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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Footnote
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of
Parviterribacter kavangonensis D16/0/H6ᵀ and P. multiflagellatus A22/0/F9₁ᵀ are
KP981370 and KP981371, respectively.

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ABSTRACT

Two Gram-positive, non-spore-forming bacteria (strains D16/0/H6\textsuperscript{T} and A22/0/F9_1\textsuperscript{T}) were isolated from Namibian semiarid savanna soils. 16S rRNA gene sequence analysis revealed an identity of 96.6% of the two strains to each other and placed them within the order Solirubrobacterales of the class Thermoleophilia. Closest validly described phylogenetic relatives were several strains of the genus Solirubrobacter and the species Conexibacter arvalis with pairwise sequence similarities of \(\leq 94.0\%\). Cells of strain D16/0/H6\textsuperscript{T} were ovoid to rod shaped whereas strain A22/0/F9_1\textsuperscript{T} formed regular rods. Cells of both strains were motile and divided by binary fission. Colonies were pink and white to pale yellowish/brownish in color, respectively. Strains D16/0/H6\textsuperscript{T} and A22/0/F9_1\textsuperscript{T} were aerobic chemoheterotrophic mesophiles with a broad temperature (13 - 43°C and 17 - 43°C, respectively) and pH (4.5 - 8.5 and 5.0 - 9.5, respectively) range of growth. Complex, proteinaceous substrates and glucose were the preferred carbon and energy source. Strain A22/0/F9_1\textsuperscript{T} also grew on various carboxylic acids. For both strains the peptidoglycan diamino acid was \textit{meso}-2,6-diaminopimelic acid. The major quinone was MK-8. As minor compound, MK-7 occurred in strain D16/0/H6\textsuperscript{T}; strain A22/0/F9_1\textsuperscript{T} also contained MK-7, MK-7(H\textsubscript{2}), and MK-8(H\textsubscript{2}). Major fatty acids of strain D16/0/H6\textsuperscript{T} were C\textsubscript{17:0} 10-methyl, \textit{iso}-C\textsubscript{16:0} and C\textsubscript{18:1} \(\omega9c\). Strain A22/0/F9_1\textsuperscript{T} contained C\textsubscript{18:1} \(\omega9c\), C\textsubscript{17:1} \(\omega8c\), C\textsubscript{17:1} \(\omega6c\) and \textit{iso}-C\textsubscript{16:0} as major components. The DNA G+C contents of strains D16/0/H6\textsuperscript{T} and A22/0/F9_1\textsuperscript{T} were 72.8 and 74.0 mol\%, respectively. Based on these characteristics, the two isolates are described as two novel species of the novel genus \textit{Parviterribacter} gen. nov., \textit{P. kavangonensis} sp. nov. strain D16/0/H6\textsuperscript{T} (= DSM 25205\textsuperscript{T} = LMG 26950\textsuperscript{T}) and \textit{P. multiflagellatus} sp. nov. strain A22/0/F9_1\textsuperscript{T} (= DSM 25204\textsuperscript{T} = LMG 26949\textsuperscript{T}). As the novel genus and species cannot be clearly assigned to an established family within the order Solirubrobacterales, the novel family \textit{Parviterribacteraceae} fam. nov. is proposed.
Recently, the former subclass *Rubrobacteridae* within the class *Actinobacteria* (Stackebrandt et al., 1997) has been dissected and its members assigned to the two newly established classes *Rubrobacteria* and *Thermoleophilia* (Ludwig et al., 2012b). The new class *Rubrobacteria* (Suzuki, 2012) encompasses the established order *Rubrobacterales* with the family *Rubrobacteraceae* (Stackebrandt et al., 1997) and the genus *Rubrobacter* (Suzuki et al., 1988). The second class *Thermoleophilia* (Suzuki & Whitman, 2012) comprises the two orders *Thermoleophilales* (Reddy & Garcia-Pichel, 2009) and *Solirubrobacterales* (Reddy & Garcia-Pichel, 2009). The order *Thermoleophilales* harbors the single family *Thermoleophilaceae* (Stackebrandt, 2004; Zhi et al., 2009) including the two thermophilic species *Thermoleophilum album* (Zarilla & Perry, 1984) and *T. minutum* (Zarilla & Perry, 1986). The *Solirubrobacterales* comprise three families of mesophilic, mostly soil derived bacteria, namely the *Solirubrobacteraceae*, *Conexibacteraceae*, and *Patulibacteraceae* (Stackebrandt, 2004; Zhi et al., 2009). The *Solirubrobacteraceae* harbor the genus *Solirubrobacter* (Singleton et al., 2003) with its currently five species *S. pauli* (Singleton et al., 2003), *S. soli* (Kim et al., 2007), *S. ginsenosidimutans* (An et al., 2011), *S. phytolaccae* (Wei et al., 2014), and *S. taibaiensis* (Zhang et al., 2014). The *Conexibacteraceae* contain the genus *Conexibacter* (Monciardini et al., 2003) with its two members *C. woesei* (Monciardini et al., 2003) and *C. arvalis* (Seki et al., 2012). The *Patulibacteraceae* comprise the genus *Patulibacter* (Takahashi et al., 2006) that so far includes the four species *P. minatonensis* (Takahashi et al., 2006), *P. americanus* (Reddy & Garcia-Pichel, 2009), *P. ginsengiterrae* (Kim et al., 2012), and *P. medicamentivorans* (Almeida et al., 2013). In addition, there is a species, *Bactoderma rosea* (Tepper & Korshunova, 1973; Yarza et al., 2013), whose 16S rRNA gene sequence was obtained only recently (Yarza et al., 2013) and that phylogenetically is affiliated to the genus *Patulibacter* (99.7% sequence similarity to *P. minatonensis*). Finally, the order *Gaiellales* (Albuquerque et al., 2011) so far has not been included in the reorganization of Ludwig et al. (2012b). It encompasses the family *Gaiellaceae* (Albuquerque et al., 2011) with the single genus *Gaiella* (Albuquerque et al., 2011) and the mesophilic species *Gaiella occulta* (Albuquerque et al., 2011) established for an isolate from a deep mineral water aquifer.

In the present study, two novel isolates are described that extend the series of soil isolates of the order *Solirubrobacterales* and represent a new family with a novel genus and two novel species.

Strain D16/0/H6\textsuperscript{T} originates from a pristine dark loam with neutral pH (6.8 and 7.2 measured in 2 mM CaCl\textsubscript{2} and in distilled water, respectively) sampled in spring 2008 at Mile
46, Northern Namibia. Strain A22/0/F9_1\textsuperscript{T} was isolated from a clayey sand soil with slightly basic pH (7.4 and 8.2 measured in 2 mM CaCl\textsubscript{2} and in distilled water, respectively). The latter soil sample was collected in spring 2009 from a pasture at the farm Erichsfelde in central Namibia. Soil suspensions were prepared in 10 mM MES, pH 5.5 for the 2008 sample and HEPPS, pH 8.0 for the 2009 sample, respectively; 100 µl aliquots were plated on agar-solidified SSE/HD 1:10 medium (Foesel et al., 2013; Supplementary Material and Methods) buffered at pH 5.5 or 8.0 with 10 mM MES or HEPPS, respectively. Pure cultures were obtained by restreaking. Unless otherwise noted SSE/HD 1:10 of the respective pH was also used for further physiological tests and biomass production.

On solid media strain D16/0/H6\textsuperscript{T} formed small colonies of ≤ 0.1 mm in diameter that were light pink in color, translucent, smooth, shiny, and convex with entire margins. The colonies of strain A22/0/F9_1\textsuperscript{T} had a size of up to 1 mm, were whitish to pale yellowish/brownish in color, translucent, smooth, shiny, and convex with entire margins. In liquid culture no particularities such as intensive coloring or aggregation were observed.

Strain D16/0/H6\textsuperscript{T} formed ovoid cells to short rods of an average size of (1.2 ± 0.2) \times (0.6 ± 0.04) µm (Fig. 1 a). In older cultures also longer rods of up to 3 µm occurred. Cells of strain A22/0/F9_1\textsuperscript{T} were regular rods with a length of (1.8 ± 0.3) and a width of (0.6 ± 0.03) µm (Fig. 1 d). Both strains were motile. They divided by binary fission and stained Gram-positive in standard procedures (Smibert & Krieg, 1994). This Gram-type was verified by the KOH-test (Buck, 1982) and corresponds to the Gram-type of all Solirubrobacterales, while the Thermoleophilales and Gaiellales stain Gram-negative (Table 1). Similar to the related taxa, capsule or spore formation assessed by India ink and malachite green staining (Beveridge et al., 2007), respectively, was not detected. For transmission electron microscopy cells were prepared as described earlier (Foesel et al., 2013). Cells of strain D16/0/H6\textsuperscript{T} had a single, lateral flagellum whereas cells of strain A22/0/F9_1\textsuperscript{T} carried multiple flagella (Fig. 1 b, e). Pili or fimbria were absent. Although flagella of strain A22/0/F9_1\textsuperscript{T} sometimes seemed to interlink cells, the formation of well organized network-like structures due to self-aggregation of flagella as shown for Conexibacter woesei (Monciardini et al., 2003) was not observed. Ultrastructural analyses confirmed the Gram-positive cell wall structure (Fig. 1 c, f). Cells of strain D16/0/H6\textsuperscript{T} contained conspicuous intracytoplasmic inclusion bodies. Furthermore a surface layer could be detected which was missing for strain A22/0/F9_1\textsuperscript{T}.

Almost full-length 16S rRNA gene fragments of strains D16/0/H6\textsuperscript{T} and A22/0F9_1\textsuperscript{T} were amplified and sequenced as described before (Foesel et al., 2013). The resulting sequences comprised 1456 (D16/0/H6\textsuperscript{T}) and 1455 (A22/0F9_1\textsuperscript{T}) unambiguous nucleotides
between *E. coli* positions 28 and 1491 (Brosius *et al.*, 1978). Together with not yet included reference sequences they were added to the 16S rRNA-based ‘All-Species Living Tree’ Project (LTP) database (Yarza *et al.*, 2008) release 119 (November 2014) using the program package ARB version 6.0 (Ludwig *et al.*, 2004). After automated alignment with the Fast aligner tool implemented in ARB, the alignment was manually refined based on secondary structure information. Phylogenetic trees were calculated using neighbor-joining, maximum-parsimony, and maximum-likelihood algorithms (40% maximum frequency filter calculated for the considered sequences resulting in 1366 valid columns between position 83 and 1406 of the *E. coli* 16S rRNA reference gene; 1000 bootstrap resamplings). All three methods assigned the two novel strains D16/0/H6\(^T\) and A22/0F9_1\(^T\) to the order *Solirubrobacterales*. In the maximum-likelihood (Fig. 2) and the neighbor-joining trees (Supplementary Fig. S1a), the two species constitute a separate, deeply branching lineage, while the maximum-parsimony algorithm (Supplementary Fig. S1b) clustered the sequences together with the family *Patulibacteraceae* within the order *Solirubrobacterales*. Based on nucleotide identity alone (ARB neighbor-joining tool without the use of an evolutionary substitution model), the closest validly described relatives of D16/0/H6\(^T\) were *Solirubrobacter ginsenosidimutans* and *S. phytolaccae* (94.0%) followed by *Conexibacter arvalis* (93.8%) and *S. soli* (93.6%). The next relatives of A22/0F9_1\(^T\) were *S. phytolaccae* (93.5%), *S. ginsenosidimutans* (93.1%), and *S. soli* (93.3%). Pairwise 16S rRNA gene sequence identity between the two novel strains was 96.6%.

For DNA-DNA hybridization cells were disrupted in a Constant Systems TS 0.75 KW (IUL Instruments, Koenigswinter, Germany), DNA was purified from the crude lysate by chromatography on hydroxyapatite (Cashion *et al.*, 1977). Hybridization was carried out according to established protocols (De Ley *et al.*, 1970; Huss *et al.*, 1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6x6 multiecell changer and a temperature controller with in situ temperature probe (Varian). Duplicate measurements yielded hybridization levels of 30.2% and 28.4% between strain D16/0/H6\(^T\) and strain A22/0F9_1\(^T\), which is far below the threshold value of 70% DNA-DNA relatedness generally accepted for the definition of a novel species (Wayne *et al.*, 1987).

Signature nucleotide patterns originally established for the former subclass *Rubrobacteridae* and its subordinate taxonomic ranks (Stackebrandt, 2004; Stackebrandt *et al.*, 1997) were adopted from the most recent revisions (Reddy & Garcia-Pichel, 2009; Zhi *et al.*, 2009) and reevaluated considering the recent taxonomic conversions within this group.
(see above) and the addition of several novel species. The updated signature nucleotides are given in the respective taxon descriptions. An overview is provided in Table 2.

For determination of the peptidoglycan composition whole cells of strains D16/0/H6\(^T\) and A22/0F9\_1\(^T\) were hydrolyzed (4N HCl, 100 °C, 15 hours) and the hydrolysates subjected to thin-layer chromatography on cellulose plates according to Protocol 1 of Schumann (2011). In both strains meso-2,6-diaminopimelic acid (meso-Dpm) was found as diagnostic diamino acid of the peptidoglycan which is a common feature of all members of the class Thermoleophilia (Table 1).

For G+C content determination cells were disrupted in a French press. The DNA was purified using hydroxyapatite (Cashion et al., 1977). After sample treatment with P1 nuclease and bovine alkaline phosphatase (Mesbah et al., 1989) the resulting deoxyribonucleosides were analyzed by high performance liquid chromatography (HPLC, Shimadzu Corporation, Kyoto, Japan) with conditions adapted from Tamaoka & Komagata (1984). The molar G+C content was calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah et al., 1989) to be 72.8 and 74.0 mol% for strains D16/0/H6\(^T\) and A22/0F9\_1\(^T\), respectively. These values range within the G+C contents reported for the members of the orders Solirubrobacterales and Gaiellales, while the G+C content of the Themoleophilales and the Rubrobacterales is lower (Tab.1).

Isoprenoid quinones were extracted from dried biomass with chloroform/methanol (2:1, v/v) (Collins & Jones, 1981) and analyzed via HPLC (Tindall, 1990). The quinone system of strains D16/0/H6\(^T\) and A22/0F9\_1\(^T\) comprised menaquinone MK-8 (87 and 67%, respectively) as major component. In addition, strain D16/0/H6\(^T\) contained MK-7 (11%). Strain A22/0F9\_1\(^T\) possessed MK-7 (12%), too, but also the partially hydrogenated menaquinones MK-7(H\(_2\)) (8%), MK-8(H\(_2\)) (11%) and MK-9(H\(_2\)) (3%). With respect to cellular quinones, the two novel strains thus resemble the Rubrobacterales, which contain MK-8 as predominant quinone (Tab.1), rather than the three families of the order Solirubrobacterales that are characterized by the dominance of MK-7(H\(_4\)) (Solirubrobacteraceae, Conexibacteraceae) and DMK-7 or MK-7(H\(_2\)) (Patulibacteraceae).

Cultures for fatty acid analysis were grown under identical conditions (SSE/HD 1:10 agar plates, pH 5.5, 18 d at room temperature). About 40 mg wet weight of fresh cells were harvested and extracted according to the standard protocol (Sasser, 1990) of the Microbial Identification System (MIDI Inc.; version 6.1) for fatty acids analysis. Compounds were identified by comparison to the TSBA40 peak naming table database. Both strains possessed straight chain, methyl or hydroxyl-branched saturated and monounsaturated fatty acids, but
differed largely in their major components and their full profiles (Supplementary Table S1).

Strain D16/0/H6T contained C17:0 10-methyl (36.0%), iso-C16:0 (21.6%) and C18:1 ω9c (10.0%) as major components. In addition, the fatty acid profile of strain D16/0/H6T showed a variety of minor peaks below 4% including several peaks potentially not identified or misidentified by the standard methods of the MIDI System (Albuquerque et al., 2011, 2014; Carreto et al., 1996). Those were indicated accordingly (Supplementary Table S1). Major fatty acids of strain A22/0F9_1T were C18:1 ω9c (35.1%), C17:1 ω9c (16.0%), C17:1 ω6c (11.9%) and iso-C16:0 (11.7%). In addition, summed feature 6 (C19:1 ω9c and/or C19:1 ω11c) and C17:0 10-methyl occurred in significant amounts (8.6% and 7.2%, respectively). The occurrence of C17:0 10-methyl as major component is a trait that strain D16/0/H6T shares with the Rubrobacterales (Albuquerque et al., 2014), while members of the order Solirubrobacterales contain these unusual fatty acids in low relative proportions at best (Almeida et al., 2013; Kim et al., 2012; Reddy & Garcia-Pichel, 2009; Sasser, 1990; Seki et al., 2012; Wei et al., 2014; Zhang et al., 2014). The high amounts of the methyl-branched fatty acid iso-C16:0 and the presence of certain monounsaturated fatty acids of different length (C18:1 ω9c, C17:1 ω8c, C17:1 ω6c) in strains D16/0/H6T and A22/0F9_1T are shared with different members of the Solirubrobacterales (Table 1).

Growth ranges and optima of temperature and pH were determined under oxic conditions in liquid SSE/HD 1:10 media. Temperature ranges of 10 - 56°C and pH values of 2.5 - 10.0 were tested. Depending on the respective pH value MES, HEPES, HEPPS, or CHES (Sigma-Aldrich, Steinheim, Germany or Applichem, Darmstadt, Germany) were used as buffer system at a final concentration of 10 mM. Since SSE/HD1:10 already contains high amounts of different salts, salt tolerance was tested in liquid DSMZ medium 830 (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium830.pdf) amended with NaCl to final concentrations of 0 - 10% (w/v). Growth was determined by following the optical density at 660 nm. Strain D16/0/H6T grew at temperatures of 13 - 43°C and pH 4.5 - 8.5. Optimal growth (defined as ≥ 75% of highest growth rate) was determined at 35 - 43°C (highest rate at 40°C) and pH 5.0 - 8.0 (highest rate at pH 6.0). Growth of strain A22/0F9_1T occurred at 17 - 43°C and pH 5.0 - 9.5. It grew optimally at 32 - 43°C (highest rate at 35°C) and pH 6.0 - 7.5 (highest rate at pH 6.5). Minimal doubling times were 9.0 and 9.2 h for strain D16/0/H6T and A22/0F9_1T, respectively. Strain D16/0/H6T grew best at NaCl concentrations of 0%, but only with reduced growth rate at 0.25% and 0.5%, while at ≥ 1.0% NaCl no growth at all occurred. Strain A22/0F9_1T tolerated NaCl concentrations of 0 - 1.0%, the highest growth rate was observed at 0.25% NaCl. In summary, both strains exhibit broad
ranges of tolerance towards temperature and pH, achieve comparable doubling times, but show different, individual minima, maxima and optima. In comparison to most of the mesophilic soil isolates within the order Solirubrobacterales the two novel isolates show elevated temperature optima and maxima, but by far do not reach the preferred growth temperatures of the Thermoleophilales and the thermophilic strains of the Rubrobacterales (Table 1).

Anaerobic growth with alternative electron acceptors was evaluated in liquid medium 830 (see above) with \( \text{N}_2 \) as the gas phase and 10 mM NaNO\(_3\), 10 mM Na\(_2\)SO\(_4\), 10 mM iron pyrophosphate (Fe\(_4\)(P\(_2\)O\(_7\))\(_3\)·x H\(_2\)O), or 2 mM NaNO\(_2\) as electron acceptor. Fermentative growth was assessed using the API20E test system (Biomérieux, Marcy L’Etoile, France).

Concentrations of electron acceptors were determined colorimetrically (Cataldo et al., 1975; Gadkari, 1984; Harrigan & Mc Cance, 1966; Tabatabai, 1992; Tamura et al., 1974). However, strains D16/0/H6\(^T\) and strain A22/0F9\(_1\)\(^T\) neither reduced nitrate, nitrite, sulfate or iron (III), nor showed fermentative growth.

The range of growth substrates utilized by strains D16/0/H6\(^T\) and strain A22/0F9\(_1\)\(^T\) was determined in microtiter plates in two parallels using soil solution equivalent (SSE) (Angle et al., 1991) amended with 50 mg L\(^{-1}\) of yeast extract as basal medium. In total 106 single substrates were tested including sugars, organic acids, keto acids, alcohols, amino acids (0.5 to 10 mM), casamino acids, casein hydrolysate, laminarin, peptone, yeast extract (0.05% w/v each), and Tween 80 (0.001% w/v). Substrate utilization was recorded as positive if the final OD\(_{660}\) surpassed the control value without addition of substrate by 1.5 fold. Growth on the polymers cellulose, chitin and starch was tested on solidified media as described before (Huber et al., 2014) with the additional staining of chitin plates with Congo Red (Thiagarajan et al., 2011). Cytochrome c-oxidase and catalase activities were monitored employing standard protocols (Cowan, 1974; Smibert & Krieg, 1994). Cytochrome c-oxidase additionally was tested by Bactident® Oxidase test strips (Merck, Darmstadt, Germany). Further physiological features (e. g. indol formation, urease activity, activity of different exoenzymes) were determined using the API20E and the API ZYM test systems (Biomérieux). Strains D16/0/H6\(^T\) and A22/0F9\(_1\)\(^T\) both grew well on glucose and protein containing, complex substrates such as casamino acids, peptone, or yeast extract (Table 1; Supplementary Table S2). Strain A22/0F9\(_1\)\(^T\) also grew on several longer-chain organic acids. In contrast, sugars were the preferred growth substrate of many of the related species (Table 1). Exoenzyme profiles of strains D16/0/H6\(^T\) and strain A22/0F9\(_1\)\(^T\) (Supplementary Table S3) on the whole resemble those known from species of the related families. Only the
Solirubrobacteraceae and Rubrobacteraceae show additional activity of several carbohydrate hydrolyzing enzymes, which largely is in congruence with the observed substrate ranges of the respective species. Additional results of the physiological tests are compiled in Supplementary Tables S2 and S3, and in the species descriptions below.

In summary, strains D16/0/H6\textsuperscript{T} and strain A22/0/F9\_1\textsuperscript{T} are affiliated with the order Solirubrobacterales, but cannot be unambiguously attributed to one of the established families Solirubrobacteraceae, Conexibacteraceae or Patulibacteraceae. Their distinct phylogenetic position and the specific combination of phenotypic traits differentiate the two isolates from the members of all existing families. Although several distinguishing features were observed between the two strains, their close phylogenetic relationship indicates that they belong to the same genus. Consequently, strains D16/0/H6\textsuperscript{T} and strain A22/0/F9\_1\textsuperscript{T} are proposed to form the novel genus Parviterribacter, including the two novel species, *P. kavangonensis* and *P. multiflagellatus* constituting the novel family Parviterribacteraceae.

Furthermore, we propose the order Gaiellales as a further, deep-branching order of the class Thermoleophilia based on its phylogenetic position, the observed signature nucleotide pattern and several phenotypic properties shared with other members of the class.

Based on its phylogenetic position the species *Bactoderma rosea* is proposed to be a member of the genus *Patulibacter*. Whether it should be assigned to its phylogenetically closest relative, the species *P. minatonensis*, or remain an autonomous species needs to be clarified by future physico-chemical characterization.

**Description of Parviterribacter, gen. nov.**

*Parviterribacter* (Par.vi.ter.ri.bac’ter. L. adj. parvus, small; L. fem. n. terra, earth; N.L. masc. n. bacter, a rod; N.L. masc. n. *Parviterribacter* small, rod-shaped bacterium isolated from soil).

Gram-positive, non-spore-forming, motile, ovoid to rod shaped cells that divide by binary fission. No capsule formation. Strictly aerobic, chemoorganotrophic mesophiles. Catalase positive, cytochrome *c*-oxidase variable. The peptidoglycan contains meso-Dpm as diagnostic diamino acid. Major fatty acids consist of mono-unsaturated and iso- and/or 10-methyl-branched fatty acids. Major quinone is MK-8. The DNA G+C content is 73 - 74%.

The type species is *Parviterribacter kavangonensis*.

**Description of Parviterribacter kavangonensis, sp. nov.**
Parviterribacter kavangonensis (ka.van.go.nen´sis. N.L. masc. adj. kavangonensis deriving from the Kavango region, Namibia).

Displays the following characteristics in addition to those given in the genus description.

Cells are 1.0 - 1.5 µm long and 0.6 - 0.7 µm in diameter. On agar plates forms small colonies of ≤ 0.1 mm in diameter that are light pink in color, translucent, smooth, shiny, and convex with entire margins. Grows at 13 - 43°C and pH 4.5 - 8.5; optimal growth at 35 - 43°C and pH 5.0 - 8.0. Minimal doubling time is 9.0 h. Tolerates NaCl concentrations of up to 0.5% (w/v); optimal growth occurs in the absence of NaCl.

Grows on glucose, mannose, lyxitol, aspartate, lysine, hydroxy-proline, acetate, butyrate, β-hydroxybutyrate, propionate, protocatechuate, pyruvate, succinate, casamino acids, casein hydrolysate, peptone, and yeast extract. No growth is observed on arabinose, cellobiose, erythrose, erythulose, fructose, fucose, galactose, lactose, lyxose, maltose, melizitose, raffinose, rhamnose, sorbose, sucrose, trehalose, xylose, glucosamine, N-acetylglucosamine, N-acetylgalactosamine, acetoin, adonitol, arabitol, dulcitol, mannotol, myo-inositol, sorbitol, xylitol, alanine, arginine, cysteine, glutamate, glycine, histidine, isoleucine, leucine, methionine, ornithine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, adipate, ascorbate, benzoate, trimethoxybenzoate, α-hydroxybutyrate, γ-hydroxybutyrate, isobutyrate, caproate, caprylate, citrate, isocitrate, crotonate, formate, fumarate, gluconate, 2-oxoglucuronate, glucuronate, 2-oxoglutarate, glycolate, glyoxylate, heptanoate, isovalerate, laevulinate, lactate, malate, malonate, nicotinic acid, oxaloacetate, shikimate, tartrate, 2-oxovalerate, butanol, 1,2-butanediol, 2,3-butanediol, ethanol, ethylene glycol, glycerol, methanol, propanol, 1,2-propanediol, fermented rumen extract, chitin, cellulose, laminarin, starch, and Tween 80.

Aesculin and gelatine are hydrolyzed, albeit gelatin only very slowly. Indol and acetoin formation is not observed. Urease and arginine dihydrolase negative. The following additional enzyme activities are present: acidic phosphatase, alkaline phosphatase, naphtol-AS-BI-phosphohydrolase, esterase (C4), esterase lipase (C8), leucine-arylamidase, cystine-arylamidase (weak), β-glucuronidase, α-mannosidase, and α-fucosidase (weak). Lipase (C14), valine-arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidase, α- and β-glucosidase, and N-acetyl-β-glucosaminidase activity is absent.

Oxygen is the sole electron acceptor. The major quinone is MK-8, in addition MK-7 occurs. The fatty acid profile is dominated by C_{17:0} 10-methyl, iso-C_{16:0}, and C_{18:1} ω9c.

The type strain is D16/0/H6^{T} (= DSM 25205^{T} = LMG 26950^{T}), and was isolated from a loamy subtropical savanna soil at Mile 46, Kavango region, Northern Namibia (18°17′14.0″S
19°15’32.3´´E, 1163 m height above sea level). The DNA G+C content of the type strain is 72.8 mol%.

**Description of Parviterribacter multiflagellatus, sp. nov.**

*Parviterribacter multiflagellatus* (mul.ti fla.gel.la’tus. L. adj. multus, many, numerous; L. n. *flagellum*, a whip; L. masc. suff. -atus, suffix denoting provided with; N.L. masc. adj. *multiflagellatus*, provided with numerous flagella).

In addition to those given in the genus description shows the following features. Cells are 1.3 - 2.3 µm long and 0.6 - 0.7 µm wide. Colonies attain a size of up to 1 mm, are whitish to pale yellowish/brownish in color, translucent, smooth, shiny, and convex with entire margins. Grows at 17 - 43°C and pH 5.0 - 9.5; optimal growth occurs at 32 - 43°C and pH 6.0 - 7.5. Minimum doubling time is 9.2 h. Tolerates NaCl concentrations of up to 1.0% (w/v); optimal growth occurs at 0.25%.

Grows on glucose, cellobiose, N-acetylglucosamine, adonitol, glutamate, butyrate, isobutyrate, caproate, caprylate, crotonate, gluconate, heptanoate, laevulinate, glycerol, protocatechuate, pyruvate, succinate, casamino acids, casein hydrolysate, peptone, yeast extract, and starch. No growth is observed on arabinose, erythrose, erythulose, fructose, fucose, galactose, lactose, lyxose, maltose, mannose, melititose, raffinose, rhamnose, sorbose, sucrose, trehalose, xylene, glucosamine, N-acetylglactosamine, acetoin, arabitol, dulcitol, lyxitol, mannitol, myo-inositol, sorbitol, xylitol, alanine, arginine, aspartate, cysteine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, hydroxyproline, threonine, tryptophan, tyrosine, valine, acetate, adipate, ascorbate, benzoate, trimethoxybenzoate, α-hydroxybutyrate, β-hydroxybutyrate, γ-hydroxybutyrate, citrate, isocitrate, formate, fumarate, 2-oxogluconate, glucuronate, 2-oxoglutarate, glycolate, glyoxylate, isovalerate, lactate, malate, malonate, nicotinic acid, oxaloacetate, propionate, shikimate, tartrate, 2-oxovalerate, butanol, 1,2-butandiol, 2,3-butandiol, ethanol, ethylene glycol, methanol, propanol, 1,2-propanediol, fermented rumen extract, chitin, cellulose, laminarin, and Tween 80.

Aesculin and gelatine are hydrolyzed. Indol and acetoin are not formed. Urease and arginine dihydrolase negative. The following additional enzyme activities are present: acidic phosphatase (weak), alkaline phosphatase (weak), naphtol-AS-BI-phosphohydrolase, esterase (C4), esterase lipase (C8), lipase (C14; weak), leucine-arylamidase (weak), cystine-arylamidase (weak), β-glucosidase, and N-acetyl-β-glucosaminidase (weak). Valine-
arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidase, α-glucosidase, β-glucuronidase, α-mannosidase, and α-fucosidase activity is absent.

Oxygen is the sole electron acceptor. The major quinone is MK-8, as further notable constituents MK-7, MK-7(H$_2$), and MK-8(H$_2$) occur. The fatty acid profile is dominated by C$_{18:1}$ ω9c, C$_{17:1}$ ω8c, C$_{17:1}$ ω6c, and iso-C$_{16:0}$.

The type strain is A22/0/F9-1$^T$ (= DSM 25204$^T$ = LMG 26949$^T$), which was isolated from a sandy subtropical savanna soil in Erichsfelde, central Namibia (21°38’15.8’’S 16°52’03.9’’E, 1497 m height above sea level). The DNA G+C content of the type strain is 74.0 mol%.

**Description of Parviterribacteriaceae, fam. nov.**

*Parviterribacteriaceae* (Par.vi.ter.ri.bac.te.ri.ce’ae. N.L. masc. n. *Parviterribacter* type genus of the family; suff. -aceae, ending denoting a family; N.L. fem. pl. n. *Parviterribacteraceae*, the *Parviterribacter* family).

The family *Parviterribacteriaceae* is a member of the order *Solirubrobacterales*. Currently, the family comprises the sole genus *Parviterribacter*. The delineation of the family is determined by its phylogenetic position of the 16S rRNA gene sequences of its members and the quinone system (MK-8 as major quinone). The detailed description is the same as that given for the genus *Parviterribacter*. Signature nucleotides in the 16S rRNA gene sequence (Escherichia coli reference gene; Brosius et al. (1978)) partially shared with other families within the order *Solirubrobacterales* are: 52:359 (C-G), 408:434 (G-C), 590:649 (U-A), 600:638 (C-G), 823:877 (A-U), and 999:1041 (U-A). Signatures differentiating the *Parviterribacteriaceae* from all other *Solirubrobacterales* families are C-G and U-G at positions 681:709 and 1115:1185, respectively. Signatures shared with all members of the order *Solirubrobacterales* or even the class *Themoleophilia* are listed in the respective taxon descriptions. The type genus of this family is *Parviterribacter*.


The description is as given by Zhi et al. (2009) with the following amendments. Gram-positive, non motile rods. Aerobic mesophiles with a preference for sugars as sole carbon source. The sole or predominant quinone is MK-7(H$_4$). Dominating fatty acids are C$_{18:1}$ ω9c and C$_{16:0}$ iso. Updated signature nucleotides in the 16S rRNA gene sequence partially shared with other *Solirubrobacterales* families are: 52:359 (C-G), 144:178 (C-G), 408:434 (G-C), 600:638 (C-G), 681:709 (U-A), 823:877 (G-C), 999:1041 (U-A), and 1115:1185 (C-G). The
signature C-G at positions 145:177 and 590:649 differentiates the Solirubrobacteriaceae from any other family of the order. Signatures shared with all members of the order Solirubrobacterales and the class Themoleophilia are listed in the respective taxon descriptions.

**Emended description of the family Conexibacteraceae Stackebrandt 2005 (effective publication Stackebrandt, 2004) emended Zhi et al. 2009**

The description is as given by Zhi et al. (2009) with the following amendments. Gram-positive, motile rods. Aerobic, psychrotolerant mesophiles. The diagnostic amino acid is meso-Dpm, the major respiratory quinone is MK-7(H₄). Shared, dominating fatty acids are C₁₇:₁ ω6c and C₁₈:₁ ω9c. Signature nucleotides in the 16S rRNA gene sequence partially shared with other Solirubrobacterales are: 590:649 (U-A), 600:638 (C-G), 681:709 (U-A), 823:877 (G-C), and 1115:1185 (C-G). Signatures differentiating the Conexibacteraceae from any other family of the order are: 52:359 (U-A), 144:178 (U-A), 145:177 (U-A), 408:434 (A-U), and 999:1041 (G-U). Signatures shared with all members of the order Solirubrobacterales or even the class Themoleophilia are listed in the respective taxon descriptions.

**Emended description of the family Patulibacteraceae Takahashi et al. 2006 emended Zhi et al. 2009**

The description is as given by Takahashi et al. (2006) and Zhi et al. (2009) with the following amendments. The main isoprenoid quinone is DMK-7 or MK-7(H₂). Updated signature nucleotides in the 16S rRNA gene sequence partially shared with other Solirubrobacterales families are: 52:359 (C-G), 144:178 (C-G), 408:434 (G-C), 590:649 (U-A), 600:638 (U-G), 681:709 (U-A), 823:877 (A-U), and 1115:1185 (C-G). Signatures shared with all members of the order Solirubrobacterales or even the class Themoleophilia are listed in the respective taxon descriptions.

**Emended description of the order Solirubrobacterales Reddy and Garcia-Pichel 2009**

The description is as given by Reddy and Garcia-Pichel (2009) with the following amendments. Contains the families Solirubrobacteraceae, Conexibacteraceae, Patulibacteraceae, and Parviterribacteraceae. Updated 16S rRNA gene sequence signature nucleotides are: 63:104 (G-C), 70:98 (G-C), 293:304 (G-C), 370:391 (C-G), 580:761 (U-A), 670:736 (A-U), 722:733 (U-A), 941:1342 (A-U), 1118:1155 (U-A), and 1311:1326 (A-U). Signatures shared with the other orders of the class Themoleophilia are given in the class description.

The description is as given by Zhi et al. (2009) with the following amendments. Gram-negative (although typical Gram-positive cell wall structure as demonstrated by TEM), non motile rods. Aerobic, moderately thermophilic strains that utilize n-alkanes as sole carbon source. Cell wall peptidoglycan is based on meso-Dpm. The updated pattern of 16S rRNA gene sequence signature nucleotides partially shared with families of the neighboring orders Solirubrobacterales and Gaiellales consists of: 52:359 (C-G), 63:104 (G-C), 70:98 (G-C), 139:224 (G-C), 144:178 (C-G), 145:177 (C-G), 377:386 (C-G), 408:434 (G-C), 590:649 (C-G), 600:638 (C-G), 681:709 (C-G), 722:733 (U-A), 823:877 (G-C), 999:1041 (A-U), and 1115:1185 (C-G). Signatures differentiating the Thermoleophilaceae from any other family within the Thermoleophilia are 293:304 (G-U), 370:391 (G-C), 580:761 (C-G), 670:736 (G-C), 941:1342 (G-C), 1118:1155 (C-G), and 1311:1326 (G-C). On order level no further signatures can be added to the above given profile. Signatures shared with all members of the class Thermoleophilia can be seen from the class description.

Emended description of the order Thermoleophilales Reddy and Garcia-Pichel 2009

The description is as given by Reddy and Garcia-Pichel (2009) with the following amendments. Updated 16S rRNA gene sequence signature nucleotides largely correspond to those given in the description of the family Thermoleophilaceae. Positions 52:359, 144:178, 408:434, 823:877, 999:1041, and 1115:1185 become meaningless on order level, positions 139:224, 145:177, 377:386, 590:649, 600:638, and 681:709 become unique features within the class Thermoleophilia. Signatures shared within the whole class Thermoleophilia are listed in the respective description.

Emended description of the family Gaiellaceae Albuquerque et al. 2012 (effective publication Albuquerque et al., 2011)

The description is as given by Albuquerque et al. (2011) with the following amendments. The family is assigned to the class Thermoleophilia as own, deep-branching order (see below). 16S rRNA gene sequence signature nucleotides partially shared with families of the neighboring orders Solirubrobacterales and Thermoleophilales consists of: 52:359 (C-G), 144:178 (C-G), 293:304 (G-C), 370:391 (C-G), 408:434 (G-C), 580:761 (U-A), 670:736 (A-U), 681:709 (U-A), 823:877 (G-C), 941:1342 (A-U), 999:1041 (A-U), 1115:1185 (C-G), 1118:1155 (U-A), and 1311:1326 (A-U). Signatures differentiating the Gaiellaceae from any other family within the Thermoleophilia are 63:104 (C-G), 70:98 (A-
G), 139:224 (C-G), 145:177 (U-G), 377:386 (G-C), 590:649 (G-C), 600:638 (G-C), and 722:733 (U-G). On order level no further signatures can be added. Signatures shared with all members of the class Thermoleophilia can be seen from the class description.

**Emended description of the order Gaiellales Albuquerque et al. 2012 (effective publication Albuquerque et al., 2011)**

The description is as given by Albuquerque et al. (2011) with the following amendments. Phylogenetically the order is a deep branching member of the class Thermoleophilia. 16S rRNA gene sequence signature nucleotide patterns are those given in the description of the family Gaiellaceae with the following changes. Signature positions 52:359, 144:178, 408:434, 823:877, 999:1041, and 1115:1185 are insignificant on order level, positions 139:224 and 681:709 are unique. Signatures shared with all members of the class Thermoleophilia are given in the class description.

**Emended description of the class Thermoleophilia Suzuki and Whitman 2013**

The description is as given by Suzuki and Whitman (2012) with the following amendments. In addition, contains the order Gaiellales. 16S rRNA gene sequence signature nucleotide patterns of the class partially shared with other classes of the phylum Actinobacteria consist of: 63:104 (C-G), 293:304 (G-U), 370:391 (C-G), 377:386 (G-C), 408:434 (G-C), 670:736 (A-U), 681:709 (C-G), 941:1342 (A-U), and 1115:1185 (C-G). Signatures differentiating the Thermoleophilia from any other class of the phylum Actinobacteria are G-C and G-A at positions 66:103 and 661:744, respectively.


The description is as given by Zhi et al. (2009) with the following amendments. Currently is the only family within the class Rubrobacteria. 16S rRNA gene sequence signature nucleotides partially shared with families of the neighboring class Thermoleophilia consist of: 63:104 (C-G), 293:304 (G-U), 370:391 (C-G), 377:386 (G-C), 408:434 (G-C), 670:736 (A-U), 681:709 (C-G), 941:1342 (A-U), and 1115:1185 (C-G). Signatures differentiating the Rubrobacteraceae from any other family compared with are: 52:359 (G-C), 66:103 (A-U), 70:98 (A-U), 139:224 (U-A), 144:178 (G-C), 145:177 (G-C), 409:433 (G-C), 438:496 (G-G), 657:749 (G-C), 661:744 (G-C), 953:1228 (U-A), 954:1226 (C-G), 1051:1207 (C-G), and 1313:1324 (U-A). Comparison on order level does not add further signature
478 positons. Additional signatures uniform within the family but only meaningful on class level
479 are listed in the description of the Rubrobacteria.

480 **Emended description of the order Rubrobacterales Rainey et al. 1997 emend. Reddy and
481 Garcia-Pichel 2009 and Zhi et al. 2009**

482 The description is as given by Reddy and Garcia-Pichel (2009) and Zhi et al. (2009)
483 with the following amendments. Known members are mesophilic or moderately thermophilic
484 to thermophilic. Updated 16S rRNA gene sequence signature nucleotides are those given in
485 the description of the family Rubrobacteraceae. Sole exception is position 408:434 that
486 becomes insignificant on order level. Further signatures acting on class level can be seen from
487 the description of the Rubrobacteria.

488 **Emended description of the class Rubrobacteria Suzuki 2013**

489 The description is as given by Suzuki (2012) with the following amendments. 16S
490 rRNA gene sequence signature nucleotide patterns of the class partially shared with other
491 classes of the phylum Actinobacteria are: 66:103 (A-U), 127:234 (G-C), 242:284 (C-G),
492 291:309 (U-A), 316:337 (C-G), 661:744 (G-C), 670:736 (A-U), 819 (A), 941:1342 (A-U),
493 955:1225 (U-A), 1313:1324 (U-A), and 1410:1490 (A-U).
494 Signatures differentiating the Rubrobacteria from all other classes of the phylum
495 Actinobacteria are: 52:359 (G-C), 70:98 (A-U), 139:224 (U-A), 144:178 (G-C), 145:177 (G-C),
496 293:304 (G-U), 409:433 (C-G), 438:496 (G-G), 502:543 (C-G), 657:749 (G-C), 681:709
497 (C-G), 953:1228 (U-A), 954:1226 (C-G), 1051:1207 (C-G), and 1115:1185 (C-G).
ACKNOWLEDGEMENTS

We thank Dr. Peter Schumann, Dr. Cathrin Spröer, Gabi Pötter, Anika Wasner, Bettina Sträubler and Birgit Grünn (all DSMZ) for analysis of cellular fatty acid composition, respiratory quinones, peptidogycan amino acid composition, and G+C-content as well as for performing DNA-DNA-hybridization. Ina Schleicher (HZI) assisted with electron microscopic preparations. Dr. Peter Schumann is also acknowledged for advice regarding peptidoglycan structure and fatty acid composition, Prof. Dr. Erko Stackebrandt for helpful thoughts on signature nucleotides. Dr. Pia Wuest is acknowledged for critically reading the manuscript.

Soils in Namibia were sampled under collection permits 1245/2008 and 1358/2009, and exported under permits ES 23744 (of March 27, 2008) and ES 24478 (of April 6, 2009). Isolation and deposit of strains D16/0/H6T and A22/0/F9_1T is under the MTA of the NBRI (National Botanical Research Institute, Namibia) of April 05, 2012. This work was supported by grants of German Federal Ministry of Science and Education to Jörg Overmann (BIOLOG/BIOTA project 01LC0621C and TFO project 01LL0912M).


Zhi, X.-Y., Li, W.-J. & Stackebrandt, E. (2009). An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the
712 TABLES
Table 1: Characteristics of D16/0/H6<sup>T</sup> and strain A22/0/F9<sub>1</sub><sup>T</sup> compared those of at the time of writing all validly described strains within related families of the orders Solirubrobacterales, Thermoleophilales, Gaiellales and Rubrobacterales.

Strains/families: 1, D16/0/H6<sup>T</sup>; 2, A22/0/F9<sub>1</sub><sup>T</sup>; 3, Solirubrobacteraceae, comprising summarized features of Solirubrobacter pauli B33D1<sup>T</sup> (Singleton et al., 2003), S. soli Gsoil 355<sup>T</sup> (Kim et al., 2007), S. ginsenosidimutans BXN5-15<sup>T</sup> (An et al., 2011), S. phytolaccae GTGR-8<sup>T</sup> (Wei et al., 2014), S. taibaiensis GTJR-20<sup>T</sup> (Zhang et al., 2014); 4, Conexibacteraceae, including Conexibacter woesei ID131577<sup>T</sup> (Monciardini et al., 2003), C. arvalis KV-962<sup>T</sup> (Seki et al., 2012); 5, Patulibacteraceae, including Bactoderma rosea (Tepper & Korshunova, 1973), Patulibacter minatonensis KV-614<sup>T</sup> (Takahashi et al., 2006), P. americanus CP1777-2<sup>T</sup> (Reddy & Garcia-Pichel, 2009), P. ginsengiterrae P4-5<sup>T</sup> (Kim et al., 2012), P. medicamentivorans I11<sup>T</sup> (Almeida et al., 2013); 6, Thermoleophilaceae, including Thermoleophilum album HS-5<sup>T</sup> (Zarilla & Perry, 1984), T. minutum YS-4<sup>T</sup> (Zarilla & Perry, 1986); 7, Gaiellaceae, including Gaiella occulta F2-233<sup>T</sup> (Albuquerque et al., 2011); 8, Rubrobacteraceae, including Rubrobacter radiotolerans IAM 12072<sup>T</sup> (Suzuki et al., 1988), R. xylanophilus PRD-1<sup>T</sup> (Carreto et al., 1996), R. taiwanensis LS-293<sup>T</sup> (Chen et al., 2004), R. bracarensis VF70612_S1<sup>T</sup> (Jurado et al., 2012), R. aplysinae RV113<sup>T</sup> (Kämpfer et al., 2014), R. calidifluminis RG-1<sup>T</sup> and R. naiadicus RG-3<sup>T</sup> (Albuquerque et al., 2014).

All strains are strictly aerobic, formation of spores or capsules is reported for none of them.

+, positive; –, negative; (+), weak growth detected, v, variable; ND, no data available.

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).
<table>
<thead>
<tr>
<th>Characteristic</th>
<th><strong>Solirubrobacterales</strong></th>
<th><strong>Thermoleophila</strong></th>
<th><strong>Gaelliales</strong></th>
<th><strong>Rubrobacterales</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolation source</strong></td>
<td>Terrestrial</td>
<td>Terrestrial (3/5), plant tissue (2/5)</td>
<td>Terrestrial (3/5), aquatic (1/5), ND (1/5)</td>
<td>Aquatic</td>
</tr>
<tr>
<td><strong>Cell shape</strong></td>
<td>Ovoid cells to rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
</tr>
<tr>
<td><strong>Gram-staining</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Motility</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Pigmentation</strong></td>
<td>Pink</td>
<td>White-yellowish/brownish</td>
<td>White (3/5), yellow (1/5), pink (1/5)</td>
<td>White-yellowish (3/5), pink (2/5)</td>
</tr>
<tr>
<td><strong>Oxidase</strong></td>
<td>+</td>
<td>+ (2/5), + (1/5), - (2/5)</td>
<td>+ (2/5), - (1/2)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Catalase</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>NaCl tolerance (%)</strong></td>
<td>≤ 0.5</td>
<td>≤ 1.0</td>
<td>≤ 0.5 ≤ 1.5</td>
<td>&lt; 2 ≤ 4</td>
</tr>
<tr>
<td><strong>Temperature range (°C)</strong></td>
<td>13-43</td>
<td>27-43</td>
<td>7-38</td>
<td>5-46</td>
</tr>
<tr>
<td><strong>pH range</strong></td>
<td>4.5-8.5</td>
<td>5.0-9.5</td>
<td>6.0-10.0</td>
<td>5.0-10.0</td>
</tr>
<tr>
<td><strong>pH optimum</strong></td>
<td>5.0-8.0</td>
<td>6.0-7.5</td>
<td>6.5-8.0</td>
<td>7.0-9.0</td>
</tr>
<tr>
<td><strong>DNA G+C-content (mol%)</strong></td>
<td>72.8</td>
<td>74.0</td>
<td>71.0-71.8</td>
<td>71.75</td>
</tr>
<tr>
<td><strong>Diamino acid</strong></td>
<td>meso-Dpm</td>
<td>meso-Dpm</td>
<td>ND</td>
<td>meso-Dpm</td>
</tr>
<tr>
<td><strong>Major quinone</strong></td>
<td>MK-8</td>
<td>MK-8</td>
<td>MK-7(H4)</td>
<td>MK-7(H4)</td>
</tr>
<tr>
<td><strong>Major fatty acids (≥ 10%)</strong></td>
<td>C17:1ω9c, C18:1ω9c, C18:1ω11c, C16:0</td>
<td>C17:1ω9c, C18:1ω9c, C18:1ω11c, C16:0</td>
<td>C16:0 (1/2), C17:1ω9c (2/2), C18:1ω9c (2/2), C16:0 ω11c (1/2)</td>
<td>C18:1ω9c (4/4), C16:0 isoleucine (3/4), C17:0 anteiso (1/4)</td>
</tr>
<tr>
<td><strong>Preferred carbon sources</strong></td>
<td>Complex proteinaceous substrates, glucose</td>
<td>Complex proteinaceous substrates, glucose, carboxylic acids</td>
<td>Sugars (3/5)</td>
<td>Sugars (1/2)</td>
</tr>
</tbody>
</table>
Given separately for the mesophilic strains *R. bracarensis* and *R. aplysinae* (first value) and the thermophilic species *R. radiotolerans, R. xylanophilus, R. taiwanensis, R. calidifluminis* and *R. naiadicus* (second value).

No or only few values exist for *Bactoderma rosea* for fatty acid profile and substrate range, respectively.

Fatty acid profile of *R. aplysinae* was not considered, as probably misinterpretation of major components occurred (see text).

Only most striking patterns observed within groups given; additionally strain-specific substrate usage from different substance classes occurred.
### Table 2: 16S rRNA gene signature nucleotide patterns for the classes *Thermoleophilia* Suzuki and Whitman 2013 and *Rubrobacteria* Suzuki 2013 and subordinate orders and families.

Adapted from Reddy and Garcia-Pichel (2009) and Zhi et al. (2009) and revised considering the recent taxonomic conversions and the addition of novel species.

Taxonomic units were taken from Ludwig et al. (2012a). Sequence patterns were compared based on the ‘All-Species Living Tree’ Project (LTP) database (Yarza et al., 2008) release 119 (November 2014) using the program package ARB version 6.0 (Ludwig et al., 2004).


Bold letters indicate signatures characteristic of the order, grey background indicates signatures characteristic of the class. Underlined letters highlight unique signatures among the families of the *Thermoleophilia*, dotted lines represent uniqueness within the *Solirubrobacterales*. A ‘v’ denotes variable signatures within the respective taxonomic unit, grey font indicates meaningless signatures within the respective taxonomic unit.

Note that validity of signatures may change from one taxon level to the other which cannot always be indicated in the table.
<table>
<thead>
<tr>
<th>Position(s)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V 1</th>
<th>VI 2</th>
<th>VI 3</th>
<th>VI 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>52:359</td>
<td>v</td>
<td>v</td>
<td>C-G</td>
<td>v</td>
<td>G-C</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
</tr>
<tr>
<td>63:104</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
</tr>
<tr>
<td>66:103</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
<td>C-G</td>
</tr>
<tr>
<td>70:98</td>
<td>v</td>
<td>A-U</td>
<td>v</td>
<td>v</td>
<td>A-U</td>
<td>G-C</td>
<td>G-C</td>
<td>G-C</td>
</tr>
<tr>
<td>139:224</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>U-A</td>
<td>G-C</td>
<td>G-C</td>
<td>G-C</td>
</tr>
<tr>
<td>144:178</td>
<td>v</td>
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FIGURE LEGENDS

Fig. 1. Phase-contrast photomicrographs of strains D16/0/H6T (a) and A22/0/F9_1T (d), transmission electron micrographs of strains D16/0/H6T (b, c) and A22/0/F9_1T (e, f). Scale bars, 10 µm (a, d), 1 µm (b), 0.5 µm (e) and 0.2 µm (c, f). Arrow heads indicate inclusion bodies, cm = cytoplasmic membrane, cw = cell wall, sf = surface layer.

Fig. 2. Rooted maximum-likelihood phylogenetic tree based on almost full-length 16S rRNA gene sequences showing the relationship of strains D16/0/H6T and A22/0/F9_1T to each other and to related taxa. Bootstrap values (expressed as percentages of 1000 replicates) 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). Bar indicates 10% nucleotide divergence.
Figure 1.
Figure 2.