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Reclassification and amended description of *Caulobacter leidyi* as *Sphingomonas leidyi* comb. nov., and emendation of the genus *Sphingomonas*

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Footnote

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 247 = DSM 25078 = LMG 26658 and DSM 4733^T = ATCC 15260^T are JN591312 and AJ227812, respectively.

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ABSTRACT

`*Caulobacter leidyi´* DSM 4733^T has been shown to be affiliated with the family *Sphingomonadaceae* instead of the *Caulobacteraceae*, and due of its poor characterization has been omitted from the current edition of Bergey's Manual of Systematic Bacteriology and removed to limbo. We isolated a novel sphingoglycolipid containing, dimorphic prosthecate bacterium from a prealpine freshwater lake. Strain 247 and `*Caulobacter leidyi´* DSM 4733^T were characterized in detail. The rod-shaped cells stain Gram-negative, are aerobic, catalase- and oxidase- positive, and form a stalk or polar flagellum. Both strains grow optimally at 28-30°C, and pH 6.0-8.0. The major fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, 11-Methyl $C_{18:1}\omega7c$. $C_{14:0}$ 2OH represents the major 2-hydroxy fatty acid. Q-10 is the major respiratory quinone and the major polar lipids are diphosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, three glycolipids, two phosphoaminolipids and two unidentified sphingoglycolipids. The major polyamine is *sym*-homospermidine. The G+C content of genomic DNA of strains 247 and DSM 4733^T are 67.6 mol% and 67.0 mol% respectively. According to 16S rRNA gene sequence analysis and DNA-DNA hybridization, strains DSM 4733^T and 247 are phylogenetically closely related (99.6% 16S rRNA gene sequence similarity, 82.9% DNA-DNA similarity) and affiliated to the genus *Sphingomonas*. The closest recognized species is *Sphingomonas aquatilis* DSM 15581^T (98.1% sequence similarity). In addition, the presence of cystine arylamidase, absence of β-galactosidase, and the lack of L-arabinose, galactose and sucrose utilization distinguish strains DSM 4733^T and 247 from most other *Sphingomonadaceae*. So far, the dimorphic life cycle that involves a prosthecate and a flagellated stage is unique for strains DSM 4733^T and 247 among all *Sphingomonadaceae*. Therefore, `*Caulobacter leidyi´* is reclassified as *Sphingomonas leidyi*, with the type strain DSM 4733^T (= ATCC 15260^T = CIP 106443^T = VKM B-1368^T) and strain 247 (DSM 25078 = LMG 26658) as an additional strain of this species.

The genus *Caulobacter* was originally described by Henrici & Johnson (1935) and later amended to comprise a variety of dimorphic prosthecate bacteria (Poindexter, 1981b, 1989; Schmidt 1981). These typically aquatic chemoorganotrophs (Poindexter, 1981a) form a cellular appendage, the prostheca or stalk, which is derived from the cell envelope and carries a holdfast at its distal tip. The daughter cell arises by asymmetric cell division at the younger, opposite cell pole that bears a single flagellum. After a period of motility, the progeny cell sheds the flagellum and develops a stalk at the originally flagellated pole (Garrity *et al.*, 2005; Poindexter, 1989). This dimorphic life cycle has been related to the oligotrophic growth mode of the bacteria (Poindexter, 1981a).

Prior to routine nucleotide sequence analysis, species of the genus *Caulobacter* were distinguished based on their cell morphology, salt tolerance, carbon substrate utilization and growth factor requirements (Poindexter, 1989). Subsequently, 16S rRNA gene sequence comparisons revealed that bacteria exhibiting a developmental life cycle with asymmetric binary fission are paraphyletic and form at least 6 phylogenetically distant groups, suggesting that prosthecate morphology developed several times, or was lost in different lineages, during the evolution of the alphaproteobacteria. Besides members of the genus *Caulobacter sensu strictu* (*Caulobacter* group III.; Garrity *et al.*, 2005) that include *Caulobacter crescentus*, *C. fusiformis*, *C. henrici* and *C. vibrioides*, dimorphic representatives are found in the related genus *Brevundimonas* (*B. alba*, *B. aurantiaca*, *B. bacteroides*, *B. intermedia*, *B. subvibrioides*, *B. variabilis*; group IV.), and in the distantly related genera *Asticacaulis* (*A. biprosthecium*, *A. excentricus*; group II.) and *Maricaulis* (*M. maris*; group V.) (Abraham *et al.*, 1997, 1999, 2001; Garrity *et al.*, 2005). More recently, a prosthecate representative of the genus *Phenylobacterium* (*P. conjunctum* FWC21^T) was described (Abraham *et al.*, 2008). A sixth lineage is represented by strain CB37^T = ATCC 15260^T = DSM 4733^T that was originally described as `*Caulobacter leidyi*´ (Poindexter, 1964). However, subsequent 16S rRNA gene sequence analyses revealed that this strain is affiliated with the family *Sphingomonadaceae* (Stahl *et al.*, 1992; Garrity *et al.*, 2005). As a consequence, `*C. leidyi*´ was excluded from the genus *Caulobacter*, omitted from Bergey's Manual of Systematic Bacteriology and removed to limbo (Garrity *et al.*, 2005).

Aside from 16S rRNA gene sequence similarity, two biochemical characteristics of the so far little characterized strain DSM 4733^T point towards a distinct taxonomic position of this strain and support an affiliation with the family *Sphingomonadaceae*. The holdfast of this strain does

not bind wheat-germ agglutinine and does not contain oligo-N-acetylglucosamine that participates in adhesion as in most *Caulobacter* spp. (Garrity *et al.*, 2005). Besides hexadecanoic acid (16:0) and octadecenoic acid (18:1 ω7c/18:1 ω9t/18:1 ω12t), strain DSM 4733^T contains 2-hydroxy myristic acid (C14:0 2-OH) as a dominant fatty acid (Abraham *et al.*, 1999). This feature is unique among all known dimorphic prosthecate bacteria but characteristic for the *Sphingomonadaceae* where glucuronosyl ceramide and 2-hydroxy myristic acid replace the lipopolysaccharides that are present in the cell wall of other bacteria (Yabuuchi & Kosako, 2005). Although the isolation of additional strains (strains WCP 2020S, CB 296 and an unnamed representative from Lake Baikal) has been reported (Garrity *et al.*, 2005; Lapteva *et al.*, 2007), no further information on their properties is currently available. Due to the limited physiological and chemotaxonomic information available for strain DSM 4733^T and the lack of additional strains, a taxonomic revision of this lineage is so far missing.

Strains DSM 4733^T, *Sphingomonas paucimobilis* DSM 1098^T, *Sphingomonas aquatilis* DSM 15581^T, *Sphingomonas wittichii* DSM 6014^T, *Sphingomonas panni* DSM 15761^T, *Sphingomonas echinoides* DSM 1805^T, and *Sphingopyxis alaskensis* DSM 13593^T were obtained from the DSMZ collections. Strain 247 was isolated from Starnberger See, a mesotrophic prealpine lake (584 m above sea level; maximum water depth 128 m). On December 20, 2007, water was collected at a water depth of 1 m from a pier located on the eastern shore near the municipality of Ammerland (47°55′N, 11°02′E) using a bilge pump (Overmann *et al.*, 1998). Primary enrichments were grown in basic synthetic freshwater medium buffered with 10 mM HEPES (Bartscht *et al.*, 1999) supplemented with 20 amino acids, glucose, pyruvate, citrate, 2-oxoglutarate, succinate (200 μM each), Tween 80 (0.001% v/v), formate, acetate and propionate (200 μM each), trace element solution SL 10, and 10-vitamin solution (Jaspers *et al.*, 2001). Signal molecules (cAMP, *N*-butyryl homoserine lactone, *N*-oxohexanoyl-DL-homoserine lactone, ATP) were added at 10 μM final for growth stimulation (Bruns *et al.*, 2002). For purification of the strains, agar plates were prepared with washed agar, basic synthetic freshwater medium and 1:10 diluted HD (0.05% casein peptone, 0.01% glucose, 0.025% yeast extract, w/v).

For comparative physiological testing, bacterial strains were routinely cultivated on DSMZ medium 830 (R2A agar) (containing 0.05% w/v yeast extract, 0.05% peptone, 0.05% casamino acids, 0.05% glucose, 0.05% starch, 0.03% sodium pyruvate, 0.03% K_2HPO_4, 0.005% $MgSO_4$,

1.5% agar; pH, 7.2) (Reasoner & Geldreich, 1985) and at an incubation temperature of 28 °C. Growth was tested between pH values of 5 to 10 (at intervals of 1 pH). Temperature dependence of growth was assessed between 8 and 45°C (at intervals of 3°C) using a temperature gradient incubator (Model TN-3, Sangyo). Cell morphology was determined by phase-contrast microscopy using agar-coated slides (Pfennig & Wagener, 1986) and transmission electron microscopy. Bacteria were fixed with 2% glutaraldehyde / 5% formaldehyde in the culture medium and negatively stained with 2% uranyl acetate applying a carbon film. Samples were examined in a TEM910 transmission electron microscope (Carl Zeiss, Oberkochen) at an acceleration voltage of 80 kV. Cell motility was evaluated using light microscopy and soft agar (0.1% w/v yeast extract, 0.01% K_2HPO_4, 0.2% Agar).

The Gram-type was determined using the Bactident Amino Peptidase kit and confirmed with the KOH test. Catalase activity was determined using 10% (v/v) H_2O_2 and oxidase activity using N, N, N′, N′-tetramethyl-ρ-phenylenediamine. Physiological and biochemical characteristics, and enzyme activities were determined with API 20NE, API ZYM, API 50CH test strips (bioMérieux) and Biolog Gen III microplates (BiOLOG, Hayward, CA, USA) according to the instructions of the manufacturer. Susceptibility to antibiotics was determined on R2A agar plates using the disc diffusion method (NCCLS, 2007) (in µg per disc): penicillin G (10), oxacillin (5), ampicillin (10), ticarcillin (75), cefalotin (30), mezlocillin (30), cefazolin (30), cefotaxim (30), aztreonam (30), chloramphenicol (30), tetracyclin (30), imipenem (10), gentamycin (10), amikacin (30), vancomycin (30), erythromycin (15), lincomycin (15), ofloxacin (5), colistin (10), norfloxacin (10), pipemidic (20), bacitracin (10), polymyxin B (300), nitrofurantoin (100), neomycin (30), kanamycin (30), doxycycin (30), clindamycin (10), ceftriaxone (30), fosfomycin (50), nystatin (100), linezolid (10), moxifloxacin (5), quinupristin/dalfopristin (15), piperacillin/tazobactam (40), and teicoplanin (30).

Fatty acids, respiratory quinones, polar lipids, and polyamines were extracted from cells grown on R2A medium. For fatty acid analysis, 40 mg wet weight of fresh cells were harvested from the agar plates, and extracted according to the standard protocol of the Microbial Identification system (MIDI; Sherlock Version 4.5, database TSBA6) (Sasser, 1990). Respiratory quinones were extracted from 200 mg freeze-dried cell material and analysed according to the method of Tindall and Collins (Tindall 1990a, b; Tindall, 2005; Collins, 1994). Quinones were

first separated by their structural classes (such as menaquinones, ubiquinones) using thin layer chromatography (TLC). The resulting bands were eluted and further separated and identified by HPLC, using a RP18 column (Tindall, 1996). Polar lipids were extracted from 100 mg freeze-dried cell material, separated by two-dimensional chromatography and identified by their R_F values in combination with their reaction with specific staining reagents (Tindall 1990a, b). Polyamines were extracted from freeze-dried cells that had been harvested at the late exponential growth phase and analysed as reported by Busse & Auling (1988). HPLC analysis was carried out applying the equipment described by Stolz *et al*. (2007).

Genomic DNA was extracted using the High Pure PCR template preparation Kit (Roche) and the almost complete 16S rRNA gene amplified with primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACT T-3') (Lane, 1991). PCR products were purified with the QIAquick PCR purification kit (Qiagen) and sequenced by the dideoxynucleotid method on an ABI Prism 3730 genetic analyzer (Applied Biosystems), employing the AmpliTaq FS Big Dye terminator cycle sequencing kit. Sequences were edited and assembled with the Vector NTI computer package (Invitrogen). Additional 16S rRNA gene sequences of the type strains of all species of *Caulobacter*, of all type species of relevant genera of the *Sphingomonadaceae*, and of the type strains of all other dimorphic prosthecate alphaproteobacteria species (in the genera *Brevundimonas*, *Phenylobacterium*, *Asticcacaulis*, *Maricaulis*) were retrieved from the GenBank database (Altschul *et al.*, 1997) and imported into the ARB program package (Ludwig *et al.*, 2004). Automated alignments of the Fast Aligner tool were corrected manually according to secondary structure information to yield an alignment of approx. 1500 bp. Phylogenetic trees were constructed with the FastdnaML maximum likelihood algorithm, neighbor joining and maximum parsimony algorithms as implemented in the ARB software package. For determination of phylogenetic distances, a full distance matrix was calculated within ARB from the neighbour joining analysis. Bootstrap values were calculated with 1000 bootstrap resamplings. Sequence accession numbers are provided in Fig. 1. The mol% G+C content of DNA was determined as described by Mesbah *et al.* (1989). For DNA-DNA hybridization (DDH), cells were disrupted by using a French pressure cell (Thermo Spectronic) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DDH was carried out as described by De

Ley *et al*. (1970) as modified by 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). All determinations were conducted in duplicate.

Strains DSM 4733^T and 247 formed transparent, colorless, circular and convex colonies on R2A agar, PYE agar and 1:10 diluted HD agar after 24 hours incubation. Individual cells of strain 247 were Gram-negative, asporogenous, rod-shaped, 0.7-1.2 µm long and 0.4-0.5 µm wide (Table 1) and typically formed rosettes (Fig. 1A). Electron microscopic investigations revealed the presence of cells bearing either a polar flagellum or a stalk (Fig. 1B,C) very similar to strain DSM 4733^T (for phase contrast and electron microscopic images of the latter, refer to Poindexter, 1989; Fig. 21.32).

Cells were aerobic, catalase- and oxidase-positive and motile by the single polar flagellum. The novel isolate grew at pH values between 5 and 10 (optimum, pH 7) and temperatures between 10 and 40°C (optimum, 28°C) (Table 1). Both strains did not reduce nitrate but were capable of using esculin, glucose, N-acetyl-glucosamine, maltose, L-arabinose, D-xylose, D-glucose, N-acetyl-glucosamine, cellobiose, trehalose and malic acid. Melibiose, β-gentiobiose and D-lyxose were weakly utilized. They produced alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase. Cells of strains DSM 4733^T and 247 were sensitive (inhibition zones >30 mm) to tetracyclin, amikacin, vancomycin, doxycycin, fosfomycin, moxifloxacin and resistant to penicillin G, oxacillin, ampicillin, ticarcillin, cefalotin, mezlocillin, cefazolin, cefotaxim, sztreonam, chloramphenicol, lincomycin, colistin, pipemidic, bacitracin, nitrofurantoin, clindamycin, ceftriaxone, nystatin, linezolid, quinupristin/dalfopristin, and piperacillin/tazobactam. Additional characteristics are listed in the species description, Table 1 and Supplementary Table S1.

The major respiratory quinone for strains DSM 4733^T and 247 was Q-10 (100% and 81.5%, respectively). The major fatty acids for strain 247 were $C_{18:1}\omega7c$ (67.2%), $C_{16:0}$ (10.7%), 11-Methyl $C_{18:1}\omega7c$ (8.4%). $C_{14:0}$ 2OH (6.7%) was the major 2-hydroxy fatty acid. For strain DSM 4733^T, the major fatty acids were $C_{18:1}\omega7c$ (70.5%), $C_{16:0}$ (14.6%), 11-Methyl $C_{18:1}\omega7c$ (2.7%), and the major hydroxy fatty acids was also $C_{14:0}$ 2OH (6.6%) (Table 2). For both strains

the major polar lipids were diphosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, three glycolipids (GL1-3), two phosphoaminolipids and two sphingoglycolipids (Supplementary Fig. S1). The dominant polyamine of strains DSM 4733^T and 247 was *sym*-homospermidine [64.7 and 73.9 µmol (g dry mass)$^{-1}$, respectively], which was accompanied by smaller amounts of putrescine [2.3 and 3.1 µmol (g dry mass)$^{-1}$, respectively] and spermidine [3.4 and 2.8 µmol (g dry mass)$^{-1}$, respectively], spermine [0.9 and 1.1 µmol (g dry mass)$^{-1}$, respectively] and traces of diaminopropane and cadaverine.

Based on the nearly complete (1493 bp) 16S rRNA gene sequence of strain 247 and the available (1443 nt long) 16S rRNA gene sequence of strain DSM 4733^T (AJ 227812), our phylogenetic analysis of all dimorphic prosthecate alphaproteobacteria and the type strains of *Sphingomonadaceae* type species placed strains DSM 4733^T and 247 unambiguously within the family *Sphingomonadaceae* (Yabuuchi *et al.*, 1990) (Fig. 2; Supplementary Fig. S2). Like the neighbour-joining tree, maximum likelihood and maximum parsimony analyses (phylogenetic trees not shown) identified *Sphingomonas aquatilis* DSM 15581^T as the closest relative of both strains. Furthermore, maximum likelihood analysis confirmed the clusters of the neighbour-joining tree that had high bootstrap support as well as most of the other shallow clusters. The sequence similarity of the 16S rRNA genes of strains DSM 4733^T and 247 was 99.6%. Within the *Sphingomonadaceae*, *Sphingomonas aquatilis* DSM 15581^T was identified as the phylogenetically closest relative with a sequence similarity of 98.1 % to strain DSM 4733^T and 97.7 % to strain 247. Sequence similarity with *Sphingomonas paucimobilis* DSM 1098^T (type strain of type species of genus *Sphingomonas*) was 94.5%.

DNA-DNA similarity between DSM 4733^T and 247 was 82.9%. In contrast, the DNA-DNA similarity was only 18.6% between DSM 4733^T and *Sphingomonas aquatilis* DSM 15581^T and only 5.2% between strain 247 and *Sphingomonas aquatilis* DSM 15581^T. Based on the threshold value of 70% DNA-DNA similarity for the definition of a bacterial species (Wayne *et al.*, 1987) strains 247 and DSM 4733^T belong to the same species that is distinct from *Sphingomonas aquatilis* DSM 15581^T.

The results of the phylogenetic analysis of 16S rRNA gene sequences and DDH therefore indicate that DSM 4733^T and strain 247 represent a novel species within the genus

Sphingomonas and the family *Sphingomonadaceae*. Both 16S rRNA gene sequences also include all 7 signature sequences of *Sphingomonadaceae* but none of those determined for *Caulobacteraceae* or other alphaproteobacteria families (Yabuuchi & Kosako, 2005). The large phylogenetic distance to species of the genus *Caulobacter* (≤ 87.9 % nucleotide sequence similarity, Fig. 1) and the affiliation with the *Sphingomonadaceae* is commensurate with the fatty acid patterns and polar lipid composition of the two strains. Whereas $C_{16:0}$ and $C_{18:1}\omega7c$/$C_{18:1}\omega9t$/$C_{18:1}\omega12t$ represent the dominant non-polar fatty acids in members of the *Caulobacteraceae* and *Sphingomonadaceae* alike, the dominance of hydroxymyristic acid ($C_{14:0}$ 2OH) and absence of 3-OH fatty acids is diagnostic for *Sphingomonadaceae* and $C_{14:0}$ 2OH is missing in the *Caulobacteraceae* (containing $C_{12:1}$ 3OH, or $C_{12:0}$ 3OH in the closely related *Brevundimonas* species) (Abraham *et al.*, 1999). The presence of sphingoglycolipids is another key characteristic that defines the genus *Sphingomonas* and the family *Sphingomonadaceae* and, together with phosphatidylcholine, distinguishes the two investigated strains from the *Caulobacteraceae* or other prosthecate alphaproteobacteria (Abraham *et al.*, 1999; Yabuuchi & Kosako, 2005).

Corroborating the close phylogenetic relationship to *Sphingomonas aquatilis*, the 16S rRNA gene sequences of DSM 4733^T and strain 247 include all six signature sequences previously determined for the genus *Sphingomonas* and contain *sym*-homospermidine as the dominant polyamine similar to other species of this genus (Takeuchi *et al.*, 2001). Characteristics that distinguish strains DSM 4733^T and strain 247 from most other *Sphingomonadaceae* include the presence of cystine arylamidase (variable in DSMZ 4733^T), absence of β-galactosidase, and the lack of L-arabinose, galactose and sucrose utilization (Table 1; Takeuchi *et al.*, 2011). So far, the dimorphic life cycle that involves a prosthecate and a flagellated stage is unique among the *Sphingomonadaceae*. Based on our detailed comparative phylogenetic, physiological and chemotaxonomic characterization of strains DSM4733^T and 247, we propose to reclassify *Caulobacter leidyi* DSM 4733^T as a species of the genus *Sphingomonas* as *Sphingomonas leidyi* comb. nov. for these unique prosthecate dimorphic bacteria within the family *Sphingomonadaceae*. With this reclassification, the family *Sphingomonadaceae* for the first time now also includes stalked bacteria. Other phylogenetically closely related, but so far morphologically uncharacterized, strains have been isolated from Lake Starnberg (Jogler *et al.*,

2011) drinking water (Donofrio *et al.*, 2010), and arsenic-contaminated mine tailings (Macur *et al.*, 2001) suggesting a broader distribution of *Sphingomonas leidyi* in the environment.

Emended description of the genus *Sphingomonas* Yabuuchi *et al.* 1990 emend. Yabuuchi *et al.* 1999, emend. Takeuchi *et al.* 2001, emend. Yabuuchi *et al.* 2002, emend. Busse *et al.* 2003

Cells are Gram-negative, non-sporulating rods measuring 0.3-0.8 x 0.7-1.9 μm. Motile or non-motile, some species exhibit a dimorphic life cycle that involves a prosthecate nonmotile and a flagellated motile cell. Colonies are yellow, off-white or colorless. Strictly aerobic and chemo-organotrophic. Catalase-positive. Respiratory quinone is predominantly Q-10. Major fatty acids are 18:1, saturated 16:0 and/or 17:1. Major 2-hydroxy fatty acids are 2-OH 14:0 or 2-OH 15:0. Glycosphingolipids are present. *sym*-Homospermidine is the major polyamine. The DNA G+C content is 62 - 68 mol%. Members of the *a*-4 subclass of *Proteobacteria*. Characteristic 16S rRNA signatures are found at positions 52:359 (C:G), 134 (G), 593 (G), 987:1218 (G:C) and 990:1215 (U:G). Type species: *Sphingomonas paucimobilis* (Holmes *et al.* 1977) Yabuuchi *et al.* 1990.

Description of *Sphingomonas leidyi* comb. nov.

Sphingomonas leidyi (lei'dy.i, N. L. gen. masc. n. leidyi, of Leidy, named for J. Leidy, who observed tufts of (bacterial) growth of fungi in insect guts in 1853)

The description of *Sphingomonas leidyi* is the same as that given for *Caulobacter leidyi* by Henrici and Abraham (Henrici & Johnson, 1935, emended by Poindexter, 1964; Abraham *et al.*, 1999), with the following additional characteristics. Cells are predominantly short, uncurved, slightly tapered, and sometimes nearly ovoid, with short stalks. Colonies are colorless. Isolates known from freshwater, millipede hindgut and soil. Nitrate is not reduced; esculin, glucose, N-acetyl-glucosamine, maltose and malic acid are hydrolysed. Cells are divided by binary division.

Produces alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase. L-arabinose, D-xylose, D-glucose, N-acetyl-glucosamine, cellobiose, maltose, and trehalose are utilized. Melibiose, β-gentiobiose and D-lyxose are weakly utilized. Cells are sensitive to tetracyclin, amikacin, vancomycin, doxycycin, fosfomycin, moxifloxacin. Resistant to penicillin G, oxacillin, ampicillin, ticarcillin, cefalotin, mezlocillin, cefazolin, cefotaxim, aztreonam,

chloramphenicol, lincomycin, colistin, pipemidic, bacitracin, nitrofurantoin, clindamycin, ceftriaxone, nystatin, linezolid, quinupristin/dalfopristin, piperacillin/tazobactam.

Q-10 is the major respiratory quinone, the major fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, 11-Methyl $C_{18:1}\omega7c$, and $C_{14:0}$ 2OH is the major 2-hydroxy fatty acids. The major polar lipids are diphosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, three glycolipids, two phosphoaminolipids and two sphingoglycolipids. The major polyamine is *sym*-homospermidine. The genomic DNA G+C content of strain DSM 4733^T is 67 mol%. The type strain is DSM 4733^T (= ATCC 15260^T = CIP 106443^T = VKM B-1368^T) and was isolated from a millipede hind-gut. Another representative strain of this species is 247 (DSM 25078 = LMG 26658) with the genomic DNA G+C content 67.6 mol%, isolated from a prealpine freshwater lake (Starnberger See, Germany); an additional strain has been isolated from Lake Baikal.

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FIGURE LEGENDS

Fig. 1. A. Phase-contrast photomicrograph of a rosette formed by cells of strain 247. Bar, 5 µm. **B.** Transmission electron micrograph of negatively stained flagellated cell of strain 247. Bar, 1 µm. **C.** Transmission electron micrograph of negatively stained stalked cell of strain 247. Bar, 1 µm.

Fig. 2. Rooted neighbour-joining phylogenetic tree, based on 16 rRNA gene sequences showing the relationships between *Sphingomonas leidyi* DSM 4733^T, strain 247 and other dimorphic prosthecate alphaproteobacteria. The type species of the respective genera were included in the analysis. Numbers at nodes indicated the level (in %) of bootstrap support based on 1000 resampled datasets. *Maricaulis maris* DSM4734^T was used as the out group. Bar indicates 1% nucleotide divergence.

LEGEND TO SUPPLEMENTARY FIGURE

Supplementary Fig. S1. Polar lipid patterns of **A.** strain DSM 4733^T and **B.** strain 247 after separation by two-dimensional thin-layer chromatography, detected with anis aldehyde (for all lipids), ninhydrin (for amino groups), cis-aconitinacid-anhydride (for phosphorus-containing lipids), meta-periodate/schiff (for vicinal hydroxy groups), molybdenum blue (for phosphor groups). DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidyldimethylethanolamine; GL1, GL2, GL3, glycolipids; SGL, sphingoglycolipids (may divide into two spots with similar RF values); L1, L2, unidentified lipids; PNL1, PNL2, phosphoaminolipids; GNL1, GNL2, glycoaminolipids.

Supplementary Fig. S2. Rooted neighbour-joining phylogenetic tree, based on 16 rRNA gene sequences showing the relationships between *Sphingomonas leidyi* DSM 4733^T, strain 247 and other dimorphic prosthecate alphaproteobacteria. Compared to Fig. 2, all presently known *Sphingomonas* strains were included in the analysis. The type species of the respective genera were included in the analysis. Numbers at nodes indicated the level (in %) of bootstrap support based on 1000 resampled datasets. *Maricaulis maris* DSM4734^T was used as the out group. Bar indicates 1% nucleotide divergence..

Table 1. Different physiological and phenotypic characteristics of strain 247, *Caulobacter leidyi* DSM 4733^T and related type strains of the genus *Sphingomonas*.

Strains: 1, strain 247; 2, *Sphingomonas leidyi* DSM 4733^T; 3, *Sphingomonas aquatilis* DSM 15581^T, 4, *Sphingomonas paucimobilis* DSM 1098^T; 5, *Sphingomonas wittichii* DSM 6014^T; 6, *Sphingomonas echinoides* DSM 1805^T; 7, *Sphingopyxis alaskensis* DSM 13593^T. +, positive; -, negative; V, weak reaction; ?, questionable reaction; Y: yellow; GW, greyish-white; CL, colorless; ND, not determined.

All strains tested negative for: α-fucosidase, α-galactosidase, α-mannosidase, β-glucuronidase, arginine dihydrolase, lipase (C14), protease, urease, Gram-staining, fermentation of glucose, indole production, nitrate reduction to nitrite, nitrite reduction to N_2, adipic acid, adonitol, D-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, L-fucose, β-gentiobiose, gluconate, glycerol, glycogen, inositol, inulin, 2-keto-gluconate, 5-keto-gluconate, mannitol, D-mannitol, β-methyl-D-xyloside, phenylacetic acid, potassium gluconate, ribose, salicin, sorbitol, L-sorbose, starch, D-tagatose, xylitol, L-xylose.

All strains tested positive for: alkaline phosphatase, arylamidase, catalase, α-glucosidase, β-glucosidase/aesculin, leucine acid phosphatase, aerobic growth, L-arabinose, cellobiose, D-glucose, maltose, trehalose and D-xylose.

Substrate or test	1	2	3	4	5	6	7
Cell width	0.4-0.5	0.4-0.5	ND	0.7	0.5-0.7	0.8	0.2-0.5
cell length (μm)	0.7-1.2	1.2	ND	1.4	1.2-2.0	1.9	0.5-3
Motility	+	+	+	+	-	+	+
G+C content (mol%)	67.6	67	63	65	ND	65.8	65
Pigmentation of colonies	CL	CL	Y	Y	Y	Y	Y
Growth in the presence of 4% NaCl	-	+	-	+	+	+	-
α-chymotrypsine	-	-	-	+	-	-	-
Cystine arylamidase	+	V	V	-	-	-	-
Esterase (C4)	V	V	-	+	-	V	V
Esterase Lipase (C8)	V	+	-	-	+	V	V
β-galactosidase	-	-	+	+	+	?	-
N-acetyl-β-glucosaminidase	-	V	V	+	+	-	-
β-glucosidase	+	+	+	+	+	+	-
Naphtol phosphohydrolase	V	+	-	-	V	V	V
Trypsine	+	V	-	V	-	-	+
Valine arylamidase	+	+	+	+	+	+	-
Oxidase	+	+	+	+	-	+	-
L-arabinose	-	-	+	+	+	+	-
D-fructose	-	-	+	+	+	-	-
Galactose	-	-	+	+	+	+	-
Lactose	-	-	V	+	+	V	-
D-lyxose	V	V	V	V	+	+	V
D-maltose	+	+	+	+	-	+	+

D-mannose	-	+	+	+	+	-	-
Melezitose	-	-	V	+	-	-	-
Melibiose	V	-	-	+	-	-	-
D-raffinose	-	-	-	+	-	-	-
Rhamnose	-	+	+	-	+	-	-
Sucrose	-	-	+	+	+	+	-
Trehalose	+	+	+	+	+	+	-
D-turanose	-	-	-	+	+	+	-
Amygdalin	-	-	-	+	+	-	-
Arbutin	-	-	V	V	+	-	-
α-methyl-D-mannopyranoside	-	-	-	+	-	-	-
α-methyl-D-glucoside	-	-	-	+	-	-	-
N-acetyl-glucosamine	+	+	+	+	+	+	-
Capric acid	-	-	-	+	-	-	-
Malic acid	+	+	+	+	+	-	+
Trisodium citrate	-	-	-	-	+	-	-

Table 2. Cellular fatty acids profiles of strain 247, *Sphingomonas leidyi* DSM 4733^T and the related species of the family *Sphingomonas*

Strains: 1, strain 247; 2, *Sphingomonas leidyi* DSM 4733^T; 3, *Sphingomonas aquatilis* DSM 15581^T (Lee *et al.*, 2001); 4, *Sphingomonas paucimobilis* DSM 1098^T; 5, *Sphingomonas wittichii* DSM 6014^T; 6, *Sphingomonas panni* DSM 15761^T; 7, *Sphingomonas echinoides* DSM 1805^T; 8, *Sphingopyxis alaskensis* DSM 13593^T. Values shown are percentages of the total fatty acids. -, not detected; tr, trace (<1%).

Strains	1	2	3	4	5	6	7	8
$C_{14:0}$	tr	-	tr	tr	1.71	tr	tr	-
$C_{14:0}$ 2OH	6.72	6.63	10.86	6.90	8.35	4.24	8.14	1.33
$C_{15:0}$	-	-	-	-	-	3.07	-	2.77
$C_{15:0}$ 2OH	-	-	-	-	-	1.28	-	8.82
Summed feature 3	tr	1.52	15.75	4.71	11.38	13.15	2.37	6.54
$C_{16:1}\omega5c$	tr	tr	tr	tr	2.04	1.84	tr	tr
$C_{16:0}$	10.73	14.61	20.58	8.75	15.9	-	12.31	4.94
$C_{16:0}$ 2OH	tr	tr	-	-	-	-	-	1.14
$C_{16:1}$ 2OH	-	-	-	-	-	-	tr	-
$C_{17:1}\omega8c$	-	-	-	-	-	1.37	-	8.52
$C_{17:1}\omega6c$	tr	1.12	-	tr	-	12.01	tr	38.8
$C_{17:0}$	-	-	-	-	-	1.52	tr	3.66
$C_{18:1}\omega7c$	67.15	70.47	51.6	75.29	53.75	51.62	64.04	19.7
$C_{18:1}\omega5c$	1.60	tr	1.51	1.77	-	tr	1.48	tr
$C_{18:0}$	tr	-	1.04	tr	-	-	tr	tr
11-Methyl $C_{18:1}\omega7c$	8.39	2.73	-	-	3.13	-	8.71	1.59
$C_{19:0}$ cyclo $\omega8c$	1.93	tr	-	-	3.73	-	tr	-
Summed feature 7	-	-	-	-	-	-	-	tr

Summed features represent groups of two or more fatty acids that could not be separated by gas chromatography with the MIDI system. Summed feature 3 contains C16:1ω7c and/or C15:0 iso 2-OH. Summed feature 7 contains C19 Cyclo ω10c and/or C19 ω6c.

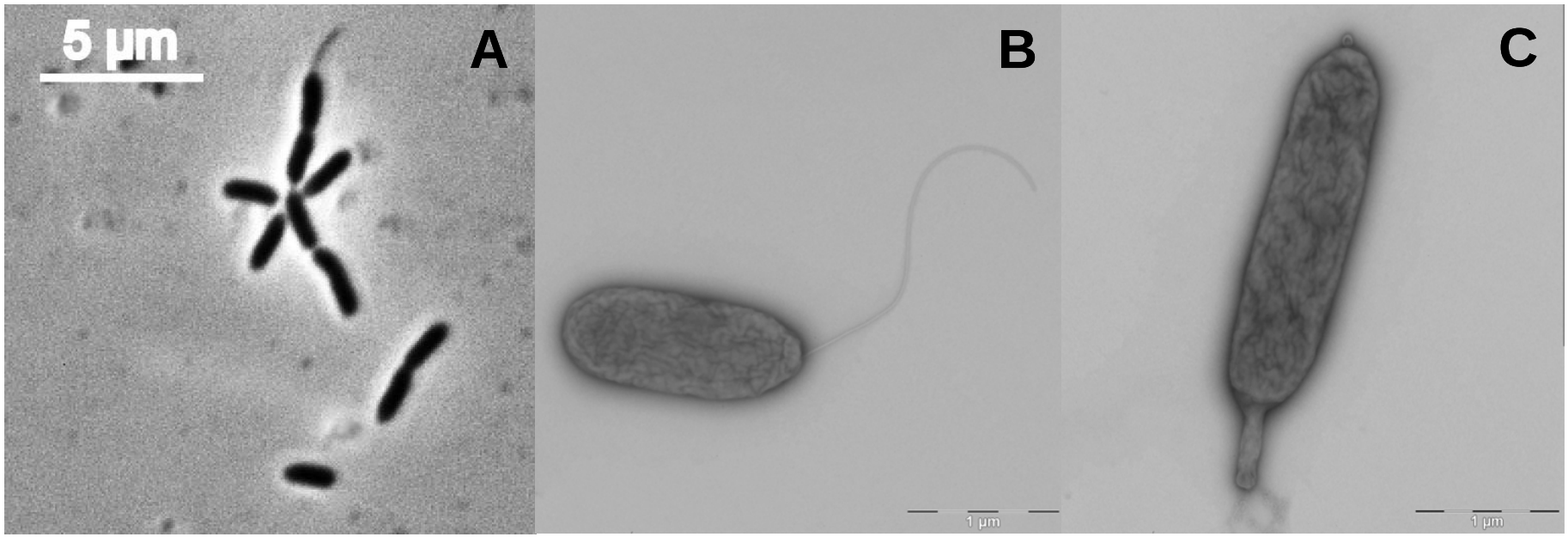

Fig. 1
Chen et al. (2011)

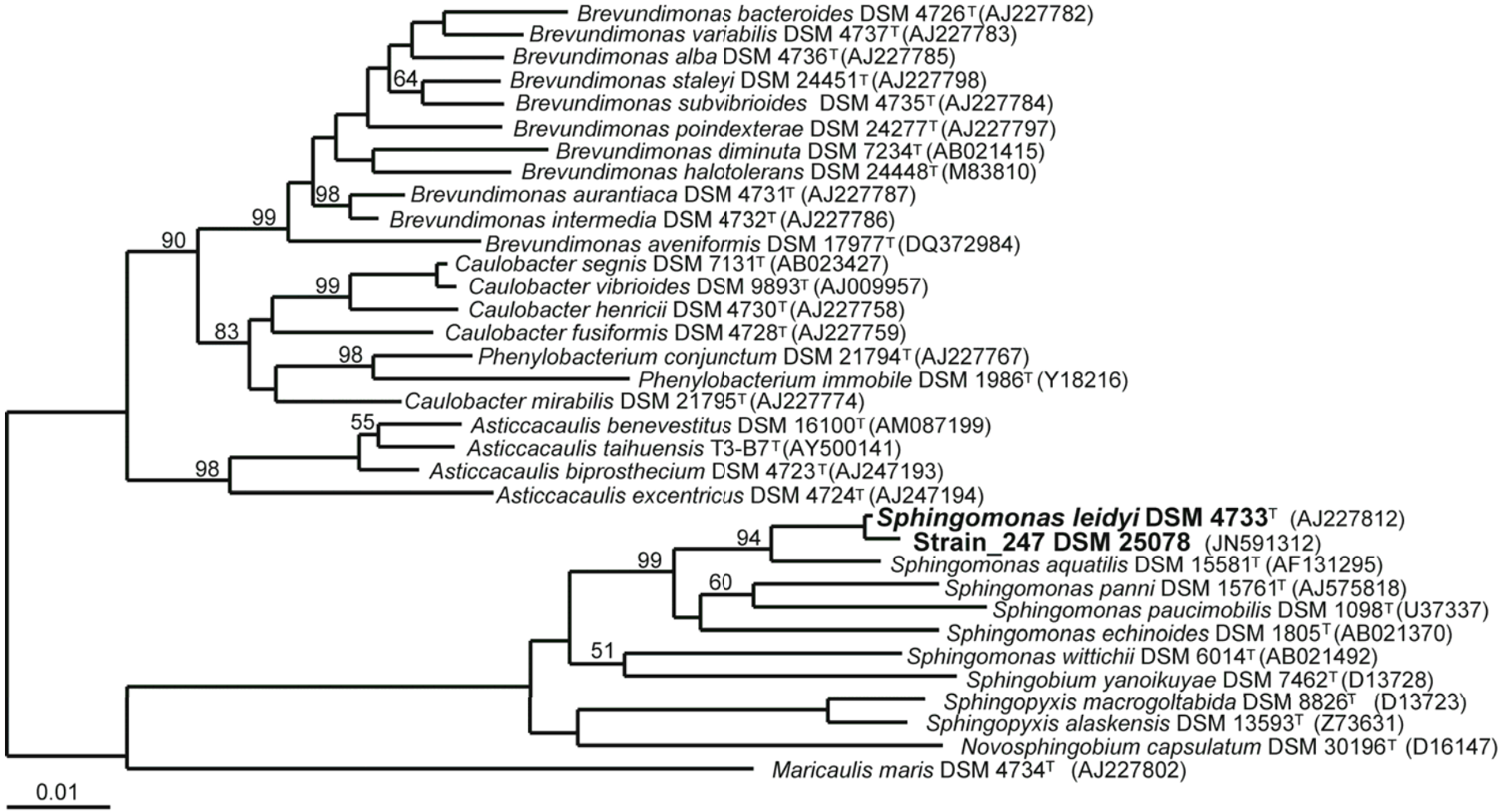

Fig. 2
Chen et al. (2011)