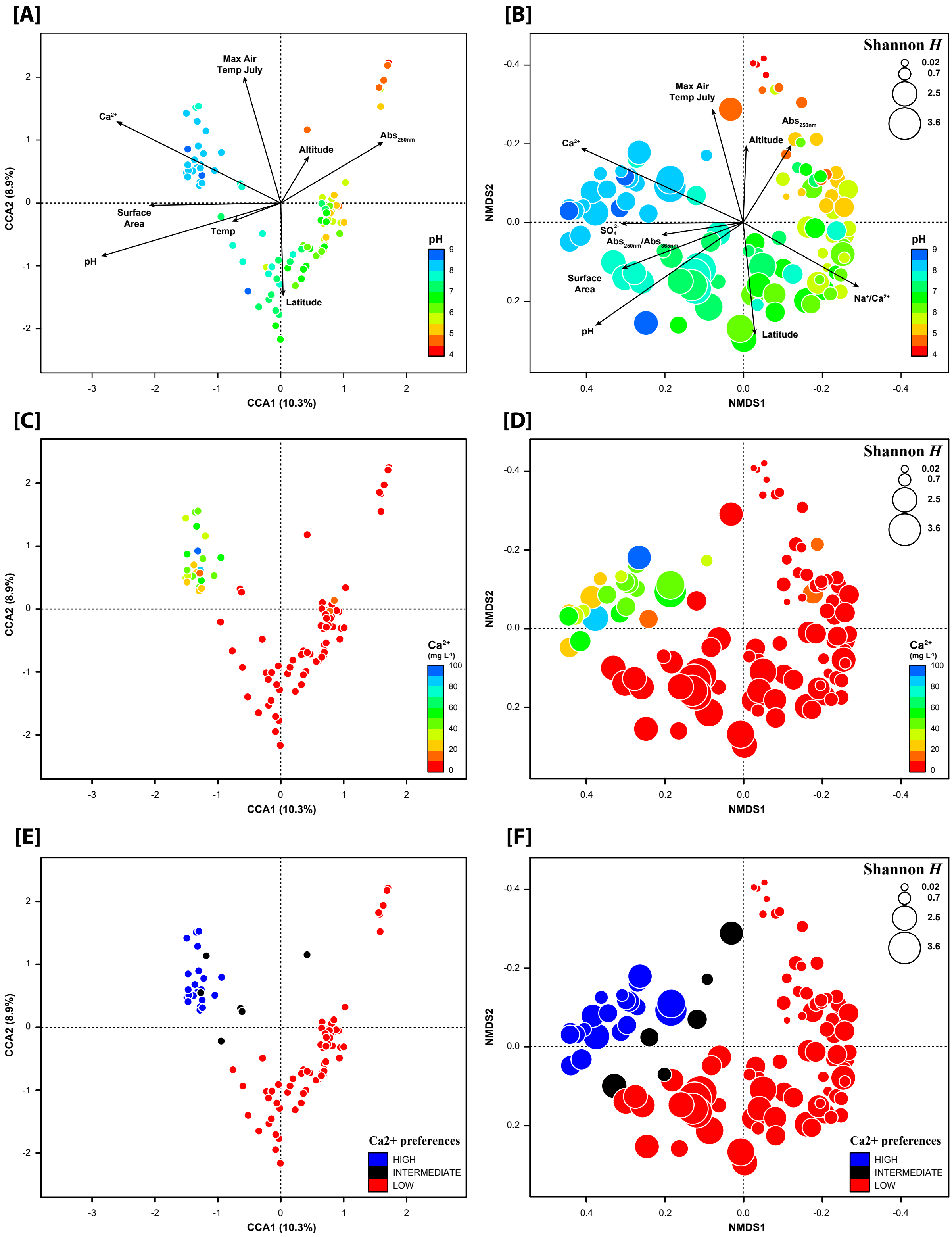
(B)



(C)



Suppl. Mat. Fig. S3. Canonical correspondance analysis (CCA, left column) and non-metric multidimensional scaling (NMDS) ordinations (right column) color-coded by pH of sample (A, B), by Ca^{2+} concentrations of samples (C, D), or by Ca^{2+}-preferences of the communities present in the different samples (E, F). The classification in the three community types corresponds to the classification shown in Fig. 4. The two plots shown in the top row are identical with those shown in Fig. 3 and are only displayed for comparison with the other plots.



Suppl. Mat. Fig. S4. Variables used for the variation partitioning analyses and results of variation partitioning analyses.

Chemical variables (Env)

pH
Conductivity (µS/cm)
Absorption, 250 nm (Abs250nm)
Na^{+} Concentration (mg/l)
Mg^{2+} Concentration (mg/L)
Ca^{2+} Concentration (mg/L)
SO_4^{2+} (sulphate) Concentration (mg/L)
Total Ions (mg/l)

Geographic and climatic variables (GeoClim)

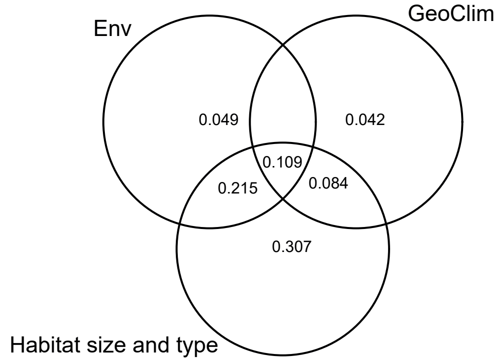
Latitude (decimal degrees)
Altitude (m)
Temperature Maximum in July (°C)
Annual Mean Temperature (°C)
Mean Temperature Warmest Quarter (°C)

Habitat properties (Habitat)

Surface Area (ha)
Habitat Type (running, standing)

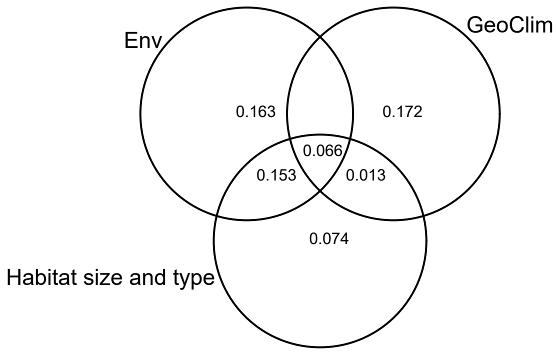
(A) OTU richness

Residuals = 0.201



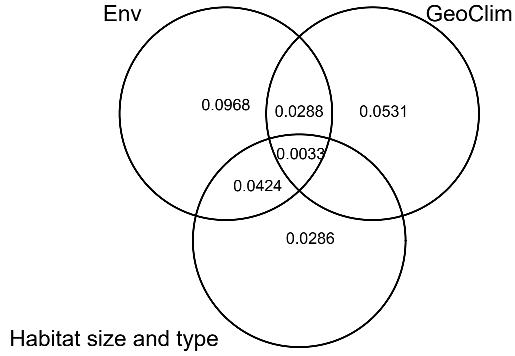
(B) Shannon diversity index

Residuals = 0.394

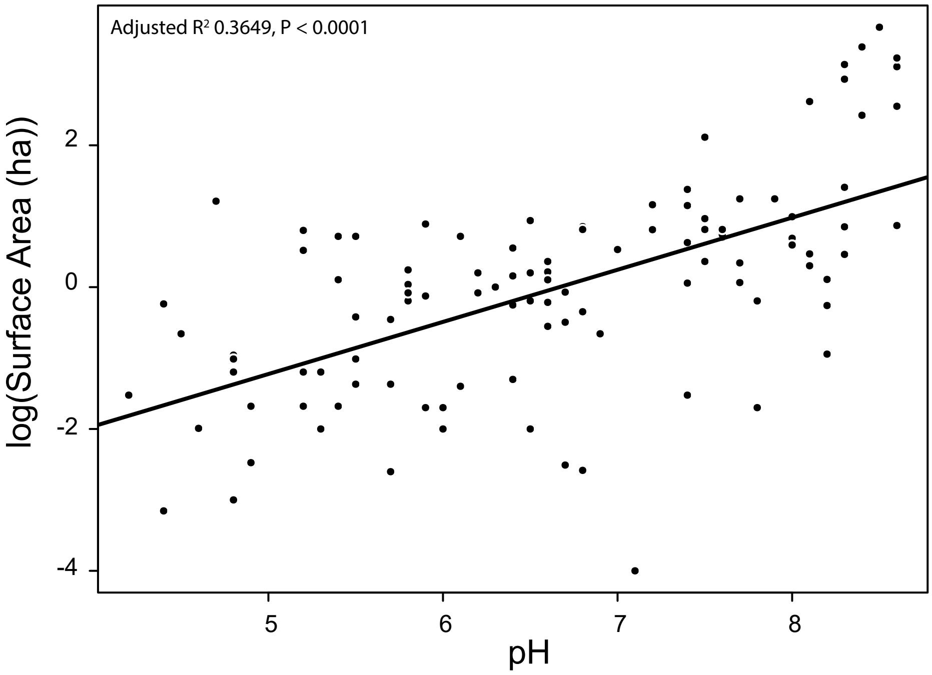


(C) Bray-Curtis community dissimilarity

Residuals = 0.7571



Suppl. Mat. Fig. S5. Top, pH of water samples and size of the investigated standing freshwater habitats (lakes, ponds and ditches). The line shows the result of a linear regression of the plotted data. **Bottom,** relationship of habitat size (surface area, hectar) and OTU richness determined by linear regression analyses. Because of the influence of pH on OTU richness (Fig. 6B), z values (slope) were determined by regressions for individual pH intervals of one pH unit, respectively. Only samples from standing waters were considered in this analysis. The obtained z values (slope of the relationship between surface area and OTU richness) are quite similar to the results previously reported by Reche et al. (2005) for entire bacterial communities (0.161 ± 0.02, results based on DGGE data).



pH_range	N	z	Std Error (z)	adjusted R^2	P value	Comment
4.0 - 5.0	10			-0.0187	0.3934	no significant linear model
4.5 - 5.5	20			-0.0133	0.4049	no significant linear model
5.0 - 6.0	23	0.1126	0.0448	0.1812	**0.0195**	*
5.5 - 6.5	27	0.1492	0.0520	0.2050	**0.0079**	**
6.0 - 7.0	27	0.1231	0.0508	0.1484	**0.0223**	*
6.5 - 7.5	30	0.1162	0.0349	0.2458	**0.0023**	**
7.0 - 8.0	25	0.1158	0.0355	0.2706	**0.0032**	**
7.5 - 8.5	29			0.0626	0.0937	no significant linear model

p < 0.05, *
p < 0.01, **
p < 0.001, ***

z values statistics

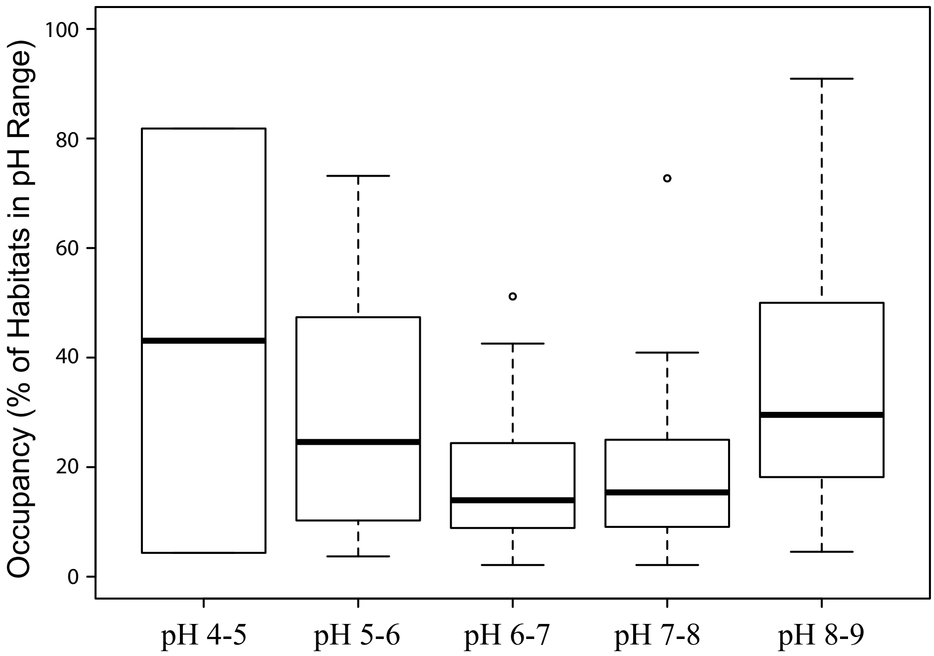
Average	0.123
SD	0.015
Range	0.113 - 0.149

Reference

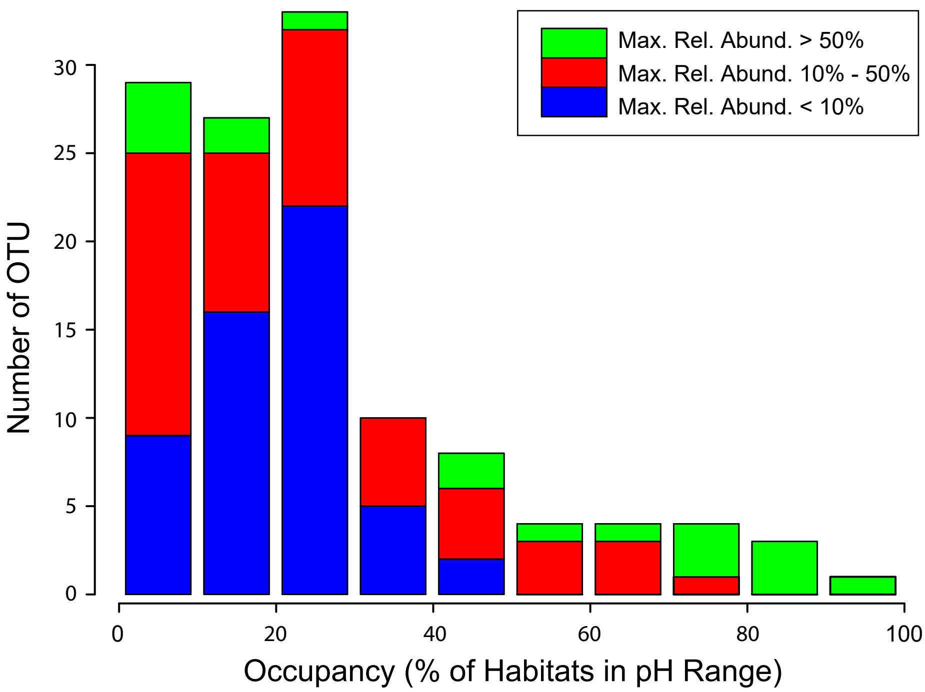
Reche, I., Pulido-Villena, E., Morales-Baquero, R., and E.O. Casamayor (2005) Does ecosystem size determine aquatic bacterial richness? Ecology 86 (7), 1715-1722

Suppl. Mat. Fig. S6. pH-related habitat occupancy of OTU. Occupancy refers to the percentage of habitats in a two pH unit range around the specific pH optimum of the respective OTU. For OTU with low and high Ca^{2+} preferences, habitat sets with low or high Ca^{2+} concentrations were considered, respectively. Only OTU with read numbers of >0.1% of total read numbers were considered. **(A)** Box plots of occupancy of OTU assigned to different pH classes according to their respective pH optimum. **(B)** Relationship of occupancy of OTUs and the respectively observed maximum relative abundance of the OTUs. The color code separates the OTUs by their maximum relative abundance in the investigated samples in three different categories.

[A]



[B]



Suppl. Mat. Table S1. Investigated environmental samples and habitats. The air temperature data were obtained from the WorldClim climate model (Fick and Hijmans, 2017).

Sample	Sampling Date	Habitat Type	Region	Latitude (°N)	Longitude (°E)	Altitude (m)	Annual Mean Air Temp. (°C)	Mean Air Temp. Warmest Quarter (°C)	Mean Air Temp. Coldest Quarter (°C)	Surface Area (ha)	Water Temperature (°C)	pH	Conductivity (µS/cm)	Ca^{2+} (mg/l)
Aci1, sample 1	09.08.2015	Lake_Pond	Lapland	69.13086	27.76061	139	-0.6	10.8	-11.3	6.40E-01	16.0	5.8	6.4	0.13
Aci1, sample 2	07.08.2018	Lake_Pond	Lapland	69.13086	27.76061	139	-0.6	10.8	-11.3	6.40E-01	18.0	6.5	5.8	0.17
Aito	20.09.2016	Ditsch	Corsica	42.26429	8.85181	1210	8.8	16.0	2.9	1.00E-04	11.5	7.1	78.5	3.41
Attersee	18.05.2016	Lake_Pond	Central_Europe	47.80096	13.48766	469	7.0	15.7	-2.2	4.62E+03	14.5	8.5	281.0	41.15
Basta	19.09.2016	Lake_Pond	Corsica	42.06541	9.13423	2093	6.1	13.6	0.0	4.25E+00	12.4	7.4	17.7	1.05
BlScho	22.10.2015	Lake_Pond	Central_Europe	48.12411	8.17377	1004	6.3	13.9	-1.3	2.20E-01	5.6	4.5	23.6	1.01
Bors	08.08.2016	Lake_Pond	Lapland	70.37676	25.71275	78	-1.6	9.9	-12.3	2.80E-01	15.4	6.6	27.9	0.62
Buhl	21.10.2015	Lake_Pond	Central_Europe	48.50129	8.24565	803	7.0	14.8	-1.0	1.27E+00	8.0	5.4	62.2	1.35
Calac	20.09.2016	Lake_Pond	Corsica	42.32719	9.00634	793	9.7	16.9	3.7	1.30E+02	22.1	7.5	54.5	3.17
Capit	22.09.2016	Lake_Pond	Corsica	42.21319	9.01330	1930	6.0	13.4	-0.1	5.50E+00	13.6	7.6	16.8	0.84
Cinto	15.07.2015	Lake_Pond	Corsica	42.37105	8.93452	2289	6.3	13.6	0.3	1.09E+00	18.0	7.4	13.5	1.02
Clou	11.06.2018	Lake_Pond	Central_Europe	47.26082	13.55083	1972	0.9	8.7	-6.9	1.00E-02	18.5	5.3	4.4	0.40
DonauLinz	18.05.2016	River	Central_Europe	48.31398	14.32776	250	9.2	18.5	-0.4		13.2	8.5	376.0	51.28
Egelsee_Attersee	18.05.2016	Lake_Pond	Central_Europe	47.83242	13.50433	624	8.2	17.1	-1.2	6.39E-01	15.0	7.8	252.0	47.88
Egelsee_Mondsee	17.05.2016	Lake_Pond	Central_Europe	47.79634	13.39688	481	8.0	16.9	-1.3	2.20E+00	10.4	7.7	336.0	46.05
EgelseeM2	03.05.2016	Lake_Pond	Central_Europe	47.96233	13.12470	592	8.2	16.9	-1.1	4.89E+00	13.0	8.0	478.0	84.26
Eibensee	17.05.2016	Lake_Pond	Central_Europe	47.80005	13.34293	952	6.9	15.6	-2.2	2.94E+00	8.4	8.1	244.0	27.63
Elch	04.08.2016	Lake_Pond	Lapland	70.48078	28.87066	326	-0.4	7.7	-7.2	8.50E-01	12.5	6.7	28.5	0.69
Elmsee	05.07.2006	Lake_Pond	Central_Europe	47.68623	13.96245	1630	1.0	8.9	-7.0	1.97E+00	17.1	8.1	123.0	27.67
Enz.4	23.05.2016	Lake_Pond	Central_Europe	47.17774	12.61940	1729	3.0	11.1	-5.5	2.00E-02	6.2	5.9	8.9	1.40
Enz.Main, sample 1	23.05.2016	Lake_Pond	Central_Europe	47.17795	12.61838	1730	3.0	11.1	-5.5	9.70E-02	13.8	5.5	11.9	0.19
Enz.Main, sample 2	28.05.2018	Lake_Pond	Central_Europe	47.17795	12.61838	1730	3.0	11.1	-5.5	9.70E-02	17.5	4.8	10.2	0.14
Feldsee	22.10.2015	Lake_Pond	Central_Europe	47.87164	8.03378	1116	4.8	12.0	-2.2	9.25E+00	9.2	7.5	18.7	2.35
Fels	10.07.2018	Lake_Pond	Central_Europe	47.26108	13.55498	1980	0.9	8.7	-6.9	2.50E-03	14.7	5.7	5.6	0.46
Fieb	17.08.2015	Lake_Pond	Lapland	70.54689	25.84572	59	-1.0	10.1	-11.2	3.80E-01	15.9	5.5	65.0	1.13
Filblingsee	30.06.2006	Lake_Pond	Central_Europe	47.78387	13.27619	1064	5.8	14.4	-3.2	1.28E+00	19.1	8.2	192.0	39.36
Fuschlsee	17.05.2016	Lake_Pond	Central_Europe	47.79726	13.29585	662	6.7	15.3	-2.4	2.65E+02	11.6	8.4	340.0	45.77
Gam1	16.08.2015	Lake_Pond	Lapland	71.05331	28.23086	27	1.4	8.7	-4.6	1.16E+00	11.1	7.7	165.0	1.66
Gerlos3	23.05.2016	Lake_Pond	Central_Europe	47.23525	12.15506	1650	2.9	10.9	-5.5	1.02E-02	17.1	4.6	14.1	0.19
Gerzinsel	28.06.2007	Lake_Pond	Central_Europe	47.45532	13.43741	1550	4.4	12.8	-4.4	7.00E-04	11.3	4.4	18.9	0.30
Grundlsee	28.06.2006	Lake_Pond	Central_Europe	47.62805	13.84672	708	4.0	12.5	-4.7	4.14E+02	13.9	8.1	180.0	38.54
Hallstaettersee	28.06.2006	Lake_Pond	Central_Europe	47.55984	13.67657	508	6.2	15.0	-3.0	8.55E+02	14.2	8.3	199.0	36.21
Hinterer Gosausee	29.06.2006	Lake_Pond	Central_Europe	47.50285	13.54884	1156	1.5	9.5	-6.6	2.56E+01	16.0	8.3	138.0	29.44
Hohl	20.10.2015	Lake_Pond	Central_Europe	48.70366	8.41889	987	6.9	14.9	-1.1	5.80E-01	6.2	4.4	32.0	0.75
Holzoestersee	25.09.2003	Lake_Pond	Central_Europe	48.05826	12.90026	463	8.3	17.0	-0.9	7.39E+00	17.9	8.6	308.0	53.31
Hunz	20.10.2015	Lake_Pond	Central_Europe	48.57475	8.34853	758	7.4	15.4	-0.8	7.50E-01	8.8	5.9	14.1	1.30
Hutt	31.07.2014	Lake_Pond	Central_Europe	47.07581	12.99057	1697	0.1	7.6	-7.4	2.00E-02	14.7	6.0	4.0	1.20
Ibm2	03.05.2016	Lake_Pond	Central_Europe	48.05462	12.96019	424	8.4	17.1	-0.9	1.00E-03	9.9	4.8	23.4	2.05
Inari2	08.08.2015	Lake_Pond	Lapland	68.93894	26.94767	160	-1.4	11.2	-13.5	5.60E-01	16.8	6.4	11.7	0.93
Irrsee	17.05.2016	Lake_Pond	Central_Europe	47.89159	13.31151	553	7.7	16.4	-1.6	3.55E+02	12.9	8.6	293.0	50.55
Jiri	25.09.2013	Lake_Pond	Central_Europe	48.61894	14.67253	895	5.2	13.9	-3.6	3.56E+00	9.9	6.4	43.3	4.24
Kaakku	21.08.2015	Lake_Pond	Lapland	66.78122	25.44906	98	0.2	13.2	-12.3	1.09E+00	20.5	5.8	6.9	0.69
Kaija	20.08.2015	Lake_Pond	Lapland	68.33044	24.10200	314	-2.3	11.2	-15.2	7.14E+00	20.3	6.8	14.3	1.47
Kara	18.08.2015	Lake_Pond	Lapland	69.38825	24.45947	304	-3.0	10.3	-15.5	6.10E-01	16.0	6.6	19.5	2.08
Lac de Creno, sample 1	16.07.2015	Lake_Pond	Corsica	42.20463	8.94588	1310	11.0	18.0	5.0	2.30E+00	25.8	6.6	43.3	1.87
Lac de Creno, sample 2	16.09.2016	Lake_Pond	Corsica	42.20463	8.94588	1310	11.0	18.0	5.0	2.30E+00	16.8	7.5	49.6	1.84
Lac de Melo, sample 1	13.07.2015	Lake_Pond	Corsica	42.21361	9.02344	1710	6.0	13.4	-0.1	6.50E+00	20.8	6.8	15.3	1.31
Lac de Melo, sample 2	22.09.2016	Lake_Pond	Corsica	42.21361	9.02344	1710	6.0	13.4	-0.1	6.50E+00	13.8	7.6	19.5	0.87
Latsch	16.06.2014	Lake_Pond	Central_Europe	47.07581	12.99057	1690	0.1	7.6	-7.4	1.00E-02	15.0	6.0	3.4	0.76
Leitensee	25.09.2003	Lake_Pond	Central_Europe	48.05883	12.96710	426	8.4	17.1	-0.9	9.84E+00	18.0	8.0	450.0	93.52
Loeckermoorsee	22.09.2009	Lake_Pond	Central_Europe	47.55441	13.54585	1391	3.9	12.3	-4.8	3.00E-02	13.6	4.2	26.7	0.30
Matthisleweiher	22.10.2015	Lake_Pond	Central_Europe	47.88514	8.08507	990	6.5	14.0	-1.1	1.64E+00	7.2	6.6	20.8	2.64
Mittersee	25.06.2006	Lake_Pond	Central_Europe	47.77300	13.44814	1370	4.6	13.1	-4.2	5.51E-01	n.d.	8.2	130.0	26.95
Moenichsee	25.06.2006	Lake_Pond	Central_Europe	47.76841	13.45378	1283	4.6	13.1	-4.2	2.89E+00	n.d.	8.3	157.0	28.26
Mondsee	17.05.2016	Lake_Pond	Central_Europe	47.82975	13.37609	483	8.0	16.9	-1.3	1.38E+03	11.8	8.3	350.0	48.56
Mumm	21.10.2015	Lake_Pond	Central_Europe	48.59680	8.20117	1037	7.1	14.9	-1.0	3.39E+00	7.0	7.0	39.7	3.41
Muodo	20.08.2015	Lake_Pond	Lapland	67.82692	23.16186	259	-1.6	11.9	-14.3	3.50E-01	17.2	5.7	10.0	1.25
Nickl1	02.02.2016	Lake_Pond	Central_Europe	47.78925	12.05854	475	8.5	17.3	-0.8	1.63E+01	6.0	4.7	28.0	0.92
Nino	14.07.2015	Lake_Pond	Corsica	42.25503	8.94043	1743	7.8	15.1	1.8	6.50E+00	24.6	7.5	30.5	2.73
NPozz1	14.09.2016	Lake_Pond	Corsica	42.25193	8.94696	1748	7.8	15.1	1.8	2.00E-02	18.7	7.8	33.7	1.09
Odin	14.08.2015	Lake_Pond	Lapland	70.55572	30.17058	85	1.5	8.8	-4.6	2.20E-01	13.7	6.9	47.6	0.44
Orient	21.09.2016	Lake_Pond	Corsica	42.22823	9.05897	2066	5.8	13.2	-0.2	1.14E+00	11.0	7.4	17.5	1.35
Ounas	31.07.2018	River	Lapland	66.79097	25.41733	86	0.2	13.2	-12.3		25.2	8.0	65.5	8.63
Ounas, technical replicate	31.07.2018	River	Lapland	66.79097	25.41733	86	0.2	13.2	-12.3		25.2	8.0	65.5	8.63
Pallasjaervi	31.07.2018	Lake_Pond	Lapland	68.03161	24.14961	278	-2.2	11.4	-15.1	1.70E+03	21.7	8.6	24.7	3.12
Pas1	12.08.2015	Lake_Pond	Lapland	69.44558	29.90306	63	1.0	10.1	-7.2	7.76E+00	15.9	5.9	27.4	2.03
Pas2, sample 1	12.08.2015	Lake_Pond	Lapland	69.50147	30.04969	84	0.7	9.6	-7.2	1.59E+00	16.5	6.2	29.5	2.52
Pas2, sample 2	05.08.2018	Lake_Pond	Lapland	69.50147	30.04969	84	0.7	9.6	-7.2	1.59E+00	19.1	6.5	25.4	1.61
Polo	20.08.2015	Lake_Pond	Lapland	67.65836	22.95736	302	-1.7	11.8	-14.4	1.27E+00	15.7	6.6	18.3	2.10
Pond Rossmoos	22.06.2006	Lake_Pond	Central_Europe	47.86786	13.47566	900	6.5	15.2	-2.6	3.10E-03	18.5	6.7	104.0	3.61
Pond-1, sample 1	27.08.2009	Lake_Pond	Central_Europe	47.73982	13.30167	1281	4.8	13.3	-4.0	6.35E-02	18.5	5.2	6.3	3.40
Pond-1, sample 2	29.10.2009	Lake_Pond	Central_Europe	47.73982	13.30167	1281	4.8	13.3	-4.0	6.35E-02	3.0	4.8	10.3	3.40
Pond-1, sample 3	15.06.2010	Lake_Pond	Central_Europe	47.73982	13.30167	1281	4.8	13.3	-4.0	6.35E-02	15.7	5.3	6.9	3.40
Pond-3, sample 1	25.06.2007	Lake_Pond	Central_Europe	47.73766	13.30236	1273	4.8	13.3	-4.0	2.09E-02	22.2	5.4	6.4	0.30
Pond-3, sample 2	23.07.2007	Lake_Pond	Central_Europe	47.73766	13.30236	1273	4.8	13.3	-4.0	2.09E-02	20.9	5.2	4.1	0.30
Pond-3, sample 3	03.11.2007	Lake_Pond	Central_Europe	47.73766	13.30236	1273	4.8	13.3	-4.0	2.09E-02	4.3	4.9	9.5	0.30
Ram, sample 1	01.06.2016	Lake_Pond	Central_Europe	47.89758	12.85235	452	8.7	17.4	-0.6	5.20E+00	17.1	6.1	31.5	5.91
Ram, sample 2	13.07.2016	Lake_Pond	Central_Europe	47.89758	12.85235	452	8.7	17.4	-0.6	5.20E+00	21.7	5.4	29.4	6.70
Ram, sample 3	30.09.2016	Lake_Pond	Central_Europe	47.89758	12.85235	452	8.7	17.4	-0.6	5.20E+00	21.1	5.5	28.8	5.11
Rama	09.08.2016	Lake_Pond	Lapland	69.41134	24.50204	348	-3.1	10.1	-15.7	2.39E+01	14.0	7.4	29.5	3.14
Reno	19.09.2016	Lake_Pond	Corsica	42.06383	9.13680	2051	6.1	13.6	0.0	2.61E-03	10.5	6.8	12.4	0.80
RePozz1	18.09.2016	Lake_Pond	Corsica	42.03356	9.14216	1783	6.6	14.1	0.5	3.00E-02	7.1	7.4	33.5	0.66
Roav1	11.08.2015	Lake_Pond	Lapland	69.47808	29.85533	125	0.6	9.7	-7.4	3.20E-01	18.6	6.7	33.5	2.43
Roav2, sample 1	11.08.2015	Lake_Pond	Lapland	69.48597	29.84117	182	0.6	9.7	-7.4	8.30E-01	16.5	5.8	19.4	0.78
Roav2, sample 2	06.08.2018	Lake_Pond	Lapland	69.48597	29.84117	182	0.6	9.7	-7.4	8.30E-01	16.8	6.2	14.9	0.46
Roav3	04.08.2016	Lake_Pond	Lapland	69.49040	29.83895	156	0.6	9.7	-7.4	8.68E+00	14.8	6.5	24.7	1.35
Roav4	06.08.2018	Lake_Pond	Lapland	69.49037	29.82632	174	0.7	9.8	-7.4	1.44E+00	17.7	6.4	18.8	0.91
Roav5	06.08.2018	Lake_Pond	Lapland	69.48740	29.82207	169	0.7	9.8	-7.4	1.00E+00	17.2	6.3	15.6	0.54
Rub	07.04.2016	Lake_Pond	Central_Europe	48.49975	14.85441	920	5.3	14.0	-3.5	1.75E+00	9.9	5.8	41.5	3.34
SaBePond1	18.07.2015	Lake_Pond	Central_Europe	46.49704	9.17225	2069	1.9	9.0	-4.8	4.00E-02	18.0	6.1	3.9	0.90
Safj	14.08.2015	Lake_Pond	Lapland	70.56206	30.20697	13	1.5	8.8	-4.6	1.00E-02	9.8	6.5	40.3	0.12
Schwarze Lacke Enz	30.06.2010	Lake_Pond	Central_Europe	47.16455	12.61700	1735	-1.5	5.8	-8.6	4.50E-01	14.5	6.8	11.3	2.51
Schwarzes Moos 1	18.05.2016	Lake_Pond	Central_Europe	48.87219	14.98022	507	7.6	16.5	-1.6	1.10E-01	15.6	4.8	45.7	3.94
Sommersbergsee	28.06.2006	Lake_Pond	Central_Europe	47.61920	13.73742	856	5.7	14.5	-3.4	2.00E+00	22.9	8.1	286.0	51.37
Stern	27.06.2016	Lake_Pond	Central_Europe	47.78275	12.04750	480	8.5	17.3	-0.8	6.31E+00	20.5	5.2	68.5	11.60
Stor3	13.08.2015	Lake_Pond	Lapland	70.54003	29.69150	211	0.4	8.1	-6.0	3.93E+00	12.4	8.0	118.9	9.76
Suisensee	25.06.2006	Lake_Pond	Central_Europe	47.77579	13.43879	1400	4.6	13.1	-4.2	1.14E-01	n.d.	8.2	128.0	30.10
Suoal	17.08.2015	Lake_Pond	Lapland	70.54800	25.85283	58	-1.0	10.1	-11.2	3.30E+00	14.5	5.2	75.2	0.96
Sving, sample 1	13.08.2015	Lake_Pond	Lapland	70.52593	29.62729	259	0.4	8.1	-6.1	1.76E+01	14.7	7.7	73.5	6.47
Sving, sample 2	05.08.2016	Lake_Pond	Lapland	70.52593	29.62729	259	0.4	8.1	-6.1	1.76E+01	13.1	7.9	73.3	4.95
Tana1	01.08.2018	River	Lapland	69.37293	24.38683	290	-3.0	10.2	-15.5		23.0	7.6	38.0	4.39
Tana2	05.08.2018	River	Lapland	70.15594	28.20871	2	0.6	9.5	-7.1		16.2	7.4	44.6	4.99
Traun	18.05.2016	River	Central_Europe	48.08901	13.87397	355	8.4	17.4	-1.0		13.1	8.5	386.0	49.76
Traunsee	23.06.2006	Lake_Pond	Central_Europe	47.89914	13.77062	422	8.1	17.1	-1.3	2.44E+03	15.8	8.4	319.0	46.56
Troeg7, sample 1	06.08.2008	Lake_Pond	Central_Europe	47.24994	13.26709	1960	1.9	9.8	-6.2	4.28E-02	19.4	5.5	5.1	0.93
Troeg7, sample 2	08.07.2018	Lake_Pond	Central_Europe	47.24994	13.26709	1960	1.9	9.8	-6.2	4.28E-02	13.8	5.7	3.2	0.93
Troeg8	06.08.2008	Lake_Pond	Central_Europe	47.24938	13.26557	1980	1.9	9.8	-6.2	3.35E-03	18.7	4.9	8.8	0.84
Tund1	16.08.2015	Lake_Pond	Lapland	70.90019	27.74119	278	-0.3	7.6	-6.8	6.46E+00	11.1	7.2	52.5	2.73
Tund3	08.08.2016	Lake_Pond	Lapland	70.87869	27.72131	276	-0.3	7.6	-6.8	1.41E+01	12.6	7.4	39.7	1.30
Tynn	16.08.2015	Lake_Pond	Lapland	70.60036	27.25928	80	-0.3	8.7	-8.1	5.07E+00	14.4	7.6	86.9	5.95
Unterer Klaffersee	04.08.2015	Lake_Pond	Central_Europe	47.29881	13.79432	2103	0.0	7.7	-7.6	3.90E+00	13.0	7.4	22.8	4.13
Wenger2	03.05.2016	Lake_Pond	Central_Europe	47.92521	13.17914	511	8.1	16.9	-1.1	5.00E-02	12.6	6.4	60.8	11.67
Wildensee	07.07.2006	Lake_Pond	Central_Europe	47.71273	13.85099	1535	3.2	11.5	-5.4	7.09E+00	17.7	8.3	130.0	17.22
Windgfaellweiher	22.10.2015	Lake_Pond	Central_Europe	47.85328	8.12417	965	6.0	13.4	-1.5	1.45E+01	9.2	7.2	69.7	5.17
Wolfgangsee	17.05.2016	Lake_Pond	Central_Europe	47.71999	13.48126	538	7.3	16.1	-1.9	1.28E+03	10.5	8.6	255.0	43.24

Suppl. Mat. Table S2. Reference strains, reference operational taxonomic units (refOTU, >98%) and environmental OTU (eOTU, >98%). The term refOTU refers to $OTU_{98\%}$ sharing with a cultured reference strain a priB sequence similarity of >98%. eOTU are $OTU_{98\%}$, which do not share with any cultured strain a priB sequence similarity >98%. The priB and genome accession numbers refer to the reference strains or reads mentioned in column 2. Reference OTU (refOTU) were named after one of the reference strains contained in the respective refOTU.

#	Reference Strain / read	$OTU_{98\%}$ (refOTU or eOTU)	*Polynucleobacter* Subcluster	Lifestyle	priB Accession Number	Genome Accession Number
1	15G-AUS-farblos	AP-Tund2-400A-C3	PnecC	free-living	MT988562	JACVOM000000000
2	30F-ANTBAC	30F-ANTBAC	PnecD	free-living	MT988563	JACVON000000000
3	31A-FELB	Species-Complex-4	PnecC	free-living	MT988564	JACVOO000000000
4	71A-WALBACH	71A-WALBACH	PnecC	free-living	MT988565	JACVOP000000000
5	73C-SIWE	Species-Complex-6	PnecC	free-living	MT988566	JACVOK000000000
6	78F-HAINBA	Species-Complex-4	PnecC	free-living	MT988567	JACVOQ000000000
7	80A-SIGWE	80A-SIGWE	PnecC	free-living	MT988568	JACVOR000000000
8	86C-FISCH	Species-Complex-1	PnecC	free-living	MT988569	JACVOL000000000
9	AM-25C3	AM-25C3	PnecC	free-living	MT988570	JACVOS000000000
10	AM-26B4	Species-Complex-5	PnecD	free-living	MT988571	JACVOT000000000
11	AM-7D1	MWH-T2W17	PnecC	free-living	MT988572	CP061319
12	AP-AinPum-20-C6	Polynucleobacter paneuropaeus	PnecC	free-living	MT988575	JAANHG000000000
13	AP-AinPum-60-G11	AP-AinPum-60-G11	PnecC	free-living	MT988576	CP061318
14	AP-Basta-1000A-D1	Species-Complex-7	PnecC	free-living	MT988577	JACVOU000000000
15	AP-Berg-20B-E5	AP-Titi-500A-B4	PnecC	free-living	MT988578	
16	AP-Blind-20-A3	AP-Blind-20-A3	PnecC	free-living	MT988579	
17	AP-Blind-40-E7	AP-Blind-40-E7	PnecC	free-living	MT988580	
18	AP-Cala-100A-B4	AP-Cala-100A-B4	PnecC	free-living	MT988581	
19	AP-Capit-er-40B-B5	Species-Complex-1	PnecC	free-living	MT988582	
20	AP-Creno-60-A2	MWH-Svant-W18	PnecC	free-living	MT988583	
21	AP-Elch-400A-B2	Species-Complex-7	PnecC	free-living	MT988584	CP061317
22	AP-Feld-500A-C5	UB-Siik-W21	PnecC	free-living	MT988585	
23	AP-Galz2-40-E9	Nonnen-W13	PnecC	free-living	MT988586	
24	AP-Holl-20A-A1	Nonnen-W13	PnecC	free-living	MT988587	
25	AP-Holl-20A-D6	Nonnen-W13	PnecC	free-living	MT988588	
26	AP-Hunz-20-A2	Polynucleobacter paneuropaeus	PnecC	free-living	MT988589	JAANGD000000000
27	AP-Ibm4-20-A2	AP-Ibm4-20-A2	PnecC	free-living	MT988590	
28	AP-Inari4-20-E4	MWH-Svant-W18	PnecC	free-living	MT988591	
29	AP-Inari4-40-G4	Polynucleobacter paneuropaeus	PnecC	free-living	MT988592	CP049645
30	AP-Jannik-300A-C4	MWH-T2W17	PnecC	free-living	MT988593	CP061316
31	AP-Kaivos-20-H2	Species-Complex-1	PnecC	free-living	MT988594	JACVOX000000000
32	AP-Kolm-20A-A1	Species-Complex-1	PnecC	free-living	MT988595	CP061315
33	AP-Latsch-80-C2	AP-Latsch-80-C2	PnecC	free-living	MT988596	JACVOY000000000
34	AP-Melu-1000-A1	Species-Complex-7	PnecC	free-living	MT988597	CP061314
35	AP-Melu-500A-A1	AP-Melu-500A-A1	PnecC	free-living	MT988599	JACVOZ000000000
36	AP-Mumm-500A-B3	AP-Mumm-500A-B3	PnecC	free-living	MT988600	JACVPA000000000
37	AP-Nickl2-20-E11	Species-Complex-1	PnecC	free-living	MT988601	
38	AP-Nino-20-F12-2016	MWH-Svant-W18	PnecC	free-living	MT988602	
39	AP-Nino-20-G2	AP-Nino-20-G2	PnecC	free-living	MT988603	CP061313
40	AP-Nino2y-300C-B4	AP-Nino2y-300C-B4	PnecC	free-living	MT988604	
41	AP-NPozz3-10B-F9	MG-52-Enon2-B4	PnecC	free-living	MT988605	
42	AP-Odin-400C-A2	Species-Complex-1	PnecC	free-living	MT988606	
43	AP-Peces-20-D6	Nonnen-W13	PnecC	free-living	MT988607	
44	AP-Peces-20-F8	Nonnen-W13	PnecC	free-living	MT988608	
45	AP-Rama-100A-F3	MG-5-Ahmo-C2	PnecC	free-living	MT988609	
46	AP-Reno-20A-C10	Species-Complex-1	PnecC	free-living	MT988610	
47	AP-RePozz3-20B-H2	AP-Nino-20-G2	PnecC	free-living	MT988611	
48	AP-Roav5-40-G5	Polynucleobacter paneuropaeus	PnecC	free-living	MT988612	CP049637
49	AP-Rub-40A-C1	Species-Complex-1	PnecC	free-living	MT988613	
50	AP-SaBe1-20-E8	Species-Complex-1	PnecC	free-living	MT988614	
51	AP-Spelu-300-2	AP-Spelu-300-2	PnecC	free-living	MT988615	
52	AP-Stechi-80A-B1	MG-5-Ahmo-C2	PnecC	free-living	MT988616	
53	AP-Stier-20B-B4	Nonnen-W13	PnecC	free-living	MT988617	
54	AP-Stier-20B-C3	Nonnen-W13	PnecC	free-living	MT988618	
55	AP-Sving-400A-A2	Species-Complex-7	PnecC	free-living	MT988619	CP061312
56	AP-Titi-500A-B4	AP-Titi-500A-B4	PnecC	free-living	MT988620	CP061311
57	AP-Titi-500C-D3	AP-Titi-500C-D3	PnecC	free-living	MT988621	
58	AP-Tund2-400A-C3	AP-Tund2-400A-C3	PnecC	free-living	MT988622	
59	AP-UKlaffer2-1000-D2	Species-Complex-7	PnecC	free-living	MT988623	
60	AP-Windg-500B-B2	AP-Windg-500B-B2	PnecC	free-living	MT988624	
61	CB	Ross2-W14	PnecC	free-living	MT988625	
62	Creno-II-W23	MG-5-Ahmo-C2	PnecC	free-living	MT988626	
63	Creno-IV-W18	UB-Siik-W21	PnecC	free-living	MT988627	
64	Creno-I-W12	Nonnen-W13	PnecC	free-living	MT988628	
65	CS-Geat-B5	Species-Complex-7	PnecC	free-living	MT988629	
66	CS-Geat-D5	JS-Mosq-20-D10	PnecC	free-living	MT988630	
67	CS-Kong-C6	Species-Complex-1	PnecC	free-living	MT988631	
68	CS-Odin-A6	CS-Odin-A6	PnecC	free-living	MT988632	JACVPD000000000
69	CS-Skans-A4	Species-Complex-1	PnecC	free-living	MT988633	
70	CS-Slettfy-B6	Species-Complex-7	PnecC	free-living	MT988634	
71	CS-Slettfy-D4	Species-Complex-1	PnecC	free-living	MT988635	

72	czRimov8-C6	Species-Complex-4	PnecC	free-living	MT988636	
73	Diet-1	Species-Complex-1	PnecC	free-living	MT988637	
74	Endosymbiont-Fsp1-4	Endosymbiont-Fsp1-4	PnecC	Endosymbiont	MT988638	LT615227
75	Endosymbiont-PPGSP-Eae1	Endosymbiont-PPGSP-Eae1	PnecC	Endosymbiont	MT988639	LT615228
76	Endosymbiont-PPGSP-Eae2	Endosymbiont-PPGSP-Eae2	PnecC	Endosymbiont	MT988640	LT606947
77	Endosymbiont-PPGSP-Eae3	Endosymbiont-PPGSP-Eae3	PnecC	Endosymbiont	MT988641	LT606948
78	Endosymbiont-PPGSP-Eae5	Endosymbiont-DACHS-Sophie	PnecC	Endosymbiont	MT988642	LT606950
79	Endosymbiont-PPGSP-Eda1	Endosymbiont-Alex-Bettina	PnecC	Endosymbiont	MT988643	LT606946
80	Endosymbiont-PPGSP-Eoc1	Endosymbiont-PPGSP-Eoc1	PnecC	Endosymbiont	MT988644	LT606951
81	Endosymbiont-PPGSP-Ewo1	Endosymbiont-PPGSP-Ewo1	PnecC	Endosymbiont	MT988645	LT606949
82	es-EL-1	es-EL-1	PnecC	free-living	MT988646	CP061310
83	es-GGE-1	UB-Siik-W21	PnecC	free-living	MT988647	JACVPE000000000
84	es-MAR-1	Species-Complex-4	PnecC	free-living	MT988648	
85	es-MAR-2	Species-Complex-1	PnecC	free-living	MT988649	CP061309
86	es-MAR-3	es-MAR-3	PnecC	free-living	MT988650	
87	es-MAR-4	es-MAR-4	PnecC	free-living	MT988651	JACVPF000000000
88	Eve-W11	Eve-W11	PnecC	free-living	MT988652	
89	FNE-F8-bin-6-1-PnecC	FNE-F8-FUKU	PnecC	free-living	MT988653	
90	Fuers-14	Species-Complex-1	PnecC	free-living	MT988654	JACVPG000000000
91	FUKU-SE-14	AP-Windg-500B-B2	PnecC	free-living	MT988655	
92	FUKU-SE-20	FUKU-SE-20	PnecC	free-living	MT988656	
93	Galti-A-W1	Galti-A-W1	PnecC	free-living	MT988657	
94	GWA2-45-21	Species-Complex-4	PnecC	free-living	MT988658	
95	JS-Aci1-20-B10	Polynucleobacter paneuropaeus	PnecC	free-living	MT988659	CP049628
96	JS-Fieb-80-E5	Species-Complex-1	PnecC	free-living	MT988660	JACVPH000000000
97	JS-Hess-40-B12	JS-Hess-40-B12	PnecC	free-living	MT988661	
98	JS-JIR24-3-B2	UB-Siik-W21	PnecC	free-living	MT988662	
99	JS-JIR-5-A7	UB-Siik-W21	PnecC	free-living	MT988663	CP061308
100	JS-Jiri-2-F12	AP-AinPum-60-G11	PnecC	free-living	MT988664	
101	JS-JIR-I-a15	JS-JIR-I-a15	PnecC	free-living	MT988665	
102	JS-JIR-I-c6	Galti-A-W1	PnecC	free-living	MT988666	
103	JS-JIR-II-b4	Species-Complex-1	PnecC	free-living	MT988667	CP061306
104	JS-JIR-II-c15	Galti-A-W1	PnecC	free-living	MT988668	
105	JS-JIR-III-a17	JS-JIR-III-a17	PnecC	free-living	MT988669	
106	JS-JIR-III-b11	Species-Complex-1	PnecC	free-living	MT988670	
107	JS-Kaija-80-A5	Species-Complex-2	PnecC	free-living	MT988671	
108	JS-Mosq-20-D10	JS-Mosq-20-D10	PnecC	free-living	MT988672	CP061305
109	JS-Mosq-20-F4	UB-Siik-W21	PnecC	free-living	MT988673	
110	JS-Polo-80-F4	Species-Complex-1	PnecC	free-living	MT988674	JACVPI000000000
111	JS-Safj-400b-B2	Species-Complex-1	PnecC	free-living	MT988675	JACVPJ000000000
112	JS-Stechi-80-C2	Species-Complex-2	PnecC	free-living	MT988676	
113	JS-Tund1-600a-C4	JS-Tund1-600a-C4	PnecC	free-living	MT988677	
114	JS-Ukon-20-H6	Species-Complex-1	PnecC	free-living	MT988678	
115	JS-Vitta2-80-B10	Species-Complex-2	PnecC	free-living	MT988679	
116	Klost2-W16	Species-Complex-1	PnecC	free-living	MT988680	
117	Landm-A-W12	Landm-A-W12	PnecC	free-living	MT988681	
118	Latsch14-1	Latsch14-1	PnecC	free-living	MT988682	
119	MG-27-Goln-C1	Galti-A-W1	PnecC	free-living	MT988685	JACVPM000000000
120	MG-27-Goln-C3	MG-27-Goln-C3	PnecC	free-living	MT988686	
121	MG-42-Nopo-D3	Species-Complex-7	PnecC	free-living	MT988687	
122	MG-43-Slet-D2	MG-43-Slet-D2	PnecC	free-living	MT988688	
123	MG-43-Slet-F1	MG-43-Slet-F1	PnecC	free-living	MT988689	
124	MG-4-Mela-E1	QLW-P1DATA-2	PnecC	free-living	MT988690	
125	MG-4-Mela-F2	AP-Blind-20-A3	PnecC	free-living	MT988691	
126	MG-52-Enon2-B4	MG-52-Enon2-B4	PnecC	free-living	MT988692	
127	MG-58-Narv-B1	MG-52-Enon2-B4	PnecC	free-living	MT988693	
128	MG-5-Ahmo-C2	MG-5-Ahmo-C2	PnecC	free-living	MT988694	CP061304
129	MG-63-Kera-F1	MG-63-Kera-F1	PnecC	free-living	MT988695	
130	MG-65-Swed-E4	Species-Complex-1	PnecC	free-living	MT988696	
131	MG-7-Harju-B2	MG-52-Enon2-B4	PnecC	free-living	MT988697	
132	MG-Unter2-18	Species-Complex-3	PnecC	free-living	MT988698	CP061302
133	MWH-Adler-W8	Species-Complex-1	PnecC	free-living	MT988700	LZFI00000000
134	MWH-Aito-300-X1	Species-Complex-1	PnecC	free-living	MT988701	
135	MWH-Aito-300-X4	Galti-A-W1	PnecC	free-living	MT988702	
136	MWH-Aus1W21	AP-Spelu-300-2	PnecC	free-living	MT988703	
137	MWH-Aus1W7	Species-Complex-1	PnecC	free-living	MT988704	
138	MWH-Berg-3C6	MWH-Berg-3C6	PnecC	free-living	MT988705	JACVPN000000000
139	MWH-Braz-FAM2G	MWH-Braz-FAM2G	PnecC	free-living	MT988706	CP061300
140	MWH-Braz-FAM2Ja2	MWH-Braz-FAM2Ja2	PnecC	free-living	MT988707	
141	MWH-Braz-FAM4A	MWH-Braz-FAM4A	PnecC	free-living	MT988708	
142	MWH-CaK5	Species-Complex-5	PnecD	free-living	MT988709	CP061299
143	MWH-Creno-3A4	MWH-Svant-W18	PnecC	free-living	MT988710	JACXSA000000000
144	MWH-Creno-4B5	Species-Complex-1	PnecC	free-living	MT988711	
145	MWH-EgelM2-3	Species-Complex-6	PnecC	free-living	MT988713	
146	MWH-Hall2	Species-Complex-6	PnecC	free-living	MT988715	
147	MWH-Hall5	MWH-Hall5	PnecC	free-living	MT988716	
148	MWH-HuK1	MWH-HuK1	PnecC	free-living	MT988717	JACVPP000000000
149	MWH-Jannik1A5	MWH-Jannik1A5	PnecC	free-living	MT988720	JACVPQ000000000
150	MWH-Jannik3A3	MWH-T2W17	PnecC	free-living	MT988721	
151	MWH-Jannik3D5	Polynucleobacter yangtzensis	PnecC	free-living	MT988722	
152	MWH-K35W1	Polynucleobacter aenigmaticus	PnecC	free-living	MT988723	NGUO00000000
153	MWH-Lacke-12-2	Species-Complex-1	PnecC	free-living	MT988724	

154	MWH-Lett3-08W15	Species-Complex-1	PnecC	free-living	MT988725	
155	MWH-LF2-54b	MWH-LF2-54b	PnecC	free-living	MT988726	
156	MWH-Loch1C5	MWH-Loch1C5	PnecD	free-living	MT988727	JACVPR000000000
157	MWH-Mekk-B1	MWH-Mekk-B1	PnecC	free-living	MT988728	JACVPS000000000
158	MWH-Mekk-C3	Species-Complex-2	PnecC	free-living	MT988729	CP061298
159	MWH-Mlynsky-W10	Nonnen-W13	PnecC	free-living	MT988730	
160	MWH-MoIso1	MWH-MoIso1	PnecC	free-living	MT988731	CP061297
161	MWH-MoK7	MWH-MoK7	PnecC	free-living	MT988734	
162	MWH-NZ4W10	AP-AinPum-60-G11	PnecC	free-living	MT988735	
163	MWH-NZ4W4	Species-Complex-4	PnecC	free-living	MT988736	
164	MWH-NZ4W7a	Nonnen-W13	PnecC	free-living	MT988737	
165	MWH-NZ7W17	Species-Complex-4	PnecC	free-living	MT988738	
166	MWH-P3-07-1	Species-Complex-2	PnecC	free-living	MT988739	CP061296
167	MWH-PoolBr-B5	Species-Complex-1	PnecC	free-living	MT988740	
168	MWH-RechtKol4	Polynucleobacter asymbioticus amplus	PnecC	free-living	MT988574	CP015017
169	MWH-S4W17	Species-Complex-1	PnecC	free-living	MT988742	CP061295
170	MWH-Salz1-W7	JS-JIR-I-a15	PnecC	free-living	MT988743	
171	MWH-Spelu-300-X3	MWH-Spelu-300-X3	PnecD	free-living	MT988744	
172	MWH-Svant-W18	MWH-Svant-W18	PnecC	free-living	MT988745	CP061293
173	MWH-T1W11	MWH-T2W17	PnecC	free-living	MT988746	
174	MWH-T2W17	MWH-T2W17	PnecC	free-living	MT988747	
175	MWH-Teich-2B6	Species-Complex-1	PnecC	free-living	MT988748	
176	MWH-UH11A	MWH-HuK1	PnecC	free-living	MT988749	
177	MWH-UH14B	MWH-UH14B	PnecC	free-living	MT988750	
178	MWH-UH14E	MWH-UH2A	PnecC	free-living	MT988751	
179	MWH-UH19D	MWH-UH14B	PnecC	free-living	MT988752	
180	MWH-UH21F	MWH-UH21F	PnecC	free-living	MT988754	
181	MWH-UH21G	MWH-UH21G	PnecC	free-living	MT988755	
182	MWH-UH23A	MWH-UH23A	PnecC	free-living	MT988756	
183	MWH-UH23C	AP-Spelu-300-2	PnecC	free-living	MT988757	
184	MWH-UH24A	MWH-UH24A	PnecB2	free-living	MT988758	CP061292
185	MWH-UH25C	MWH-UH21B	PnecC	free-living	MT988759	
186	MWH-UH25E	MWH-UH25E	PnecC	free-living	MT988760	
187	MWH-UH2A	MWH-UH2A	PnecC	free-living	MT988761	
188	MWH-UH35A	MWH-UH35A	PnecC	free-living	MT988762	
189	MWH-UH36A	MWH-UH14B	PnecC	free-living	MT988763	
190	MWH-UH38A	Species-Complex-1	PnecC	free-living	MT988764	
191	MWH-UH38Ckl	MWH-UH38Ckl	PnecC	free-living	MT988765	
192	MWH-UK1W16	Polynucleobacter paneuropaeus	PnecC	free-living	MT988766	QMCG00000000
193	Nino-II-W8	MWH-Svant-W18	PnecC	free-living	MT988769	
194	Nino-IV-W14	Nonnen-W13	PnecC	free-living	MT988770	
195	Nonnen-W13	Nonnen-W13	PnecC	free-living	MT988771	JACVPU000000000
196	Nonnen-W15	UB-Siik-W21	PnecC	free-living	MT988772	
197	MWH-PoolGreenA3	Polynucleobacter acidiphobus	PnecB2	free-living	MT988741	CP023277
198	LimPoW16	LimPoW16	PnecC	free-living	MT988683	CP028941
199	UK-Long2-W17	UK-Long2-W17	PnecC	free-living	MT988803	CP028940
200	QLW-P1DMWA-1	Polynucleobacter asymbioticus simplex	PnecC	free-living	MT988781	CP000655
201	MWH-Feld-100	Polynucleobacter campilacus	PnecC	free-living	MT988714	NGUP00000000
202	MWH-MoIso2	Species-Complex-5	PnecD	free-living	MT988732	NJGG00000000
203	AM-8B5	Polynucleobacter difficilis	PnecB1	free-living	MT988573	CP023276
204	MWH-MoK4	Species-Complex-3	PnecC	free-living	MT988733	CP007501
205	MWH-EgelM1-30-B4	Species-Complex-6	PnecC	free-living	MT988712	NAIA00000000
206	AP-Melu-1000-B4	Polynucleobacter meluiroseus	PnecC	free-living	MT988598	OANS00000000
207	STIR1 (Endosymbiont)	Polynucleobacter necessarius	PnecC	Endosymbiont	MT988784	NC_010531
208	MG-25-Pas1-D2	Polynucleobacter paneuropaeus	PnecC	free-living	MT988684	CP030085
209	MT-CBb6A5	Polynucleobacter rarus	PnecA	free-living	MT988699	NTGB00000000
210	MWH-HuW1	Polynucleobacter sinensis	PnecC	free-living	MT988718	LOJJ00000000
211	MWH-Weng1-1	Polynucleobacter sphagniphilus	PnecC	free-living	MT988768	MPIY00000000
212	MWH-UH21B	MWH-UH21B	PnecC	free-living	MT988753	CP028942
213	MWH-VikM1	Polynucleobacter victoriensis	PnecD	free-living	MT988767	
214	QLW-P1FAT50C-4	Polynucleobacter wuianus	PnecC	free-living	MT988774	CP015922
215	MWH-JaK3	Polynucleobacter yangtzensis	PnecC	free-living	MT988719	LOJI00000000
216	QLW-P1DATA-2	QLW-P1DATA-2	PnecC	free-living	MT988773	LZMQ00000000
217	QLW-P2FAT50C-1	AP-Blind-20-A3	PnecC	free-living	MT988775	
218	Ross1-W21	Species-Complex-1	PnecC	free-living	MT988776	
219	Ross1-W9	Species-Complex-1	PnecC	free-living	MT988777	JACVPW000000000
220	Ross2-W14	Ross2-W14	PnecC	free-living	MT988778	
221	Ross6-W10	Ross6-W10	PnecC	free-living	MT988779	
222	SCGC-AAA027-C02	ncmg	PnecC	free-living	MT988780	
223	SM1-W1	Species-Complex-1	PnecC	free-living	MT988782	
224	SP-AinPum-045-mR2A-3A	Species-Complex-1	PnecC	free-living	MT988783	
225	Tro7-14-1	Latsch14-1	PnecC	free-living	MT988785	
226	Tro8-14-1	Species-Complex-1	PnecC	free-living	MT988786	JACVPX000000000
227	Tro8F10W12	Species-Complex-1	PnecC	free-living	MT988787	
228	Trout-Bog-Hypolimnion-pan-assembly	Trout-Bog	PnecC	free-living	MT988788	
229	TSB-Sco08W16	TSB-Sco08W16	PnecC	free-living	MT988789	CP061291
230	TSB-Sco09W6	MG-5-Ahmo-C2	PnecC	free-living	MT988790	
231	UB-Chica-W24	LimPoW16	PnecC	free-living	MT988791	
232	UB-Domo-W1	UB-Domo-W1	PnecC	free-living	MT988792	PGTX00000000
233	UB-Kamb-W5	MWH-UH14B	PnecC	free-living	MT988793	
234	UB-Kamb-W7a	UB-Kamb-W7a	PnecC	free-living	MT988794	
235	UB-Kamb-W7b	UB-Kamb-W7b	PnecC	free-living	MT988795	

236	UB-Piko-W3	UB-Piko-W3	PnecC	free-living	MT988796	JACVPY000000000
237	UB-Siik-W21	UB-Siik-W21	PnecC	free-living	MT988797	CP061289
238	UB-Somero-W24	UB-Domo-W1	PnecC	free-living	MT988798	
239	UB-Tiil-W10	Species-Complex-1	PnecC	free-living	MT988799	JACVPZ000000000
240	UK3W23Kol1	UK3W23Kol1	PnecC	free-living	MT988800	
241	UK-Gri1-W3	Species-Complex-1	PnecC	free-living	MT988801	JACVQA000000000
242	UK-Kesae-W10	UK-Kesae-W10	PnecC	free-living	MT988802	JACVQB000000000
243	UK-Mo-2m-Kol15	UK-Mo-2m-Kol15	PnecC	free-living	MT988804	JACVQC000000000
244	UK-Mo-2m-Kol30	UK-Mo-2m-Kol30	PnecC	free-living	MT988805	
245	UK-Piela-W13	UB-Siik-W21	PnecC	free-living	MT988806	
246	UK-Piela-W22	UK-Piela-W22	PnecC	free-living	MT988807	
247	UK-Pondora-W13	UK-Pondora-W13	PnecC	free-living	MT988808	
248	UK-Pondora-W15	UK-Pondora-W15	PnecC	free-living	MT988809	JACVQD000000000
249	UK-Ruja-W24	UB-Siik-W21	PnecC	free-living	MT988810	
250	UK-Tiil2-W2	Species-Complex-1	PnecC	free-living	MT988811	
251	VK13	VK13	PnecA	free-living	MT988812	
252	VK25	Species-Complex-1	PnecC	free-living	MT988813	CP061288
253	VK50	Species-Complex-1	PnecC	free-living	MT988814	
254	VK65	Species-Complex-1	PnecC	free-living	MT988815	
255	687f09b49fb046710dcb0ea04b0389f5	eOTU0001	PnecC		MT988816	
256	c12c0dce79d78b6b14a5993834635788	eOTU0002	PnecC		MT988817	
257	a3827ec34e720937162779a2fb9d8d2c	eOTU0003	PnecC		MT988818	
258	c57b74085a9d509d8ace347885dffce3	eOTU0004	PnecC		MT988819	
259	e75ca2ee70a42d31fe76e0492e6fea6d	eOTU0005	PnecC		MT988820	
260	f8d7757735b57c9f0d7798b31271a266	eOTU0006	PnecC		MT988821	
261	5a007b457d79197df6570f93f28f88ed	eOTU0007	PnecC		MT988822	
262	262821f7ed7a71ee2c5c07de27f62f5e	eOTU0008	PnecC		MT988823	
263	3c2ba3a1bcb631c26a1a6bfc74ef917f	eOTU0009	PnecC		MT988824	
264	4a68c44f9ff1a403a995945112b1b9e1	eOTU0010	PnecC		MT988825	
265	42cd91fb135df2aeb0fd4a19e41aa35c	eOTU0011	PnecC		MT988826	
266	2326290d523535efaa199246d0da1076	eOTU0012	PnecC		MT988827	
267	c220ba572ceccdd05b909a772e1e10b5	eOTU0013	PnecC		MT988828	
268	553de0ab7280e01d0b2486236990a86d	eOTU0014	PnecC		MT988829	
269	7a066b8faa8fe0a701cb411bdcb032a0	eOTU0015	PnecC		MT988830	
270	bace9c36c82bb1eb9825dadcb99285ee	eOTU0016	PnecC		MT988831	
271	090607ee7a3acde99e757da2b7f77d95	eOTU0017	PnecC		MT988832	
272	a645c9aedda824d508a0a04735afd748	eOTU0018	PnecC		MT988833	
273	af2832af6cdc4bc717425e04f45349f7	eOTU0019	PnecC		MT988834	
274	6937b407d678cb0f8b85e4fd7ccc4886	eOTU0020	PnecC		MT988835	
275	ab3f1e417f495ef97423e70d92378326	eOTU0021	PnecC		MT988836	
276	a2a7a1853abe7aa83307631d46ceb05b	eOTU0022	PnecC		MT988837	
277	58d617cd537617465217614435c87199	eOTU0023	PnecC		MT988838	
278	fcabf9885aecdccf231ebb05bcfa2a37	eOTU0024	PnecC		MT988839	
279	1b7ef7152aacfea74b135ee3330cc22c	eOTU0025	PnecC		MT988840	
280	204a86feae61b05bf5af39af4085614e	eOTU0026	PnecC		MT988841	
281	0f2dc8409a7be84565a6f82c318753ab	eOTU0027	PnecC		MT988842	
282	f55f49b14c3c97ac59c0fed99364df00	eOTU0028	PnecC		MT988843	
283	b9374496f82af5aa3ed09d946aa24ec2	eOTU0029	PnecC		MT988844	
284	c36a83a181672b31059dd0a28dd9247f	eOTU0030	PnecC		MT988845	
285	a4f9499cbe24918d7df334fce57ecc54	eOTU0031	PnecC		MT988846	
286	52b6324d2305b1a5661daef61c1f473d	eOTU0032	PnecC		MT988847	
287	393fc12371babaef3421646d47ed56cc	eOTU0033	PnecC		MT988848	
288	606d84607c16ad6f6f6c27c7d4bb98f7	eOTU0034	PnecC		MT988849	
289	7a829642098c2ffc094b271c62feeaa9	eOTU0035	PnecC		MT988850	
290	81f0152d40b637b046e217159cf36098	eOTU0036	PnecC		MT988851	
291	feac96371c6c3f53790417e695b0d38f	eOTU0037	PnecC		MT988852	
292	cc984779219d2c4b565aeee0a0335550	eOTU0038	PnecC		MT988853	
293	c643918e5dc8e9ff816a77fc7d2117f3	eOTU0039	PnecC		MT988854	
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728	26aa8b40755dfac6e5e568b260c37dbd	eOTU0474	PnecC	MT989289
729	2c6a03ea1e02eb2354a4547ccd7c8282	eOTU0475	PnecB1	MT989290
730	4d7ef9e87bd1dabd6f4bb51a3369b0cc	eOTU0476	PnecC	MT989291
731	53668e2aa290ee7683cd765fd60bef63	eOTU0477	PnecC	MT989292
732	6e089e70cab621772b79aa05bba46b2e	eOTU0478	PnecC	MT989293
733	7addfe432da3badf92b9e6c73241d95d	eOTU0479	PnecC	MT989294
734	d824fea75d67994a5f383211691f28e3	eOTU0480	PnecC	MT989295
735	dcdba3b17957f0b99e4954a912bf54f3	eOTU0481	PnecC	MT989296
736	ddec905eddbda2365aeaa0b73821d3e8	eOTU0482	PnecB1	MT989297
737	e6ebbf44720cfbffbc812eed98900cec	eOTU0483	PnecC	MT989298
738	06261f86f92b3a822b09bcfb87a0a0ff	eOTU0484	PnecC	MT989299
739	0aa136ac2f4fd629f0ca041bdfcb8641	eOTU0485	PnecC	MT989300
740	1538fada88fc99739a4c119d313d4784	eOTU0486	PnecC	MT989301
741	28a05f2b2671937546b540cffe597d1d	eOTU0487	PnecC	MT989302
742	39008eb4ca6995e96827ef198c30b33e	eOTU0488	PnecC	MT989303
743	52b4fcd51af392713a655b093e98ea0b	eOTU0489	PnecB1	MT989304
744	6fc987d9bda76d98586d05259b4f691d	eOTU0490	PnecC	MT989305
745	8f3e02cb7f496d7bcca01a3f330aa406	eOTU0491	PnecC	MT989306
746	91df0877ab5b4e42b44e9b6ebefb566d	eOTU0492	PnecC	MT989307
747	97b387797cdb4ca87593736dad922f65	eOTU0493	PnecC	MT989308
748	a042e0324d339592f5b48a64bcf9a491	eOTU0494	PnecB1	MT989309
749	bbb1a2fe829dccaeccf95059ac60c96e	eOTU0495	PnecB1	MT989310
750	dd4fceb29a2997d85af85a26d2dfaeef	eOTU0496	PnecC	MT989311
751	1362bd1abe445a43efc78b64b480c6b5	eOTU0497	PnecC	MT989312
752	178b658c2af7fa2e83baf2a3f7e5c51e	eOTU0498	PnecC	MT989313
753	44e2c3049369bea0e7b1ff1aa50f46d8	eOTU0499	PnecC	MT989314
754	53ecf4967daf3ca15a2fe2dc8070182d	eOTU0500	PnecC	MT989315
755	549f135f4426b83c4106d54af6ae3c78	eOTU0501	PnecB1	MT989316
756	654734264c4c1702583e5042faa6ae0b	eOTU0502	PnecC	MT989317
757	7cf9757f0a3c30e0bbf428f0ee9c7da0	eOTU0503	PnecC	MT989318
758	7e8fe8db3b8cc6be1727f5dba7c5b922	eOTU0504	PnecC	MT989319
759	b257f3a6557df9df232d1455ec3a1a66	eOTU0505	PnecC	MT989320
760	b65e03e274401d53bf5b80dd36831013	eOTU0506	PnecC	MT989321
761	c9a339c759dcf0dd58920f76aa49aa74	eOTU0507	PnecB1	MT989322
762	e47f3adf34a78fe64b3ad204b4a4d9e5	eOTU0508	PnecC	MT989323
763	f2a757678969decf47ad77276eff6401	eOTU0509	PnecC	MT989324
764	176575d3c69b46285584eedf9264c980	eOTU0510	PnecC	MT989325
765	23c760b7568018c1f2b721fa35ac29d5	eOTU0511	PnecC	MT989326
766	39350c5973f66a40449c1e51b434af60	eOTU0512	PnecC	MT989327
767	87dcbceef849afb415bcad58ffce857d	eOTU0513	PnecC	MT989328
768	9bde41995886312ff409c8e2420179d8	eOTU0514	PnecC	MT989329
769	a3bc5528434cb0c399e8cb5664bc0605	eOTU0515	PnecB1	MT989330
770	d9170bddf5c1233610c60a38475961ee	eOTU0516	PnecC	MT989331
771	dcd95b82f9e037ed20a046a695baec85	eOTU0517	PnecC	MT989332
772	e0ca185a60b352db40c3d7cce32e945a	eOTU0518	PnecC	MT989333
773	e4dc13f59527cbf7aa3bd8f5e4936cb8	eOTU0519	PnecB1	MT989334
774	f2ec76d12de7f56359568ff6eb099cad	eOTU0520	PnecB1	MT989335
775	fbe033771e5269704275b540465d41b9	eOTU0521	PnecB1	MT989336

Suppl. Mat. Table S3. Mantel tests and partial Mantel tests (Spearman rank correlations, 9999 permutations) were used to analyse if dissimilarity between *Polynucleobacter* communities increase with geographic distance or with differences in pH or other environmental or climatic variables. Environmental distances represent Euclidean distances calculated by the dist() function of the vegan package.
All environmental data but pH were log-transformed prior to distance calculations. BC, Bray-Curtis dissimilarity; BC_lowCa, only low Ca^{2+} samples; geoDist, geographic distance; pHDist, pH distance between samples; CaDist, Ca^{2+} concentration distance between samples; envDist, distance based on four environmental variables§, EnvGeoClimDist, distance based on geographic, climatic and environmental variables§§.

Test	No control	Control for pH (pHDist)	Control for Ca^{2+} (CaDist)	Control for envDist§	Control for EnvGeoClimDist§§	Control for geoDist
BC vs geoDist	r = -0.0132 p = 0.6553	r = 0.0081 p = 0.3727	r = 0.0463 p = 0.0531	r = 0.0110 p = 0.3411	r = -0.0596 p = 0.9687	
BC_lowCa vs geoDist	r = 0.1237 p = 2e-04	r = 0.0728 p = 0.0073	r: 0.1283 p = 1e-04	r = 0.1102 p = 5e-04	r = 0.0514 p = 0.0602	
BC vs pHDist	r = 0.5267 p = 1e-04		r = 0.4725 p = 1e-04			r = 0.5266 p = 1e-04
BC vs CaDist	r = 0.3722 p =1e-04	r = 0.2722 p = 1e-04				r = 0.3745 p = 1e-04
BC vs envDist§	r = 0.3552 p = 1e-04					r = 0.3551 p = 1e-04
BC vs EnvGeoClimDist§§	r =0.1837 p = 2e-04					r = 0.1463 p = 0.003

§ Ca^{2+}, pH, Abs_{250nm}, Na^{+}
§§ geographic distance, altitude, climate, envDist, habitat type

p > 0.05
P < 0.01
p < 0.001