Supplementary Information

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Complete ecological isolation and cryptic diversity in *Polynucleobacter* bacteria not resolved by 16S rRNA gene sequences

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Suppl. Mat. Table S1. Strains, their affiliation with *Polynucleobacter* lineages, and accession numbers for ribosomal and genome sequences. ND, not determined

Strain	Lineage[§]	Habitat of Origin	Type of Habitat	Accession number			Reference
				SSU rRNA and/or ITS	glnA	**Genome**	
QLW-P1DMWA-1	F10	Pond-1	acidic pond	AJ879783	FN823098	CP000655	(Hahn et al 2005); (Hahn et al 2012)
P1-05-13	F10	Pond-1	acidic pond	HE646561	HE646447	ND	(Hahn et al 2012)
MWH-RechtKolB	F10	Pond Rechteckteich	acidic pond	FN429668	FN823145	ND	(Jezbera et al 2011)
QLW-P1FAT50C-4	F4	Pond-1	acidic pond	AJ879778	FN823104	ND	(Hahn et al 2005)
QLW-P1DATA-2	F5	Pond-1	acidic pond	AJ879801	FN823102	ND	(Hahn et al 2005)
MWH-UK1W16	F15	Lake Unterer Klaffersee	circum-neutral softwater lake	AM110086	FN823097	ND	(Jezbera et al 2011)
MWH-MoK4	F17	Lake Mondsee	alkaline hardwater lake	AJ550654	FN823084	CP007501	(Hahn 2003)
MWH-MoK7	F17	Lake Mondsee	alkaline hardwater lake	AJ550673	FN823083	ND	(Hahn 2003)
MWH-MoIso1	-[§§]	Lake Mondsee	alkaline hardwater lake	AJ550671	FN823082	ND	(Hahn 2003)

[§] presence of a diagnostic lineage-specific sequence in the 16S-23S ITS

[§§] closely related to lineage F17 but lacking the diagnostic sequence identifying F17 strains

Supplementary Materials Table S2. Parameters characterizing the habitats of origin of strains compared in ecophysiological experiments, as well as the garden pond used for two transplantation experiments. The mentioned detections refer to investigations using a cultivation independent method (Jezbera et al 2011).

| Parameter	Lake Mondsee	Garden pond	Pond-1
Geographic coordinates	N 47.841° E13.367°	N 47.861° E 13.340°	N 47.740° E 13.302°
pH	8.6	7.8	5.2
Conductivity (μS cm^{-1})	306	190	8.6
F10 lineage detected	-	-	+
F17 lineage detected	+	+	-

Suppl. Mat. Table S3. Compared *Polynucleobacter* genomes. The genome sequence of Beta proteobacterium SCGC AAA027-C02 was obtained by single cell genome sequencing and represents most likely an incomplete genome. The genome of the obligately endosymbiotic strain STIR1 is known for erosive genome evolution (Boscaro et al 2013).

Strain	DNA scaffolds	Genome Size (Mbp)	Gene Count	GC (%)	RefSeq / IMG Genome ID	Reference
Polynucleobacter QLW-P1DMWA-1	1	2.16	2136	45	NC_009379.1	(Meincke et al 2012)
Polynucleobacter MWH-MoK4	1	2.03	2107	45	NZ_CP007501.1	This study
Beta proteobacterium CB	1	2.05	2152	46	NC_020417.1	(Hao et al 2013)
Polynucleobacter necessarius STIR1 (Endosymbiont)	1	1.56	1782	46	NC_010531.1	(Boscaro et al 2013)
Polynucleobacter sp. FNE-F8	20	2.33	2394	45	2596583565	(Garcia et al 2013)
Beta proteobacterium SCGC AAA027-C02	63	1.30	1444	44	NZ_ATZV00000000.1	(Tatusova et al 2014)

Supplementary Materials Figure S1. 16S rRNA gene tree depicting the phylogenetic position of the six genome-sequenced PnecC strains (see Suppl. Mat. Table S3).
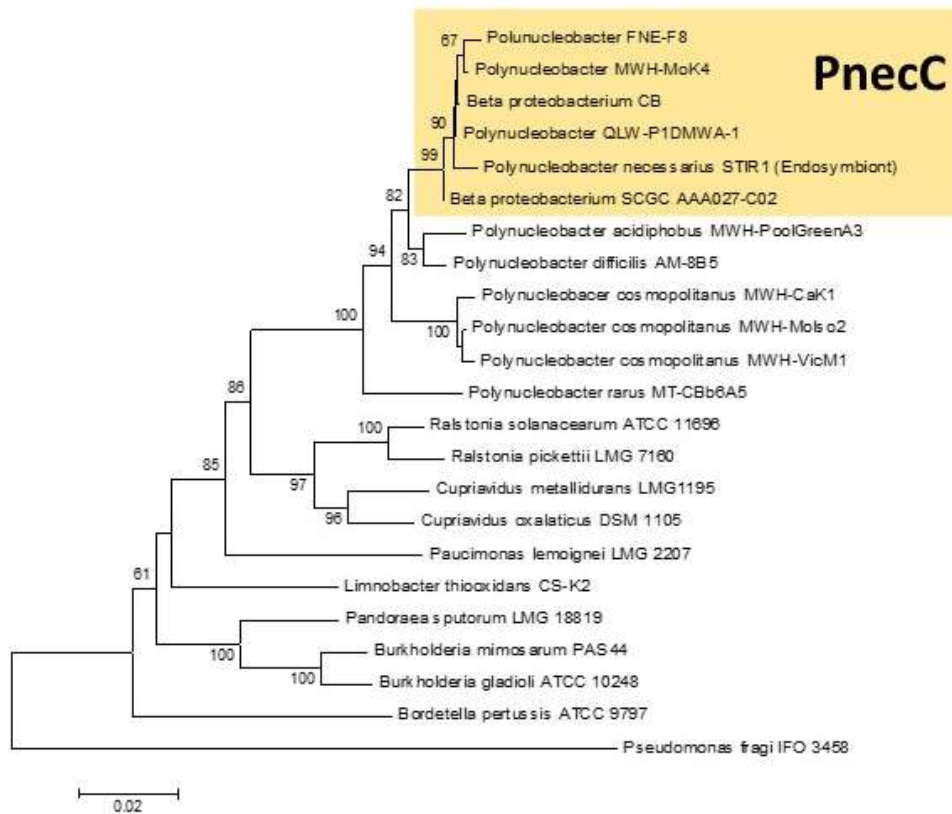
Supplementary Materials Figure S2. Detection of feoB (Fe^{2+}) and ABC type Fe^{3+} transporter genes (fpbB) in PnecC strains isolated from habitats differing in pH values by PCR.
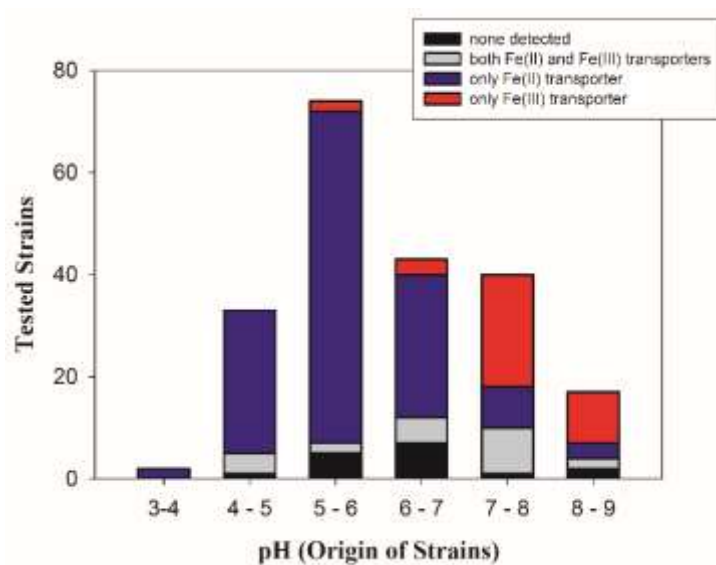
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