Zavarzinella formosa gen. nov., sp. nov., a novel stalked, *Gemmata*-like planctomycete from a Siberian peat bog

Irina S. Kulichevskaya,¹ Olga I. Baulina,² Paul L. E. Bodelier,³ W. Irene C. Rijpstra,⁴ Jaap S. Sinninghe Damsté⁴ and Svetlana N. Dedys¹

Correspondence
Svetlana N. Dedys
dedys@mail.ru

¹S. N. Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letya Otyabrya 7/2, Moscow 117312, Russia

²M. V. Lomonosov Moscow State University, Faculty of Biology Science, GSP-2, Leninskie Gory, Moscow 119992, Russia

³Netherlands Institute of Ecology, NL-3631 AC Nieuwersluis, The Netherlands

⁴NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, PO Box 59, NL-1790 AB Den Burg, The Netherlands

An aerobic, pink-pigmented, budding and rosette-forming bacterium was isolated from an acidic *Sphagnum* peat bog and designated strain A10^T. The 16S rRNA gene sequence analysis showed that strain A10^T was a member of the order *Planctomycetales* and belonged to a phylogenetic lineage defined by the genus *Gemmata*, with 90 % sequence similarity to that of *Gemmata obscuriglobus*, the only taxonomically described organism of this group. Ellipsoid-shaped cells of strain A10^T were uniformly covered with crateriform pits and possessed long (up to 10–15 µm) and unusually thick (0.5–0.7 µm) stalks of a unique ultrastructure. Thin sections revealed a complex intracellular membrane system compartmentalizing the cells. Strain A10^T was a moderately acidophilic, mesophilic organism capable of growth at pH values between 3.8 and 7.2 (with an optimum at pH 5.5–6.0) and at temperatures between 10 and 30 °C (with an optimum at 20–25 °C). The major fatty acids were C_{18:0}, C_{18:1ω5c} and C_{16:1ω5c} and the major quinone was MK-6. Cells of strain A10^T contained high amounts of bound saturated and monounsaturated C₂₆–C₃₂ (ω-1) hydroxy fatty acids. The G + C content of the DNA was 62.5 mol%. The unique cell morphology, the capability of growth in acidic conditions and a number of chemotaxonomic and genotypic characteristics served to differentiate strain A10^T from *G. obscuriglobus*. Based on these data, the novel isolate should be considered as representing a novel genus and species of planctomycetes, for which the name *Zavarzinella formosa* gen. nov., sp. nov. is proposed. The type strain is A10^T (=DSM 19928^T=VKM B-2478^T).

Recently, members of the order *Planctomycetales* were identified as one of the most numerous bacterial groups detectable by fluorescence *in situ* hybridization in acidic *Sphagnum*-dominated northern wetlands (Dedys *et al.*, 2006; Kulichevskaya *et al.*, 2006; Ivanova & Dedys, 2006). Several peat-inhabiting, acidophilic planctomycetes were isolated in pure culture and described as representatives of two novel genera and species, *Schlesneria paludicola* and

Singulisphaera acidiphila (Kulichevskaya *et al.*, 2007, 2008). These newly described planctomycetes belonged to phylogenetic lineages defined by the genera *Planctomyces* and *Isosphaera*, respectively. Apart from these recently characterized strains, our set of isolates included another peat-inhabiting planctomycete, designated strain A10^T, which belonged to a phylogenetic lineage defined by the genus *Gemmata* (Dedys *et al.*, 2006). The 16S rRNA gene sequence of strain A10^T showed 90 % similarity to that of the type strain of *Gemmata obscuriglobus* (Franzmann & Skerman, 1984), the only taxonomically described organism of this group. The morphology of the peat bog isolate A10^T was strikingly different from the morphology described for representatives of the genus *Gemmata*. In contrast to single, spherical and stalk-less cells typical of *Gemmata*, the ellipsoid-like cells of strain A10^T possessed

Abbreviation: PLFA, phospholipid fatty acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A10^T is AM162406.

A comparison of the fatty acid compositions of strain A10^T and *Gemmata obscuriglobus* DSM 5831^T and electron micrographs of negatively stained preparations of cells of strain A10^T are available as supplementary material with the online version of this paper.

thick stalks and were assembled in large rosette-like cell clusters. In this paper, we report the characteristics of the isolate from a peat bog and propose a novel genus and species to accommodate this morphologically unique bacterium.

Strain A10^T was isolated from peat soil (pH 4.0) sampled at a depth of 10–20 cm (with a water table at a depth of approximately 15 cm) of the *Sphagnum* peat bog Bakchar, Tomsk region, West Siberia (56° 51' N 82° 50' E) in July 2004. The procedure used for the isolation of strain A10^T was described by Dedysh *et al.* (2006). Briefly, microbial biofilms enriched with cells of peat-inhabiting planctomycetes were obtained by using the water agar-coverslip technique developed by Hirsch *et al.* (1977). When development of rosette-forming, budding bacteria with characteristic cell morphology was observed on a coverslip taken for microscopic examination, replicate coverslips were used for agar streak isolation. The latter was performed using agar medium M31 (modification of medium 31 described by Staley *et al.*, 1992) containing (per litre distilled water): 0.1 g KH₂PO₄, 20 ml Hutner's basal salts, 1.0 g *N*-acetylglucosamine, 0.2 g ampicillin (sodium salt), 0.1 g peptone, 0.1 g yeast extract and 15 g agar-agar (Difco), pH 5.8. The plates were incubated at 20 °C for 6 weeks. Colonies appearing on the plates were picked randomly for microscopic examination and fluorescence *in situ* hybridization-based identification of planctomycetes. At the stage of isolation, colonies of strain A10^T did not exceed 0.5 mm in diameter. The isolate was purified by successive restreaking on agar medium M31. Once obtained as a pure culture, the bacterium was maintained on agar medium M31 and was subcultured at 2 month intervals. *G. obscuriglobus* DSM 5831^T (Franzmann & Skerman, 1984) was used as a reference strain. It was maintained on medium 629, recommended by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) for cultivation of this bacterium.

Morphological observations and cell size measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). Preparation of ultrathin sections was performed as described previously (Kulichevskaya *et al.*, 2008). For scanning electron microscopy, the bacteria were fixed in 2% (w/v) glutaraldehyde in liquid medium M31 without carbon sources for 0.5–1 h and in some cases were post-fixed in 1% (w/v) osmium tetroxide in the same medium for 4 h. The samples were then dehydrated through an ethanol series, left in absolute acetone overnight, critically point-dried (Dryer HCP-2), coated with the Au-Pd alloy (IB-3 Ion Coater) and examined with a JEOL JSM-6380LA scanning electron microscope. The absorption spectra of acetone/methanol (1:1, v/v) extracts of cells of strain A10^T were determined between 250 and 1000 nm with a SPH-56 spectrophotometer. For these measurements, cells were grown in liquid medium M31 and harvested at approximately early stationary growth phase.

Physiological tests were performed in batch cultures grown in liquid medium M31 (in tightly closed 120 ml serum bottles containing 10 ml medium). Due to the formation of aggregates in liquid cultures of strain A10^T, its growth could not be monitored by using nephelometry. Therefore, we followed the growth by measuring the rate of CO₂ production in liquid cultures incubated for 14–21 days under a variety of conditions, including temperatures of 4–37 °C, pH 3.8–8.0 and NaCl concentrations of 0–1.0% (w/v). CO₂ was measured by using gas chromatography with a thermoconductivity detector. With the exception of the temperature experiment, the flasks were incubated on a rotary shaker at 120 r.p.m. and at 24 °C. Variations in pH were achieved by mixing 0.1 M solutions of H₂SO₄ and KOH. Carbon source utilization was determined using liquid mineral medium MM supplemented with respective carbon sources (0.05%, w/v). Medium MM contained (per litre distilled water): 0.1 g KH₂PO₄, 0.1 g (NH₄)₂SO₄, 0.1 g MgSO₄·7H₂O, and 1 ml metal salt solution '44' (Staley *et al.*, 1992), the pH being adjusted to 5.8. The capability to degrade various biopolymers was examined as described for *Schlesneria paludicola* (Kulichevskaya *et al.*, 2007). Oxidative and fermentative utilization of carbohydrates was determined by using an API 20NE kit (bioMérieux). Enzymic activities were examined by using an API ZYM kit (bioMérieux). Nitrogen sources were tested using liquid MM medium with 0.05% glucose in which (NH₄)₂SO₄ was replaced with one of the following compounds at a concentration of 0.05% (w/v): KNO₃, KNO₂, urea, or one of the amino acids listed in Table 1. Cultures were tested for growth under anaerobic conditions in anaerobic jars by using AnaeroGen anaerobic system envelopes (Oxoid). The ability to grow in microaerobic conditions was examined by cultivating strain A10^T in tightly closed 120 ml serum bottles containing 115 ml liquid medium MM with 0.05% glucose. Susceptibility to antibiotics was determined on M31 agar plates using discs containing the following antibiotics: ampicillin (10 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (10 µg), novobiocin (30 µg), streptomycin (10 µg), chloramphenicol (30 µg) and lincomycin (10 µg) (Oxoid).

For fatty acid, neutral lipid and quinone analyses, cells of strain A10^T were grown in liquid medium M31 and harvested at the late exponential growth phase. Phospholipid fatty acid (PLFA) and isoprenoid quinone analyses were performed as described for *Schlesneria paludicola* (Kulichevskaya *et al.*, 2007). For lipid analyses the procedures described by Goossens *et al.* (1989) were used. Genomic DNA of the novel isolate was extracted as described by Marmur (1961). The G+C content of the DNA was determined by means of thermal denaturation using a Unicam SP1800 spectrophotometer (UK) at a rate of 0.5 °C min⁻¹ and calculated according to Owen *et al.* (1969). The 16S rRNA gene sequence of strain A10^T was determined previously (Dedysh *et al.*, 2006). Phylogenetic analysis was carried out using the ARB program package (Ludwig *et al.*, 2004). The 16S rRNA gene sequence was

Table 1. Phenotypic characteristics of strain A10^T in comparison to *Gemmata obscuriglobus*

Both strains utilize glucose, galactose, lactose, cellobiose, maltose, melezitose, rhamnose, ribose, sucrose, trehalose, xylose and *N*-acetylglucosamine. Both strains hydrolyse laminarin, pectin, chondroitin sulfate, aesculin, starch, gelatin, lichenan and xylan. The two strains do not utilize fructose, fucose, glycerol, gluconate, methanol, ethanol, galacturonate, acetate, benzoate, caproate, citrate, formate, formaldehyde, fumarate, glutarate, lactate, malate, succinate, propionate, mannitol, tartrate, alanine, arginine, asparagine, aspartate, cysteine, cystine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. The two strains do not hydrolyse casein, chitin or cellulose. Both strains utilize nitrate, ammonia, *N*-acetylglucosamine, Bacto peptone and Bacto yeast extract as nitrogen sources. ND, Not determined.

Characteristic	Strain A10 ^T	<i>G. obscuriglobus</i> DSM 5831 ^T
Cell shape	Ellipsoidal	Spherical to ovoid
Cell size (µm)	2.0–2.5 × 2.5–3.2	1.4–3.0 × 1.4–3.0
Pigmentation	Pink	Rose
pH range for growth (optimum)	3.8–7.2 (5.5–6.0)	7.8–8.8 (ND)
Temperature range for growth (optimum) (°C)	10–30 (20–25)	16–35 (ND)
Oxidase	+	–
Carbon source		
Pyruvate	+	–
Raffinose	+	–
Mannose	–	+
Sorbitose	+	–
Enzymic activity		
β-Galactosidase	–	+
α-Galactosidase	–	+
α-Mannosidase	–	+
DNA G + C content (mol%)	62.5	64.4

added to the ARB database SSU_04 and aligned manually. The trees were constructed using distance-based (neighbour-joining), maximum-likelihood (DNAML) and maximum-parsimony methods. The significance levels of interior branch points obtained in the neighbour-joining analysis were determined by bootstrap analysis (based on 1000 data resamplings) using PHYLIP (Felsenstein, 1989).

On agar medium M31, strain A10^T formed highly raised, pink, circular colonies with an entire edge and a smooth surface. Approximately 3 weeks of incubation were needed before the colonies were visible by the naked eye. Six-week-old colonies of these bacteria did not exceed 1 mm in diameter. Part of the colony grew into the agar layer, so that it was difficult to remove a colony using a loop. When grown in liquid media, cells of strain A10^T showed a tendency to attach to the walls of glass flasks. Liquid cultures displayed light-pink turbidity and were non-homogeneous due to the formation of cellular conglomerates. The main absorption maxima detected in the acetone/methanol extracts of strain A10^T occurred at 471, 496, 522 and 559 nm, suggesting the presence of carotenoids.

Mature cells of strain A10^T were non-motile ellipsoids, 2.0–2.5 µm in width and 2.5–3.2 µm in length (Fig. 1a, b). The surface of negatively stained whole cells was uniformly covered with crateriform pits (Fig. 1c). The cells reproduced by budding. A young culture of strain A10^T contained both non-motile and highly motile cells. The

latter were smaller in size and apparently represented buds separated from the mother cells. In most cases, one sinusoidal, 16–22 nm thick flagellum per cell was observed (Supplementary Fig. S1a, available in IJSEM Online). On ageing, the buds transformed into non-motile cells that occurred singly or were arranged in rosette-like clusters (Fig. 1a). The number of cells in these rosettes varied depending on cultivation conditions, but some rosettes in old cultures encompassed up to 50 or more cells. Examination of cultures grown on agar media revealed the presence of an amorphous ‘holdfast’ substance excreted from the poles of many cells (Fig. 1b). This holdfast substance enabled the cells to attach to surfaces or to form rosette-like clusters. Liquid cultures of strain A10^T contained cells with long (up to 10–15 µm) and thick (0.5–0.7 µm) stalks that were clearly visible using phase-contrast microscopy (Fig. 1d). According to our observations, the stalk formation by these bacteria was most pronounced in liquid media depleted in available growth substrates. A bundle of fine fibrils was present on the apical tip of the stalk (Supplementary Fig. S1b in IJSEM Online). Presumably, these fibrils served as a holdfast, as many cells in liquid cultures were joined together at the tips of their stalks (Fig. 1d). Large rosettes of cells connected to each other by clearly visible stalks have often been observed by microbiologists in diverse aquatic environments and have been classified as belonging to the genus *Planctomyces* (Starr & Schmidt, 1984; Staley *et al.*, 1992). However, none

Fig. 1. Phase-contrast micrographs of cells of strain A10^T grown for 3 weeks (a) and for 1.5 months (b) on agar medium. White arrows show amorphous holdfast substance excreted by the cells grown on agar medium. *h*, Holdfast substance; bars, 10 μ m. (c) Electron micrograph of negatively stained cells displaying crateriform pits scattered over the whole cell surface. Bar, 1 μ m. (d) Phase-contrast micrograph showing the formation of long stalks by cells growing in liquid medium. *S*, Stalk; bar, 10 μ m. (e) Scanning electron micrograph of cells of strain A10^T grown in liquid culture. Bar, 1 μ m. (f) Electron micrograph of a negatively stained preparation showing the multifibrillar ultrastructure of a stalk and regularly spaced vesicle-like inclusions [enlarged view is shown in (g)]. *S1*, Fibrils; *S2*, vesicle-like inclusions; bars, 0.2 μ m.

of these organisms with striking morphology were obtained in an axenic culture. The stalks of cultivated members of the genus *Planctomyces* are typically 0.05–0.1 μ m wide and cannot be discerned without the use of electron microscopy (Bauld & Staley, 1976; Hirsch & Müller, 1985; Schlesner, 1989). In addition to the unusual length and thickness, the stalks of strain A10^T displayed a unique ultrastructure. Primary examination of cells in liquid cultures using phase-contrast microscopy suggested the presence of regularly spaced, phase-dark inclusions or structures inside the stalks (Fig. 1d). This was further confirmed by using scanning electron microscopy, which demonstrated the beaded morphology of stalks in strain

A10^T (Fig. 1e). Finally, examination of negatively stained cells using electron microscopy showed that the stalks possessed remarkable structural complexity and were composed of two main components: (i) numerous longitudinally aligned thin fibrils and (ii) chains of vesicle-like bodies, 0.3 $\times$ 0.5 μ m in size (Fig. 1f, g). Based on the morphological observations, we hypothesize that this chaplet-like structure may represent a result of a budding-like process, in which vesicle-like bodies are formed and separated one after another inside a growing fibrillar sheath. However, the exact nature of these structures and the mechanism behind their formation remain unclear and require further investigation.

Thin-sectioned cells of strain A10^T (not shown) displayed a pattern of cell compartmentalization typical of *Gemmata*-like planctomycetes (Lindsay *et al.*, 2001; Fuerst, 2005; Ward *et al.*, 2006). No peptidoglycan layer was visible in the cell wall of the novel isolate. The cells were surrounded by an outer membrane; its thickness ranged from 7 to 9.5 nm in different cells.

Strain A10^T was an obligately aerobic chemoheterotroph. In contrast to other peat-inhabiting planctomycetes of the genera *Schlesneria* and *Singulisphaera* (Kulichevskaya *et al.*, 2007, 2008), strain A10^T was not capable of growth under microaerobic conditions.

The carbon substrates tested in our study and the results are given in the species description (see below) or in Table 1. Most sugars and *N*-acetylglucosamine were the preferred growth substrates. With the exception of pyruvate, organic acids were not utilized. Strain A10^T was capable of hydrolysing laminarin, pectin, chondroitin sulfate, aesculin, starch, gelatin, lichenan and xylan, but not casein, chitin or cellulose. The strain was catalase- and cytochrome oxidase-positive, but urease-negative. Dissimilatory nitrate reduction was negative. Ammonia, nitrate, *N*-acetylglucosamine, Bacto peptone, Bacto yeast extract, Casamino acids, alanine, aspartate, asparagine, glutamine, leucine, threonine and tryptophan were utilized as nitrogen sources. The following enzymic activities (API ZYM) were detected in strain A10^T: alkaline and acid

phosphatase, esterase, leucine arylamidase, cystine arylamidase, valine arylamidase, phosphohydrolase, *N*-acetyl- β -glucosaminidase and α -glucosidase. The following enzymic activities were not detected: β -galactosidase, lipase, trypsin, chymotrypsin, α -galactosidase, β -glucuronidase, α -fucosidase and α -mannosidase. All isolates were resistant to ampicillin, streptomycin, chloramphenicol, lincomycin, and novobiocin, but sensitive to kanamycin, neomycin and gentamicin.

Strain A10^T grew at pH 3.8–7.2, with an optimum at pH 5.5–6.0 (Table 1). The temperature range for growth was 10–30 °C, with an optimum at 20–25 °C (Table 1). Strain A10^T was highly sensitive to salt stress. Growth inhibition of 30–70 % was observed in the presence of NaCl in the medium at concentrations of 0.1–0.5 % (w/v), and NaCl at concentrations above 0.6 % (w/v) completely inhibited growth. *G. obscuriglobus* DSM 5831^T displayed similar sensitivity to salt stress, but complete inhibition of growth was observed in the presence of NaCl at concentrations above 1 % (w/v).

Similar to other described planctomycetes (Sittig & Schlesner, 1993; Ward *et al.*, 2006; Kulichevskaya *et al.*, 2007, 2008), strain A10^T contained menaquinone-6 (MK-6) as the predominant isoprenoid quinone. The major fatty acids of strain A10^T were C_{18:0}, C_{18:1} ω 5c and C_{16:1} ω 5c (Table 2). These are also the dominant components of the PLFA profile (Supplementary Table S1, available in IJSEM

Table 2. Fatty acid and neutral lipid compositions of strain A10^T and *G. obscuriglobus* DSM 5831^T

Percentages of total sum are reported. Minor components (making up <0.1 % of the total) were not included. Major fatty acids are shown in bold. A detailed list of PLFAs is given in Supplementary Table S1 (available in IJSEM Online).

Compound	Strain A10 ^T		<i>G. obscuriglobus</i> DSM 5831 ^T	
	Free	Residually bound*	Free	Residually bound
Fatty acid				
nC _{14:0}	0.8	1.5		
iC _{15:0}	1.8	2.3		
nC _{16:1} ω 5c	13.1	12.2	3.5	17.6
nC _{16:0}	5.4	5.4	1	2.7
nC _{18:1} ω 5c	45.1	21.2		2.0
nC _{18:0}	9.4	15.8	17.3	59.1
nC _{20:0}				1.7
ω OH-C _{24:0}				16.9
(ω -1)OH-C _{26:0}		1.0		
(ω -1)OH-C _{28:1}		9.0		
(ω -1)OH-C _{28:0}	1.1	8.4		
(ω -1)OH-C _{30:1}		18.7		
(ω -1)OH-C _{30:0}	0.6	3.0		
(ω -1)OH-C _{32:1}		1.5		
Neutral lipid				
nC _{31:9}	22.7		53.5	
Lanosterol			4.4	
Parkeol			20.6	

*Released after base hydrolysis of the residue after extraction of the cell material.

Online). The high content of $C_{18:1\omega5c}$ makes the PLFA profile of our isolate clearly distinct among known planctomycetes. Bound (i.e. released after base hydrolysis of cell material) saturated and mono-unsaturated C_{26} – C_{32} (ω -1) hydroxy fatty acids form a characteristic group of lipids of strain A10^T (Table 2). The neutral lipids of strain A10^T were dominated by a polyunsaturated C_{31} hydrocarbon (Table 2) that has been detected in many other planctomycetes (Kulichevskaya *et al.*, 2007, 2008).

Comparative sequence analysis of the 16S rRNA gene placed strain A10^T into a phylogenetic cluster defined by members of the genus *Gemmata* (Fig. 2). The closest phylogenetic relative (96 % sequence identity) was the taxonomically uncharacterized planctomycete, *Gemmata*-like strain JW10-3f1, which was isolated from a eutrophic lake (Wang *et al.*, 2002). The closest and the only taxonomically described organism in this phylogenetic cluster was a freshwater budding bacterium *G. obscuriglobus* (90 % sequence similarity). The DNA G + C content of strain A10^T was 62.5 mol%. The corresponding value reported for *G. obscuriglobus* was 64.4 mol% (Franzmann & Skerman, 1984).

Morphologically and phenotypically, strain A10^T was clearly distinct from *G. obscuriglobus* DSM 5831^T (Table 1). The cells of strain A10^T possessed stalks and were assembled in rosette-like clusters. Despite using a large variety of cultivation conditions, we failed to observe

stalks in cultures of *G. obscuriglobus* DSM 5831^T. In contrast to the latter bacterium, strain A10^T was capable of growth in acidic conditions. It possessed distinct fatty acid and neutral lipid profiles, and did not synthesize the rather unique sterols, parkeol and lanosterol produced by *G. obscuriglobus* DSM 5831^T (Pearson *et al.*, 2003). We therefore propose that strain A10^T should be classified as representing a novel genus and species, for which the name *Zavarzinella formosa* gen. nov., sp. nov. is proposed. The characteristics that differentiate the genus *Zavarzinella* from the genus *Gemmata* are summarized in Table 3.

Description of *Zavarzinella* gen. nov.

Zavarzinella (Za.var.zi.nel'la. N.L. fem. dim. n. *Zavarzinella*, named in honour of the famous Russian microbiologist George A. Zavarzin, for his outstanding contribution to increasing our knowledge of microbial diversity).

Ellipsoid cells occur singly or are assembled in large rosette-like clusters. Reproduce by budding. Adult cells are non-motile. Daughter cells are motile by means of one or two flagella. Produce unusual type of stalk-like structures. Cells attach to surfaces by means of amorphous holdfast material. Chemo-organotrophic aerobe. Moderately acidophilic and mesophilic. The major quinone is MK-6. Major fatty acids are $C_{18:0}$, $C_{18:1\omega5c}$ and $C_{16:1\omega5c}$. Member of the phylum *Planctomycetes*, order *Planctomycetales*, family *Planctomycetaceae*. The type species is *Zavarzinella formosa*.

Fig. 2. 16S rRNA gene-based neighbour-joining tree (Jukes–Cantor correction) showing the phylogenetic position of strain A10^T in relation to *G. obscuriglobus* and some other representatives of the phylum *Planctomycetes*. The tree was constructed based on 1216 nucleotide positions. Bootstrap values (1000 data resamplings) >50 % are shown. The 16S rRNA gene sequence of *Chlorobium limicola* (Y10642) (not shown) was used as an outgroup. Bar, 0.1 substitutions per nucleotide position. The same tree topology was obtained using maximum-likelihood and maximum-parsimony methods.

Table 3. Major characteristics that distinguish *Zavarzinella* gen. nov. from the genus *Gemmata*

Characteristic	<i>Zavarzinella</i>	<i>Gemmata</i>
Cell shape	Ellipsoidal	Spherical to ovoid
Rosette formation	+	—
Presence of stalks	+	—
Flagellation of swarm cells	Monotrichous	Polytrichous
Growth below pH 5.0	+	—
Growth above 30 °C	—	+
Major fatty acids	C _{18:1} ω5c, C _{16:1} ω5c, C _{18:0}	C _{18:0} , C _{16:1} ω5c
Sterol synthesis	—	+
DNA G + C content (mol%)	62.5	64.4

Description of *Zavarzinella formosa* sp. nov.

Zavarzinella formosa (for.mo'sa. L. fem. adj. *formosa* beautiful, beautifully formed, finely formed).

Exhibits the following properties in addition to those given in the genus description. Colonies are pink. Mature cells are 2.0–2.5 × 2.5–3.2 µm in size. Crateriform pits are scattered over the whole cell surface. Catalase- and cytochrome oxidase-positive, but urease-negative. Dissimilatory nitrate reduction is negative. Contains high amounts of bound saturated and mono-unsaturated C₂₆–C₃₂ (ω-1) hydroxy fatty acids. Carbon sources (0.05 %, w/v) include glucose, galactose, lactose, cellobiose, maltose, melezitose, raffinose, rhamnose, ribose, sucrose, trehalose, sorbose, xylose, *N*-acetylglucosamine, pyruvate and salicin. Does not utilize fructose, mannose, fucose, glycerol, gluconate, methanol, ethanol, galacturonate, acetate, benzoate, caproate, citrate, formate, formaldehyde, fumarate, glutarate, lactate, malate, succinate, propionate, mannitol, tartrate, alanine, arginine, asparagine, aspartate, cysteine, cystine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. Capable of hydrolysing laminarin, pectin, chondroitin sulfate, aesculin, starch, gelatin, lichenan and xylan. Does not hydrolyse casein, chitin or cellulose. Has the following enzymic activities: alkaline and acid phosphatase, esterase, leucine arylamidase, cystine arylamidase, valine arylamidase, phosphohydrolase, *N*-acetyl-β-glucosaminidase and α-glucosidase (API ZYM). Nitrogen sources (0.05 %, w/v) are nitrate, ammonia, *N*-acetylglucosamine, Bacto peptone, Bacto yeast extract, Casamino acids, alanine, aspartate, asparagine, glutamine, leucine, threonine and tryptophan. Growth factors are required. Resistant to ampicillin, streptomycin, chloramphenicol, lincomycin, and novobiocin, but sensitive to kanamycin, neomycin and gentamicin. Growth occurs at pH 3.8–7.2 (optimum, pH 5.5–6.0) and at temperatures between 10 and 30 °C (optimum, 20–25 °C). Highly sensitive to NaCl. Growth is inhibited at concentrations of NaCl above 0.6 % (w/v). The G + C content of the DNA of the type strain is 62.5 mol%.

Acidic wetlands are the main habitat. The type strain, A10^T (=DSM 19928^T=VKM B-2478^T), was isolated from the *Sphagnum* peat bog Bakchar, Tomsk region, West Siberia.

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