INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Kulichevskaya *et al.*, *Int. J. Syst. Evol. Microbiol.* 2020;70:1240–1249

DOI 10.1099/ijsem.0.003904

MICROBIOLOGY SOCIETY

OPEN MICROBIOLOGY

Limnoglobus roseus gen. nov., sp. nov., a novel freshwater planctomycete with a giant genome from the family *Gemmataceae*

Irina S. Kulichevskaya[1], Daniil G. Naumoff[1], Kirill K. Miroshnikov[1], Anastasia A. Ivanova[1], Dmitriy A. Philippov[2], Anna Hakobyan[3], W. Irene C. Rijpstra[4,5], Jaap S. Sinninghe Damsté[4,5], Werner Liesack[3] and Svetlana N. Dedysh[1,*]

Abstract

The family *Gemmataceae* accommodates aerobic, chemoorganotrophic planctomycetes, which inhabit various freshwater ecosystems, wetlands and soils. Here, we describe a novel member of this family, strain PX52^T, which was isolated from a boreal eutrophic lake in Northern Russia. This isolate formed pink-pigmented colonies and was represented by spherical cells that occurred singly, in pairs or aggregates and multiplied by budding. Daughter cells were highly motile. PX52^T was an obligate aerobic chemoorganotroph, which utilized various sugars and some heteropolysaccharides. Growth occurred at pH 5.0–7.5 (optimum pH 6.5) and at temperatures between 10 and 30 °C (optimum 20–25 °C). The major fatty acids were $C_{18:1}\omega7c$, $C_{18:0}$ and βOH-$C_{16:0}$; the major intact polar lipid was trimethylornithine, and the quinone was MK-6. The complete genome of PX52^T was 9.38 Mb in size and contained nearly 8000 potential protein-coding genes. Among those were genes encoding a wide repertoire of carbohydrate-active enzymes (CAZymes) including 33 glycoside hydrolases (GH) and 87 glycosyltransferases (GT) affiliated with 17 and 12 CAZy families, respectively. DNA G+C content was 65.6 mol%. PX52^T displayed only 86.0–89.8% 16S rRNA gene sequence similarity to taxonomically described *Gemmataceae* planctomycetes and differed from them by a number of phenotypic characteristics and by fatty acid composition. We, therefore, propose to classify it as representing a novel genus and species, *Limnoglobus roseus* gen. nov., sp. nov. The type strain is strain PX52^T (=KCTC 72397^T=VKM B-3275^T).

The family *Gemmataceae* belongs to the order *Planctomycetales* and accommodates strictly aerobic, chemoorganotrophic planctomycetes with spherical or ellipsoidal cells, which occur singly, in pairs, or are assembled in large rosette-like clusters and dendriform-like structures [1]. This family currently includes five described genera, i.e., *Gemmata* [2, 3], *Zavarzinella* [4], *Telmatocola* [5], *Fimbriiglobus* [1] and *Tuwongella* [6]. Daughter cells of species of the genera *Gemmata* and *Zavarzinella* are highly motile, while members of the genera *Telmatocola, Fimbriiglobus* and *Tuwongella* possess non-motile swarm cells. A unique morphological feature of members of the genera *Zavarzinella* and *Telmatocola* is formation of thick stalks, which are only observed in old cultures. All planctomycetes of this family are characterized by large genome sizes, which vary between 9.0 - 9.2 Mb in species of the genus *Gemmata* and 12.3 Mb in *Fimbriiglobus ruber* SP5^T. The latter is the largest among all currently determined planctomycete genomes [7].

Representatives of the family *Gemmataceae* are commonly found in various freshwater habitats [8, 9], boreal and subarctic wetlands [10, 11], and soils [12, 13]. The ecological functions of these bacteria have not yet been fully understood. The spectrum of preferred growth substrates of *Gemmataceae*-affiliated planctomycetes includes various sugars and some heteropolysaccharides, such as xylan, laminarin, lichenin and starch [11]. The ability to degrade cellulose, however, has only been demonstrated for *Telmatocola sphagniphila* [5], while the ability to utilize chitin has only been reported for

Author affiliations: [1]Winogradsky Institute of Microbiology, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow 119071, Russia; [2]Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok 152742, Russia; [3]Max-Planck-Institut für terrestrische Mikrobiologie, D-35043 Marburg, Germany; [4] NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, The Netherlands; [5]Utrecht University, Faculty of Geosciences, Department of Earth Sciences, Geochemistry, Utrecht, The Netherlands.

***Correspondence:** Svetlana N. Dedysh, dedysh@mail.ru

Keywords: *Planctomycetes*; family *Gemmataceae*; *Limnoglobus roseus* gen. nov., sp. nov.; large genome.

Abbreviations: ANI, average nucleotide identity; CAZymes, carbohydrate-active enzymes; GH, glycoside hydrolases; GT, glycosyltransferases.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Limnoglobus roseus* PX52^T is MN209796. The annotated genome sequence of strain PX52T has been deposited in NCBI GenBank under the accession number CP042425.

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Fimbriiglobus ruber [7]. In this study, we describe a freshwater planctomycete, strain PX52^T, which was isolated from a boreal lake and affiliated with the family *Gemmataceae*.

Strain PX52^T was isolated in August 2016 from a water sample collected from the upper oxic layer (0–10 cm) of the boreal eutrophic lake Morotskoye (Vologda region, European North Russia, 58° 43′ 28.5″ N, 37° 39′ 07″ E). The lake area is 6.24 km^2 and its maximum depth is 2.1 m. Site-specific parameters are as follows (range given in parenthesis): water conductivity (30–50 µS cm^{-1}), dissolved organic carbon (25.0–37.5 mg l^{-1}), total nitrogen (2.0–3.7 mg l^{-1}), and total phosphorus (45–77 µg l^{-1}). The pH is 7.5–7.8.

The enrichment strategy applied the use of agar medium, which was prepared with original lake water and contained 0.05 g l^{-1} carbenicillin (sodium salt). An aliquot of lake water (10 ml) was spread onto this medium, the plates were placed in bags to prevent drying, and incubated at room temperature. Microbial cell masses that developed on plates after four weeks of incubation were enriched with microorganisms having planctomycete-like morphology. Aliquots (20 µl) of these enrichment cultures were spread plated onto the medium MPYVG (modification of medium 621 DSMZ) solidified with 10 g phytagel (Sigma–Aldrich) and containing 0.1 g peptone (Fluka), 0.25 g yeast extract, 0.1 g NH_4NO_3 and 20 ml Hutner's basal salts [14] per litre distilled water. After sterilization, the medium was complemented with 5 ml of 5% (w/v) glucose solution, 1 ml Staley's vitamin solution [14], 0.05 g carbenicillin (sodium salt), pH 6.5. The plates were then incubated at 22 °C for four weeks. Colonies that developed on plates were screened microscopically for the presence of budding cells with planctomycete-like morphology. Selected cell material was re-streaked onto the same medium MPYVG, supplemented with 0.05% (w/v) glucose. Once obtained in pure culture, the isolate was maintained on MPYVC medium and was sub-cultured at 2 month intervals.

Morphological observations and cell size measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). For negative staining, cells were dried onto grids and treated with 1% (w/v) phosphotungstic acid. The specimen samples were examined with a JEM-1011 (JEOL) transmission electron microscope.

Physiological tests were performed in liquid MPYVG medium. Growth of PX52^T was monitored by nephelometry at 600 nm in an Eppendorf BioPhotometer for 2–3 weeks across gradients of the following parameters: temperature (4–37 °C), pH (3.8–8.0), and NaCl [0–3.0% (w/v)]. The incubations across the temperature range were made under static conditions in triplicate; OD_{600} was determined after two weeks of incubation. Variations in the pH were achieved by mixing 0.1 M solutions of H_2SO_4 and KOH. Carbon source utilization was determined using mineral medium M1 [1], supplemented with 0.005% yeast extract and the individual carbon sources given in the species description in a concentration of 0.05% (w/v). Cultivation was done in 120 ml flasks containing 10 ml medium. Cultures were incubated at 22 °C for two to three weeks on a shaker at 100 r.p.m. All experiments were performed in triplicate. Oxidative and fermentative utilization of carbohydrates was determined as described for the Hugh–Leifson test [15]. Nitrogen sources were tested using liquid medium M1, in which $(NH_4)_2SO_4$ was replaced with one of the following compounds at a concentration of 0.01% (w/v): KNO_3, KNO_2, urea, *N*-acetylglucosamine, Bacto peptone, Bacto yeast extract or one of the amino acids listed in Table 1. Analyzes of enzymatic profiles, oxidase test, gelatin and urease hydrolysis were made with API ZYM and API 20NE kits (bioMérieux). Catalase test was carried out using the standard method [15]. Cultures were tested for growth under anoxic conditions in anaerobic jars using AnaeroGen anaerobic system envelopes (Oxoid), which absorb atmospheric oxygen with the simultaneous generation of CO_2 (up to 9–13%, v/v). Susceptibility to antibiotics was determined on solid MPYVG medium using discs (Oxoid) containing the following antibiotics: ampicillin (10 µg), gentamycin (10 µg), kanamycin (30 µg), neomycin (10 µg), novobiocin (30 µg), streptomycin (10 µg), chloramphenicol (30 µg), rifampicin (10 µg), tetracycline (10 µg) and lincomycin (10 µg). Growth of PX52^T and occurrence of growth inhibition zones around these discs were assessed after 4 weeks of incubation at 22 °C. Only the inhibition zones exceeding 2 mm were taken into account.

For lipid analysis, cells of PX52^T were grown in liquid MPVYG medium and harvested in the late exponential growth phase. Fatty acids were analyzed after acid hydrolysis of whole cells following procedure described elsewhere [16]. The main intact polar lipids (IPLs) in PX52^T were analyzed using procedures reported previously [17].

Isoprenoid quinones were extracted according to the protocol of Collins [18] and analyzed using a tandem-type mass spectrometer LCQ ADVANTAGE MAX and a Finnigan Mat 8430 ionization mass spectrometer. The mass spectra were first recorded in MS9 mode and then analyzed using MS/MS mode.

PCR-mediated amplification of the 16S rRNA gene from positions 28 to 1491 (numbering according to the International Union of Biochemistry nomenclature for *Escherichia coli* 16S rRNA) was performed using primers 9 f and 1492 r and reaction conditions described by Weisburg *et al.* [19]. The 16S rRNA gene amplicons were purified using QIAquick spin columns (Qiagen) and sequenced on an ABI Prism 377 DNA sequencer using BigDye terminator chemistry, as specified by the manufacturer (PE Applied Biosystems). Phylogenetic analysis was carried out using the ARB program package [20]. 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 [21].

For genome analysis, cells of PX52^T were grown in liquid MPYVG medium with 0.5% glucose and harvested after two weeks of incubation at 22 °C. Genome sequencing of PX52^T was performed at the Max Planck Genome Centre Cologne (MP-GCC; Cologne, Germany), using the PacBio RSII platform (Pacific Biosciences). *De novo* assembly was done using the hierarchical genome-assembly process (HGAP2) via the

Table 1. Major characteristics that distinguish strain PX52^T from described *Gemmataceae* planctomycetes. Strains: 1, PX52^T; 2, *Fimbriiglobus ruber* SP5^T [1]; 3, *Telmatocola sphagniphila* SP2^T [5]; 4, *Zavarzinella formosa* A10^T [4]; 5, *Gemmata obscuriglobus* DSM 5831^T [2, 4]; 6, *Tuwongella immobilis* MBLW1 ^{T} [6]. +, Positive; –, negative; w weakly positive; ND, not determined; R, resistant; S, susceptible

Characteristic	1	2	3	4	5	6
Cell shape	Spherical	Spherical	Spherical	Ellipsoidal	Spherical to ovoid	Spherical
Cell size (μm)	1.5–2.3	1.6–2.8×1.4-2.8	1.2–2.0	2.5–3.2×2.0–2.5	1.4–3.0×1.4–3.0	2.2–3.1
Motile swarm cells	+	–	–	+	+	–
Flagellation	Multitrichous	–	–	Monotrichous	Multitrichous	–
Fimbriae formation	+	+	–	–	ND	+
Rosette formation	+	–	+	+	–	–
Stalk formation	+	–	+	+	–	–
Colony colour	Pink	Dark pink to red	Pink	Pink	Rose	Pink
Salinity tolerance	< 0.5%	< 0.1%	< 0.1%	< 0.6%	< 0.6%	< 0.4%
pH growth range	5.0–7.5	4.0–6.8	4.0–7.0	3.8–7.2	7.8–8.8	6.0–10.5
pH optimum	6.5	5.5–6.0	5.0–5.5	5.5–6.0	ND	7.5–8.0
Temperature range, °C	10–30	10–30	10–30	10–30	16–35	20–40
Temperature optimum, °C	20–25	20–25	20–26	20–25	ND	32–36
Oxidase test	–	–	–	+	–	+
Catalase test	+	+	+	+	+	–
Urease test	–	–	–	–	–	+
Carbon source utilization:						
Sucrose	+	+	+	+	+	–
Xylose	+	+	+	+	+	–
Lactose	+	+	–	+	+	
Mannose	+	+	+	–	+	ND
Sorbose	–	–	–	+	–	ND
Raffinose	+	+	+	+	–	ND
N-acetylglucosamine	+	+	+ (<0.01%)	+	+	+
Pyruvate	–	+	–	+	–	ND
Chondroitin sulfate	–	–	–	+	+	ND
Xylan	w	+	+	+	ND	ND
Pectin	w	–	–	+	+	ND
Carboxymethylcellulose	–	–	+	–	–	ND
Chitin	–	+	–	–	ND	ND
Enzymatic activities:						
α-Galactosidase	–	–	–	–	+	ND
Cystine arylamidase	–	–	+	+	–	ND
Valine arylamidase	+	+	+	+	–	ND
N-acetyl-β-glucosaminidase	+	+	w	+	+	ND
α-Mannosidase	–	+	–	–	+	ND

Continued

Table 1. Continued

Characteristic	1	2	3	4	5	6
Antibiotic susceptibility						
Gentamicin	R	S	S	S	S	R
Neomycin	R	S	R	S	S	S
Kanamycin	R	S	S	S	S	S
DNA G+C content, mol%	65.6*	64.2*	58.5	59.1*	64.0–67.2*	57*

*Data are based on genome sequence analysis [3, 6, 7, 40].

SMRT Portal v.2.0 offered by Pacific Biosciences. Initial automated genome annotation was carried out using RAST v. 2.0 (Rapid Annotation using Subsystem Technology) with default parameters [22–24]. Subsequent inspection was done using the PROKKA package [25] including all dependencies such as PRODIGAL v 2.6.2 [26], HMMER server [27], RNAMMER [28], BLAST+ [29] and ARAGORN [30]. Annotation with PROKKA was performed against both the UNIPROT database [31] and a manually constructed database that includes all available annotated genome sequences of *Isosphaeraceae*- and *Gemmataceae*-affiliated planctomycetes. The analysis of PX52^T genome sequence using the Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed applying the GhostKOALA tool [32]. The DNA–DNA hybridization values between the genome of PX52^T and the genomes of phylogenetically related planctomycetes were estimated using formula 2 of the Genome-to-Genome-Distance-Calculator [33, 34]. The average nucleotide identity (ANI) values were determined using ANI calculator (http://enve-omics.ce.gatech.edu/ani/). The phylogenomic tree was reconstructed based on the comparative sequence analysis of 120 ubiquitous single-copy proteins using the Genome Taxonomy Database toolkit (GTDB-Tk) [35], release 04-RS89 (https://github.com/Ecogenomics/GTDB-Tk) with further decorating in MEGA [36] applying the maximum-likelihood method. The annotated genome sequence of PX52^T has been deposited in the GenBank database under BioProject number PRJNA556575, accession number CP042425.

On phytagel-solidified MPYVG medium, PX52^T formed small (1–2 mm in diameter after three weeks of incubation), circular, pink-pigmented colonies with entire edges and smooth surfaces. These colonies were composed of non-motile, spherical cells, which varied in size from 1.5 to 2.3 µm and reproduced by budding (Fig. 1a). The buds separated from the mother cells were highly motile. Old (4–5 weeks) cultures contained large aggregates or rosettes of cells, which were connected to each other by short stalk-like structures (Fig. 1b). Examination of negatively stained cells of PX52^T using electron microscopy showed the presence of flagella, numerous fimbriae and crateriform pits, which were uniformly scattered over the cell surface (Fig. 1c). Liquid cultures displayed pink turbidity.

PX52^T was capable of growth at pH values between 5.0 and 7.5 (with an optimum at pH 6.5), and at temperatures between 10 and 30 °C (with an optimum at 20–25 °C). NaCl inhibited growth at concentrations above 0.5% (w/v). PX52^T was an obligately aerobic chemoorganotroph. It was not capable of growth under anoxic conditions. The preferred growth substrates were various sugars, including *N*-acetylglucosamine, and some polymeric compounds, such as aesculin, arabinogalactan, dextrin, laminarin, locust bean gum, gelatin, lichenin, pullulan, starch and xanthan (see Table 1 and the species description). Weak growth was also observed on xylan and pectin. Organic acids and alcohols were not utilized. Cellulose, casein, chondroitin sulfate, chitosan and chitin were not hydrolyzed. Yeast extract (100 mg l^{-1}) was required for growth. PX52^T was resistant to ampicillin, chloramphenicol, gentamicin, kanamycin, neomycin and streptomycin, but sensitive to lincomycin, novobiocin, rifampicin and tetracycline.

The major fatty acids (FA) detected in PX52^T were $C_{18:1}\omega7c$ and $C_{18:0}$ (Table 2). Notably, the FA $C_{18:1}\omega7c$, which is by far the most abundant FA in PX52^T, was absent from *Zavarzinella formosa* A10^T, *Telmatocola sphagniphila* SP2^T [1], and *Tuwongella immobilis* MBLW1^T [6], and was present only in minor amounts in *Fimbriiglobus ruber* SP5^T (0.4%) and *Gemmata obscuriglobus* UQM2246^T (1.6%) [6]. The fatty acid βOH-$C_{16:0}$ comprised 11.6% of total FAs in PX52^T and was also present in the acid hydrolysates of *Fimbriiglobus ruber* SP5^T (8.2%) and *Telmatocola sphagniphila* SP2^T (4.9%) (Table 2). The βOH-fatty acids are derived from the different intact polar ornithine lipids and differ in composition. The presence of polyunsaturated hydrocarbon $C_{31:9}$ (Table 2) is characteristic for all members of this family [1].

Trimethylornithine was the major component of the IPL profile of PX52^T, although minor amounts of phosphatidylglycerol, dimethylphosphatidylethanolamine, monomethylornithine and dimethylornithine, as well as phosphatidylcholine were also detected. This IPL composition was most similar to that reported for *Fimbriiglobus ruber* SP5^T [1].

Similar to other members of the order *Planctomycetales* [37], PX52^T contained menaquinone-6 (MK-6) as the only isoprenoid quinone.

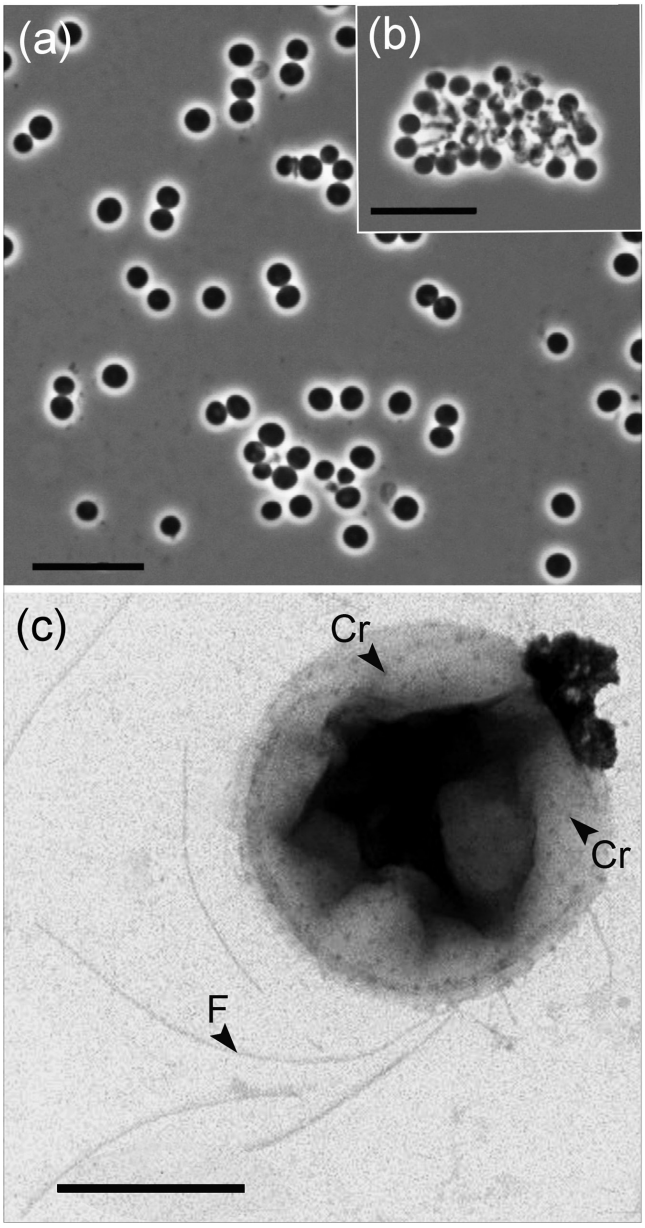

Fig. 1. (a) Phase-contrast image of cells of strain PX52^T grown for 14 days on solid medium MPVGY; (b) Stalk-like structures observed in 1-month-old culture of strain PX52^T; bar, 10 µm; (c) Electron micrograph of a negatively stained cell displaying crateriform pits (Cr) scattered all over cell surface and several flagella (F); bar, 1 µm.

Table 2. Fatty acids and hydrocarbons (percentages; normalized on their sum) present in the acid hydrolysate of cell material of PX52^T (1) in comparison to those in *Fimbriiglobus ruber* SP5^T (2) and *Telmatocola sphagniphila* SP2^T (3). The data presented for (2) and (3) are unpublished results, which were previously obtained for these planctomycetes using the same experimental procedure. Only fatty acids comprising ≥0.4% of the total are shown. Major components (>5%) are given in bold type

Lipids	1	2	3
Fatty acids			
$C_{16:1}\omega9$		8.2	
$C_{16:1}\omega5$			**28.5**
$C_{16:0}$	1.4	**16.7**	7.3
$isoC_{17:0}$			1.2
$anteisoC_{17:0}$			0.6
$C_{17:1}\omega6$	0.4		
$C_{17:0}$	1.1		0.7
$C_{18:1}\omega9$		2.0	
$C_{18:1}\omega7c$	**53.9**	0.4	
$C_{18:1}\omega7t$	0.9		
$C_{18:1}\omega5$	0.5		**25.7**
$C_{18:0}$	**16.1**	1.4	8.4
$C_{19:0}$	1.0		
$C_{20:1}\omega9$		**52.6**	
$C_{20:0}$		4.2	
Hydroxy-fatty acids			
$\beta OH\text{-}C_{16:0}$*	**11.6**	8.2†	4.9
$iso\text{-}\beta OH\text{-}C_{17:0}$*			**7.8**
$anteiso\text{-}\beta OH\text{-}C_{17:0}$*			2.6
$\beta OH\text{-}C_{17:0}$*			0.7
$\beta OH\text{-}C_{18:1}$*			1.1
$\beta OH\text{-}C_{18:0}$*	6.2	0.4	3.1
$(\omega-1)OH\text{-}C_{28:1}\omega8$	4.5		
$(\omega-1)OH\text{-}C_{28:0}$		0.3	
$(\omega-1)OH\text{-}C_{30:1}$		2.3	4.1
$(\omega-1)OH\text{-}C_{32:1}$			0.5
Hydrocarbons			
$C_{31:9}$	2.4	3.3	2.8

*Partly present as lyso-monomethylornithine.
†Partly present as lyso-ornithine lipids.

The genomic assembly obtained for PX52^T represented a single contig of 9378099 bp (read coverage of 495×), with an average DNA G+C content of 65.6 mol%. A total of 7728 potential protein-coding genes, three rRNA operons and 88 tRNA genes were identified. Two of the three 16S rRNA gene copies in PX52^T were identical (and also matched to the 16S rRNA gene sequence determined by the Sanger sequencing of PCR-amplified gene fragment, GenBank accession number MN209796), but displayed four mismatches to the third copy. The same phenomenon has been reported previously for several members of the family *Isosphaeraceae* [38]. The phylogenetic position of PX52^T based on the results of comparative 16S rRNA gene sequence analysis is shown in Fig. 2. PX52^T displayed 89.8% 16S rRNA gene sequence

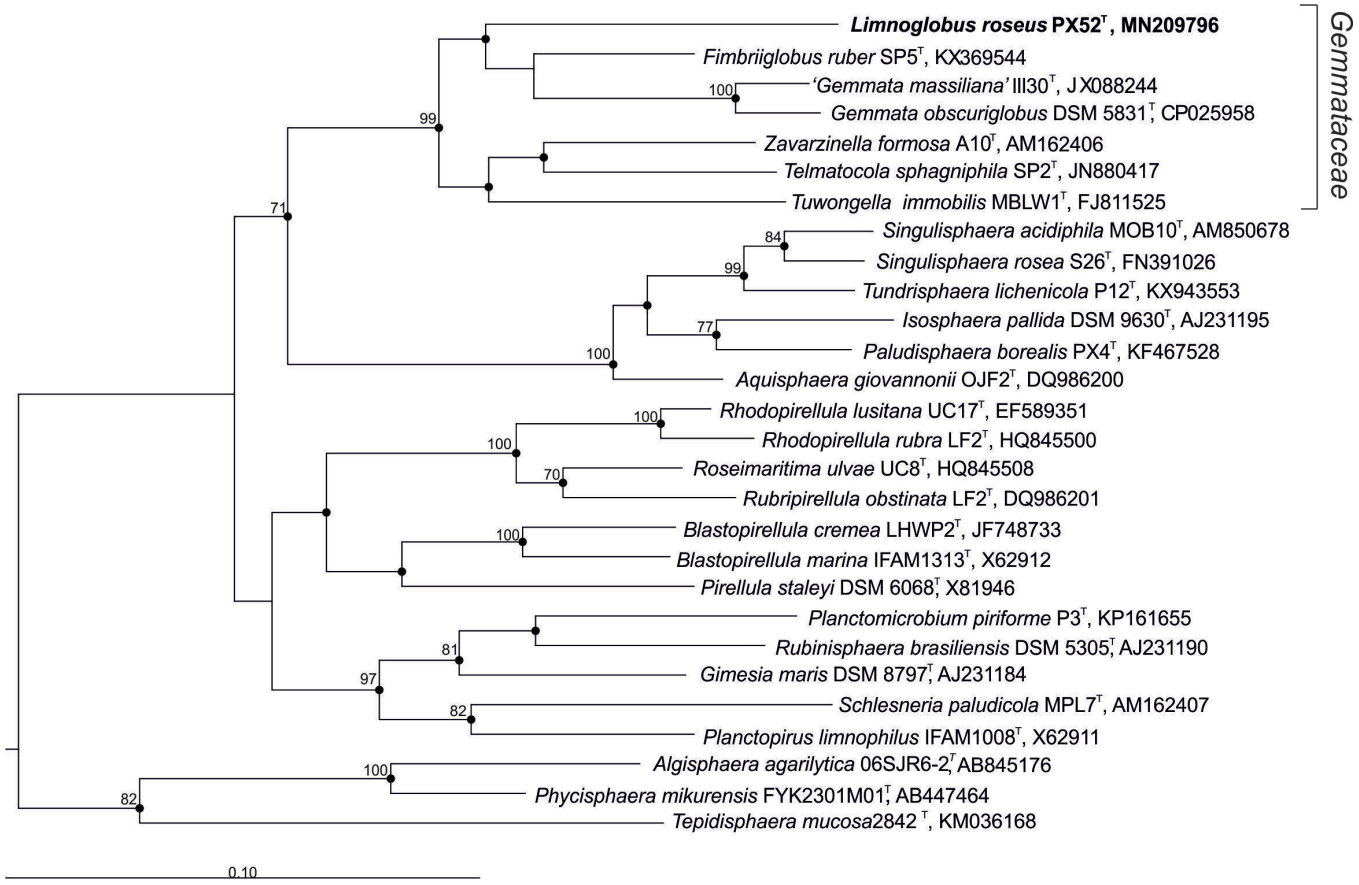

Fig. 2. 16S rRNA gene-based neighbour-joining tree (Jukes–Cantor correction) showing the phylogenetic position of *Limnoglobus roseus* PX52^T.The significance levels of interior branch points obtained in neighbor-joining analysis were determined by bootstrap analysis (1000 data re-samplings). Bootstrap values of over 70% are shown. Filled circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The root (not shown) is composed of five 16S rRNA gene sequences from anammox planctomycetes (AF375994, AF375995, AY254883, AY257181, and AY254882). Bar, 0.1 substitutions per nucleotide position.

similarity to *Fimbriiglobus ruber* SP5^T, 87.6% to *Gemmata obscuriglobus* UQM2246^T, 86.6% to *Tuwongella immobilis* MBLW1^T and *Zavarzinella formosa* A10^T and 86.0% to *Telmatocola sphagniphila* SP2^T. The phylogenomic tree, which was reconstructed based on the comparative sequence analysis of 120 ubiquitous single-copy proteins (Fig. 3) confirmed the distinct phylogenetic position of PX52^T.

Following the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes [39], we determined both the overall genome similarity and the average nucleotide identity (ANI) between PX52^T and closely related planctomycetes. PX52^T shared 19.4±2.3% overall genome similarity with *Gemmata obscuriglobus* UQM2246^T and 19.3±2.3% genome similarity with *Fimbriiglobus ruber* SP5^T. These values are in the range generally calculated for members of different genera [40]. Accordingly, the ANI values shared between the genome of PX52^T and the genomes of *Gemmata obscuriglobus* UQM2246^T and *Fimbriiglobus ruber* SP5^T were also low, i.e. 76% in both cases.

KEGG-based annotation of the PX52^T genome sequence identified 1983 proteins that could be classified into 18 major functional categories. The genes encoding metabolic pathways common for chemoorganotrophic bacteria, such as glycolysis, the citrate cycle, the pentose-phosphate pathway and oxidative phosphorylation were present in the genome of PX52^T. This planctomycete has the genomic potential for synthesis of all amino acids. The number of ABC transporters based on PROKKA predictions is 31. Also, three fructose-type sugar-specific subunits of phosphotransferase system could be found in PX52^T. All genes essential for chemotaxis were identified in the genome of PX52^T. These include *cheA, cheB, cheR, cheC, cheY* and *cheW*. High representation of genes responsible for flagellar assembly (23 genes) agrees well with the fact that PX52^T produces motile swarmer cells. A wide array of genes (approximately 80 genes) coding for type IV pilus-based twitching motility was also revealed in the genome of PX52^T. This included pilins (Flp), assembly ATPase (PilB/TadA), inner membrane core proteins (PilC/

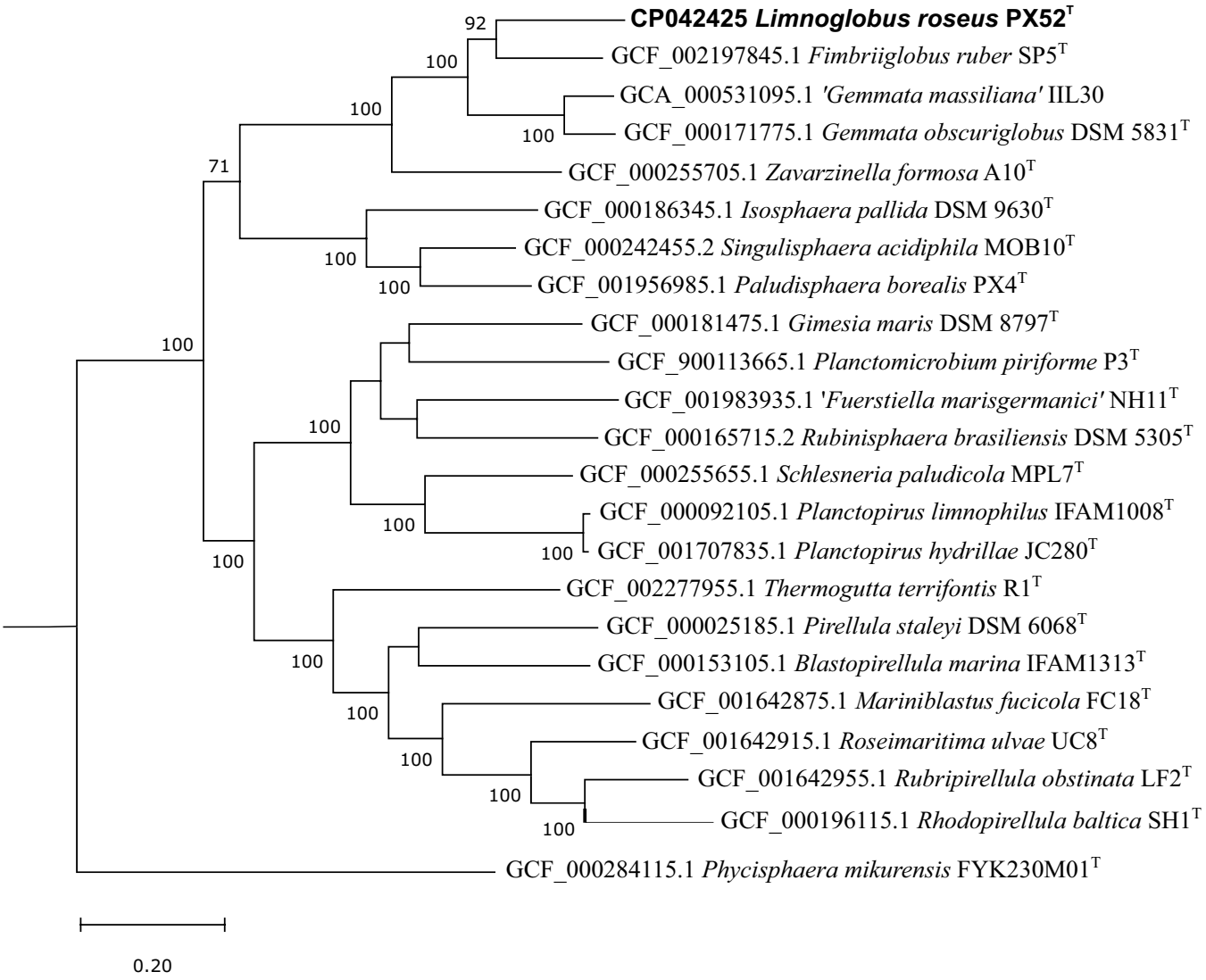

Fig. 3. Phylogenomic tree showing the phylogenetic position of *Limnoglobus roseus* PX52^T based on the comparative sequence analysis of 120 ubiquitous single-copy proteins. The tree was reconstructed using the Genome Taxonomy Database toolkit [35], Release 04-RS89. The significance levels of interior branch points obtained in maximum-likelihood analysis were determined by bootstrap analysis (100 data re-samplings). Bootstrap values of over 70% are shown. The root (not shown) was composed of 14 genomes of members of the Candidate division *Poribacteria*. Bar, 0.2 substitutions per amino acid position.

TadB and TadC), prepilin peptidase (PilD/TadV), outer membrane secretin (PilQ/RcpA), retraction ATPase (PilT), inner membrane proteins (PilM, PilN and PilO) and regulatory proteins. The cytoskeletal protein MreB, whose gene has a patchy presence among the *Gemmataceae* planctomycetes, i.e., it is absent in *Gemmata obscuriglobus* UQM2246^T and *Zavarzinella formosa* A10^T but is present in *Fimbriiglobus ruber* SP5^T, is also found in PX52^T. Several, but not all, genes involved in peptidoglycan biosynthesis, including *murB* and *murG*, were detected.

Homology analysis of all proteins potentially encoded in the genome of PX52^T was performed in order to reveal the complete set of carbohydrate-active enzymes. As a result, 33 glycoside hydrolases, 87 glycosyltransferases, 4 polysaccharide lyases and 15 carbohydrate esterases belonging to 17, 12, 3 and 7 CAZy families, respectively, were detected (Table 3). The CAZyme repertoire in PX52^T was more diverse than that in *Gemmata obscuriglobus* DSM 5831^T, the only taxonomically described member of the family *Gemmataceae* currently represented in the CAZy database. The range of expressed hydrolytic capabilities of PX52^T (Table 1) was in good agreement with the genome-encoded hydrolytic potential of this bacterium (Table 3). Two β-fructofuranosidases from the GH32 family (PX52LOC_04107 and PX52LOC_05985) are responsible for utilization of sucrose, raffinose and probably melezitose.

Table 3. Families of carbohydrate-active enzymes encoded in the genome of PX52^T in comparison with those in *Gemmata obscuriglobus* DSM 5831^T

Family	PX52^T	DSM 5831^T	Family	PX52^T	DSM 5831^T
GH1	0	1	GT9	2	2
GH2	3	2	GT19	1	1
GH5	1	0	GT20	1	2
GH10	1	1	GT30	1	1
GH13	6	8	GT32	0	1
GH15	1	2	GT35	2	1
GH16	1	1	GT41	1	2
GH18	1	0	GT83	5	4
GH24	0	1	GT84	1	1
GH31	1	0	PL1	2	0
GH32	2	1	PL9	0	1
GH33	4	3	PL10	1	0
GH37	0	1	PL11	1	0
GH39	2	0	CE1	5	2
GH43	3	0	CE6	1	0
GH77	1	1	CE9	2	2
GH94	1	1	CE11	1	1
GH127	2	0	CE12	2	0
GH133	1	1	CE13	0	0
GH140	2	1	CE14	2	1
GT1	2	0	CE15	2	1
GT2	44**	30	TIGR02604*	20	–
GT4	29**	44	DUF1080*	17	–
GT5	1	1	PF01408*	33	–

Data for *Gemmata obscuriglobus* DSM 5831^T are given according to the CAZy database [41].

*TIGR02604, DUF1080 and PF01408 are families of putative glycoside hydrolases, which are not yet represented in the CAZy database. TIGR02604 family contains putative β-propeller-type glycoside hydrolases of unknown function; for one protein (GenPept, AGW45552.1), however, the β-galactosidase activity has been demonstrated [38, 42, 43]. The DUF1080 family contains putative β-jelly-roll-type glycoside hydrolases of unknown function [38]; endoxanthanase activity was recently suggested for some of these enzymes based on the results of a combined application of genomic and transcriptomic approaches [44]. The PF01408 family contains putative Rossmann-fold-type glycoside hydrolases of unknown function and belongs to the CL0063 clan from the PFAM database [38]. **Three glycosyltransferases from PX52^T (PX52LOC_01329, PX52LOC_04115 and PX52LOC_05866) have two catalytical domains belonging to GT2 and GT4 families. They are included in statistics for both families.

Lactose is hydrolyzed by putative β-galactosidases from GH2 and/or TIGR02604 families. Weak growth on xylan and pectin can be explained by existence of two β-xylosidases (PX52LOC_02955 and PX52LOC_08045) from GH39 family and three pectate lyases (PX52LOC_02870, PX52LOC_04966 and PX52LOC_04964), respectively. Utilization of cellobiose, aesculin and salicin can be performed by putative β-glucosidases from GH2 and/or GH5 families. Arabinogalactan is hydrolyzed by α-L-arabinofuranosidase (PX52LOC_06319, GH43), two β-L-arabinofuranosidases (PX52LOC_01987 and PX52LOC_04962, GH127) and multiple β-galactosidases (GH2 and TIGR02604). Enzymes from the GH13, GH15 and GH133 families can be responsible for trehalose (the first two families) and dextrin (all three) utilization. Laminarin and lichenin are most likely hydrolyzed by a putative laminarinase (PX52LOC_05018, GH16) and a putative licheninase (GH5 or GH16), respectively. Enzymes from the GH2, GH5 and GH31 families can be responsible for locust bean gum utilization. Pullulan and starch are hydrolyzed by some enzymes from GH13 family. Xanthan gum is hydrolyzed by multiple putative endoxanthanases (DUF1080), putative α-mannosidase (GH31), putative β-mannosidase (GH2 or GH5) and putative β-glucuronidase (GH2). Enzymes from the GH13 and GH31 families can be responsible for maltose cleavage. According to the results of growth tests, PX52^T cannot utilize cellulose, chitin, chitosan and chondroitin sulfate. These data are in good agreement with the results of genome analysis.

In summary, PX52^T was phylogenetically (Figs 2 and 3) and phenotypically (Table 1) distinct from other described members of the family *Gemmataceae,* i.e., members of the genera *Fimbriiglobus, Gemmata, Zavarzinella*, *Telmatocola* and *Tuwongella*. The presence of motile swarm cells distinguished PX52^T from members of the genera *Telmatocola* and *Tuwongella*. The ability to form stalk-like structures differentiated it from members of the genera *Fimbriiglobus, Gemmata* and *Tuwongella*. The possession of $C_{18:1}\omega7c$ and βOH-$C_{16:0}$ as the major fatty acids distinguished it from all previously described members of the family *Gemmataceae*. These differences indicate that PX52^T should be considered as representing a novel genus and species of planctomycetes, *Limnoglobus roseus* gen. nov., sp. nov.

DESCRIPTION OF *LIMNOGLOBUS* GEN. NOV.

Limnoglobus (Lim.no.glo′bus. Gr. fem. n. *limne*, lake; L. masc. n. *globus*, ball; N.L. masc. n. *Limnoglobus*, lake ball, referring to the isolation source of the type species).

Spherical cells that occur singly, in pairs, or are assembled in large aggregates during growth on solid or liquid media. Reproduce by budding. Daughter cells are motile by means of several flagella. Chemoorganotrophic aerobe. Mildly acidophilic and mesophilic. The major fatty acids are $C_{18:1}\omega7c$, $C_{18:0}$ and βOH-$C_{16:0}$. The major polar lipid is trimethylornithine. The major quinone is MK-6. Member of the order *Planctomycetales,* family *Gemmataceae*. The type species is *Limnoglobus roseus*.

DESCRIPTION OF *LIMNOGLOBUS ROSEUS* SP. NOV.

Limnoglobus roseus (ro′se.us. L. masc. adj. *roseus* rose colored, referring to the pigmentation of the bacterium).

Exhibits the following properties in addition to those given in the genus description. Colonies are pink. Mature spherical cells are non-motile and 1.6–2.3 µm in size. Catalase-positive and cytochrome oxidase and urease-negative. Dissimilatory nitrate reduction and glucose fermentation are negative. Carbon sources (0.05 % w/v) include glucose, galactose, cellobiose, lactose, maltose, melezitose, mannose, rhamnose, raffinose, ribose, sucrose, trehalose, xylose, pyruvate, salicin and *N*-acetylglucosamine. Capable of hydrolyzing aesculin, arabinogalactan, dextrin, laminarin, locust bean gum, gelatin, lichenin, pullulan, starch and xanthan. Cannot utilize arabinose, fructose, sorbose, 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, and valine. Cannot hydrolyze casein, chondroitin sulfate, chitosan, chitin, and cellulose. Shows the following enzyme activities: alkaline and acid phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, phosphohydrolase, *N*-acetyl-β-glucosaminidase, β-glucosidase, and β-galactosidase (API ZYM test). Utilizes nitrate, ammonia, Bacto Yeast Extract, glutamine, asparagine, proline, glycine and serine as nitrogen sources. Growth factors are required. Growth occurs at pH 5.0–7.5 (optimum, pH 6.5) and at temperatures between 10 and 30 °C (optimum, 20–25 °C). NaCl inhibits growth at concentrations above 0.5% (w/v).

The type strain is strain PX52^T (=KCTC 72397^T=VKM B-3275^T), which was isolated from a boreal mesotrophic lake (Vologda region, European North Russia). The DNA G+C content of the type strain is 65.6 mol%. The EMBL/GenBank accession number for the 16S rRNA gene sequence is MN209796.The EMBL/GenBank accession number for the genome is CP042425.

Funding information
This study was supported by the Russian Science Foundation (project No 16-14-10210). J S S D received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement number 694569 – MICROLIPIDS). J S S D also receives funding from the Soehngen Institute for Anaerobic Microbiology (SIAM) though a gravitation grant from the Dutch ministry for Education, Culture and Science (grant number 024.002.002).

Acknowledgements
The authors thank N.E. Suzina for electron microscopy analysis.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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