Parviterribacter kavangonensis and *Parviterribacter multiflagellatus*, a novel genus and two novel species within the order *Solirubrobacterales* and emended description of the classes *Thermoleophilia* and *Rubrobacteria* and its orders and families

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Running title: *Parviterribacter kavangonensis* gen. nov., sp. nov. and *P. multiflagellatus*, sp. nov.

Subject category: New Taxa, subsection *Actinobacteria*

Footnote

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Parviterribacter kavangonensis* D16/0/H6^T and *P. multiflagellatus* A22/0/F9_1^T are KP981370 and KP981371, respectively.

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Isolation medium SSE/HD1:10 (1 L):
(see also DSMZ medium 1426; www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium1426.pdf)

Peptone	0.50 g
Yeast extract	0.25 g
Glucose	0.10 g
MES (10 mM final concentration)	1.95 g
SSE (double concentrated)[a]	500 ml
Distilled water	500 ml (less the volume of the solutions added after autoclaving)

Adjust to pH 5.5 and autoclave.

After cooling add the following sterile filtered solutions:

Trace element solution SL-10[b]	1.0 ml
Vitamin solution[c]	1.0 ml

[a] SSE (Soil Solution Equivalent) after Angle *et al.* (1991) - double concentrated:

$CaCl_2 \times 2H_2O$	0.2938 g
NH_4Cl	0.1069 g
$MgCl_2 \times 6H_2O$	0.2036 g
$(NH_4)_2SO_4$	0.1983 g
$MgSO_4 \times 7H_2O$	0.7390 g
$CaSO_4 \times 2H_2O$	0.8606 g
$Ca(NO_3)_2 \times 4H_2O$	0.2360 g
$NaNO_3$	0.4240 g
KH_2PO_4 (100 mM solution)	0.5000 ml
$FeSO_4 \times 7H_2O$	0.0111 g
K_2SO_4	0.0870 g
Distilled water	1000 ml

Stir over night and use directly or autoclave and store for future use.

[b] Trace element solution SL-10 (Tschech & Pfennig, 1984):

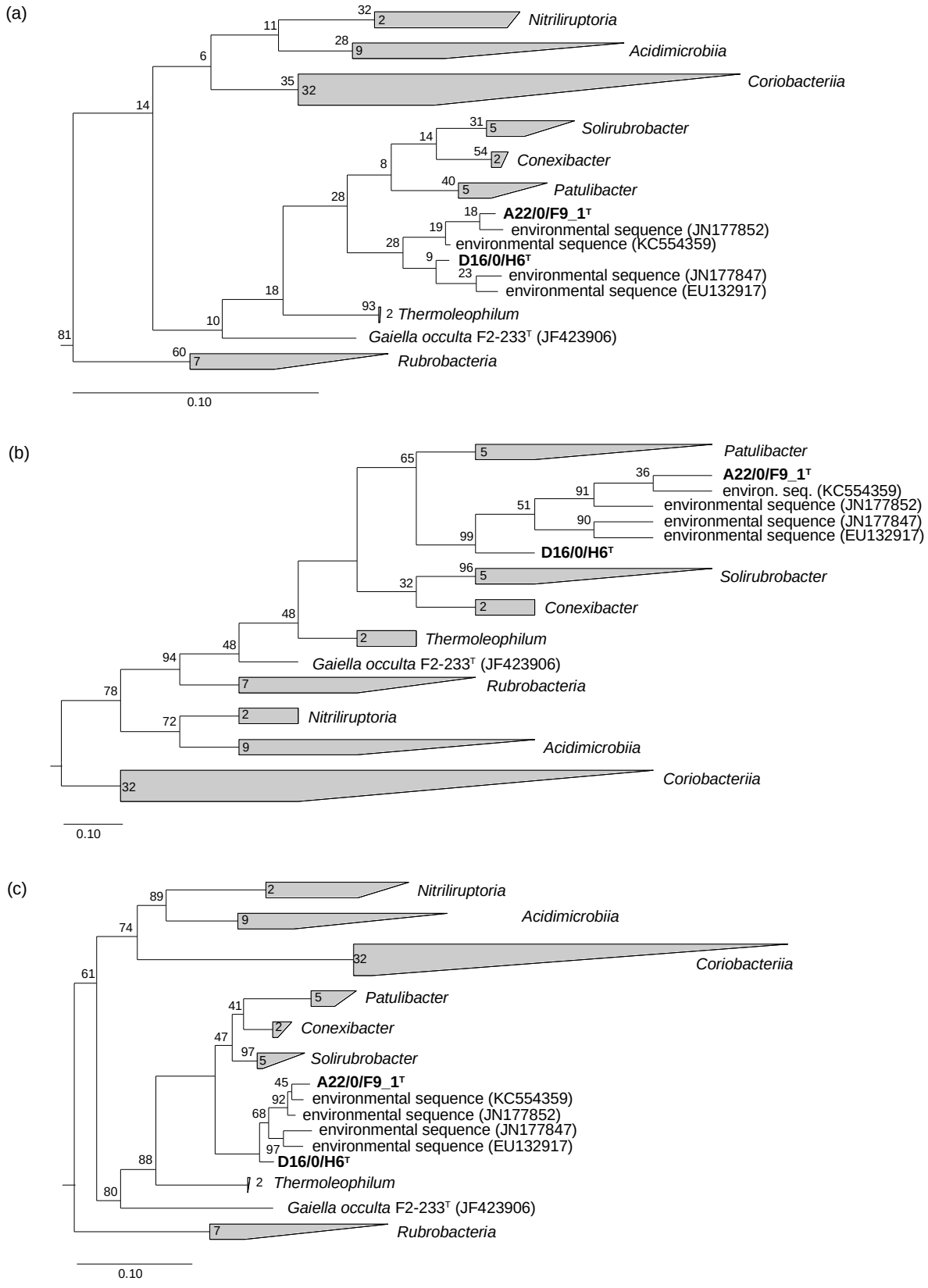
HCl (25%; 7.7 M)	10 ml
$FeCl_2 \times 4H_2O$	1.50 g
$CoCl_2 \times 6H_2O$	190.0 mg
$MnCl_2 \times 4H_2O$	100.0 mg
$ZnCl_2$	70.0 mg
$Na_2MoO_4 \times 2H_2O$	36.0 mg
$NiCl_2 \times 6H_2O$	24.0 mg
H_3BO_3	6.0 mg
$CuCl_2 \times 2H_2O$	2.0 mg
Distilled water	990 ml

First dissolve $FeCl_2$ in HCl, then dilute with water, add and dissolve the other salts. Finally make up to 1000 ml.

[c] Balch's vitamin mixture (Balch *et al.*, 1979):

Biotin	2 mg
Folic acid	2 mg
Pyridoxine hydrochloride	10 mg
Thiamine hydrochloride	5 mg
Riboflavin	5 mg
Nicotinic acid	5 mg
DL-Ca-pantothenate	5 mg
Vitamin B_{12}	0.1 mg
p-Aminobenzoic acid	5 mg
Lipoic acid	5 mg
Distilled water	1000 ml

Supplementary Figure 1. Phylogenetic trees based on almost full-length 16S rRNA gene sequences showing the relationship of strains D16/0/H6^{T} and A22/0/F9_1^{T} to each other and to further related taxa calculated with (a) the neighbor-joining, (b) the maximum-parsimony and (c) the maximum-likelihood algorithm and (d) a consensus tree combining trees a-c. Bootstrap values (expressed as percentages of 1000 replicates) below 100% are indicated at the respective branching points. The following sequences were used as outgroup: *Chloroflexus aggregans* DSM 9485 (CP001337), *Chloroflexus aurantiacus* J-10-fl (D38365) and *Roseiflexus castenholzii* DSM 13941 (CP000804). Bars indicate 10% nucleotide divergence.


(a)
Nitriliruptoria
Acidimicrobiia
Coriobacteriia
Solirubrobacter
Conexibacter
Patulibacter
A22/0/F9_1T
environmental sequence (JN177852)
environmental sequence (KC554359)
D16/0/H6T
environmental sequence (JN177847)
environmental sequence (EU132917)
Thermoleophilum
Gaiella occulta F2-233T (JF423906)
Rubrobacteria
0.10
(b)
Patulibacter
A22/0/F9_1T
environ. seq. (KC554359)
environmental sequence (JN177852)
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0.10
(c)
Nitriliruptoria
Acidimicrobiia
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Patulibacter
Conexibacter
Solirubrobacter
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environmental sequence (KC554359)
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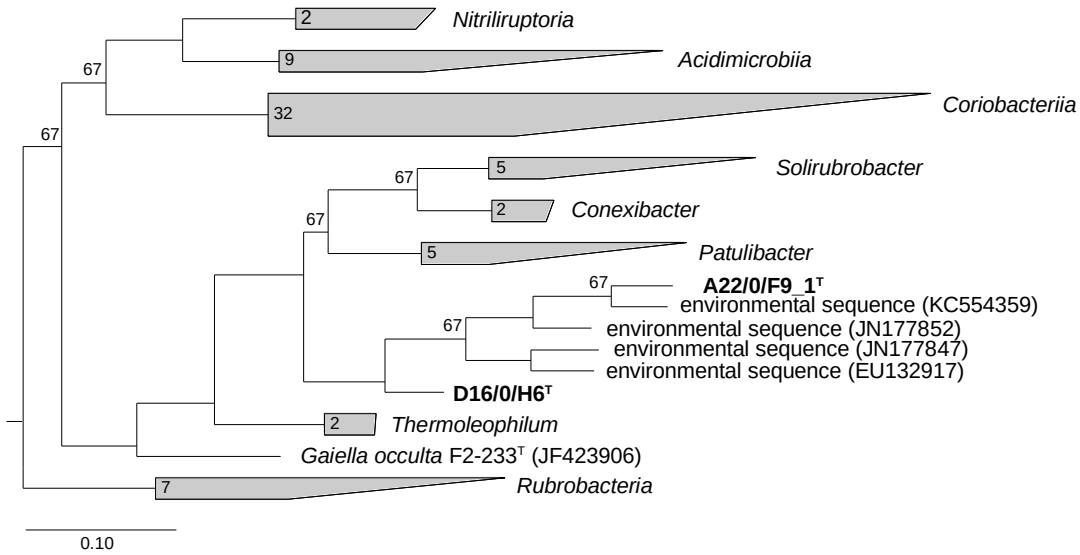
D16/0/H6T
Thermoleophilum
Gaiella occulta F2-233T (JF423906)
Rubrobacteria
0.10

(d)


2
Nitriliruptoria
9
Acidimicrobiia
67
32
Coriobacteriia
67
5
Solirubrobacter
67
2
Conexibacter
67
5
Patulibacter
67
A22/0/F9_1T
environmental sequence (KC554359)
67
environmental sequence (JN177852)
environmental sequence (JN177847)
environmental sequence (EU132917)
D16/0/H6T
2
Thermoleophilum
Gaiella occulta F2-233T (JF423906)
7
Rubrobacteria
0.10

Supplementary Table 1.

Full cellular fatty acid profile of strains D16/0/H6^{T} and A22/0/F9_1^{T}.

Percentages of total fatty acids identified with standard methods of the MIDI System are outlined in the table. Summed features represent two fatty acids that could not be separated by GLC with the MIDI system.
Summed feature 3 contained 16:1 ω7c and/or 15:0 iso 2-OH, feature 4 contained 17:1 iso I and/or 17:1 anteiso B, feature 6 contained 19:1 ω9c and/or 19:1 ω11c, and feature 7 contained 19:0 cyclo ω10c and/or 19:1 ω6c; bold, major components ≥ 10%; -, not detected.

Fatty acid	D16/0/H6^{Ta,d}	A22/0/F9_1^{T}
Saturated		
14:0	0.25	0.27
15:0	0.33	0.78
16:0	0.47	0.83
17:0	-	0.40
18:0	-	0.25
Unsaturated		
15:1 ω6c	0.25	-
17:1 ω6c	2.86	**11.91**
17:1 ω8c	3.89	**16.03**
18:1 ω7c	**-**	0.36
18:1 ω9c	**9.99**	**35.11**
18:3 ω6c (6,9,12)^{b,d}	0.27	-
Methyl-branched		
14:0 iso	1.21	-
15:0 iso	0.38	0.66
15:0 anteiso	0.31	-
16:0 iso	**21.58**	**11.74**
16:1 iso H	1.07	-
17:0 iso	1.66	0.53
17:0 anteiso	3.70	0.36
17:0 10-methyl	**35.97**	**7.22**
17:1 iso ω9c	1.65	-
18:0 iso	3.47	1.26
18:0 10-methyl (TBSA)	1.29	0.57
18:1 iso H	0.65	-
19:0 10-methyl	0.84	-
20:0 iso	0.36	-
Hydroxy		
14:0 2-OH	-	0.30
15:0 2-OH	-	0.48
15:0 3-OH^{c,d}	0.49	-

Summed Feature		
3	3.86	2.31
4	0.49	-
6	2.46	**8.62**
7	0.26	-

[a] Contains further, not identified minor peaks at ECLs 16.049, 16.084 and 18.017 that can be interpreted as *iso*-$C_{16:0}$ 10-methyl, $C_{17:0}$ alcohol and $C_{19:0}$ alcohol, respectively (Albuquerque *et al.*, 2011; Carreto *et al.*, 1996).

[b] Potential erroneous interpretation by the MIDI System, might rather be $C_{18:0}$ alcohol (Albuquerque *et al.*, 2014; Carreto *et al.*, 1996).

[c] Potential erroneous interpretation by the MIDI system, might rather be $C_{16:0}$ 12-methyl (Albuquerque *et al.*, 2014; Carreto *et al.*, 1996).

[d] Verification of doubtful peaks would require further analysis via GC-MS. However, for most of these minor components this is not feasible as their low concentration would not allow for analyzable mass spectra.

Supplementary Table 2.

Substrate range of strains D16/0/H6^T and A22/0/F9_1^T.

Both strains grew on glucose, butyrate, protocatechuate, pyruvate, succinate, casamino acids, casein hydrolysate, peptone, and yeast extract. Substrates tested but not utilized are given in the respective species descriptions.

Carbon source	D16/0/H6^T	A22/0/F9_1^T
Cellobiose	-	+
Mannose	+	-
N-acetyl-glucosamine	-	+
Adonitol	-	+
Lyxitol	+	-
Aspartate	+	-
Glutamate	-	+
Lysine	+	-
Hydroxy-Proline	+	-
Acetate	+	-
β-Hydroxybutyrate	+	-
Isobutyrate	-	+
Caproate	-	+
Caprylate	-	+
Crotonate	-	+
Gluconate	-	+
Heptanoate	-	+
Laevulinate	-	+
Propionate	+	-
Glycerol	-	+
Starch	-	+

Supplementary Table 3.

Enzyme activities of strains D16/0/H6^T and A22/0/F9_1^T and species from related taxa as determined by the API ZYM test system (Biomérieux).

Strains/families: **1**, D16/0/H6^T; **2**, A22/0/F9_1^T; **3**, *Solirubrobacteraceae*, comprising summarized features of *Solirubrobacter pauli* B33D1^T (Singleton *et al.*, 2003), *S. soli* Gsoil 355^T (Kim *et al.*, 2007), *S. ginsenosidimutans* BXN5-15^T (An *et al.*, 2011), *S. phytolaccae* GTGR-8^T (Wei *et al.*, 2014), *S. taibaiensis* GTJR-20^T (Zhang *et al.*, 2014); **4**, *Conexibacteracae*, including *Conexibacter woesei* ID131577^T (Monciardini *et al.*, 2003), *C. arvalis* KV-962^T (Seki *et al.*, 2012); **5**, *Patulibacteraceae*, including *Bactoderma rosea* (Tepper & Korshunova, 1973), *Patulibacter minatonensis* KV-614^T (Takahashi *et al.*, 2006), *P. americanus* CP1777-2^T (Reddy & Garcia-Pichel, 2009), *P. ginsengiterrae* P4-5^T (Kim *et al.*, 2012), *P. medicamentivorans* I11^T (Almeida *et al.*, 2013); **6**, *Thermoleophilacae*, including *Thermoleophilum album* HS-5^T (Zarilla & Perry, 1984), *T. minutum* YS-4^T (Zarilla & Perry, 1986); **7**, *Gaiellaceae*, including *Gaiella occulta* F2-233^T (Albuquerque *et al.*, 2011); **8**, *Rubrobacteraceae*, including *Rubrobacter radiotolerans* IAM 12072^T (Suzuki *et al.*, 1988), *R. xylanophilus* PRD-1^T (Carreto *et al.*, 1996), *R. taiwanensis* LS-293^T (Chen *et al.*, 2004), *R. bracarensis* VF70612_S1^T (Jurado *et al.*, 2012), *R. aplysinae* RV113^T (Kämpfer *et al.*, 2014), *R. calidifluminis* RG-1^T and *R. naiadicus* RG-3^T (Albuquerque *et al.*, 2014).

+, positive; -, negative; (+), weak enzyme activity detected; ND, no data.

When characteristics differ among strains, numbers in brackets give the number of strains showing the respective feature (first number) compared to the number of all strains considered (second number).

	Thermoleophilia							*Rubrobacteria*
	Solirubrobacterales					*Thermoleophilales*	*Gaiellales*	*Rubrobacterales*
Enzyme	1	2	3[a]	4	5[b]	6[c]	7	8[d]
Alkaline phosphatase	+	(+)	+	+(1/2), - (1/2)	+(2/3), - (1/3)	+	ND	+
Acid phosphatase	+	(+)	+	+	+	+	ND	+(2/3), - (1/3)
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	+	+	ND	+
Esterase (C 4)	+	+	+	+	+	+	ND	+
Esterase Lipase (C 8)	+	+	+	+	+	+	ND	+
Lipase (C 14)	-	(+)	+(3/5), - (2/5)	+(1/2), - (1/2)	+	-	ND	-
Leucine arylamidase	+	(+)	+	+	+	-	ND	+
Valine arylamidase	-	-	+(4/5), (+) (1/5)	+(1/2), - (1/2)	-	-	ND	+(2/3), - (1/3)
Cysteine arylamidase	(+)	(+)	+(4/5), - (1/5)	+(1/2), - (1/2)	+(2/3), - (1/3)	-	ND	+
Trypsin	-	-	-	+(1/2), - (1/2)	-	-	ND	+(2/3), - (1/3)
α-Chymotrypsin	-	-	+(2/5), - (3/5)	-	-	-	ND	+(2/3), - (1/3)
α-Galactosidase	-	-	-	+(1/2), - (1/2)	-	-	ND	-
β-Galactosidase	-	-	+(3/5), - (2/5)	-	-	-	ND	+
β-Glucuronidase	+	-	-	-	-	-	ND	ND(2/3), - (1/3)
α-Glucosidase	-	-	+	-	-	-	ND	+
β-Glucosidase	-	+	+	-	+(1/3), - (2/3)	-	ND	+
N-Acetyl-β-glucosaminidase	-	(+)	+(2/5), - (3/5)	-	-	-	ND	+(1/3), - (2/3)
α-Mannosidase	+	-	+(3/5), - (2/5)	-	-	-	ND	+(2/3), - (1/3)
α-Fucosidase	(+)	-	+(1/5), - (4/5)	-	-	-	ND	-

[a] Data for *S. pauli*, *S. soli*, and *S. ginsenosidimutans* not from original description, but from Wei *et al.* (2014).

[b] No data for *B. rosea* and *P. americanus.*

[c] No data for *T. album.*

[d] No data for *R. radiotolerans, R. xylanophilus, R. taiwanensis, and R. aplysinae.*