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Polynucleobacter sphagniphilus sp. nov. a planktonic freshwater bacterium isolated from an acidic and humic freshwater habitat

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Abstract

Strain MWH-Weng1-1^T, isolated from an acidic freshwater habitat located in the Wenger bog, Austria, was characterized by investigation of phenotypic, chemotaxonomic and genomic traits. Phylogenetic analyses based on 16S rRNA gene sequences placed the strain in the cryptic species complex PnecC within the genus *Polynucleobacter*. The strain has a genome of 2.04 Mbp with a G +C content of 45.6 mol%. The major fatty acids of the strain were $C_{16:1}$ ω7c, $C_{16:0}$, and $C_{18:1}$ ω7c. In order to resolve the systematic position of the strain within the species complex PnecC, concatenated partial sequences of eight housekeeping genes were used for phylogenetic analyses. The obtained trees did not place strain MWH-Weng1-1^T close to any of the six previously described species within this cryptic species complex. Pairwise whole genome average nucleotide identity (gANI) comparisons with genome sequences of strains representing the six previously described species of the subcluster resulted throughout in values < 78%, which clearly suggested that strain MWH-Weng1-1^T (DSM 24018^T =CIP 111099^T) represents a novel species. We propose the name *Polynucleobacter sphagniphilus* sp. nov. and strain MWH-Weng1-1^T as the type strain for this new species.

Keywords

Polynucleobacter; cryptic species complex; *Burkholderiaceae*; genome; whole genome average nucleotide identity (gANI); freshwater

The genus *Polynucleobacter* and the species *Polynucleobacter necessarius* were described by Klaus Heckmann and Helmut J. Schmidt [1] for bacterial endosymbionts of benthic freshwater ciliates affiliated with the genus *Euplotes*. Later, it was discovered that free-living strains, closely related to endosymbiotic strains, represent important planktonic freshwater bacteria [2–5]. Analyses of 16S rRNA gene sequences suggested that the genus

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Conflicts of interest
The authors declare the absence of any conflict of interest.

Ethical statement
The presented study does not include any experimental work with humans or vertebrates.

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Polynucleobacter can be subdivided in at least four subclusters designated PnecA, PnecB, PnecC, and PnecD [4]. Subcluster PnecC, which currently includes the endosymbiotic *P. necessarius* and five free-living species, was recognized as being a cryptic species complex, which means that this seemingly very species-rich subcluster cannot be resolved by 16S rRNA gene phylogenies [6, 7]. Taxonomic research on strains affiliated with subcluster PnecC is hampered by the lack of a type strain of *P. necessarius* or another pure culture representing this endosymbiotic specie [6]. Reference material of the type species of the genus was contained in the *Euplotes aediculatus* 'stock 15' culture (=E24 =ATCC 30859) (Heckmann and Schmidt, 1987), which is unfortunately not any more available. However, recently, it was shown [6] that the lost endosymbionts used for the species description are well represented by another endosymbiotic *P. necessarius* strain, i.e. STIR1, for which a complete genome sequence is available [8].

Here we describe strain MWH-Weng1-1^T affiliated with subcluster PnecC, which was isolated from a small acidic freshwater pond, and propose to establish for this strain the species name *P. sphagniphilus* sp. nov.

Strain MWH-Weng1-1^T was isolated from an acidic water body located at the margins of Wenger bog (approximate geographic coordinates 47.93 N 13.18 E) near Salzburg in Austria. The sampled water had a pH of 4.0, a conductivity of 63.4 µS cm^{-1}, and a temperature of 5.8 °C. The water was strongly stained by humic substances (OD_{250nm} of 0.2µm-filtered water of 1.77). The strain was isolated by using the filtration-acclimatization method and NSY medium [9, 10].

Cells of strain MWH-Weng1-1^T are short rods of small size (Table 1). The strain forms small circular, convex, colourless colonies with shiny surface on NSY agar plates. Growth at different temperatures and growth under anoxic conditions in an anaerobic chamber were examined by using NSY agar plates as described previously [11]. Salinity (NaCl) tolerance was determined using NSY agar supplemented with various NaCl concentrations as described previously [11]. The strain showed no anaerobic growth, grew at temperatures up to 31°C and tolerated salt concentrations up to 0.4% (Table 1).

Utilization of various substrates was investigated in the same way as for previously described *Polynucleobacter* species [11–15]. Briefly, growth enabled by utilization of a specific substrate was determined by comparison of OD at 575 nm established in liquid one tenth-strength NSY medium (0.3 g l^{-1}) with and without 0.5 g l^{-1} test substrate, respectively. Differences of < 10 %, 10–50% and >50% of the OD obtained in the test treatments compared to the OD obtained without test substrate (i.e. in 0.3 g l^{-1} NSY medium) were scored after 10 days of growth as no utilization (-), weak utilization (w) and good utilization (+), respectively (Table 1).

The analysis of the whole-cell fatty acid composition (Table 2) was carried out as described previously [12]. Biomass was harvested from cultures on R2A agar plates, which were inoculated with 1 ml cell suspension, incubated while keeping the agar surface moist and inspected for growth daily, starting the third day after inoculation. Once a biomass film was well visible, the cell mass was harvested.

Genome sequencing and analyses were performed for further characterization of strain MWH-Weng1-1^T. DNA used for sequencing was extracted from biomass grown in liquid NSY medium as described previously [16]. A fragment library was mate-pair sequenced by a Roche GS FLX system by using Titanium chemistry (Beckman Coulter Genomics SA, Grenoble, France). Sequencing resulted in 237 963 reads and *de novo* assembly of 234 897 reads by using the software MIRA [17] resulted in 20 contigs with a sequencing coverage of 38x. Three gaps could be closed by PCR amplification of gap regions and subsequent Sanger sequencing of amplicons. These efforts resulted in a reduction of the contig number to seventeen. The obtained genome sequence has a total length of 2.04 Mbp and a G+C content of 45.6 mol% (Table 3). The genome sequence was annotated using the IMG/ER annotation pipeline [18], as well as by the NCBI Prokaryotic Genome Annotation Pipeline. The annotation obtained by the latter pipeline was deposited in DDBJ/EMBL/GenBank (Accession Number MPIY01000000), while the analyses presented here refer to the IMG annotation (IMG genome ID 2574179738). Gene finding resulted in 2103 protein coding and 49 RNA genes.

Strain MWH-Weng1-1^T shares with the five other free-living type strains representing species affiliated with subcluster PnecC a genome size in the range of 2.0-2.3 Mbp (Table 3), as well as a G+C value of DNA of about 45%. On the other hand, the genomes of the six free-living type strains differ largely in gene content (Table 4). Interestingly, regarding the listed selected accessory genes, the gene content of strain MWH-Weng1-1^T is most similar to *P. wuianus* QLW-P1FAT50C-4^T and a little less similar to *P. asymbioticus* QLW-P1DMWA-1^T, but much more distinct from the genomes of the type strains of the other three free-living species affiliated with subcluster PnecC. The genomes of strains MWH-Weng1-1^T and QLW-P1FAT50C-4^T differ only in two of the gene content features considered in Table 4, but the former genome differed in absence/presence from the genome of strain MWH-MoK4^T in 12 of the 16 considered accessory genes and gene clusters. It is worth mentioning that the two genomes most similar in gene content to strain MWH-Weng1-1^T share with this strains the origin from acidic freshwater systems, while the other three type strains considered in Table 4 were isolated from alkaline freshwater systems.

The partial glutamine synthetase gene (glnA) and the 16S-23S internal transcribed spacer (ITS) sequences of strain MWH-Weng1-1^T determined by previous PCR amplicon sequencing (accession numbers FN823191 and FN429716, respectively) and by genome sequencing were identical, respectively.

Phylogenetic reconstructions based on 16S rRNA gene sequences place strain MWH-Weng1-1^T in subcluster PnecC [4] of the genus *Polynucleobacter* (data not shown). As reported previously, strains affiliated with this species complex share 16S rRNA genes with sequence similarities ≥ 99%. In order to better resolve the phylogenetic position of strain MWH-Weng1-1^T a tree based on concatenated multilocus sequences was calculated (Fig. 1). Partial sequences of eight loci (rpoB, trpE, icdA, glnA, mdh, fbp, msbA, and gyrA) defined previously [6] were extracted from genome sequences (Table 3), concatenated and aligned by using the software MEGA7 [19]. This resulted in a total alignment length of 6359 bp. The same software was used for calculation of neighbour-joining (NJ), maximum likelihood and maximum parsimony trees (Fig. 1). In previous pairwise comparisons, sequence

similarity values of these concatenated sequences correlated well with average nucleotide identify (ANI) values of whole genome sequences [6]. This suggests that this locus selection is somehow representative for the entire genomes. The phylogenetic reconstruction based on this multilocus sequence set also placed strain MWH-Weng1-1^T in subcluster PnecC but did not suggest that the strain is affiliated with any previously described species.

Polynucleobacter strains affiliated with subcluster PnecC are known to either be obligate endosymbionts of ciliates [1, 20] or free-living organisms possessing a planktonic lifestyle [6, 21]. Due to the isolation of strain MWH-Weng1-1^T from a small freshwater system, due to its ability to grow in artificial media in absence of a potential host [20], and due to the lack of a large number of pseudogenes in the genome of the strain [8], it is assumed that this strain represents a free-living bacterium with a planktonic lifestyle. Cultivation-independent investigations on 56 European freshwater systems obtained priB sequences identical with the reference sequence of strain MWH-Weng1-1^T with high copy numbers from several acidic systems but no sequences or only very small copy numbers from all investigated alkaline systems (Huemer, A., Schmidt, J. & M.W. Hahn, unpublished data). Habitats in which the taxon represented by strain MWH-Weng1-1^T was detected with high abundance usually represented small bog ponds or humic lakes receiving water from neighbouring bogs. Typically, these habitats were characterized by the presence of peat moss (*Sphagnum* spp.) forming floating mats along the shore lines (Schwingrasen) and/or occurring in the catchment with high abundance. The pattern of environmental detection of the taxon suggests that the strain and the taxon represented by the strain are adapted to acidic freshwater systems. These detections fit very well to the environmental conditions in the home habitat of the type strain. A preference of strain MWH-Weng1-1^T for acidic habitats is also suggested by the presence of genes putatively encoding a Fe(II) transporter (Table 4) and the absence of genes encoding Fe(III) transporters (Hahn et al., 2016b). Furthermore, the above mentioned much higher similarity in gene content between the three PnecC strains obtained from acidic systems suggests a common adaptation to acidic environments.

We tested if strain MWH-Weng1-1^T has to be considered to be affiliated with one of the five previously described free-living species affiliated with subcluster PnecC [22] by performing average nucleotide identity (gANI) analyses with whole genome sequences by using the IMG/ER system [18]. This resulted in all pairwise comparisons in gANI values of 76.7 - 77.1% (Table 5), which suggests that the strain is not affiliated with any of these four species [23–27]. A comparison of strain MWH-Weng1-1^T with the type material of the species *P. necessarius* cannot be performed due to the lack of a genome sequence or pure genomic DNA of the endosymbionts, however, previous investigations suggested that the genome sequence of the endosymbiont *P. necessarius* STIR1 has to be considered to share a highly similar genome sequence with the endosymbiont described as *P. necessarius* [6]. An ANI comparison of the genomes of MWH-Weng1-1^T and STIR1 resulted in an ANI value of 79.6%, which is very similar to those values obtained for the four free-living type strains (Fig. 1). Thus, strain MWH-Weng1-1^T has to be considered to represent a new species affiliated with subcluster PnecC of the genus *Polynucleobacter*.

Strain MWH-Weng1-1^T can be discriminated from the type strains of *Polynucleobacter* species not affiliated with subcluster PnecC by chemotaxonomic traits. As for other species

affiliated with subcluster PnecC, strain MWH-Weng1-1^T can be discriminated from the type strains of *P. rarus* [14], *P. acidiphobus* [15] and *P. difficilis* [12] based on the G+C content of their DNA [6]. The discrimination of strain MWH-Weng1-1^T from *P. cosmopolitanus* is possible by the absence of the fatty acid $C_{12:0}$3-OH [13], which was so far only found in *Polynucleobacter* strains affiliated with the species *P. cosmopolitanus*. Furthermore, strain MWH-Weng1-1^T, like all other PnecC strains, contains the signature sequence 5'-GAGCCGGTGTTTCTTCCC-3' at *Escherichia coli* position 445–463 of the 16S rRNA, which is absent in *Polynucleobacter* strains not affiliated with this subcluster [6]. A feature distinguishing strain MWH-Weng1-1^T from all previously described type strains of species affiliated with subcluster PnecC is the combination of negative results in assimilation tests with both oxaloacetate and L-aspartate (Table 1). The other type strains showed at least for one of these two substrates positive growth. Furthermore, strain MWH-Weng1-1^T is the only type strain within subcluster PnecC with a clearly positive result on growth with D-galacturonic acid.

Description of *Polynucleobacter sphagniphilus* sp. nov

Polynucleobacter sphagniphilus (sphag.ni'phi.lus. N.L. m. *Sphagnum*, generic name of sphagnum moss; N.L. adj. philus -a -um (from Gr. adj. philos -ê -on), friend, loving; N.L. masc. adj. *sphagniphilus*, *Sphagnum*-loving).

Contains free-living *Polynucleobacter* strains dwelling in the water body of acidic freshwater systems frequently characterized by *Sphagnum* moss growing along the shore lines and the terrestrial neighbourhood.

Cells are short sometimes slightly curved rods, 0.65-1.0 μm in length and 0.3–0.5 μm in width, depending on cultivation conditions. Chemo-organotrophic, aerobic, anaerobic growth was not observed. Colonies grown on NSY agar are non-pigmented, circular and convex with smooth surface. Growth occurs up to 31 °C and in 0–0.4% (w/v) NaCl. Assimilates acetate, pyruvate, malonate, malate, fumarate, succinate, D-galacturonic acid, L-glutamate, and L-alanine. Does not assimilate glycolate, glyoxylate, oxalate, oxaloacetate, citrate, D-glucose, D-galactose, D-lyxose, L-leucine, L-histidine, L-aspartate, L-asparagine, L-serine, or betaine. Major fatty acids of the strain are $C_{16:1}$ ω7c, $C_{16:0}$, and $C_{18:1}$ ω7c. The type strain is MWH-Weng1-1^T (=DSM 24018^T = CIP 111099^T), which was isolated from a small acidic bog pond located in Austria. The genome of the type strain is characterized by a size of 2.04 Mbp and a G+C content of 45.6 mol%. Genome and 16S rRNA gene sequences characterizing the type strain are available under the accession MPIY01000000.

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Abbreviations

gANI	whole genome average nucleotide identity
NJ	neighbour-joining
OD	optical density

References

1. Heckmann K, Schmidt HJ. *Polynucleobacter necessarius* gen. nov., sp. nov., an obligately endosymbiotic bacterium living in the cytoplasm of *Euplotes aediculatus*. Int J Syst Bacteriol. 1987; 37(4):456–7.
2. Bahr M, Hobbie JE, Sogin ML. Bacterial diversity in an arctic lake: A freshwater SAR11 cluster. Aquat Microb Ecol. 1996; 11(3):271–7.
3. Zwart G, Crump BC, Agterveld M, Hagen F, Han SK. Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol. 2002; 28(2):141–55.
4. Hahn MW. Isolation of strains belonging to the cosmopolitan *Polynucleobacter necessarius* cluster from freshwater habitats located in three climatic zones. Appl Environ Microbiol. 2003; 69(9): 5248–54. [PubMed: 12957910]
5. Percent SF, Frischer ME, Vescio PA, Duffy EB, Milano V, McLellan M, et al. Bacterial community structure of acid-impacted lakes: What controls diversity? Appl Environ Microbiol. 2008; 74(6): 1856–68. [PubMed: 18245245]
6. Hahn MW, Schmidt J, Pitt A, Taipale SJ, Lang E. Reclassification of four *Polynucleobacter necessarius* strains as *Polynucleobacter asymbioticus* comb. nov., *Polynucleobacter duraquae* sp. nov., *Polynucleobacter yangtzensis* sp. nov., and *Polynucleobacter sinensis* sp. nov., and emended description of the species *Polynucleobacter necessarius*. Int J Syst Evol Microbiol. 2016; 66:2883–92. [PubMed: 27064460]
7. Hahn MW, Jezberová J, Koll U, Saueressig-Beck T, Schmidt J. Complete ecological isolation and cryptic diversity in *Polynucleobacter* bacteria not resolved by 16S rRNA gene sequences. ISME J. 2016; 10:1642–55. [PubMed: 26943621]
8. Boscaro V, Felletti M, Vannini C, Ackerman MS, Chain PSG, Malfatti S, et al. *Polynucleobacter necessarius*, a model for genome reduction in both free-living and symbiotic bacteria. Proc Natl Acad Sci USA. 2013; 110(46):18590–5. [PubMed: 24167248]
9. Hahn MW, Stadler P, Wu QL, Pockl M. The filtration-acclimatization method for isolation of an important fraction of the not readily cultivable bacteria. J Microbiol Methods. 2004; 57(3):379–90. [PubMed: 15134885]
10. Hahn MW, Pockl M, Wu QL. Low intraspecific diversity in a *Polynucleobacter* subcluster population numerically dominating bacterioplankton of a freshwater pond. Appl Environ Microbiol. 2005; 71(8):4539–47. [PubMed: 16085847]
11. Hahn MW, Lang E, Brandt U, Wu QL, Scheuerl T. Emended description of the genus *Polynucleobacter* and the species *Polynucleobacter necessarius* and proposal of two subspecies, *P. necessarius* subsp. *necessarius* subsp. nov. and *P. necessarius* subsp. *asymbioticus* subsp. nov. Int J Syst Evol Microbiol. 2009; 59:2002–9. [PubMed: 19567561]
12. Hahn MW, Minasyan A, Lang E, Koll U, Sproer C. *Polynucleobacter difficilis* sp. nov., a planktonic freshwater bacterium affiliated with subcluster B1 of the genus *Polynucleobacter*. Int J Syst Evol Microbiol. 2012; 62:376–83. [PubMed: 21441373]
13. Hahn MW, Lang E, Brandt U, Luensdorf H, Wu QL, Stackebrandt E. *Polynucleobacter cosmopolitanus* sp. nov., free-living planktonic bacteria inhabiting freshwater lakes and rivers. Int J Syst Evol Microbiol. 2010; 60:166–73. [PubMed: 19648339]
14. Hahn MW, Lang E, Tarao M, Brandt U. *Polynucleobacter rarus* sp. nov., a free-living planktonic bacterium isolated from an acidic lake. Int J Syst Evol Microbiol. 2011; 61:781–7. [PubMed: 20435748]

15. Hahn MW, Lang E, Brandt U, Sproeer C. *Polynucleobacter acidiphobus* sp. nov., a representative of an abundant group of planktonic freshwater bacteria. Int J Syst Evol Microbiol. 2011; 61:788–94. [PubMed: 20435747]

16. Meincke L, Copeland A, Lapidus A, Lucas S, Berry KW, Del Rio TG, et al. Complete genome sequence of *Polynucleobacter necessarius* subsp. *asymbioticus* type strain (QLW-P1DMWA-1^T). Stand Genomic Sci. 2012; 6(1):74–83. [PubMed: 22675600]

17. Chevreux B, Pfisterer T, Drescher B, Driesel AJ, Muller WE, Wetter T, et al. Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. Genome Res. 2004; 14(6):1147–59. [PubMed: 15140833]

18. Markowitz VM, Chen IMA, Palaniappan K, Chu K, Szeto E, Grechkin Y, et al. IMG: the integrated microbial genomes database and comparative analysis system. Nucleic Acids Res. 2012; 40(D1):D115–D22. [PubMed: 22194640]

19. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016; 33:1870–4. [PubMed: 27004904]

20. Vannini C, Poeckl M, Petroni G, Wu QL, Lang E, Stackebrandt E, et al. Endosymbiosis in statu nascendi: close phylogenetic relationship between obligately endosymbiotic and obligately free-living *Polynucleobacter* strains (*Betaproteobacteria*). Environ Microbiol. 2007; 9(2):347–59. [PubMed: 17222133]

21. Wu QL, Hahn MW. Differences in structure and dynamics of *Polynucleobacter* communities in a temperate and a subtropical lake, revealed at three phylogenetic levels. FEMS Microbiol Ecol. 2006; 57(1):67–79. [PubMed: 16819951]

22. Hahn MW, Huymann LR, Koll U, Schmidt J, Lang E, Hoetzinger M. *Polynucleobacter wuianus* sp. nov., a free-living freshwater bacterium affiliated with the cryptic species complex PnecC. Int J Syst Evol Microbiol. 2017; 67(2):379–85. [PubMed: 27902302]

23. Kim M, Oh H-S, Park S-C, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol. 2014; 64(Pt 2):346–51. [PubMed: 24505072]

24. Konstantinidis K, Tiedje J. Genomic insights that advance the species definition for prokaryotes. Proc Natl Acad Sci USA. 2005; 102:2567–72. [PubMed: 15701695]

25. Konstantinidis KT, Ramette A, Tiedje JM. The bacterial species definition in the genomic era. Philosophical Transactions of the Royal Society B: Biological Sciences. 2006; 361(1475):1929–40.

26. Konstantinidis KT, Tiedje JM. Prokaryotic taxonomy and phylogeny in the genomic era: advancements and challenges ahead. Curr Opin Microbiol. 2007; 10(5):504–9. [PubMed: 17923431]

27. Rosselló-Móra R, Amann R. Past and future species definitions for Bacteria and Archaea. Syst Appl Microbiol. 2015; 38(4):209–16. [PubMed: 25747618]

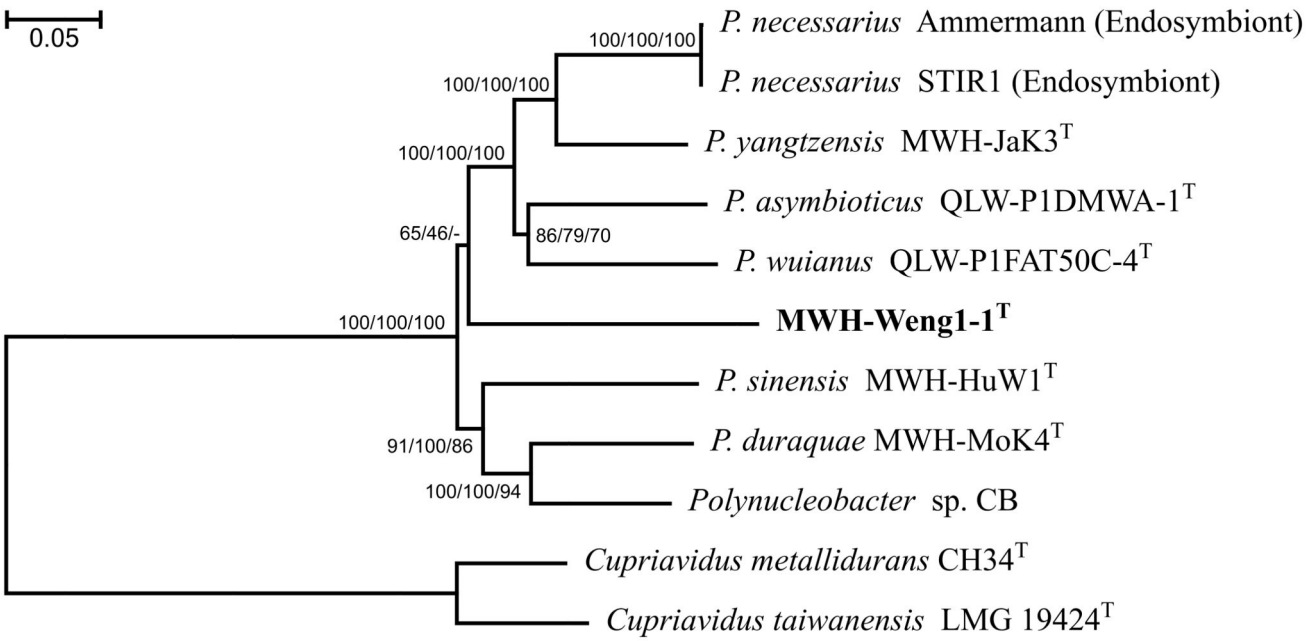

Fig. 1.

Phylogenetic position of strain MWH-Weng1-1^T. Neighbour-joining (NJ) tree calculated with concatenated multilocus sequences of eight protein-encoding genes. The tree was rooted with sequences of two *Cupriavidus* strains. All included *Polynucleobacter* strains are affiliated with subcluster PnecC. Bootstrap values are shown from left to right for NJ, maximum likelihood, and maximum parsimony trees calculated with the same sequence set.

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Table 1

Traits characterizing the six *Polynucleobacter* type strains affiliated with subcluster PnecC. All strains have the following characteristics in common. Motility not detectable; assimilation of acetic acid, pyruvic acid, and succinic acid; no assimilation of glycolic acid, oxalic acid, citric acid, and L-serine. 1, *P. sphagniphilus* sp. nov. MWH-Weng1-1^T; 2, *P. wuianus* QLW-P1FAT50C-4^T; 3, *P. asymbioticus* QLW-P1DMWA-1^T; 4, *P. duraquae* MWH-MoK4^T; 5, *P. sinensis* MWH-HuW1^T; 6, *P. yangtzensis* MWH-JaK3^T. +, increase in optical density (OD); w, weak increase in OD; -, no significant increase in OD.

	1	2	3	4	5	6
Cell morphology	short rods	short rods	short rods	curved rods	short curved rods	short rods
Cell length (µm)	0.6-1.0	0.6-1.7	0.7-1.2	0.9-2.9	0.6-1.4	0.5-1.5
Cell width (µm)	0.3-0.5	0.3-0.6	0.4-0.5	0.4-0.5	0.4-0.5	0.3-0.5
Temperature range of growth (°C)	5 - 31	5 - 34	5 - 34(w)	5 - 30	5 - 35	5 - 35
NaCl tolerance (%NaCl, w/v)	0 - 0.4	0 - 0.5	0 - 0.5(w)	0 - 0.3	0 - 0.5	0 - 0.3(w)
Anaerobic growth	-	-	+	-	-	+
Assimilation of:						
Glyoxylic acid	-	**w**	w	-	-	-
Propionic acid	**w**	**+**	+	-	+	+
Malonic acid	+	**w**	w	-	+	w
Oxaloacetic acid	-	**+**	-	+	+	+
Malic acid	+	**+**	+	w	+	+
Fumaric acid	+	**+**	+	w	+	+
Levulinic acid	**w**	w	w	-	-	-
D-Galacturonic acid	+	w	w	w	w	w
D-Mannose	w	-	w	-	-	-
D-Glucose	-	-	w	w	-	-
D-Galactose	-	-	w	-	-	-
D-Lyxose	-	-	w	w	-	w
D-Fructose	w	w	w	w	-	w
L-Fucose	w	-	w	-	-	w
D-Sorbitol	w	-	w	-	-	-
L-Glutamate	+	**+**	+	-	+	-
L-Histidine	-	**+**	*n.d.*	*n.d.*	*n.d.*	*n.d.*
L-Aspartate	-	**+**	+	-	-	-
L-Cysteine	+	w	+	+	w	+
L-Alanine	+	**+**	w	-	-	-
L-Asparagine	-	**w**	w	-	-	-
L-Leucine	-	-	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Betaine	-	-	w	-	-	-

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Table 2

Major fatty acid compositions of *Polynucleobacter* strains representing subcluster PnecC. Strains were grown on R2A agar. Data for strains QLW-P1DMWA-1^T, MWH-HuW1^T, MWH-JaK3^T were taken from [11], data for MWH-MoK4^T from Hahn *et al.* (2016b), and data for QLW-P1FAT50C-4^T from Hahn *et al.* (2017).

Fatty acid	MWH-Weng1-1^T DSM 24018^T	QLW-P1FAT50C-4^T DSM 24008^T	QLW-P1DMWA-1^T DSM 18221^T	MWH-MoK4^T DSM 21495^T	MWH-HuW1^T DSM 21492^T	MWH-JaK3^T DSM 21493^T
$C_{12:0}$	4.4	3.3	3.4	3.8	5.5	3.7
$C_{14:0}$	1.4	0.3	0.9	0.3	0.3	1.2
$C_{15:0}$	-	0.2	0.3	-	0.3	-
$C_{16:0}$	26.2	18.6	22.2	15.9	29.6	15.5
$C_{17:0}$	-	-	-	-	0.5	-
$C_{18:0}$	0.9	0.5	1.2	0.5	2.4	0.5
$C_{20:0}$	-	-	1.1	-	-	-
$C_{14:1}$ ω5c	0.5	-	-	-	0.2	0.6
$C_{15:1}$ ω6c	-	-	-	-	0.6	-
$C_{16:1}$ ω5c	0.4	0.4	0.9	0.4	-	0.4
$C_{16:1}$ ω7c	35.8	40.8	41.3	38.6	45.0	35.6
$C_{18:1}$ ω9c	-	-	-	0.3	0.4	-
$C_{18:1}$ ω7c	15.1	20.4	12.9	19.8	1.1	20.4
11-methyl $C_{18:1}$ ω7c	4.3	4.6	3.1	4.2	1.1	8.1
$C_{12:0}$ 2-OH	1.9	0.3	2.5	1.3	1.3	2.2
$C_{16:1}$ 2-OH	0.5	1.5	-	1.8	-	v
Feature 1	1.3	-	0.4	-	1.0	0.5
Feature 2	7.35	7.1	9.6	11.9	9.9	9.2
Feature 7	-	1.2	0.4	-	0.3	2.0

Summed features represent groups of two fatty acids which could not be separated by GLC and the MIDI system, such as summed feature 2 containing $C_{16:1}$ isoI and $C_{14:0}$-3OH and summed feature 7 containing $C_{19:1}$ ω6c and an unknown compound with an ECL of 18.846, or represent compounds with uncertain identity such as summed feature 1 (equivalent chain length 10.921) probably containing modified $C_{12:0}$.

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Table 3

Genome characteristics of the investigated *Polynucleobacter* strains.

Species	Strain	Life style	Genome size (Mbp)	Scaffolds	G+C content (mol%)	DDBJ/EMBL/GenBank accession number	Reference
P. sphagniphilus sp. nov.	MWH-Weng1-1^T (=DSM 24018^T)	FL	2.04	17	45.6	MPIY01000000	This study
P. wuianus	QLW-P1FAT50C-4^T (=DSM 24008^T)	FL	2.23	1	44.9	CP015922	Hahn *et al.*, in press
P. asymbioticus	QLW-P1DMWA-1^T (=DSM 18221^T)	FL	2.16	1	44.8	CP000655	Meincke *et al.*, 2012
P. duraquae	MWH-MoK4^T (=DSM 21495^T)	FL	2.03	1	45.2	CP007501	Hahn *et al.*, 2016b
P. sinensis	MWH-HuW1^T (=DSM 21492^T)	FL	2.32	19	45.5	LOJJ01000000	Hahn *et al.*, 2016a
P. yangtzensis	MWH-JaK3^T (=DSM 21493^T)	FL	2.05	42	45.4	LOJI01000000	Hahn *et al.*, 2016a
P. necessarius	STIR1 [host, *Euplotes aediculatus*]	E	1.56	1	45.6	CP001010	Boscaro *et al.*, 2013

FL, free-living; E, endosymbiotic.

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Table 4

Comparison of the presence and absence of selected genes.

Genes putatively encoding	*P. sphagniphilus* sp. nov. MWH-Weng1-1^T	*P. wuianus* QLW-P1FAT50C-4^T	*P. asymbioticus* QLW-P1DMWA-1^T	*P. duraquae* MWH-MoK4^T	*P. sinensis* MWH-HuW1^T	*P. yangtzensis* MWH-JaK3^T
Inorganic nutrients						
ABC-type Fe^{3+} transport system	-	-	-	+	+	+
feoAB genes (uptake of Fe^{2+})	+	+	+	-	+	+
ABC-type Nitrate/Nitrite/Cyanate transporter	+	+	+	-	-	+
Nitrate reductase (assimilatory)	+	+	+	-	-	+
Nitrite reductase (assimilatory)	+	+	+	-	-	+
Cyanate lyase (releases NH_3 and CO_2 from cyanate)	+	+	+	-	-	+
Urease and ABC-type urease transporter	+	+	+	-	-	-
Oxidative phosphorylation/Energy metabolism						
Cytochrome bd-I terminal oxidase (CydAB)	+	+	+	-	+	-
Fumarate reductase	+	+	-	+	-	+
Carbon monoxide dehydrogenase	+	+	-	2 clusters	-	+
Acetate permease actP	+	-	+	-	-	-
Anoxygenic photosynthesis						
Photosynthesis gene cluster	-	+	-	+	-	-
Motility						
Flagella genes	-	-	-	+	-	-
Oxidative stress						
Catalase	1 gene	-	2 genes	-	-	1 gene
Other						
Cellulose synthase operon protein C	-	-	+	-	-	-
Cellulose synthase catalytic subunit [UDP-forming]	-	-	+	-	-	-

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Table 5

Whole genome Average Nucleotide Identity (gANI) values of strain MWH-Weng1-1^T with the genomes of the five free-living type strains of species affiliated with subcluster PnecC and the endosymbiont *P. necessarius* STIR1, which is also affiliated with this subcluster. Analyses were performed by using the IMG/ER system [18]. Exchanging of subject and query genome resulted in all pairwise calculations in identical gANI values, however the obtained Alignment Fractions (AF) differed when query and reference genomes were exchanged.

Strain	gANI (%)	AF (%)
P. asymbioticus QLW-P1DMWA-1^T	76.7	65[a]/68[b]
P. yangtzensis MWH-JaK3^T	76.9	64[a]/64[b]
P. sinensis MWH-HuW1^T	76.9	55[a]/63[b]
P. duraquae MWH-MoK4^T	76.7	63[a]/62[b]
P. wuianus QLW-P1FAT50C-4^T	77.1	63[a]/68[b]
P. necessarius STIR1 (Endosymbiont)	76.9	71[a]/54[b]

[a] genome of MWH-Weng1-1^T served as subject genome

[b] genome of MWH-Weng1-1^T served as query genome