This is a pre- or post-print of an article published in
Jogler, M., Chen, H., Simon, J., Rohde, M., Busse, H.-J.,
Klenk, H.-P., Tindall, B.J., Overmann, J.
Description of *Sphingorhabdus planktonica* gen. nov., sp.
nov. and reclassification of three related members of the
genus *Sphingopyxis* in the genus *Sphingorhabdus* gen. nov
(2013) International Journal of Systematic and
Evolutionary Microbiology, 63 (PART4), pp. 1342-1349.

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4 **Description of *Sphingorhabdus planktonica* gen. nov., sp. nov., and**
5 **reclassification of three related *Sphingopyxis* species as members of the novel**
6 **genus *Sphingorhabdus***

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22 **Running title:** *Sphingorhabdus planktonica* gen. nov., sp. nov.

23 **Subject category:** New Taxa, subsection *Proteobacteria*
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26 **Footnote**

27 The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain
28 G1A_585^T = DSM 25081^T = LMG 26646^T is JN381068.
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Abstract

A previously undescribed aerobic, non-sporulating bacterium, strain G1A_585^T, was isolated from an oligotrophic freshwater lake in Bavaria (Germany). The rod-shaped cells were Gram-negative and non-motile. Based on 16S rRNA gene similarity, strain G1A_585^T is a member of the family *Sphingomonadaceae* and shares less than 95.2 % similarity with all type strains of the most closely related genus *Sphingopyxis*. Phylogenetically, it shares a common root with the three marine species *Sphingopyxis flavimaris* DSM 16223^T, *Sphingopyxis marina* DSM 22363^T, and *Sphingopyxis litoris* DSM 22379^T. Polar lipids of strain G1A_585^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidyltrimethyl-ethanolamine, phosphatidylcholine, sphingoglycolipids, three glycolipids, and one unknown lipid. Ubiquinone-10 represents the dominant quinone (93.1 %) and is accompanied by ubiquinone-9 (6.5 %). The major cellular fatty acids are C_{18:1}ω7c and/or C_{18:1}ω6c (summed feature 8) (38.2%); C_{16:1}ω7c (33.6%) and C_{14:0} 2-OH (17.8%). As the major polyamine spermidine and traces of 1,3-diaminopropane, putrescine and spermine were detected. Strain G1A_585^T has a DNA G+C content of 55.7 mol% and tested positive for oxidase and catalase. Based on the phylogenetic relationship, its low G+C content compared to most other *Sphingopyxis* species, and the presence of signature nucleotides in the 16S rRNA gene sequence, the novel genus and species *Sphingorhabdus planktonica* gen. nov., sp. nov. is proposed with strain G1A_585^T (= DSM 25081^T = LMG 26646^T) as the type strain. Since *Sphingopyxis flavimaris* DSM 16223^T (= KCTC 12232^T), *Sphingopyxis marina* DSM 22363^T (= IMSNU 14132^T = KCTC 12763^T = JCM 14161^T) and *Sphingopyxis litoris* DSM 22379^T (= IMSNU 14133^T = KCTC 12764^T = JCM 14162^T) form a phylogenetic group together with strain G1A_585^T that is clearly separated from all other known *Sphingopyxis* strains and shares signature nucleotides, these three *Sphingopyxis* strains are reclassified as members of the proposed novel genus *Sphingorhabdus*.

Members of the family *Sphingomonadaceae* represent typical freshwater bacteria (Gich *et al.*, 2005; Glöckner *et al.*, 2000; Zwart *et al.*, 2002), but also occur widespread in the marine environment, in pristine and contaminated soils, the rhizosphere and in clinical specimen (Balkwill *et al.*, 2006; Kim *et al.*, 2007; Takeuchi *et al.*, 2001). The genus *Sphingomonas* was established in 1990 (Yabuuchi *et al.*, 1990) and later divided into the four genera *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis* (Takeuchi *et al.*, 2001). Phylogenetically related genera are *Blastomonas* and *Zymomonas*, as well as *Sandaracinobacter* (Yurkov *et al.*, 1997), *Sphingosinicella* (Maruyama *et al.*, 2006), *Sandarakinorhabdus* (Gich & Overmann, 2006), and the most recently described *Stakelama* (Chen *et al.*, 2010) and *Sphingomicrobacterium* (Kämpfer *et al.*, in press). *Sphingomonadaceae* are characterized by harboring glycosphingolipids (Yabuuchi *et al.*, 1990) and are physiologically highly diverse. Several members are capable of aerobic anoxygenic photosynthesis (Kim *et al.*, 2007). Many sphingomonads degrade polycyclic aromatic compounds and xenobiotics (Basta *et al.*, 2005), including toxic dioxin pollutants, which makes them useful for industrial wastewater treatment (Wittich *et al.*, 1992). Whereas most *Sphingomonadaceae* are typically aerobic, *Zymomonas mobilis* subspecies are facultative anaerobes that produce ethanol via fermentation (Kalnenieks, 2006).

A survey of bacterioplankton in an oligotrophic alpine freshwater lake (Walchensee, near Kochel in Bavaria, Germany; located 802 m above sea level) revealed that *Sphingomonadaceae* constituted the major fraction of alphaproteobacteria. Among the *Sphingomonadaceae*, one previously unknown 16S rRNA gene sequence type alone accounted for 25% of the sequences of alphaproteobacteria that were recovered. This phylotype was identified to be physiologically active throughout the year not only in the oligotrophic Walchensee but also in nearby mesotrophic Starnberger See (Jogler *et al.*, 2011). A strain, G1A_585^T, representing this sequence type was isolated from Walchensee and subsequently characterized.

Water samples were obtained from Walchensee on December 20, 2007, from a boat at a distance of 30 m from the western shore (47°35'N, 11°20'E). Strain G1A_585^T was enriched in microtiter plates employing diluted growth medium and the high throughput multidrop technique (Bruns *et al.*, 2003). The medium consists of basic synthetic freshwater medium buffered with 10 mM HEPES (Bartscht *et al.*, 1999) supplemented with 20 canonical amino acids, glucose, pyruvate, citrate, 2-oxoglutarate, succinate (200 µM each), Tween 80 (0.001% v/v) and a fatty acid mixture containing formate, acetate and propionate (200 µM each) (Jaspers *et al.*, 2001). Trace element solution SL 10 (1 ml·l⁻¹), 10-vitamin solution (10 ml·l⁻¹) and the inducers cAMP, *N*-butyryl

homoserine lactone and *N*-oxohexanoyl-DL-homoserine lactone at final concentrations of 10 μ M were added (Bruns *et al.*, 2002).

Strain G1A_585^T was purified from the enrichment cultures by streaking on agar plates containing artificial freshwater, 1:10 diluted HD medium (HD medium consisting of 0.05 % casein peptone, 0.01 % glucose, 0.025 % yeast extract; w/v), and 10 mM HEPES buffer (pH 7). The agar was washed three times with distilled water and applied at 1% (w/v) concentration. Growth was also tested on plates containing 1:10 diluted HD medium but without artificial freshwater, as well as on standard R2A agar (Reasoner & Geldreich, 1985), and Nutrient and CASO agar (Oxoid), but no growth was observed on either of the latter three media. Only poor growth of strain G1A_585^T was detected in liquid 1:10 diluted HD medium without artificial freshwater.

Colonies on agar plates were yellowish-orange in color and transparent. Carotenoids were extracted from 10 ml of liquid culture in the early stationary phase. The culture was centrifuged and the cell pellet mixed with 1 ml pure acetone. Absorption spectra were monitored with a Lambda 25 spectrometer (Perkin Elmer). Within the spectrum a shoulder at 427 nm and two peaks at 453 nm and 481 nm were detected indicating the presence of carotenoids in strain G1A_585^T. Typical carotenoids of *Sphingomonadaceae* are zeaxanthin and/or nostoxanthin. A shoulder positioned between 420 nm and 430 nm is typical for other *Sphingomonadaceae* species and attributed to unidentified pigments (Asker *et al.*, 2007; Busse *et al.*, 2003; Denner *et al.*, 2001; Gich & Overmann, 2006; Jenkins *et al.*, 1979; Stolz *et al.*, 2000).

The almost complete 16S rRNA gene was amplified by PCR using the primers 8f and 1492r (Lane, 1991). The resulting PCR products were purified with the QIAquick PCR purification kit (Qiagen) and sequenced by the dideoxynucleotid method on an AB 3730 DNA Analyzer (Applied Biosystems) using the AmpliTaq FS Big Dye terminator cycle sequencing kit. Sequences were edited and assembled with the Vector NTI computer package (Invitrogen). Sequences were automatically aligned with the integrated Fast Aligner tool of the ARB software package (Ludwig *et al.*, 2004), and the alignment was corrected manually according to secondary structure information. Phylogenetic analyses with the Neighbor Joining and Maximum Parsimony algorithm were conducted using the ARB package with 1,000 bootstrap resamplings. Maximum Likelihood phylogenetic trees were generated with PhyML (Guindon *et al.*, 2010) and bootstrap values were calculated with 100 resamplings. The 16S rRNA gene sequence of strain G1A_585^T obtained in the present study was deposited in the GenBank database under accession number JN381068.

1 Within the phylogenetic trees, strain G1A_585^T constitutes a separate branch, yet shares a root
2 with a cluster of the three marine species *Sphingopyxis flavimaris* DSM 16223^T, *Sphingopyxis*
3 *marina* DSM 22363^T, and *Sphingopyxis litoris* DSM 22379^T (Fig. 1). Strain G1A_585^T shares 93.6 -
4 94.2% 16S rRNA gene sequence similarity with the three *Sphingopyxis* species, whereas 16S rRNA
5 gene sequence similarities between *Sphingopyxis flavimaris* DSM 16223^T, *Sphingopyxis marina*
6 DSM 22363^T and *Sphingopyxis litoris* DSM 22379^T ranged between 97.9 - 98.5%. The common root
7 with the latter species has bootstrap supports of 91%, 75% and 49% in Neighbor Joining, Maximum
8 Parsimony and Maximum Likelihood phylogenetic trees, respectively. The second branch separating
9 the four strains from all other *Sphingopyxis* species has an even higher bootstrap support (97% /
10 78% / 89%; Fig. 1). All type strains of *Sphingopyxis* showed less than 95.2% 16S rRNA gene
11 similarity to strain G1A_585^T. In addition, the signature nucleotides at positions 990:1215 that are
12 used to differentiate *Sphingomonadaceae* genera (Takeuchi *et al.*, 2001) were found to differ
13 between all other *Sphingopyxis* species (except *Sphingopyxis witflariensis*, which is phylogenetically
14 very distant; compare Fig. 1) and the phylogenetically separated cluster of G1A_585^T, *Sphingopyxis*
15 *flavimaris* DSM 16223^T, *Sphingopyxis marina* DSM 22363^T, and *Sphingopyxis litoris* DSM 22379^T
16 (Tab. 1).

17 Cells of strain G1A_585^T were rod-shaped and on average were (1.17 ± 0.20) µm long and
18 (0.47 ± 0.07) µm wide (Fig. S1a; supplementary material) as determined with an Axio Imager M2
19 microscope (Zeiss) equipped with a AxioCam MRm camera and AxioVision 4.8.2. software (Zeiss).
20 In contrast to members of all validated *Sphingopyxis* species, strain G1A_585^T is immotile. For
21 negative staining, cells were fixed with 2 % glutaraldehyde and 5 % formaldehyde in growth
22 medium, washed with TE buffer and negatively stained with 4% aqueous uranyl acetate using a
23 carbon film on mica. Parallel samples for routine transmission electron microscopy were fixed with
24 2% glutaraldehyde and 5% formaldehyde in cacodylate buffer, treated with 1% aqueous osmium and
25 then dehydrated with a graded series of acetone on formvar-coated grids. For contrasting, 2% uranyl
26 acetate in 70% acetone was used. Samples were examined in a TEM910 transmission electron
27 microscope (Carl Zeiss, Oberkochen) at an acceleration voltage of 80 kV. Transmission and
28 scanning electron microscopy revealed no flagella (Fig. S1b, c; supplementary material).

29 Cells were Gram-negative and positive for cytochrome *c* oxidase and catalase as tested with
30 standard methods (Gerhardt *et al.*, 1994). For determination of the optimal pH for growth, artificial
31 freshwater medium with 1:10 diluted HD was prepared at different pH using appropriate buffers.
32 MES was employed for pH values of 5, 6 and 6.5; HEPES for 7, 7.5 and 8, HEPPS for 8.5 and

CHES for values of pH 9 and 10. Strain G1A_585^T was able to grow between pH 6.5 and pH 9, with optimal growth detected between pH 7 and 8. The temperature optimum of growth was at 18°C to 24°C, whereas no growth was detected below 11°C and above 27°C. The G+C content of genomic DNA of strain G1A_585^T was determined as described previously (Cashion *et al.*, 1977; Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984) and was 55.7 mol%. By comparison, species within the genus *Sphingopyxis* have higher DNA G+C contents between 62.3-69.2 mol% (Vancanneyt *et al.*, 2001; Yoon *et al.*, 2005; Zhang *et al.*, 2010; Godoy *et al.*, 2003; Lee *et al.*, 2008; Kim *et al.*, 2005; Takeuchi *et al.*, 2001; Srinivasan *et al.*, 2010; Choi *et al.*, 2010; Sharma *et al.*, 2010), with the exception of *Sphingopyxis flavimaris* DSM 16223^T, *Sphingopyxis marina* DSM 22363^T and *Sphingopyxis litoris* DSM 22379^T (Tab. 2). The DNA G+C contents for the latter three strains were determined in this study and ranged between 52.6-57.8 mol%.

Quinones and polar lipids were extracted from 0.1 g of lyophilized biomass (Tindall, 1990a, b). Strain G1A_585^T contains ubiquinone-10 (93.1 %) in addition to minor amounts of ubiquinone-9 (6.5 %). Polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidyltrimethyl-ethanolamine, phosphatidylcholine, sphingoglycolipids, three glycolipids and one unknown lipid (Fig. S2; supplementary material). Polyamines were extracted from cells in the stationary growth phase according to the procedure described previously (Busse & Auling, 1988). Analysis was carried out using the HPLC equipment described by Stolz *et al.* (2007). Spermidine was the major polyamine [21.1 µmol (g dry biomass)⁻¹]. In addition, traces [<0.6 µmol (g dry biomass)⁻¹] of 1,3-diaminopropane, putrescine and spermine were detected.

For fatty acid profiles (Stead *et al.*, 1992) cells of strain G1A_585^T, of the phylogenetically related *Sphingopyxis* species *Sphingopyxis flavimaris* DSM 16223^T, *Sphingopyxis marina* DSM 22363^T, and *Sphingopyxis litoris* DSM 22379^T, as well as cells *Sphingopyxis witflariensis* DSM 14551^T were analyzed. In addition, type strains of the type species of seven related genera (*Sphingopyxis macrogoltabida* DSM 8826^T; *Sphingosinicella microcystinivorans* DSM 19791^T; *Sphingomonas paucimobilis*^T DSM 1098; *Sphingobium yanoikuyae* DSM 7462^T; *Novosphingobium capsulatum* DSM 30196^T; *Sandarakinorhabdus limnophila* DSM 17366^T and *Blastomonas natatoria* DSM 3183^T) were analyzed. 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, which were identified against the TSBA40 peak naming table database. The fatty acid profile has been shown to vary for cells grown under different conditions

(Sikorski *et al.*, 2008; Takeuchi *et al.*, 2001). Therefore all strains were grown under similar conditions (artificial freshwater with 1:10 HD, 20°C) with the exception of *Sphingopyxis flavimaris* (DSM 16223^T), *Sphingopyxis marina* (DSM 22363^T), and *Sphingopyxis litoris* (DSM 22379^T). The latter three strains are not capable of growing in artificial freshwater with 1:10 diluted HD and hence had to be cultivated in Bacto Marine Broth (DIFCO 2216) which in turn, however, was not suitable for growing strain G1A_585^T. As described above, all attempts to identify a common growth medium suitable for cultivation of all four strains failed.

The dominant cellular fatty acids for strain G1A_585^T were C_{18:1}ω7c and/or C_{18:1}ω6c (summed feature 8) reaching 38.2 %. C_{16:1}ω7c, C_{16:1}ω6c and/or C_{15:0} iso 2-OH (summed feature 3) reached 33.6 %. Based on a combination of the absolute retention times, the biosynthetic pathways involved in fatty acid biosynthesis and the information provided by MIDI Inc., the fatty acid contained in the summed feature 3 was identified as C_{16:1}ω7c and will be referred to as such in this text. The major 2-hydroxy fatty was C_{14:0} 2-OH (17.8 %). In addition, C_{16:0} (8.0 %), C_{16:1} 2-OH (1.7 %), C_{14:0} (0.7 %), C_{17:0} (1.5 %), C_{17:1}ω6c (1.1 %), C_{16:1}ω9c (0.9 %), C_{16:0} 2-OH (0.7 %) and C_{15:0} (0.2 %) were detected. Interestingly, strain G1A_585^T showed an exceptionally high amount of C_{14:0} 2-OH (17.8 %) compared to related *Sphingopyxis* species (Tab. 2; Tab. S2; supplementary material) and all tested genus type strains (Tab. S1; supplementary material). Furthermore C_{16:1}ω9c was not detected in related *Sphingopyxis* species and strains of type species of the other genera.

Physiological and biochemical characteristics were determined in artificial freshwater without 1:10 diluted HD using API 20E galleries according to the instructions of the manufacturer (bioMérieux) and are given in the species description. Most notably, strain G1A_585^T produced the enzymes β-glucosidase and β-galactosidase and assimilates D-glucose as the sole carbon source (Tab. 2).

Taken together, strain G1A_585^T is a representative of the *Sphingomonadaceae* that exhibits typical characteristics of this family, including ubiquinone-10 as the major quinone, the presence of the polar lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidyl dimethylethanolamine, phosphatidylcholine, sphingoglycolipids and glycolipids. The detected polyamine composition of strain G1A_585^T is characteristic for the genera *Sphingopyxis*, *Novosphingobium* and *Sphingomonas*. However, strain G1A_585^T forms a separated phylogenetic branch that clearly diverges from most members of the genus *Sphingopyxis*. The phylogenetic divergence is supported by the existence of characteristic signature nucleotides at positions 990:1215. Distinct properties of strain G1A_585^T include a

specific fatty acid composition, the presence of β -glucosidase and β -galactosidase, and a very narrow spectrum of carbon substrates utilized for growth.

Queries of the GenBank 16S rRNA gene sequence database by BLAST (Altschul, 1997) revealed that environmental sequences identical to the 16S rRNA gene sequence of G1A_585^T have been repeatedly detected also in other freshwater lakes. Based on its distinct properties and wide distribution, we propose a novel genus and species, *Sphingorhabdus planktonica* for the novel type of bacterium, with strain G1A_585^T (= DSM 25081^T = LMG 26646^T) as the type strain. The genomic DNA G+C contents for *Sphingopyxis flavimaris* DSM 16223^T, *Sphingopyxis marina* DSM 22363^T and *Sphingopyxis litoris* DSM 22379^T as determined in this study ranged between 52.6 - 57.8 mol% (Table 2), whereas type strains of the other species within the genus *Sphingopyxis* exhibited higher DNA G+C contents between 62.3 - 69.2 mol%. Based on the phylogenetic relationship, their lower G+C content, and the characteristic signature nucleotides, a reclassification of *Sphingopyxis flavimaris* DSM 16223^T, *Sphingopyxis marina* DSM 22363^T and *Sphingopyxis litoris* DSM 22379^T as members of the proposed novel genus *Sphingorhabdus* is recommended.

Description of *Sphingorhabdus* gen. nov.

Sphingorhabdus [Sphin.go.rhab'dus. N.L. n. *sphingosinum* (from Gr. gen. n. *sphingos*, of sphinx, and suff.-*ine*), sphingosine; N.L. pref. *sphingo*-, pertaining to sphingosine; Gr. fem. n. *rhabdos*, rod; N.L. fem. n. *Sphingorhabdus*, a sphingosine-containing rod].

Cells are motile or non-motile, gram negative, aerobic, non-spore forming rods. They contain ubiquinone-10 as major respiratory quinone and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and sphingoglycolipids as major polar lipids. As major polyamine spermidine was detected. Cells are positive for oxidase and catalase. Major non polar fatty acids are C_{18:1}ω7c and/or C_{18:1}ω6c (summed feature 8) (38.2%) and C_{16:1}ω7c (33.6%) (summed feature 3). The signature nucleotides at position 990:1215 are U:A.

The type species of the genus is *Sphingorhabdus planktonica* with the type strain G1A_585^T (DSM 25081^T = LMG 26646^T).

Description of *Sphingorhabdus planktonica* sp. nov.

Sphingorhabdus planktonica [plank.to'ni.ca N.L. fem. adj. *planktonica* (from Gr. adj. *planktos*, wandering), living in the plankton, planktonic].

General characteristics are the same as those given in the description of the genus. Colonies are yellowish-orange and transparent. Cells are $1.17\ \mu\text{m} \pm 0.20\ \mu\text{m}$ long and $0.47\ \mu\text{m} \pm 0.07\ \mu\text{m}$ wide. Cells absorb at 427 nm, 453 nm and 481 nm, indicating the presence of carotinoids. The G+C content of the genomic DNA is 55.7 mol%. Strain G1A_585^T is able to grow between pH 6.5 and pH 9, with optimal growth between pH 7 and 8. The temperature optimum is 18°C to 24°C. No growth detected below 11°C and above 27°C. D-glucose is utilized for growth, whereas L-arabinose, D-mannose, D-mannitol, N-acetyl-glocosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not utilized. Nitrate was not reduced, indole was not produced and fermentation of D-glucose is not observed. Arginine dihydrolase, urease and protease are absent, but β -glucosidase and β -galactosidase are produced.

The type strain is G1A_585^T (DSM 25081^T = LMG 26646^T) and was isolated from the freshwater lake Walchensee (near Kochel, Bavaria, Germany).

Description of *Sphingorhabdus marina* comb. nov.

Sphingopyxis marina (ma.ri'na. L. fem. adj. marina of the sea, marine).

The description of *Sphingorhabdus marina* is the same as published for *Sphingopyxis marina* (Kim *et al.*, 2008). The G+C content of the type strain is 56.5 mol%.

The type strain is FR1087^T (DSM 22363^T = IMSNU 14132^T = KCTC 12763^T = JCM 14161^T).

Description of *Sphingorhabdus litoris* comb. nov.

Sphingopyxis litoris (li.to'ris. L. gen. n. litoris of the seashore, of the coast).

The description of *Sphingorhabdus litoris* is the same as that given for *Sphingopyxis litoris* (Kim *et al.*, 2008). The G+C content of the type strain is 52.6 mol%.

The type strain is FR1093^T (DSM 22379^T = IMSNU 14133^T = KCTC 12764^T = JCM 14162^T).

Description of *Sphingorhabdus flavimaris* comb. nov.

Sphingopyxis flavimaris (fla.vi.ma'ris. L. adj. flavus yellow; L. gen. n. maris of the sea; N.L. gen. n. flavimaris of the Yellow Sea).

The description of *Sphingorhabdus flavimaris* is the same as published for *Sphingopyxis flavimaris*. . The G+C content of the type strain is 57.8 mol%.

The type strain is SW-151^T (DSM 16223^T = KCTC 12232^T).

Acknowledgements

We thank Dr. Peter Schumann and Birgit Grün for the determination of the G+C content, and Prof. Peter Kämpfer (Justus-Liebig-University, Giessen) and Dr. Elke Lang (DSMZ, Braunschweig) for providing *Sphingomonadaceae* type strains. Technical assistance during the determination of quinones and fatty acids was provided by Gabriele Pötter (DSMZ, Braunschweig), and advice during API testing by Anja Frühling and cultivation assistance by Anja Stieler (DSMZ, Braunschweig). This work was funded by grants of the Deutsche Forschungsgemeinschaft to J. Overmann (grants no. OV 20/17-1 and OV 20/19-1).

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Figures

Fig. 1. Neighbor Joining phylogenetic tree of *Sphingomonadaceae*. Bootstrap values are given in % and only shown for nodes that had high support (in the order Neighbor Joining, Maximum Parsimony, Maximum Likelihood). The phylogenetic trees calculated with different algorithms had a similar topology, but *Sphingopyxis baekryungensis* DSM 16222^T showed an unstable position within the *Sphingomonadaceae*. Therefore the sequence of this strain was excluded from the analysis. In order to achieve the highest resolution, sequences of the most closely related *Rhizobium* strains (*R. hainanense* I66, accession number RHU71078; *R. gallicum*, RGU86343; *R. yanglingense* SH2262, AF003375; *R. sullae* IS123^T, Y10170; *R. alarii* GBV016^T, AM931436; *R. leguminosarum* IAM 1260, D12782; *R. etli* CFN 42, EU488751; *R. tibeticum* CCBAU85039, EU256404; *R. soli* DS-42, EF363715) were used as outgroup. Bar represents 1% fixed point mutations per nucleotide.

Supplementary figures

Fig. S1. (a) Phase-contrast photomicrograph, (b) transmission electron micrograph of negatively stained cells and (c) scanning electron micrograph of cells of strain G1A_585^T.

Fig. S2. Polar lipid profiles of (a) strain G1A_585^T, (b) *Sphingopyxis marina* (DSM 22363^T) and (c) *Sphingopyxis litoris* (DSM 22379^T) after separation with two-dimensional thin layer chromatography. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidylmethylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipids; L1, unidentified lipid; GL1- GL4, glycolipids.

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Fig. 1

Table 1. 16S rRNA nucleotide signatures used for the differentiation of *Sphingomonadaceae* (Takeuchi *et al.*, 2001).

The consensus of each genus is highlighted by grey shading. Species with divergent nucleotides (marked in bold and underlined) at signature positions are given. The complete set of analysed species are shown for *Sphingopyxis* and *Sphingorhabdus*.

Nucleotide position	52:359	134	593	987:1218	990:1215
<i>Sphingomonas</i>	C:G	G	G	G:C	U:G
<i>adhaesiva</i>	C:G	G	G	G: <u>U</u>	U:G
<i>rosa</i>	C:G	<u>A</u>	G	G:C	U:G
<i>suberifaciens</i>	C:G	<u>A</u>	G	G:C	U:G
<i>Sphingobium</i>	U:A	G	U	A:U	U:G
<i>vulgare</i>	U:A	<u>A</u>	U	A:U	U:G
<i>olei</i>	U:A	G	U	A: <u>C</u>	U:G
<i>abikonense</i>	U:A	G	U	A: <u>C</u>	U:G
<i>lactosutens</i>	U:A	G	U	A: <u>C</u>	U:G
<i>Novosphingobium</i>	C:G	G	U	G:C	U:A
<i>Sphingopyxis</i>	C:G	G	U	G:C	U:G
<i>alaskensis</i>	C:G	G	U	G:C	U:G
<i>bauzanensis</i>	C:G	G	U	G:C	U:G
<i>chilensis</i>	C:G	G	U	G:C	U:G
<i>ginsengisoli</i>	C:G	G	U	G:C	U:G
<i>macrogoltabida</i>	C:G	G	U	G:C	U:G
<i>panaciterrae</i>	C:G	G	U	G:C	U:G
<i>panaciterrulae</i>	C:G	G	U	G:C	U:G
<i>soli</i>	C:G	G	U	G:C	U:G
<i>taejonensis</i>	C:G	G	U	G:C	U:G
<i>terrae</i>	C:G	G	U	G:C	U:G
<i>ummariensis</i>	C:G	G	U	G:C	U:G
<i>granuli</i>	C:G	<u>A</u>	<u>C</u>	G:C	U:G
<i>witflariensis</i>	C:G	G	U	G:C	U: <u>A</u>
<i>Sphingorhabdus</i>	C:G	G	U	G:C	U: <u>A</u>
<i>planktonica</i>	C:G	<u>A</u>	U	G:C	U: <u>A</u>
<i>litoris</i>	N:G	G	<u>A</u>	G:C	U: <u>A</u>
<i>marina</i>	N:G	G	U	G:C	U: <u>A</u>
<i>flavimaris</i>	C:G	G	U	G:C	U: <u>A</u>

Table 2. Differential phenotypic characteristics of isolate G1A_585^T and related *Sphingopyxis* species.

1, G1A_585^T (this study); 2, *Sphingopyxis marina* DSM 22363^T (Kim *et al.*, 2008); 3, *Sphingopyxis litoris* DSM 22379^T (Kim *et al.*, 2008); 4, *Sphingopyxis flavimaris* DSM 16223^T (Yoon & Oh, 2005); 5, *Sphingopyxis macrogoltabida* DSM 8826^T (Takeuchi *et al.*, 1993; Lee *et al.*, 2008; Srinivasan *et al.*, 2010); 6, *Sphingopyxis witflariensis* DSM 14551^T (Zhang *et al.*, 2010; Srinivasan *et al.*, 2010). All strains were positive for catalase. All strains were negative for reduction of nitrates, indole production, glucose fermentation, D-mannitol, D-mannose and phenylacetic acid. +, positive; –, negative; w, weakly positive; ND, not determined.

	1	2	3	4	5	6
color	yellowish-orange transparent ^a	yellow ^b	yellow ^b	yellow ^b	yellowish white ^a	yellow transparent ^a
motility	-	+	+	+	+	+
oxidase	+	+	ND	+	+	+
DNA G+C content (mol%)	55.7	56.5	52.6	57.8	63.9	ND
arginine dihydrolase	-	+	+	ND	-	-
polar lipids ^c	DPG, PE, PG, PMME, PDME, PC, SGL, GL, L ^a	DPG, PE, PG, PC, SGL, GL ^b	DPG, PG, PE, PC, SGL ^b	DPG, PE, PMME, PDME, PG, SGL, GL, L	ND	DPG, PE, PG, PMME, PC, SGL
major fatty acids	summed feature 8, summed feature 3, C _{14:0} 2-OH ^a	summed feature 3, summed feature 8 ^b	summed feature 3, summed feature 8, C _{16:0} , C _{18:1} ω7 ^c 11-methyl ^b	summed feature 3, summed feature 8 ^b	summed feature 3, summed feature 8 ^a	summed feature 3, summed feature 8 C _{17:1} ω6 ^c
major polyamine	spermidine	spermidine, agmatine	spermidine	ND	ND	spermidine
urease	-	+	+	-	-	-
β-glucosidase	+	w	w	ND	+	-
protease	-	+	-	-	-	-
β-galactosidase	+	-	+	ND	+	-
utilization of:						
glucose	+	+	-	+	+	+
L-arabinose	-	+	-	-	+	-
N-acetyl-glucosamine	-	-	-	ND	-	-
D-maltose	-	-	-	-	+	+
potassium gluconate	-	-	-	ND	-	-
capric acid	-	-	-	ND	+	+

adipic acid	-	+	-	ND	-	-
malic acid	-	+	+	-	-	-
trisodium citrate	-	+	+	-	-	-

^aanalyzed in this study after growth in 1:10 HD medium with artificial freshwater.

^banalysed in this study after growth on Bacto Marine Broth (DIFCO 2216).

^cDPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidylmethylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipids; L, unidentified lipid; GL, glycolipids.