Occallatibacter riparius gen. nov., sp. nov. and *O. savannae* sp. nov. two novel acidobacterial species isolated from Namibian soils and emended description of the family *Acidobacteriaceae*

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Footnote

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Occallatibacter riparius* 277^T and 307 and *Occallatibacter savannae* A2-1c^T are HQ995659, HQ995660, and HQ995661, respectively.

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ABSTRACT

Three Gram-negative, non-spore-forming, encapsulated bacteria were isolated from a Namibian river bank soil (strains 277^T and 307) and a semiarid savanna soil (strain A2-1c^T). 16S rRNA gene sequence analyses placed them within *Acidobacteria* subdivision 1 and revealed 100% similarity between strain 277^T and 307, 98.2% between A2-1c^T and the former two. The closest relatives with validly published names were *Telmatobacter bradus*, *Acidicapsa borealis*, and *A. ligni* (94.7 - 95.9% similarity). Cells of all three strains were rod shaped, motile and divided by binary fission. Ultrastructural analyses revealed a thick cell envelope resulting mainly from a thick periplasmic space. Colonies of strains 277^T and 307 were white to cream and light pink colored, respectively, while strain A2-1c^T displayed a bright pink color. All three strains were aerobic chemoheterotrophic mesophiles with a broad temperature range of growth and a moderately acidic pH optimum. Sugars and complex proteinaceous substrates were the preferred carbon and energy source. A few polysaccharides were degraded. The major quinone in all three strains was MK-8. As minor compound MK-7 occurred in strain A2-1c^T. Major fatty acids were *iso*-$C_{15:0}$ and *iso*-$C_{17:1}$ *ω7c*. In addition, *iso*-$C_{17:0}$ occurred in significant amounts. The DNA G+C content of strains 277^T, 307, and A2-1c^T was 59.6, 59.9, and 58.5 mol%, respectively. Based on these characteristics, the three isolates are described as two novel species of the novel genus *Occallatibacter* gen. nov., *O. riparius* sp. nov. strain 277^T (= DSM 25168^T = LMG 26948^T) and strain 307 (= DSM 25169 = LMG 26947) and *O. savannae* sp. nov. strain A2-1c^T (= DSM 25170^T = LMG 26946^T). Together with several other recently described taxa the novel isolates provide the basis for an emended description of the established family *Acidobacteriaceae*.

Although the description of the type species of the genus *Acidobacterium*, *Acidobacterium capsulatum*, dates back to 1991 (Kishimoto *et al.*, 1991a), the family *Acidobacteriaceae* was described based on phylogenetic analysis of 16S rRNA gene sequences and then validated only 20 years later (Thrash & Coates, 2011b, 2012a, b). To date, it constitutes the sole family of the order *Acidobacteriale*s within the class *Acidobacteria* (Cavalier-Smith, 2002). The latter is commonly known as subdivision 1 of the currently 26 phylogenetically coherent groups within the phylum *Acidobacteria* (Barns *et al.*, 2007; Thrash & Coates, 2011a, 2012a, b). At the time of its original description, the family *Acidobacteriaceae* included the three genera *Acidobacterium* (1 species; Kishimoto *et al.*, 1991a, b), *Edaphobacter* (2 species; Koch *et al.*, 2008), and *Terriglobus* (1 species; Eichorst *et al.*, 2007). However, over the past five years the list of described genera of *Acidobacteria* subdivision 1 was extended to currently 8 genera, additionally including *Acidicapsa* (2 species; Kulichevskaya *et al.*, 2012), 'Acidipila' (1 species; Okamura *et al.*, 2011), *Bryocella* (1 species; Dedysh *et al.*, 2012), *Granulicella* (9 species; Männistö *et al.*, 2012, Pankratov & Dedysh, 2010, Yamada *et al.*, 2014), and *Telmatobacter* (1 species; Pankratov *et al.*, 2012). Four additional species were described for the genus *Terriglobus* (Baik *et al.*, 2013; Männistö *et al.*, 2011, Pascual *et al.*, 2015, Whang *et al.*, 2014). Moreover the *Candidatus* species 'Koribacter versatilis' (Ward *et al.*, 2009) affiliates with this group. All isolates described so far are chemoorganoheterotrophic mesophiles with an acidic to moderately acidic pH optimum that were mostly isolated from terrestrial environments (Table 1). Here we describe the isolation and characterization of three novel strains that extend the number of slightly acidophilic soil isolates of the subdivision 1 *Acidobacteria* and represent a novel genus with two novel species.

Strains 277^T and 307 were isolated from a sandy river bank soil with moderately acidic pH (5.1 in distilled water) from the Okavango River, Northern Namibia (17°51'59"S, 19°54'24"E, 1065 m elevation above sea level) sampled in spring 2010. At the time of sampling the site was covered by water (approximately 30 cm in deph) and grown with the wild rice species *Oryza longistaminata*. Strain A2-$1c^T$ originated from a loamy sand of similar pH (5.0 and 6.1 measured in 2 mM $CaCl_2$ and in distilled water, respectively) sampled in spring 2009 at Erichsfelde in central Namibia (21°36'41.4" S, 16°52'13.4" E, 1481 m elevation above sea level). The vegetation of this site was a typical open thornbush savanna dominated by *Acacia melifera* and the grass *Stipagrostis uniplumis* which receives an average annual precipitation of approximately 360 mm during the rainy season in summer.

All three isolates were gained from high throughput cultivation experiments set up in sterile 96-well microtiterplates per well containing 180 µl of soil solution equivalent (SSE)/Cmix medium buffed at pH 5.5 (Erichsfelde samples) or 5.8 (Okavango samples) with MES (Supplementary Material and Methods). After 6-8 weeks of incubation at 15 (strains 277^T and 307) or 25 °C (strain A2-1c^T) in the dark as static cultures, grown wells were screened for the presence of *Acidobacteria* by group specific PCR using the primer pair Acido31f (Barns *et al.*, 1999) and 907r (Lane, 1991). *Acidobacteria*-positive cultures from the Okavango cultivation experiment were transferred and subcultured in 1:10 diluted liquid HD medium (DSMZ medium 1124; https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium1124.pdf). Strains 277^T and 307 were purified by plating, picking and restreaking on a low nutrient isolation medium described earlier (Foesel *et al.*, 2013), which was solidified with purified agar (Oxoid, Basingstoke, UK) instead of gellan gum. Of the cultures obtained from Erichsfelde samples, 10 µl aliquots of *Acidobacteria*-positive wells were directly streaked on SSE/HD1:10 medium solidified with 1.5% (w/v) purified agar (Foesel *et al.*, 2013) and a pure culture of A2-1c^T was obtained by restreaking. Unless otherwise noted, SSE/HD 1:10 (pH 5.5) was also used for further physiological tests and biomass production.

On solid media within 1-2 weeks strain 277^T and 307 formed soft and slimy colonies of up to 2 mm in diameter that were translucent, smooth, shiny, and convex with entire margins. While colonies of strain 277^T were white to cream-colored, those of strain 307 developed a light pink color and older colonies occasionally turned brown. With a diameter of at most 0.5 mm, colonies of strain A2-1c^T were smaller than those of the other two strains. They were of very rigid consistency, displayed a bright pink color and were translucent, smooth, shiny, and convex to hemispherical with entire margins.

Cells of all three strains were rod shaped with a size of (1.0 - 2.0) x (0.6 - 0.7) µm (277^T, 307; Fig. S1 a, d) and (1.0 - 2.5) x (0.5 - 0.7) µm (A2-1c^T; Fig. S1 g). Depending on the growth phase of cultures, also longer rods and bent cells were observed. All three strains performed non-directional tumbling movements. They divided by binary fission and were Gram-negative in standard staining procedures (Smibert & Krieg, 1994) as well as the KOH-test (Buck, 1982). Similar to many of the *Acidobacteria* subdivision 1 members the novel strains formed capsules (India ink staining), while spore formation (malachite green staining) (Beveridge *et al.*, 2007) was not observed (Tab. 1). For scanning and transmission electron micrographs cells were fixed chemically with glutaraldehyde/formaldehyde and further processed as described earlier (Huber *et al.*, 2014). Cells seemed to shrink strongly during the

chemical fixation procedure for scanning and negative-stained samples. Therefore, images of ultrathin sections were taken from high-pressure frozen and cryosubstituted cells (Wanner *et al.*, 2008).

Cells of strains 277^T and 307 possessed single fimbria-like structures, whereas cells of strain A2-1c^T carried multiple fimbria-like structures (arrow heads in Fig. S1 c, g, k); for strains 277^T and 307 the presence of capsules was evident in negative-stained samples and FESEM images (arrows in Fig. S1 b, c, f, g). Scanning electron micrographs of strain 307 revealed possible self-aggregation through the fimbria-like structures (Fig. 1S f). Flagella could not be detected. Therefore it is speculated that the unusual tumbling movements observed under the light microscope might be caused by winding and unwinding of the fimbria-like structures to higher-molecular structures (stars in Fig. S1 d, h, l) and back. Ultrastructural analyses confirmed the Gram-negative cell wall structure of all strains (Fig. 1) by an outer and inner membrane, and demonstrated an exceptionally thick periplasmic space of up to approx. 50 nm with a well defined peptidoglycan layer (e.g. Fig. 1 b). Cells of all strains contained electron dense granules presumably representing polyphosphate (e.g. Fig. 1 f). In all strains a large portion of ribosomes was affiliated with the cytoplasma membrane. Strain A2-1c^T showed many, very conspicuous granules that most likely represent different stages of phage capsids (Fig. 1 e, f). To a lesser extent such structures were also detected in strain 277 and strain 307.

Almost full-length 16S rRNA gene fragments of strains 277^T, 307, and A2-1c^T were amplified and sequenced as described before (Foesel *et al.*, 2013). The resulting sequences comprised 1463 (277^T, 307) and 1461 (A2-1c^T) unambiguous nucleotides between *Escherichia coli* positions 28 and 1491 (Brosius *et al.*, 1978). Sequences 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, resulting in 1380 valid columns between position 44 and 1455 of the *E. coli* 16S rRNA reference gene; 1000 bootstrap resamplings). All three methods placed the three novel isolates within subdivision 1 of the *Acidobacteria* where they constitute a stable cluster together with *Telmatobacter bradus* (Pankratov *et al.*, 2012), *Acidicapsa borealis*, and *A. ligni* (Kulichevskaya *et al.*, 2012) (Fig. 2; Supplementary Fig. S2). The pairwise 16S rRNA gene sequence similarity (ARB neighbor-joining tool without

the use of an evolutionary substitution model) between strains 277^T and 307 was 100.0%. Strain A2-1c^T shared 98.2% of its 16S rRNA gene sequence with the former two. The closest validly described relatives of strains 277^T, 307, and A2-1c^T were *T. bradus* (95.9% and 95.2%, respectively), *A. ligni* (95.8% and 95.3%, respectively), and *A. borealis* (95.1% and 94.7%, respectively). In addition, *Bryocella elongata* (Dedysh *et al.*, 2012) exhibited an identity of 95.1% (277^T, 307) and 95.0% (A2-1c^T) to the novel isolates, but belonged to a different phylogenetic clade (Fig. 2). Together with all other validly or effectively described *Acidobacteria* subdivision 1 members the novel isolates form a coherent cluster with a minimal similarity of > 91% between the most distantly related 16S rRNA sequences which is close to the proposed threshold of 92% for family definition (Rosselló-Móra & Amann, 2015). Just '*Candidatus* K. versatilis' showed 16S rRNA gene similarity values of only around 89-92% to the above mentioned sequence cluster.

As the calculated 16S rRNA gene sequence similarities between strains A2-1c^T, 277^T, and 307 were within or above the threshold values of 98.2-99.0% for which DNA-DNA hybridization (DDH) is considered to be mandatory according to the latest findings (Meier-Kolthoff *et al.*, 2013), DDH experiments were carried out to verify the status of A2-1c^T as a distinct species and of 277^T and 307 as two strains of the same species. Therefore cells were disrupted in a Constant Systems TS 0.75 KW (IUL Instruments, Koenigswinter, Germany) and 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 multicell changer and a temperature controller with in situ temperature probe (Varian). Duplicate measurements in 2 x saline-sodium citrate (SSC) at 70 ºC yielded hybridization values of 67.3% and 73.8% between strains 277^T and 307, of 9.1% and 13.0% between strains 277^T and A2-1c^T, and of 16.1% and 12.2% between strains 307 and A2-1c^T. Thus, hybridization levels for strain A2-1c^T with the two other strains are far below the threshold value of 70% DNA-DNA relatedness generally accepted for the definition of a novel species (Wayne *et al.*, 1987). Hybridization values between strains 277^T and 307 were around 70%. Taking a conservative approach, these two strains were therefore included in one species.

For G+C content determination cells were disrupted and the DNA was purified as described for DDH (see above). 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 59.6, 59.9, and 58.5 mol% for strains 277^T, 307 and A2-1c^T, respectively. These values range within the G+C contents reported for all other *Acidobacteria* SD1 members (Tab.1) and are only little higher than those of the next relatives *T. bradus* TPB6017^T (57.6 mol%), *A. borealis* KA1^T (54.1 mol%), and *A. ligni* WH120^T (51.7 mol%).

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 the three novel strains comprised menaquinone MK-8 as sole assignable (277^T and 307; 99.9% and 96.8%, respectively) or as major (A2-1c^T; 93.3%) component. Strain A2-1c^T additionally contained MK-7 (6.7%). With respect to cellular quinones, the three novel strains thus resemble the other described subdivision 1 *Acidobacteria*, which all contain MK-8 as predominant quinone (Table 1).

Fatty acid profiles were acquired for the three novel strains together with their closest phylogenetic relatives *T. bradus*, *A. borealis*, and *A. ligni*. As far as growth requirements permitted, cultures were grown under identical conditions (SSE/HD 1:10 agar plates, pH 5.5, 18 d at 20 °C). Only *T. bradus* DSM 23630^T was grown in static liquid culture instead of agar plates to obtain enough biomass. 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). Compounds were identified by comparison to the TSBA40 and 60 peak naming table databases. All three strains possessed straight chain or methyl-branched saturated and monounsaturated fatty acids while hydroxyl groups only played a marginal role. Their full profiles only varied slightly in the amount of particular compounds and the presence/absence of a few minor compounds < 2% (Tab. S1). Major fatty acids of strains 277^T, 307, and A2-1c^T were *iso*-$C_{15:0}$ (61.9, 61.6, and 54.8%) and *iso*-$C_{17:1}$ *ω7c* (29.0, 28.0, and 30.7%). In addition, *iso*-$C_{17:0}$ (5.3, 7.6, and 9.5%) occurred in significant amounts. This is in congruence with the fatty acid profiles of the next relatives *T. bradus* and the two *Acidicapsa* species. While all characterized members of *Acidobacteria* subdivision 1 possess *iso*-$C_{15:0}$ as most abundant component, the second most abundant fatty acid in the genera 'Acidipila', *Bryocella*, *Edaphobacter*, *Granulicella*, and *Terriglobus* is $C_{16:1}$ *ω7c* followed by $C_{16:0}$ as third most relevant component (Table 1). Furthermore high amounts of the unusual membrane-spanning lipid, 13,16-dimethyl octacosanedioic acid (*iso*-diabolic acid) were previously reported in the tree novel isolates and all other subdivision 1 *Acidobacteria* studied

after direct acid hydrolysis of the cell material (Kulichevskaya *et al.*, 2012; Sinninghe Damsté *et al.*, 2011).

Growth ranges and optima of temperature and pH were determined under oxic conditions in liquid SSE/HD 1:10 media. Temperature ranges of 11 - 56°C and pH values of maximally 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 contained high amounts of different salts, salt tolerance was tested in liquid medium HD1:10 (DSMZ medium 1124; see above) amended with NaCl to final concentrations of 0 - 10% (w/v). Growth was determined by following the optical density at 660 nm. All three strains grew at temperatures of approximately 11 - 40°C (Table 1) and also displayed very similar temperature optima (defined as ≥ 75% of highest growth rate) of 26 - 37°C (strain 277^T), 28 - 36°C (strain 307), and 29 - 40°C (strain A2-1c^T). Their pH ranges of growth reached from acidic to around neutral (strains 277^T and A2-1c^T) or slightly basic (strain 307). Strains 277^T and 307 grew optimally at pH 5.0 - 6.5; strain A2-1c^T showed a slightly lower pH optimum of 4.0 - 5.5. Minimal doubling times were 8.9, 14.0, and 7.1 h for strains 277^T, 307, and A2-1c^T, respectively. While strains 277^T and 307 tolerated NaCl concentrations of ≤ 1.0% and showed best growth at 0.5% NaCl, strain A2-1c^T grew best in the absence of NaCl and tolerated only 0.5% NaCl. In comparison to the other *Acidobacteria* subdivision 1 species, the three novel isolates show elevated temperature ranges and optima of growth. Sole exception is the recently described isolate *T. albidus* which reaches similar values (Pascual *et al.*, 2015). This might be explained by the origin of those isolates from sub-Saharan Africa, while the majority of the other isolates originates from soils of the temperate and the boreal zone. In contrast, preferential growth at acidic to moderately acidic pH values is a feature the novel isolates share with all other characterized *Acidobacteria* subdivision 1 members. No or only low salt tolerance is a further common characteristic of this group.

Anaerobic growth with alternative electron acceptors was evaluated in liquid medium HD1:10 (see above) with N_2 as the gas phase and 10 mM $NaNO_3$, 10 mM Na_2SO_4, 10 mM iron pyrophosphate ($Fe_4(P_2O_7)_3 \cdot x\ H_2O$), 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). All three strains neither reduced nitrate, nitrite, sulfate or iron (III), nor showed fermentative growth. The novel isolates thus resemble the majority of the strictly aerobic *Acidobacteria* subdivision

1 members, whereas their closest phylogenetic relative *T. bradus* TPB6017^T grew only under reduced oxygen tension or anaerobically (Pankratov *et al.*, 2012).

The range of growth substrates utilized by strains 277^T, 307, and A2-1c^T was determined in microtiterplates in two parallels using soil solution equivalent (SSE) (Angle *et al.*, 1991) with an elevated iron content (Foesel *et al.*, 2013) and amended with 50 mg L^{-1} of yeast extract as basal medium. In total, 72 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). In the same manner degradation of pectin, xylan, and lignin was assessed by staining with aqueous solutions of ruthenium red (Cruickshank & Wade, 1980; Gainvors *et al.*, 1994), Congo red (Scheirlinck *et al.*, 1990; Teather & Wood, 1982), and ferric chloride/ferric cyanide (Sundman & Nase, 1971; Tekere *et al.*, 2001), respectively. Laccase activity was evaluated on plates containing 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as substrate analog (Soden *et al.*, 2002; Tekere *et al.*, 2001). Cytochrome *c*-oxidase and catalase activities were monitored employing standard protocols (Cowan, 1974; Smibert & Krieg, 1994). Cytochrome *c*-oxidase was additionally tested by Bactident® Oxidase test strips (Merck, Darmstadt, Germany). Other physiological features (e. g. indol formation, urease activity, activity of different exoenzymes) were determined using the API20E and the API ZYM test systems (bioMérieux).

All three strains preferentially grew on sugars which is a characteristic shared with all described *Acidobacteria* subdivision 1 species (Table 1; Tab. S2). Several single amino acids and the complex, protein containing substrates casamino acids, caseine hydrolysate, peptone, and yeast extract were also utilized. Polysaccharide degradation is a capability that has been studied and is prevalent in most *Acidobacteria* subdivision 1 members (Tab. S3) and that is particularly pronounced in *T. bradus*, *A. borealis*, *B. elongata*, *T. saanensis*, and *Granulicella* spp. inhabiting peat and tundra soils (Dedysh *et al.*, 2012; Kulichevskaya *et al.*, 2012; Männistö *et al.*, 2011, 2012; Pankratov & Dedysh, 2010; Pankratov *et al.*, 2012; Rawat *et al.*, 2012). Of all polysaccharides tested in the present work strains 277^T and 307 only degraded starch. Strain A2-1c^T was positive for pectin degradation and weakly positive for chitin degradation. Furthermore strains 277^T and 307 showed significant laccase activity, whereas

strain A2-1c^T was weakly positive. Like most members of *Acidobacteria* subdivision 1 the three novel strains express almost the whole variety of exoenzymes included in the API ZYM test system (Tab. S4). While strain 277^T at least weakly expressed all enzymes tested, strain 307 lacked N-Acetyl-β-glucosaminidase, strain A2-1c^T missed N-Acetyl-β-glucosaminidase, esterase lipase (C 8), and β-glucosidase. Additional physiological characteristics are detailed in the species descriptions below.

In summary, strains 277^T, 307, and A2-1c^T are aerobic, chemoorganoheterophic, white to pink pigmented mesophiles. Albeit fitting well into the series of moderately acidophilic soil isolates of *Acidobacteria* subdivision 1, they can clearly be differentiated from their closest relatives *Telmatobacter* and *Acidicapsa* based on their phylogeny, morphology, and physiology and therefore are proposed to form the novel genus *Occallatibacter*, including the two novel species, *O. riparius* and *O. savannae*.

Description of *Occallatibacter*, gen. nov.

Occallatibacter (Oc.cal.la.ti.bac'ter. L. masc. adj. *occallatus*, thick-skinned, which refers to the thick cell envelope; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Occallatibacter*, rod-shaped bacterium with thick cell envelope).

Gram-negative, non-spore-forming, rod-shaped cells that divide by binary fission. Perform non-directional tumbling movements, although flagella were not detected. Capsules are formed. Strictly aerobic, chemoorganotrophic mesophiles. Catalase positive, cytochrome *c*-oxidase negative. Sugars are the preferred carbon and energy source. Several single amino acids and complex, protein containing substrates are also utilized. Individual polysaccharides are degraded. Major fatty acids after Blight-Dyer extraction are *iso*-$C_{15:0,}$ *iso*-$C_{17:1}\omega 7c$, and *iso*-$C_{17:0.}$ After direct acid hydrolysis of cell material high amounts of 13,16-dimethyl octacosanedioic acid (*iso*-diabolic acid) are found. Major quinone is MK-8. The DNA G+C content is 59 - 60%. The type species is *Occallatibacter riparius*.

Description of *Occallatibacter riparius*, sp. nov.

Occallatibacter riparius (ri.pa'ri.us. N.L. masc. adj. *riparius*, that inhabits the banks of rivers which refers to the isolation source of the type strain).

Displays the following characteristics in addition to those given in the genus description. Cells are 1.0 - 2.0 µm long and 0.6 - 0.7 µm in diameter. On solid media forms soft and slimy colonies of up to 2 mm in diameter that are white to cream or light pink in color, translucent, smooth, shiny, and convex with entire margins. Grows at 11 - 40°C and pH 3.5 - 8.5; optimal

growth at 26 - 37°C and pH 5.0 - 6.5. Minimal doubling time is 8.9 h. Tolerates NaCl concentrations of up to 1.0% (w/v); optimal growth occurs at 0.5% NaCl.

Grows on cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, melizitose, raffinose, rhamnose, sucrose, trehalose, xylose, aspatate, glutamate, ornithine, lysine, tyrosine, gluconate, succinate, glycerol, casamino acids, casein hydrolysate, pepton, yeast extract, and starch. Strain specific growth occurs on fucose, sorbitol, arginine, acetate, and laminarin. No growth is observed on arabinose, lyxose, sorbose, adonitol, arabitol, mannitol, myo-inositol, xylitol, alanine, cysteine, glycine, histidine, leucine, isoleucine, methionine, phenylalanine, proline, hydroxy-proline, serine, threonine, tryptophan, valine, butyrate, citrate, crotonate, formate, fumarate, glycolate, isovalerate, lactate, malate, malonate, nicotinic acid, oxaloacetate, propionate, pyruvate, tartrate, butanol, ethanol, methanol, propanol, chitin, cellulose, pectin, Tween 80, and xylan.

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

Oxygen is the sole electron acceptor. The sole quinone is MK-8.

The type strain is 277^T (= DSM 25168^T = LMG 26948^T). An additional strain is 307 (= DSM 25169^T = LMG 26947). Both were isolated from a sandy river bank soil from Okavango River, Northern Namibia (17°51'59"S, 19°54'24"E, 1065 m elevation above sea level). The DNA G+C content of the type strain is 59.6 mol%.

Description of *Occallatibacter savannae*, sp. nov.

Occallatibacter savannae (sa.van'nae. N.L. fem. adj. *savannae*, derived from savanna (soil) referring to the isolation source of the type strain).

In addition to those given in the genus description shows the following features. Cells are 1.0 - 2.5 µm long and 0.5 – 0.7 µm wide. Colonies reach a size of at most 0.5 mm, are of very firm consistency, display a bright pink color, are translucent, smooth, shiny, and convex to hemispherical with entire margins. Grows at 11 - 40°C and pH 3.5 - 6.5; optimal growth

occurs at 29 - 40°C and pH 4.0 - 5.5. Minimum doubling time is 7.1 h. Tolerates NaCl concentrations of up to 0.5% (w/v); optimal growth occurs in the absence of NaCl.

Grows on cellobiose, fucose, fructose, galactose, glucose, lactose, maltose, mannose, melizitose, raffinose, rhamnose, sucrose, trehalose, xylose, xylitol, alanine, ornithine, phenylalanine, proline, tyrosine, gluconate, pyruvate, succinate, glycerol, casamino acids, casein hydrolysate, pepton, yeast extract, chitin, laminarin, and pectin. No growth is observed on arabinose, lyxose, sorbose, adonitol, arabitol, mannitol, myo-inositol, sorbitol, arginine, aspatate, cysteine, glutamate, glycine, histidine, leucine, isoleucine, lysine, methionine, hydroxy-proline, serine, threonine, tryptophan, valine, acetate, butyrate, citrate, crotonate, formate, fumarate, glycolate, isovalerate, lactate, malate, malonate, nicotinic acid, oxaloacetate, propionate, tartrate, butanol, ethanol, methanol, propanol, cellulose, starch, Tween 80, and xylan.

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

Oxygen is the sole electron acceptor. The major quinone is MK-8, as further constituent MK-7 occurs.

The type strain is A2-1c^{T} (= DSM 25170^{T} = LMG 26946^{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 58.5 mol%.

Emended description of the family *Acidobacteriaceae* Thrash and Coates 2012

The description is as given by Thrash and Coates (2011b) with the following amendments. In addition to the genera *Acidobacterium* (type genus), *Edaphobacter*, and *Terriglobus* the family currently includes the genera *Acidicapsa*, 'Acidipila', *Bryocella*, *Granulicella*, *Telmatobacter*, and the novel genus *Occallatibacter*. All members are Gram-negative cocci to rods. Mostly catalase positive and oxidase negative. Capsule formation is prevalent, motility is variable. Members are aerobic or facultatively anaerobic, in some cases cold-adapted, mesophiles with a preference for sugars as carbon and energy source. Polysaccharide degradation is a common feature. The sole or predominant quinone is MK-8.

After Blight-Dyer extraction characteristic combinations of major fatty acids are (i) *iso*-$C_{15:0}$, $C_{16:1}\omega7c$ and $C_{16:0,}$ (ii) *iso*-$C_{15:0,}$ *iso*-$C_{17:1}\omega7c$ mostly accompanied by *iso*-$C_{17:0}$, or (iii) *iso*-$C_{15:0}$ and $C_{18:1}\omega9c_{.}$ After direct acid hydrolysis of cell material high amounts of 13,16-dimethyl octacosanedioic acid (*iso*-diabolic acid) are found. The G+C-content ranges from 51.7 to 62.1 mol%.

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605 **Table 1:** Characteristics of the novel genus *Occallatibacter including strains 277^T, 307, and strain A2-1c^T compared to all described genera within*
606 *Acidobacteria subdivision* 1: **1**, ***Occallatibacter,*** including strains (a) 277^T, (b) 307, and (c) A2-1c^T (all this study); **2**, ***Telmatobacter***, including *T.*
607 *bradus* TPB6017^T (Pankratov *et al.*, 2012); **3**, ***Acidicapsa***, including *A. borealis* KA1^T and *A. ligni* WH120^T (Kulichevskaya *et al.*, 2012); **4**,
608 **'Acidipila'**, including 'A. rosea' AP8^T (Okamura *et al.*, 2011); **5**, ***Acidobacterium***, including *A. capsulatum* DSM 11244^T (Kishimoto *et al.*, 1991a);
609 **6**, ***Edaphobacter***, including *E. modestus* Jbg-1^T and *E. aggregans* Wbg-1^T (Koch *et al.*, 2008); **7**, ***Terriglobus***, including *T. roseus* KBS63^T
610 (Eichorst *et al.*, 2007), *T. saanensis* SP1PR4^T (Männistö *et al.*, 2011), *T. tenax* DRP 35^T (Whang *et al.*, 2014), *T. aquaticus* 03SUJ4^T (Baik *et al.*,
611 2013), and *T. albidus* Ac_26_B10^T ; **8**, ***Granulicella***, including *G. paludicola* OB1010^T, *G. pectinivorans* TPB6011^T, *G. aggregans* TPB6028^T, *G.*
612 *rosea* TPO1014^T (Pankratov & Dedysh, 2010), *G. arctica* MP5ACTX2^T, *G. mallensis* MP5ACTX8^T, *G. tundricola* MP5ACTX9^T, *G. sapmiensis*
613 S6CTX5A^T, *G. pectinivorans* DSM 21001^T, *G. rosea* DSM 18704^T (Männistö *et al.*, 2012), and *G. cerasi* Sakura1^T (Yamada *et al.*, 2014); **9**,
614 ***Bryocella***, including *B. elongata* SN10^T (Dedysh *et al.*, 2012).
615 All strains are Gram-negative, non-spore-forming cocci to rods, divide by binary fission, prefer sugars as growth substrates, and to variable extent
616 degrade polysaccharides. The major quinone is MK-8. All grow aerobically, albeit *T. bradus* only very weakly/slowly at full oxygen tension. +,
617 positive; -, negative; (+), weak activity/reaction detected; ND, no data.

Characteristic	1a	1b	1c	2	3	4	5	6	7	8	9
Isolation source	River bank soil	River bank soil	Semiarid savanna soil	*Sphagnum* peat	*Sphagnum* peat/ decaying wood	Acidic mine drainage/ soil	Acidic mine drainage	Alpin and forest soil	Different soils/ termite hindgut/ freshwater reservoir	Peat soil/ tundra soil/ Cladonia/ Cerry bark	*Sphagnum peat*
Cell shape	Rods	Rods	Rods	Rods	Short rods	Cocci	Rods	Short rods	Cocci to rods	Rods	Rods
Oxidase	-	-	-	-	-	ND	-	+/-	+/-	+/-	-
Calase	+	+	+	(+)	+	ND	+	+	+/(+)	+/-	+
Capsule formation	+	+	+	ND	+	+	+	-	-/ND	ND	+
Motility	(+)[a]	(+)[a]	(+)[a]	+	-	-	+	+/-	-	-	-
Pigmentation	White-cream	White-cream	Bright pink	White/ beige	Colorless/ Pink	Pink	Orange	Beige	Pink/ white/ None	White/ pink/ red	Pink
NaCl tolerance (%)	≤ 1.0	≤ 1.0	≤ 0.5	≤0.1	≤2.0	≤1.0	<3.5	ND	ND/ ≤1.0	<1.0-5.0	≤3.0
Temperature range (°C)	11-40	12-39	11-40	4-35	10-33	22-37	20-37	15-37	4-45	2-33	6-32
Temperature optimum (°C)	26-37	28-36	29-40	20-28	22-28	30	ND	30	25-37	15-30	20-24
pH range	3.5-7.5	3.5-8.5	3.5-6.5	3.0-7.5	3.5-7.3	3.0-6.0	3.0-6.0	4.0-7.0	3.5-10.0	3.0-8.5	3.2-6.6
pH optimum	5.0-6.5	5.0-6.5	4.0-5.5	4.5-5.0	4.0-5.5	4.5	ND	5.5	5.0-7.0	3.8-5.5	4.7-5.2
DNA G+C-content (mol%)	59.6	59.9	58.5	57.6	51.7-54.1	59.5	59.7-60.8	55.8-56.9	57.3-63.2	56.0-61.2	60.7
Major fatty acids	C15:0 iso, C17:1iso ω7c[b], C17:0 iso	C15:0 iso, C17:1iso ω7c[b], C17:0 iso	C15:0 iso, C17:1iso ω7c[b], C17:0 iso	C15:0 iso, C17:1iso ω7c[b], C17:0 iso	C15:0 iso, C17:1iso ω7c[b], C16:0	C15:0 iso, C16:1 ω7c[c], C16:0	C15:0 iso, C18:1 ω9c	C15:0 iso, C16:1 ω7c[c], C16:0	C15:0 iso, C16:1 ω7c[c, d], C16:0	C15:0 iso, C16:1 ω7c[c], C16:0	C15:0 iso, C16:1 ω7c[c], C16:0
Anaerobic growth	-	-	-	+	-	-	+[e]	-	-	-	-

[a] Cells showed a non-directional tumbling movement, although flagella could not be detected.

[b] Determined as *iso*-$C_{17:1}$ *ω9c* by the DSMZ service unit according to the MIDI System, but identified as *iso*-$C_{17:1}$ *ω7c* via Gas Chromatography/Mass Spectronomy (GC/MS) where the position of the double bound has been checked by DMDS adduction (Kulichevskaya *et al.*, 2012; Sinninghe Damsté *et al.*, 2011). However, this fatty acid had been erroneously identified as *iso*-$C_{17:1}$ *ω8c* instead of *iso*-$C_{17:1}$ *ω7c* in this latter work (Irene Rijpstra and Jaap Sinninghe Damsté, personal communication). To date it is unclear whether in the MIDI system *iso*-$C_{17:1}$ *ω9c* is named incorrectly or whether *iso*-$C_{17:1}$ *ω7c* was ever included in the reference data set. Yet, a similar confusion involving *iso*-$C_{17:1}$ *ω9c* (according to MIDI annotation) and *iso*-$C_{17:1}$ *ω7c* (according to GC/MS) has been reported earlier for the genus *Chryseobacterium* (Montero-Calasanz *et al.*, 2013; Montero-Calasanz *et al.*, 2014).

[c] Determined as summed feature 3 (16:1 ω7c and/or 15:0 iso 2-OH) according to the MIDI System, but identified as $C_{16:1}$ *ω7c* using GC/MS for several strains (Kulichevskaya *et al.*, 2012; Männistö *et al.*, 2011, 2012; Sinninghe Damsté *et al.*, 2011).

[d] Differing from the definition given in [c] for *T. aquaticus* 03SUJ4^T summed feature 3 was given as 16:1 ω7c and/or 16:1 ω6c in the original description (Baik *et al.*, 2013).

[e] According to Pankratov *et al.* (2012).

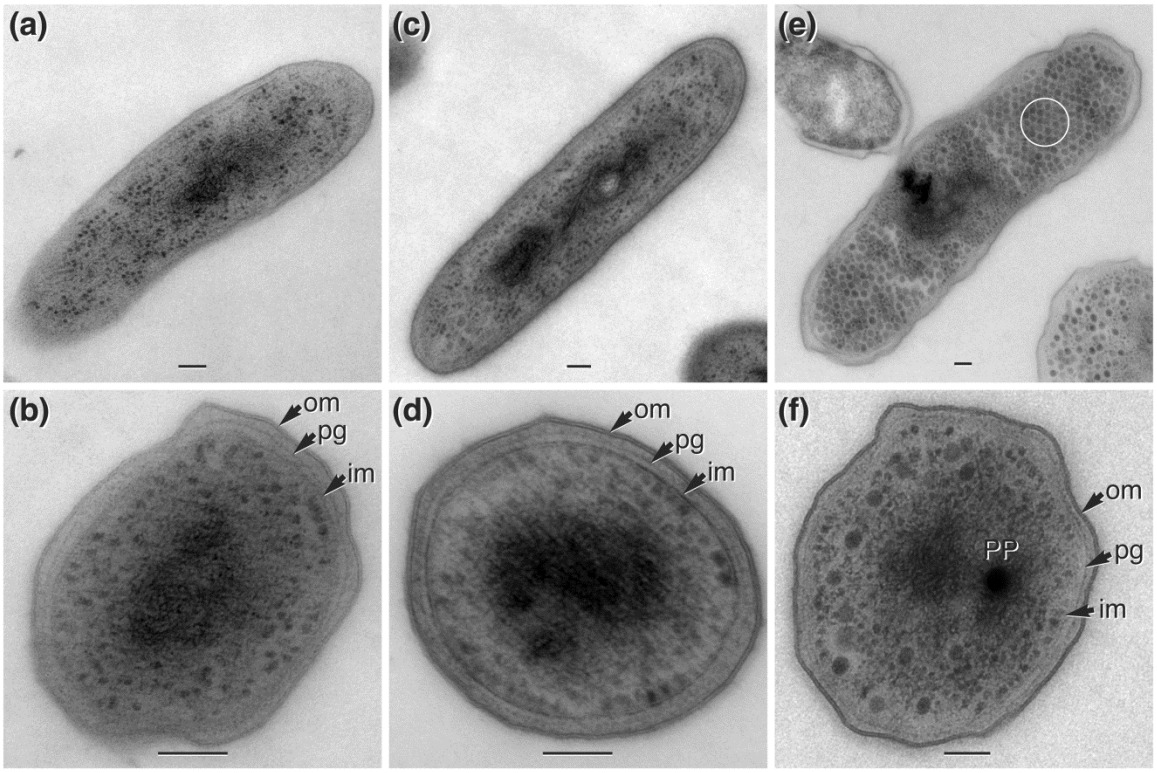
632 FIGURE LEGENDS

633 **Fig. 1.** Electron micrographs of ultrathin sections from high-pressure frozen and
634 cryosubstituted cells of strains 277^T (a, b), 307 (c, d), and A2-1c^T (e, f). All strains show
635 typical Gram-negative cell envelopes with an outer membrane (om) a thin, electron dense
636 peptidoglycan layer (pg) and an inner membrane (im). Cells of all strains contain electron
637 dense granules presumably representing polyphosphate (f, PP). Strain A2-1c^T containes
638 polygonal particles in dense package obviously representing phage capsides (e, circle). Scale
639 bar represents 100 nm.

640 **Fig. 2.** Rooted neighbor-joining phylogenetic tree (Felsenstein correction) based on almost
641 full-length 16S rRNA gene sequences showing the relationship of strains 277^T, 307, and A2-
642 1c^T to each other and to further *Acidobacteria* subdivision 1 taxa. Bootstrap values (expressed
643 as a percentages of 1000 replicates) below 100% are indicated at the respective branching
644 points. The following sequences were used as outgroup: *Rubinisphaera brasiliensis* DSM
645 5305^T (AJ231190), *Gimesia maris* DSM 8797^T (AJ231184), and *Planctopirus limnophilus*
646 DSM 3776^T (X62911). Bar indicates 1% nucleotide divergence.

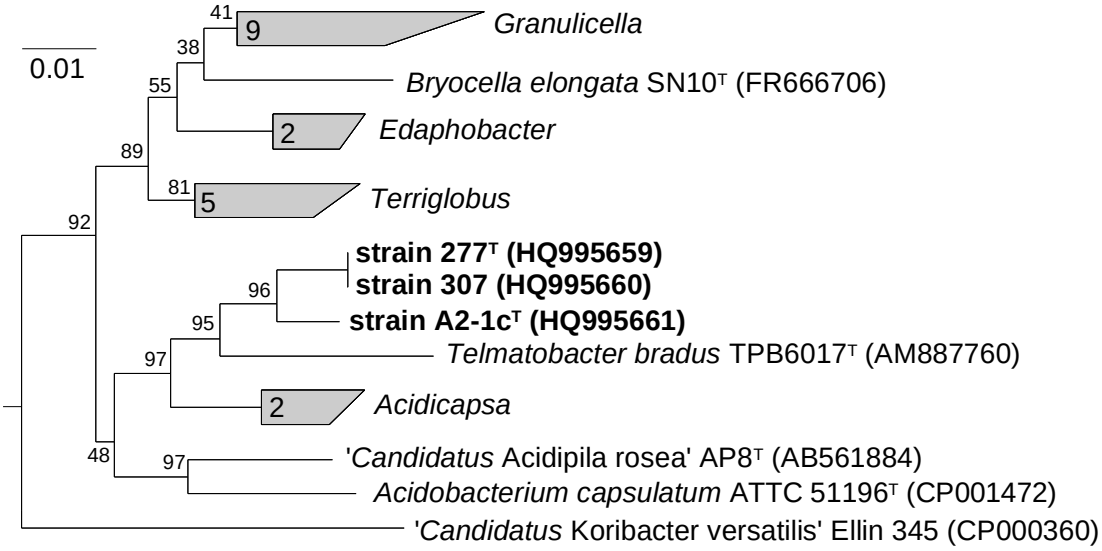
647

648 Figure 1.



649

650 Figure 2.



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