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Tianweitania sediminis gen. nov., sp. nov., a member of the family *Phyllobacteriaceae*, isolated from subsurface sediment core

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A bacterial strain, designated Z8^T, was isolated from the terrestrial sediment of the Mohe Basin in north-east China. Phylogenetic analyses of 16S rRNA genes showed that this strain belonged to the family *Phyllobacteriaceae*, and was most closely related to *Phyllobacterium bourgognense*, with a sequence similarity of 96.9 %. The major cellular fatty acids were summed feature 4 (iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$). The major respiratory quinone was ubiquinone-10. The three major polar lipids of strain Z8^T consisted of glycolipids, phosphatidylethanolamine and phosphatidylmethylethanolamine. The DNA G+C content was 59.6 mol%. The chemotaxonomic characteristics of strain Z8^T differed in some respects from those of members of the family *Phyllobacteriaceae*. Based on phylogenetic, phenotypic and chemotaxonomic data, strain Z8^T is considered to represent a novel species of a novel genus within the family *Phyllobacteriaceae*, for which the name *Tianweitania sediminis* gen. nov., sp. nov. is proposed. The type strain is Z8^T (=CGMCC 1.12944^T=JCM 30358^T).

The family *Phyllobacteriaceae* (order *Rhizobiales*, *Alphaproteobacteria*) was originally described by Knösel (1984). At the time of writing, the family comprised ten genera with validly published names: *Phyllobacterium* (Knösel, 1962), *Aminobacter* (Urakami *et al.*, 1992), *Mesorhizobium* (Jarvis *et al.*, 1997), *Aquamicrobium* (Bambauer *et al.*, 1998), *Pseudaminobacter* (Kämpfer *et al.*, 1999), *Nitratireductor* (Labbé *et al.*, 2004), *Hoeflea* (Peix *et al.*, 2005), *Chelativorans* (Doronina *et al.*, 2010), *Pseudahrensia* (Jung *et al.*, 2012) and *Lentilitoribacter* (Park *et al.*, 2013).

In August 2011, a sample of terrestrial sediment was collected from a borehole that lay in the Mohe Basin (53° 28′ 37.80′ N, 122° 19′ 23.40′ E; depth 358.5 m) of north-east China. Microbial contamination was eliminated by fluorescent microspheres and the sediment was then split and ground to a powder under frozen and sterile conditions as described previously (Han *et al.*, 2014). A novel bacterium, designated strain Z8^T, was isolated by using the standard dilution plating method on R2A agar after incubation at 25 °C for one week. The isolate was routinely cultured on R2A and preserved as 15 % (v/v) glycerol suspensions at −80 °C.

Genomic DNA was extracted with a Bacterial DNA kit (OMGEA) according to the manufacturer's instructions. The 16S rRNA gene was amplified using the primers 27F/1492R and sequenced using a ABI 3730XL 96-capillary DNA analyser (Applied Biosystems) as described previously (Tan *et al.*, 2014). The *atpD* gene was amplified using the primers atpD-273F and atpD-771R, as described by Gaunt *et al.* (2001). The sequences obtained and related sequences were retrieved from the GenBank database using the BLASTN search program (Altschul *et al.*, 1990) and 16S rRNA gene sequences were also compared with those from the EzTaxon-e server (Kim *et al.*, 2012). For sequences of the 16S rRNA gene, alignment treatment was performed

Abbreviations: ML, maximum likelihood; MP, maximum parsimony; NJ, neighbour joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *atpD* gene sequences of strain Z8^T are KJ577584 and KT949416, respectively.

Twelve supplementary figures and three supplementary tables are available with the online Supplementary Material.

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using ARB release 6.0.2 (Ludwig *et al.*, 2004) based on the complete SSU rRNA 'All-Species Living Tree' Project (LTP) database (Yarza *et al.*, 2008) release 121 (July 2015). Sequences not included in the LTP database were imported into the LTP database and automatically aligned with the Fast Aligner tool. The alignment of sequences was controlled manually based on information of secondary structure predictions. An ARB parsimony method was used to add the aligned sequences to the All-Species Living Tree. Sequences of the *atpD* gene were aligned using CLUSTAL X software (Thompson *et al.*, 1997). The position of Z8^T in various phylogenetic trees was determined by reconstructing them using the maximum-likelihood (ML) (Felsenstein 1981), neighbour-joining (NJ) (Saitou & Nei 1987) and maximum-parsimony (MP) (Fitch 1971) methods in MEGA release 6.06 (Tamura *et al.*, 2013). Bootstrap analysis with 1000 replications (Felsenstein, 1985) was used to estimate the stability of the clades in the trees. Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1980).

The sequenced, nearly full-length 16S rRNA gene fragment of strain Z8^T (GenBank accession number KJ577584) was a stretch of 1447 unambiguous nucleotides between *E. coli* positions 8 and 1511.

The nearest relative, based on 16S rRNA gene sequence similarity values, was *Phyllobacterium bourgognense* LMG 22837^T (96.9 %), followed by *Phyllobacterium myrsinacearum* LMG 2.2^T (96.7 %) (Table S1, available in the online Supplementary Material). ARB analysis placed strain Z8^T within the family *Phyllobacteriaceae*, closely neighboured by the genera *Phyllobacterium* and *Mesorhizobium* (Fig. S1). Trees of all species of the family *Phyllobacteriaceae* reconstructed by the ML, NJ and MP methods showed strain Z8^T belonged to the family *Phyllobacteriaceae* (Figs S2, S3 & S4), but Z8^T could not be assigned to any genus of this family. In every case, the resulting branching pattern was not supported by high bootstrap values, and the topology structure was largely dependent on the dataset or algorithm used for tree reconstruction, however, strain Z8^T was always close to the branch of the genus *Phyllobacterium*. The genera, *Phyllobacterium* and *Mesorhizobium*, the two closest genera to strain Z8^T within the family *Phyllobacteriaceae*, were chosen to reconstruct phylogenetic trees by the ML, NJ and MP methods, showing that strain Z8^T formed a robust monophyletic clade with the two genera (Figs S5, S6 & S7) (bootstrap values >50 %). Results based on the phylogenetic analysis of the *atpD* gene showed strain Z8^T shared highest similarity with *Mesorhizobium alhagi* CCNWXJ12-2^T (89.6 %) and *Mesorhizobium camelthorni* CCNWXJ 40-4^T (88.8 %). Phylogenetic trees of the *atpD* gene (Figs S8, S9 & S10) show that strain Z8^T formed a coherent clade with *M. alhagi* CCNWXJ12-2^T and *M. camelthorni* CCNWXJ 40-4^T. However, strain Z8^T shared the lowest 16S rRNA gene similarities within the genus *Mesorhizobium* with *M. alhagi* CCNWXJ12-2^T and *M. camelthorni* CCNWXJ 40-4^T (both 95.3 %) (data not shown) and was distant from them genetically (Figs S1, S2, S3 & S4). Irrespective of the two members of the genus *Mesorhizobium*, strain Z8^T would be placed in the cluster demarcation of the two genera *Phyllobacterium* and *Mesorhizobium* (data not shown).

P. bourgognense LMG 22837^T, *P. myrsinacearum* LMG 2.2^T (the type species of the genus *Phyllobacterium*) and *Mesorhizobium loti* LMG 6125^T (the type species of the genus *Mesorhizobium*) were selected as reference strains and were evaluated together with strain Z8^T under identical experimental conditions. The cell morphology was examined by scanning electron microscopy (Hitachi; SU1510) with cells grown for 5 days at 28 °C on yeast mannitol agar (YMA; Vincent, 1970). Colonial morphology was observed on YMA after incubation at 28 °C for 5 days. Strain Z8^T was grown in YMA for 72 h at 28 °C to check for motility by phase-contrast microscopy using the hanging drop method. Gram-reaction testing was determined by using the non-staining (KOH) method, as described by Buck (1982). Anaerobic growth was assessed by the semisolid agar method (Smibert & Krieg, 1994). Oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine (oxidase test strips; bioMe'rieux). Catalase activity was detected by bubble production in 3 % (v/v) H_2O_2. Growth was also tested in Luria–Bertani (LB) broth (Difco, US) and R2A broth (Difco, US). The temperature range for growth and the optimum growth temperature were determined in YMB incubated for 3–15 days at 0, 4, 10, 20, 25, 28, 30, 33 and 37 °C. The pH range for growth was tested at pH 4.0–10.0 (at intervals of pH 1.0) by adjusting the pH of YMB with filter-sterilized 0.1 M NaOH or HCl. Growth at different temperatures and pH values was determined in YMB and growth with NaCl was examined in R2A broth containing 0–5.0 % (w/v, at intervals of 0.5 %) NaCl by measuring the OD_{600} (UV-2450, Shimadzu). The hydrolysis of starch (0.2 %, w/v), aesculin (0.1 %, w/v), pectin (1 %, w/v),CM-cellulose(0.5 %, w/v), gelatin (10 %, w/v), Tween 80 (1 %, w/v) and urea (2 %, w/v) was tested by using YM as the basal medium (containing 1^{-1}: 10 g mannitol, 1 g yeast extract, 0.2 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 20 g agar) according to the methods of Smibert & Krieg (1994). Susceptibility to antibiotics was tested at 28 °C for 5 days on YMA plates using antibiotic discs containing the following antibiotics (mg per disc, unless otherwise stated): amikacin (30), ampicillin (10), carbenicillin (100), cefalexin (30), cefazolin (30), cefoperazone (75), cefradine (30), ceftazidime (30), ceftriaxone (30), cefuroxime (30), chloramphenicol (30), ciprofloxacin (5), clindamycin (2), doxycycline (30), erythromycin (15), furazolidone (300), gentamicin (10), kanamycin (30), midecamycin (30), minocycline (30), neomycin (30), norfloxacin (10), ofloxacin (5), oxacillin (1), penicillin G (10 U), piperacillin (100), polymyxin B (300U), sulfamethoxazole/trimethoprim (23.75/1.25), tetracycline(30) and vancomycin (30), and the zone of inhibition was used as the criterion to separate susceptible and resistant phenotypes.

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Other physiological and biochemical properties were determined by using API 20NE, API ZYM and API 50CH kits (inoculated with cell suspensions in CHB/E medium) (bioMérieux), according to the manufacturer's instructions.

Cells of strain Z8^T were Gram-stain-negative, strictly aerobic, rod-shaped and motile. They grew well in R2A broth and YMB, but did not grow in LB broth. The strain was highly mucoid on YM medium. A scanning electron micrograph of cells of strain Z8^T is available (Fig. S11). Other phenotypic features of strain Z8^T are summarized in the species description and the properties differentiating the isolate from the reference strains are detailed in Table S2.

Chemotaxonomic analyses were performed as described previously (Zhang *et al.*, 2014). For quantitative analysis of the cellular fatty acid composition, strain Z8^T and the three reference strains were grown in the same conditions on YMA medium at 28 °C and cell mass was harvested after 5 days at the late-exponential phase. The fatty acids of strain Z8^T and the three reference strains were obtained from 100 mg of freeze-dried cells by saponification, methylation and extraction, according to the protocols of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed by GC (Agilent Technologies 7890A GC system) and identified using the TSBA6 database of the microbial Identification System (Sasser, 1990). The major fatty acids (>10 % of the total fatty acids) detected in strain Z8^T were summed feature 4 and summed feature 8, which differed from the reference strains. The relative abundance of $C_{16:0}$ and $C_{16:0}$ 3-OH as well as that of $C_{19:0}$ cyclo$\omega 8c$ and $C_{18:1}\omega 7c$ 11-methyl clearly distinguished strain Z8^T from the type strains of *P. bourgognense* (LMG 22837^T), *P. myrsinacearum* (LMG 6738^T) and *M. loti* (LMG 6125^T) (Table S3). The predominant isoprenoid quinone detected in strain Z8^T was ubiquinone-10. Polar lipids were extracted from approximately 100 mg of the freeze-dried organisms as described by Minnikin *et al.* (1984) and were identified using two dimensional TLC (Minnikin *et al.* 1984; Da Costa *et al.* 2011). The solvent for the first dimension was chloroform/methanol/water (65 : 25 : 4, by vol.) and the solvent for the second dimension was chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.). Ethanol phosphomolybdic acid (5 %, v/v) was used to detect all polar lipids, while other spray reagents were used as follows: ninhydrin for aminolipids (110 °C); ninhydrin for glycolipids (140 °C); molybdenum blue for phospholipids and α-naphthol for glycolipids (110 °C). The three major polar lipids of strain Z8^T consisted of glycolipids, phosphatidylethanolamine and phosphatidylmethylethanolamine (Fig. S12). The polar lipid profile of strain Z8^T was similar to those of *P. bourgognense* LMG 22837^T, *P. myrsinacearum* LMG 2.2^T and *M. loti* LMG 6125^T, but could be differentiated from those of the three reference strains in that phosphatidylinositol and several unidentified polar lipids were absent (Fig. S12). The DNA G+C content of strain Z8^T was determined by using HPLC, according to the method described by Mesbah *et al.* (1989), to be 59.6 mol%.

According to phylogenetic trees based on various methods, strain Z8^T formed a monophyletic clade with other genera of the family *Phyllobacteriaceae*. The fatty acid profile, polar lipid content and other features differentiated strain Z8^T from other genera in the family *Phyllobacteriaceae* (Table 1). Therefore, strain Z8^T could be classified as a representative of a novel species and genus in the family *Phyllobacteriaceae*, for which the name *Tianweitania sediminis* gen. nov., sp. nov. is proposed.

Description of *Tianweitania* gen. nov.

Tianweitania [Tian.wei.ta'ni.a. N.L. fem. n. *Tianweitania* named after professor Tianwei Tan, an accademician of the China Engineering Academy, who has achieved excellent accomplishments in the fields of bio-energy, biomaterials research, bio-based chemicals etc].

Cells are Gram-stain-negative, strictly aerobic, rod-shaped and chemoheterotrophic. The major respiratory quinone is ubiquinone-10. The major polar lipids are glycolipids, phosphatidylethanolamine and phosphatidylmethylethanolamine. The major fatty acids are summed feature 4 (iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B) and summed feature 8 ($C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$). Phylogenetically, the genus *Tianweitaniais* is a member of the family *Phyllobacteriaceae* in the phylum *Bacteroidetes*. The type species is *Tianweitania sediminis*.

Description of *Tianweitania sediminis* sp. nov.

Tianweitania sediminis (se.di'mi.nis. L. gen. n. *sediminis* of sediment).

Gram-stain-negative aerobic rods and motile. Cells are 0.4–0.5 × 2.0–3.0 μm in size after incubation on YMA for 5 days. Colonies are less than 2 mm in diameter and white in YMA at 28 °C after 72 h incubation. The temperature range for growth is 20–35 °C (optimal growth occurs at 28 °C). The pH range for growth is 6.0–8.0 (optimal growth occurs at pH 7.0). Grows in the presence of NaCl concentrations up to 3 % (w/v). The oxidase test is positive and the catalase test is negative. Does not hydrolyse starch, aesculin, urea, Tween 80, pectin, cellulose or gelatin. Positive for glucose acidification. Negative for nitrate reduction, indole production and arginine dihydrolase activity. Assimilates glucose, caprate, adipate, citrate, mannitol and phenylacetate, but does not assimilate, arabinose, *N*-acetylglucosamine, maltose, gluconate, malate or mannose. Esterase (C4), esterase lipase (C8), β-galactosidase, α-mannosidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, trypsin, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-glucosidase, β-glucosidase, cystine arylamidase, chymotrypsin and β-fucosidase

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Table 1. Differential characteristics between strain Z8[T] and related genera in the family *Phyllobacteriaceae*

Taxa: 1, strain Z8[T]; 2, *Phyllobacterium* (Valverde *et al.*, 2005; Mantelin *et al.*, 2006; Yabe *et al.*, 2012; Flores-Félix *et al.*, 2013; Sánchez *et al.*, 2014); 3, *Mesorhizobium* (de Lajudie *et al.*, 1998; Han *et al.*, 2008; Zhou *et al.*, 2010; Yabe *et al.*, 2012); 4, *Aquamicrobium* (Lipski & Kämpfer, 2012; Yabe *et al.*, 2012; Wu *et al.*, 2014); 5, *Nitratireductor* (Labbé *et al.*, 2004; Kang *et al.*, 2009; Kim *et al.*, 2009; Yabe *et al.*, 2012); 6, *Aminobacter* (Urakami *et al.*, 1992; Maynaud *et al.*, 2012; Yabe *et al.*, 2012); 7, *Pseudaminobacter* (Kämpfer *et al.*, 1999; Yabe *et al.*, 2012); 8, *Hoeflea* (Peix *et al.*, 2005; Biebl *et al.*, 2006; Palacios *et al.*, 2006; Stevenson *et al.*, 2011; Yabe *et al.*, 2012); 9, *Chelativorans* (Doronina *et al.*, 2010; Yabe *et al.*, 2012); 10, *Pseudahrensia* (Jung *et al.*, 2012); 11, *Lentilitoribacter* (Park *et al.*, 2013). +, Positive; −, negative; +, weakly positive; ND, Not determined. PC, Phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; OH-PE, hydroxyphosphatidylethanolamine; GL, unknown glycolipid; PL, unidentified phospholipid; PME, phosphatidylmonomethylethanolamine; PI, phosphatidylinositol; DPG, diphosphatidylglycerol; SDG, sulphoquinovosyldiacylglyceride; PDE, phosphatidyldimethylethanolamine; NPL, ninhydrin-positive phospholipid.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Colony colour	White	White	White/beige	White/yellow	White/light yellow	White/light yellow	Beige	White/beige/light brown	White	White	Whitish-yellow
Torance to NaCl (w/v)	<3	<3	<5	<7	<8	<2	ND	<11.8	<4	<10	1–5
Oxidase	+	+	±	+	+	+	+	±	+	+	+
Catalase	−	+	+	+	+	+	+	+	+	+	+
DNA G+C content (mol%)	59.6	51–61.3	57.9–65.1	56–65	56.7–63	62.5–63.8	62.9–63.9	53.1–59.7	60.8–63.4	60.1	49.3
Phospholipids	GL, PE, PME	PE, PME, PI, GL	PE, PME, PI, GL	PG, PC, DPG, PE, ornithine lipid	PC, PG, PE, PME, DPG	PC, PG, PE, PDE, PME, DPG	PC, PG, PE, PDE, PME, DPG	PG, PE, PME, DPG, SDG, PL, GL, PC	PC, PG, PE, OH-PE, PDE, PME, DPG, PI, GL, NPL	PC, PG, DPG, PE	PG, PME
Major cellular fatty acids (>10 %)*	Summed feature 8, summed feature 4	$C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo, $C_{16:0}$, $C_{16:0}$ 3-OH, $C_{18:1}$ 2-OH, summed feature 8, summed feature 4	$C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo, $C_{16:0}$, summed feature 7	$C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo, 11-methyl $C_{18:1}\omega7c$	$C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo, summed feature 7	11-methyl $C_{18:1}\omega7c$, summed feature 7	$C_{19:0}\omega8c$ cyclo, summed feature 7	$C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo, $C_{16:0}$, summed feature 7, summed feature 8	$C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo, $C_{18:0}$	$C_{18:1}\omega7c$,	$C_{18:1}\omega7c$, 11-methyl $C_{18:1}\omega7c$, summed feature 3

*Summed feature 3 contained iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; summed feature 4 contained iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B; summed feature 7 contained $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and/or $C_{18:1}\omega12t$; summed feature 8 contained $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

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activities are absent. Acid is produced from glycerol, erythritol, L-arabinose, D-ribose, D-xylose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose, dulcitol, inositol, D-mannitol, amygdalin, salicin, sucrose, trehalose, raffinose, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, aesculin, L-rhamnose, cellobiose, maltose and glycogen. Susceptible to amikacin, ampicillin, carbenicillin, cefalexin, cefazolin, cefoperazone, cefradine, ceftazidime, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, furazolidone, kanamycin, midecamycin, minocycline, norfloxacin, ofloxacin, penicillin G, piperacillin, tetracycline, sulfamethoxazole/trimethoprim and vancomycin, but resistant to gentamicin, polymyxin B, oxacillin, neomycin and clindamycin. In addition to glycolipids, phosphatidylethanolamine and phosphatidylmethylethanolamine, one unidentified phospholipid and four unidentified polar lipids are also detected. In addition to the major fatty acids, summed feature 4 (iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), significant amounts (<5 % of the total fatty acids) of $C_{16:0}$ and $C_{19:0}$ cyclo$\omega8c$ are also present.

The type strain, Z8^T (=CGMCC 1.12944^T=JCM 30358^T), was isolated from subsurface sediment collected from the Mohe Basin in north-east China. The DNA G+C content of the type strain is 59.6 mol%.

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Bambauer, A., Rainey, F. A., Stackebrandt, E. & Winter, J. (1998). Characterization of *Aquamicrobium defluvii* gen. nov. sp. nov., a thiophene-2-carboxylate-metabolizing bacterium from activated sludge. *Arch Microbiol* **169**, 293–302.

Biebl, H., Tindall, B. J., Pukall, R., Lünsdorf, H., Allgaier, M. & Wagner-Döbler, I. (2006). *Hoeflea phototrophica* sp. nov., a novel marine aerobic alphaproteobacterium that forms bacteriochlorophyll a. *Int J Syst Evol Microbiol* **56**, 821–826.

Buck, J. D. (1982). Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

Da Costa, M. S., Albuquerque, L., Nobre, M. & Wait, R. (2011). The identification of polar lipids in prokaryotes. *Methods Microbiol* **38**, 165–181.

de Lajudie, P., Willems, A., Nick, G., Moreira, F., Molouba, F., Hoste, B., Torck, U., Neyra, M., Collins, M. D. & other authors (1998). Characterization of tropical tree rhizobia and description of *Mesorhizobium plurifarium* sp. nov. *Int J Syst Bacteriol* **48**, 369–382.

Doronina, N. V., Kaparullina, E. N., Trotsenko, Y. A., Nörtemann, B., Bucheli-Witschel, M., Weilenmann, H. U. & Egli, T. (2010). *Chelativorans multitrophicus* gen. nov., sp. nov. and *Chelativorans oligotrophicus* sp. nov., aerobic EDTA-degrading bacteria. *Int J Syst Evol Microbiol* **60**, 1044–1051.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Flores-Félix, J. D., Carro, L., Velázquez, E., Valverde, Á., Cerda-Castillo, E., García-Fraile, P. & Rivas, R. (2013). *Phyllobacterium endophyticum* sp. nov., isolated from nodules of *Phaseolus vulgaris*. *Int J Syst Evol Microbiol* **63**, 821–826.

Gaunt, M. W., Turner, S. L., Rigottier-Gois, L., Lloyd-Macgilp, S. A. & Young, J. P. (2001). Phylogenies of *atpD* and *recA* support the small subunit rRNA-based classification of rhizobia. *Int J Syst Evol Microbiol* **51**, 2037–2048.

Han, T. X., Han, L. L., Wu, L. J., Chen, W. F., Sui, X. H., Gu, J. G., Wang, E. T. & Chen, W. X. (2008). *Mesorhizobium gobiense* sp. nov. and *Mesorhizobium tarimense* sp. nov., isolated from wild legumes growing in desert soils of Xinjiang, China. *Int J Syst Evol Microbiol* **58**, 2610–2618.

Han, L., Wu, S. J., Qin, C. Y., Zhu, Y. H., Lu, Z. Q., Xie, B. & Lv, J. (2014). *Hymenobacter qilianensis* sp. nov., isolated from a subsurface sandstone sediment in the permafrost region of Qilian Mountains, China and emended description of the genus Hymenobacter. *Antonie van Leeuwenhoek* **105**, 971–978.

Jarvis, B. D. W., Van Berkum, P., Chen, W. X., Nour, S. M., Fernandez, M. P., Cleyet-Marel, J. C. & Gillis, M. (1997). Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. *Int J Syst Bacteriol* **47**, 895–898.

Jung, Y. T., Park, S., Lee, J. S., Oh, T. K. & Yoon, J. H. (2012). *Pseudahrensia aquimaris* gen. nov., sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* **62**, 2056–2061.

Kämpfer, P., Müller, C., Mau, M., Neef, A., Auling, G., Busse, H. J., Osborn, A. M. & Stolz, A. (1999). Description of *Pseudaminobacter* gen. nov. with two new species, *Pseudaminobacter salicylatoxidans* sp. nov. and *Pseudaminobacter defluvii* sp. nov. *Int J Syst Bacteriol* **49**, 887–897.

Kang, H. S., Yang, H. L. & Lee, S. D. (2009). *Nitratireductor kimnyeongensis* sp. nov., isolated from seaweed. *Int J Syst Evol Microbiol* **59**, 1036–1039.

Kim, K. H., Roh, S. W., Chang, H. W., Nam, Y. D., Yoon, J. H., Jeon, C. O., Oh, H. M. & Bae, J. W. (2009). *Nitratireductor basaltis* sp. nov., isolated from black beach sand. *Int J Syst Evol Microbiol* **59**, 135–138.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Knösel, D. (1962). Prufung von Bakterien auf Fahigkeit zur Sternbildung. *Zentralbl Bakteriol Parasirtenk Infektionskr Hyg Abt* **2**, 79–100.

Knösel, D. H. (1984). Genus IV. *Phyllobacterium* (ex Knösel 1962) nom. rev. (*Phyllobacterium* Knösel 1962, 96). In *Bergey's Manual of*

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Systematic Bacteriology, vol. 1, pp. 254–256. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Labbé, N., Parent, S. & Villemur, R. (2004). *Nitratireductor aquibiodomus* gen. nov., sp. nov., a novel α-proteobacterium from the marine denitrification system of the Montreal Biodome (Canada). *Int J Syst Evol Microbiol* **54**, 269–273.

Lipski, A. & Kämpfer, P. (2012). *Aquamicrobium ahrensii* sp. nov. and *Aquamicrobium segne* sp. nov., isolated from experimental biofilters. *Int J Syst Evol Microbiol* **62**, 2511–2516.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Mantelin, S., Saux, M. F., Zakhia, F., Béna, G., Bonneau, S., Jeder, H., de Lajudie, P. & Cleyet-Marel, J. C. (2006). Emended description of the genus *Phyllobacterium* and description of four novel species associated with plant roots: *Phyllobacterium bourgognense* sp. nov., *Phyllobacterium ifriqiyense* sp. nov., *Phyllobacterium leguminum* sp. nov. and *Phyllobacterium brassicacearum* sp. nov. *Int J Syst Evol Microbiol* **56**, 827–839.

Maynaud, G., Willems, A., Soussou, S., Vidal, C., Mauré, L., Moulin, L., Cleyet-Marel, J. C. & Brunel, B. (2012). Molecular and phenotypic characterization of strains nodulating Anthyllis vulneraria in mine tailings, and proposal of *Aminobacter anthyllidis* sp. nov., the first definition of Aminobacter as legume-nodulating bacteria. *Syst Appl Microbiol* **35**, 65–72.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinines and polar lipids. *J Microbiol Methods* **2**, 233–241.

Palacios, L., Arahal, D. R., Reguera, B. & Marín, I. (2006). *Hoeflea alexandrii* sp. nov., isolated from the toxic dinoflagellate Alexandrium minutum AL1V. *Int J Syst Evol Microbiol* **56**, 1991–1995.

Park, S., Lee, J. S., Lee, K. C. & Yoon, J. H. (2013). *Lentilitoribacter donghaensis* gen. nov., sp. nov., a slowly-growing alphaproteobacterium isolated from coastal seawater. *Antonie van Leeuwenhoek* **103**, 457–464.

Peix, A., Rivas, R., Trujillo, M. E., Vancanneyt, M., Velázquez, E. & Willems, A. (2005). Reclassification of *Agrobacterium ferrugineum* LMG 128 as *Hoeflea marina* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **55**, 1163–1166.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sánchez, M., Ramírez-Bahena, M. H., Peix, A., Lorite, M. J., Sanjuán, J., Velázquez, E. & Monza, J. (2014). *Phyllobacterium loti* sp. nov. isolated from nodules of *Lotus corniculatus*. *Int J Syst Evol Microbiol* **64**, 781–786.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*., MIDI Technical Note 101 Newark, DE: MIDI Inc.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Stevenson, B. S., Suflita, M. T., Stamps, B. W., Moore, E. R. B., Johnson, C. N. & Lawson, P. A. (2011). *Hoeflea anabaenae* sp. nov., an epiphytic symbiont that attaches to the heterocysts of a strain of Anabaena. *Int J Syst Evol Microbiol* **61**, 2439–2444.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.

Tan, X., Zhang, R. G., Meng, T. Y., Liang, H. Z. & Lv, J. (2014). *Taibaiella chishuiensis* sp. nov., isolated from freshwater. *Int J Syst Evol Microbiol* **64**, 1795–1801.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Urakami, T., Araki, H., Oyanagi, H., Suzuki, K. & Komagata, K. (1992). Transfer of *Pseudomonas aminovorans* (den Dooren de Jong 1926) to *Aminobacter* gen. nov. as *Aminobacter aminovorans* comb. nov. and description of *Aminobacter aganoensis* sp. nov. and *Aminobacter niigataensis* sp. nov. *Int J Syst Bacteriol* **42**, 84–92.

Valverde, A., Velázquez, E., Fernández-Santos, F., Vizcaíno, N., Rivas, R., Mateos, P. F., Martínez-Molina, E., Igual, J. M. & Willems, A. (2005). *Phyllobacterium trifolii* sp. nov., nodulating *Trifolium* and *Lupinus* in Spanish soils. *Int J Syst Evol Microbiol* **55**, 1985–1989.

Vincent, J. M. (1970). The cultivation, isolation and maintenance of rhizobia. In *A Manual for the Practical Study of Root-Nodule*, pp. 1–13. Edited by J. M. Vincent. Oxford: Blackwell Scientific Publications.

Wu, Z. G., Wang, F., Gu, C. G., Zhang, Y. P., Yang, Z. Z., Wu, X. W. & Jiang, X. (2014). *Aquamicrobium terrae* sp. nov., isolated from the polluted soil near a chemical factory. *Antonie van Leeuwenhoek* **105**, 1131–1137.

Yabe, S., Aiba, Y., Sakai, Y., Hazaka, M. & Yokota, A. (2012). *Thermovum composti* gen. nov., sp. nov., an alphaproteobacterium from compost. *Int J Syst Evol Microbiol* **62**, 2991–2996.

Yarza, P., Richter, M., Peplies, J., Euzeby, J., Amann, R., Schleifer, K. H., Ludwig, W., Glöckner, F. O. & Rosselló-Móra, R. (2008). The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst Appl Microbiol* **31**, 241–250.

Zhang, R. G., Tan, X., Zhao, X. M., Deng, J. & Lv, J. (2014). *Moheibacter sediminis* gen. nov., sp. nov., a member of the family *Flavobacteriaceae* isolated from sediment, and emended descriptions of *Empedobacter brevis*, *Wautersiella falsenii* and *Weeksella virosa*. *Int J Syst Evol Microbiol* **64**, 1481–1487.

Zhou, P. F., Chen, W. M. & Wei, G. H. (2010). *Mesorhizobium robiniae* sp. nov., isolated from root nodules of *Robinia pseudoacacia*. *Int J Syst Evol Microbiol* **60**, 2552–2556.

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