Kulichevskaya, I.S.; Ivanova, A.A.; Suzina, N.E.; Sinninghe Damsté, J.S.; Dedysh, S.N. (2022). *Anatilimnocola floriformis* sp. nov., a novel member of the family *Pirellulaceae* from a boreal lake, and emended description of the genus *Anatilimnocola*. *Antonie van Leeuwenhoek 115*: 1253-1264. DOI: 10.1007/s10482-022-01769-x

Published version: https://dx.doi.org/10.1007/s10482-022-01769-x

NIOZ Repository: http://imis.nioz.nl/imis.php?module=ref&refid=355204

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Anatilimnocola floriformis sp. nov., a novel member of the family *Pirellulaceae* from a boreal lake, and emended description of the genus *Anatilimnocola*

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Running title: *Anatilimnocola floriformis* sp. nov.

The annotated genome and 16S rRNA gene sequences of strain PX40^T have been deposited in NCBI GenBank under the accession numbers JAMLFW000000000 and OP020302, respectively.

ABSTRACT

Planctomycetes of the family *Pirellulaceae* are commonly addressed as budding aquatic bacteria with a complex lifestyle. Although this family is well represented by cultured and taxonomically characterized isolates, nearly all of them were obtained from brackish or marine habitats. The examples of described freshwater *Pirellulaceae* planctomycetes are limited to two species only, *Pirellula staley* and '*Anatilimnocola aggregata*'. In this study, we characterized a novel freshwater planctomycete of the genus '*Anatilimnocola*', strain PX40^T, which was isolated from a boreal eutrophic lake. Strain PX40^T was represented by budding, unpigmented, ellipsoidal to pear-shaped cells, which often occurred in characteristic flower-like rosettes. Cells were covered by bundles of fimbriae; crateriform-like structures were localized on a reproductive cell pole only. These planctomycetes were obligately aerobic, heterotrophic bacteria that utilized various sugars and some polysaccharides, and were highly sensitive to NaCl. Growth occurred in the pH range 5.0 - 7.5 (with an optimum at pH 6.5-7.0), and at temperatures between 15 and 30ºC (with an optimum at 22-25ºC). The major fatty acids of strain PX40^T were C18:1ω9c, C16:0, and 16:1ω7c; cells also contained a wide variety of hydroxy- and dihydroxy-fatty acids and a C31:9 alkene. The major intact polar lipids were diacylglyceryl-(N,N,N)-trimethylhomoserines. The 16S rRNA gene sequence of strain PX40^T displayed 96.6% similarity to that of '*Anatilimnocola aggregata*' ETA_A8^T. The genome of strain PX40^T was 8.93 Mb in size and contained one copy of rRNA operon, 76 tRNA genes and 7092 potential protein-coding genes. The DNA G+C content was 57.8%. The ANI value between strain PX40^T and '*Anatilimnocola aggregata*' ETA_A8^T was 78.3%, suggesting that these planctomycetes represent distinct species. We, therefore,

propose a novel species of the genus '*Anatilimnocola*', '*A. floriformis'* sp. nov., with strain PX40^T (=KCTC 92369^T = VKM B-3621^T= UQM 41463^T) as the type strain.

Keywords: freshwater planctomycetes; family *Pirellulaceae*; *Anatilimnocola*; cultivation studies; boreal lake.

INTRODUCTION

The *Planctomycetota* (formerly, *Planctomycetes*) is one of the morphologically distinct, widespread, and most puzzling phyla of the domain *Bacteria*. Characterized planctomycetes belong to the classes *Planctomycetia* and *Phycisphaerae*, which accommodate heterotrophic bacteria capable of utilizing a wide range of organic compounds and possessing diverse environmental adaptations. The third class-level group within the *Planctomycetota*, "*Candidatus* Brocadiia", is represented by the litho-autotrophic anammox planctomycetes. Members of the *Planctomycetia* divide by budding, while representatives of the *Phycisphaerae* and "*Candidatus* Brocadiia" multiply by binary fission. The class *Planctomycetia* (Ward and Dedysh 2022) comprises most characterized representatives, with the largest characterized diversity kept in the order *Pirellulales*, the family *Pirellulaceae*. This family accommodates Gram-stain negative, budding, chemoheterotrophic planctomycetes with ovoid, ellipsoidal, pear- or teardrop-shaped cells, which frequently form eye-catching rosette-like clusters (Dedysh et al. 2020). Cells of *Pirellulaceae* planctomycetes do not possess a stalk but may form a fibrillar holdfast. Members of this family are aerobic and facultatively anaerobic, neutrophilic bacteria that grow on various sugars and polysaccharides. These microorganisms are most abundant in marine and freshwater habitats and, therefore, are commonly addressed as aquatic bacteria (Schlesner 1994; Fuerst 1995; Brümmer et al. 2004; Bondoso et al. 2014, 2015, 2017;

71 Spring et al. 2018; Wiegand et al. 2020, 2021). However, *Pirellulaceae* planctomycetes also
72 occur in peatlands, soils, activated sludge and various other environments (Buckley et al. 2006;
73 Chouari et al. 2015; Dedysh and Ivanova 2019; Kallscheuer et al. 2020b; Rensink et al. 2020).
74 The type genus of this family is the genus *Pirellula* (Schlesner and Hirsch 1987). Other
75 described genera in the family *Pirellulaceae* with validly published names are *Aureliella* (Rast
76 et al. 2020), *Blastopirellula* (Schlesner et al. 2004), *Bremerella* (Rensink et al. 2020),
77 *Lignipirellula* (Peeters et al. 2020a), *Crateriforma* (Peeters et al. 2020b), *Mariniblastus* (Lage et
78 al. 2017), *Novipirellula* (Kallscheuer et al. 2020c), *Rhodopirellula* (Schlesner et al. 2004;
79 Kallscheuer et al. 2020d), *Roseimaritima* (Bondoso et al. 2015), *Rosistilla* (Waqqas et al.
80 2020), *Rubripirellula* (Bondoso et al. 2015) and *Stieleria* (Kallscheuer et al. 2020a). Two
81 additional described genera in this family with pending validation status are '*Anatilimnocola*'
82 (Kallscheuer et al., 2021) and '*Roseiconus'* (Kumar et al. 2021). The isolates representing most
83 of the above listed genera have been obtained from marine and brackish habitats such as natural
84 or artificial marine surfaces, macroalgae, shallow-sea hydrothermal vents and others. The only
85 currently described freshwater *Pirellulaceae* planctomycetes belong to the genera *Pirellula* and
86 '*Anatilimnocola*'. Each of these genera is represented by a single species. *Pirellula staley*, the
87 first cultured and described organism in the family, was isolated from the Lake Lansing,
88 Michigan, USA (Staley 1973; Schlesner and Hirsch 1984; Schlesner and Hirsch 1987).
89 '*Anatilimnocola aggregata*' ETA_A8^{T} was isolated from a municipal duck pond in
90 Wolfenbüttel, Germany, during a phytoplankton bloom (Kallscheuer et al. 2021). Notably,
91 these planctomycetes form a common phylogenetic lineage in the 16S rRNA gene-based tree of
92 *Pirellulaceae* planctomycetes and display significant phenotypic similarity being represented
93 by mesophilic, neutrophilic, obligately aerobic heterotrophic bacteria. The major morphological
94 differences noticed between the *Pirellula* and strain ETA_A8^{T} was the absence of a holdfast

and crateriform structures in the latter bacterium, which was also slightly larger in size (Kallscheuer et al. 2021). Neither the range of growth substrates, nor the chemotaxonomic characteristics were reported for '*Anatilimnocola aggregata*' ETA_A8^T. In this study, we isolated one additional representative of the genus '*Anatilimnocola*' from a freshwater ecosystem, i.e., a boreal eutrophic lake in Northern Russia, determined its genome sequence and described morphological, phenotypic and chemotaxonomic characteristics. Based on these results, we emend description of the genus '*Anatilimnocola*' and propose a novel species within this genus, *A. floriformis* sp. nov.

MATERIALS AND METHODS

Sampling site. Strain PX40^T was isolated from water 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) in August 2017. Its surface 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 and 3.7 mg L^{-1}), and total phosphorus (45-77 µg L^{-1}). The pH is 7.5-7.8.

Isolation and cultivation. The enrichment culture was obtained using an agar medium prepared with original lake water and containing 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 as recommended by Hirsch et al. (1977). Aliquots (20 µl) of the microbial cell masses that developed on these plates after 4 weeks of incubation were spread plated onto the MPYVG medium (modification of medium 621 DSMZ), solidified with 10 g phytagel (Sigma - Aldrich) and containing (per liter distilled water): 0.1 g peptone (Fluka), 0.25 g yeast extract, 0.1 g NH_4NO_3, 20 ml Hutner's basal salts (Staley et al. 1992). After sterilization, the medium was supplemented with 5 ml L^{-1} 5% glucose

119 solution, 1 ml L^{-1} Staley's vitamin solution (Staley et al. 1992), 0.05 g carbenicillin (sodium
120 salt) L^{-1}, and maintained at pH 7.5. The plates were subsequently incubated at 22°C for 4
121 weeks. Colonies that developed on plates were screened microscopically for the presence of
122 budding cells with a planctomycete-like morphology. The selected cell material was re-streaked
123 onto the same medium MPYVG, supplemented with 0.05% (w/v) glucose. This procedure
124 resulted in isolation of two strains, PX69^T and PX40^T. The former strain was described in an
125 earlier publication (Dedysh et al. 2020), while the characterization of the later isolate is
126 described here. Once obtained in pure culture, strain PX40^T was maintained on MPYVC
127 medium and was sub-cultured at 2 month intervals.

128 **Microscopic studies**. Morphological observations and cell size measurements were made with
129 a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss, Germany). For the electron
130 microscopy of negatively stained preparations, cells suspension was placed onto grids coated
131 with formvar film, dried and treated with 0.3% aqueous solution of uranyl acetate (pH 4.0). The
132 specimen samples were examined with JEM-1400 transmission electron microscope (JEOL,
133 Japan) at the UNIQEM Collection Core Facility, Research Center of Biotechnology of the
134 Russian Academy of Sciences, at an accelerating voltage of 80 kV.

135 **Physiological tests.** Physiological tests were performed in liquid MPYVG medium. Growth of
136 strain PX40^T was monitored by nephelometry at 600 nm in an Eppendorf BioPhotometer for 2-
137 3 weeks under a variety of conditions, including temperatures of 4-37ºC, pH 3.8-8.0 and NaCl
138 concentrations of 0-3.0 % (w/v). Incubations at various temperatures were made under static
139 conditions in triplicate; OD_{600} was determined after 2 weeks of incubation. Variations in the pH
140 were achieved by using MES (pH 4.0-6.5) and MOPS (pH 6.5–7.9) buffer systems. Carbon
141 source utilization was determined using mineral medium M1 (Kulichevskaya et al. 2017),

supplemented with 0.005% yeast extract and the individual carbon sources given in the species description in a concentration 0.05 % (w/v). Cultivation was done in 120 ml flasks containing 20 ml medium. Cultures were incubated at 22°C for 2-3 weeks on a shaker. All experiments were performed in triplicate.

The ability to grow under anoxic conditions was tested in tightly closed 120 ml serum flasks containing 80 ml of liquid medium MPYVG. Before autoclaving, these flasks were flushed for 5 min with a mixture of CO_2 and N_2 (7: 93). Growth was assessed by measuring OD_{600} after 3 weeks of incubation.

Oxidative and fermentative utilization of carbohydrates was determined as described for the Hugh-Leifson test (Gerhardt 1981). 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 by standard method (Gerhardt 1981).

Lipid analyses. For lipid analysis, cells of strain PX40^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 the procedure described elsewhere (Sinninghe Damsté et al. 2011). The main intact polar lipids (IPLs) in strain PX40^T were analyzed following procedures reported previously (Moore et al. 2013).

Genome sequencing, annotation and analysis. Genomic DNA was isolated from strain PX40^T using the phenol-chloroform assay with addition of hexadecyltrimethylammonium bromide (Wilson 2001). The sequencing library for Illumina sequencing was prepared using the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs, USA) following the manufacturer's instructions. The sequencing of this library was performed on the Illumina HiSeq2500 platform using HiSeq Rapid Run v2 sequencing reagents. Primer sequences were

removed from the Illumina reads using Cutadapt v.1.17 (Martin 2011) with the default settings, and low quality read ends were trimmed using Sickle v.1.33 (option q=30) (https://github.com/najoshi/sickle). For Nanopore sequencing the library was prepared using the 1D ligation sequencing kit (SQK-LSK108, Oxford Nanopore, UK). Sequencing of this library was carried out in an R9.4 flow cell (FLO-MIN106) using MinION device. Hybrid assembly of Illumina and Nanopore reads was performed using Unicycler v. 0.4.8 (Wick et al. 2017).

Gene search and annotation were performed using PROKKA (Seemann 2014) and GhostKoala (Kanehisa et al. 2016) packages. Search for the secondary metabolites was carried out with AntiSmash (Blin et al. 2019). The overall genome similarity (GGDC) between strain PX40^T and closely related planctomycetes was determined using an online distance calculator available at DSMZ webpage. The average nucleotide identity (ANI) values were determined using an online calculator available on the server http://enve-omics.ce.gatech.edu/ani/.

The annotated genome and 16S rRNA gene sequences of strain PX40^T have been deposited in NCBI GenBank under the accession numbers JAMLFW000000000 and OP020302, respectively.

Phylogenetic analysis. The 16S rRNA gene-based phylogenetic analysis was carried out using the MEGAX (Kumar et al. 2018). The phylogenetic tree was built applying the maximum-likelyhood statistical method. Visualization of the tree was performed on iTOL platform (Letunic and Bork 2019).

In addition, the GTDB-Tk v. 2.0.0 toolkit (Parks et al. 2018) was used to identify 120 single-copy, phylogenetically informative bacterial marker genes used in the Genome Taxonomy Database (GTDB) classification system in the genome of strain PX40^T. These were used to construct a multiple alignment of concatenated single-copy gene sequences, comprising

188 those from PX40^T and all related species from the GTDB. A selected part of the multiple
189 alignment built in GTDB-Tk was used to construct a phylogenetic tree in MEGAX (Kumar et
190 al. 2018) with default parameters. The significance levels of interior branch points obtained in
191 maximum- likelihood analysis were determined by bootstrap analysis (100 data resamplings).

192 **RESULTS AND DISCUSSION**

193 **Cell morphology and physiology.** On phytagel-solidified medium MPYVG, strain PX40^T
194 formed small (2-3 mm), circular, unpigmented colonies with an entire edge and a smooth
195 surface. These colonies were composed of bacteria with ellipsoidal to pear-shaped cells, 0.7 ±
196 0.2 μm wide and 1.9 ± 0.5 μm long, which multiplied by budding and occurred singly or in
197 flower-shaped rosettes (Fig. 1a, 1c). Daughter cells were highly motile by means of one polar
198 flagellum (Fig. 1b). Examination of negatively stained cells of strain PX40^T using electron
199 microscopy showed the presence of bundles of fimbriae and crateriform-like structures, which
200 were distributed over a reproductive cell pole only (Fig. 1c).

201 Strain PX40^T was capable of growth at pH values between 5.0 and 7.5 (with an
202 optimum at pH 6.5-7.0), and at temperatures between 15 and 30ºC (with an optimum at 22-
203 25ºC). NaCl inhibited growth at concentrations >0.3 % (w/v). Strain PX40^T grew on various
204 sugars, including N-acetylglucosamine, and some polysaccharides, such as aesculin,
205 arabinogalactan, dextrin, laminarin, locust bean gum, starch, xanthan gum, xylan and gelatin,
206 but did not assimilate amino acids or organic acids (see Table 2 and the species description).
207 Pullulan, chondroitin sulfate, cellulose, carboxymethyl cellulose, casein, chitin and chitosan
208 were not hydrolyzed. Anaerobic growth was not observed.

209 Strain PX40^T was found to be catalase- and cytochrome oxidase-positive but urease-
210 negative. Nitrate was reduced to nitrite. The test for the activity of arginine dihydrolase was
211 positive (API 20NE strip). In the API ZYM strip, alkaline phosphatase, esterase (C4), esterase

lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, α-glucosidase and α-galactosidase were detected. The following enzyme activities were not detected: lipase, trypsin, chymotrypsin, phosphohydrolase, N-acetyl-β-glucosaminidase, β-galactosidase, cystine arylamidase, β-glucuronidase, α-fucosidase and α-mannosidase.

Lipid composition. The major fatty acids of strain PX40^T were C18:1ω9c, C16:0, and 16:1ω7c (Table 1). Cells also contained a wide variety of hydroxy- and dihydroxy-fatty acids and a C31:9 polyunsaturated *n*-alkene, which is often present in planctomycetes (Kulichevskaya et al. 2016; Dedysh et al. 2020). The major intact polar lipids were diacylglyceryl-(N,N,N)-trimethylhomoserine (DGTS) lipids.

Genome characteristics. The genome of strain PX40^T was sequenced using a combination of Illumina and Nanopore technique. Combined assembly yielded 17 contigs with a total length of 8,932,541 bp. The DNA G+C content was 57.8%. One copy of rRNA operon and 76 tRNA genes were identified. Annotation of the genome sequence revealed 7,092 potential protein-coding genes of which 2,088 could be functionally assigned.

The examination of the strain PX40^T genome revealed a set of genes encoding a bacterial flagellar machinery and chemotaxis functions, consistently with observed morphological characteristics. 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 strain PX40^T. Also, 30 genes encoding proteins with significant similarity to sulfatases were found in the genome. High number of sulfatase-encoding genes is typical for the genomes of *Pirellulaceae* planctomycetes (Wegner et al., 2013). Thus, the genome of *Pirellula staley* ATCC27377^T contains 34 sulfatase-encoding genes, while the number of these genes in *Rhodopirellula*

genomes exceeds one hundred. This large diversity of sulfatases in the genomes of *Pirellulaceae* planctomycetes was interpreted as a response to the diversity of sulfated compounds in nature and especially in the marine environment (Wegner et al., 2013).

Genome mining of gene clusters that encode biosynthetic pathways for secondary metabolites in strain PX40^T revealed 6 clusters. Four clusters encode terpenes, one cluster encodes polyketide synthases (PKS) of type III and the last one codes for a non-ribosomal peptide synthetase (NRPS). The closest gene homologs to all terpene clusters from strain PX40^T belong to the planctomycete '*Anatilimnocola aggregata*' ETA_A8^T. The closest homologs for PKS type III cluster and NRPS belong to *Pirellula*-like planctomycete strain SH-Sr6A and *Mycobacterium shinjukuense* JCM 14233.

Phylogenetic placement of strain PX40^T. The 16S rRNA gene sequence of strain PX40^T was affiliated with the family *Pirellulaceae* and grouped with several environmental clone sequences obtained from a freshwater lake (GenBank accession number DQ787725), an alpine wetland (FJ801184), wastewater treatment systems (LR634621, LR642682, LR645593), and a rhizosphere soil (FJ444676) (Fig. 2). Among the taxonomically characterized planctomycetes, the highest 16S rRNA gene similarity (96.6%) was observed to that of '*Anatilimnocola aggregata*' ETA_A8^T.

To determine the genome-based phylogenetic position of strain PX40^T within the *Planctomycetes,* a phylogenetic tree based on concatenated sequences of conservative marker genes was constructed. This placed strain PX40^T within the family *Pirellulaceae* (Fig. 3). Following the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes (Chun et al. 2018), we calculated both the overall genome similarity and the average nucleotide identity (ANI) between PX40^{T}and closely related planctomycetes. The ANI value between strain PX40^T and its closest relative with determined genome sequence,

‘*Anatilimnocola aggregata*’ ETA_A8^T, was 78.3%. According to ANI thresholds recently proposed for taxonomic delineation (i.e. 65–95% for the same genus and 95–100% for the same species (Konstantinidis et al. 2017)), these two bacteria represented different species in one genus. The ANI value between strain PX40^T and *Pirellula staley* ATCC27377^T was 75.6%. The overall genome similarity between PX40^T and ‘*A. aggregata’* ETA_A8^T was 20.8±2.5%, while the genome similarity between PX40^T and *Pirellula staley* ATCC27377^T was 19.3±2.3%.

Based on the phylogenetic divergence between strain PX40^T and ‘*A. aggregata’* ETA_A8^T and several other characteristics that differentiate these planctomycetes (Table 2), we propose placing strain PX40^T in a novel species of the genus ‘*Anatilimnocola’*, *A. floriformis* sp. nov. We also include additional characteristics to the earlier published description of this genus.

Emended description of the genus *Anatilimnocola* Kallscheuer *et al*. 2021.

The description of the genus ‘*Anatilimnocola*’ is as given by Kallscheuer et al. (2021) with the following modifications. Crateriform-like structures are distributed on a reproductive cell pole; holdfasts are present. Carbon substrates are sugars and some polysaccharides. Sensitive to NaCl. The major fatty acids are C18:1ω9c, C16:0, and 16:1ω7c. The major polar lipid are diacylglyceryl-(N,N,N)-trimethylhomoserines. Main habitats are eutrophic freshwater bodies.

Description of *Anatilimnocola floriformis* sp. nov.

Anatilimnocola floriformis (flo.ri.for'mis. L. masc. n. *flos*, a flower; L. masc./fem. stuff. *-formis*, in the shape of (from L. fem. n. *forma*, shape); N.L. fem. adj. *floriformis*, referring to the characteristic of the cells to form flower-shaped aggregates).

Exhibit the following properties in addition to those given in the genus description. Colonies are small (2–3 mm in diameter), circular and unpigmented. The cells are 0.7 ± 0.2 μm

wide and 1.9 ± 0.5 μm long, occur as single cells or flower-shaped rosettes and reproduce by polar budding. The temperature range for growth is 15 - 30°C with the optimum at 22 - 25°C. The pH range for growth is 5.0 - 7.5 with 6.5-7.0 as the optimum. Growth does not occur at NaCl concentrations >0.3% (w/v). Chemoheterotrophic, aerobic, catalase- and cytochrome oxidase-positive but urease-negative. N-acetylglucosamine, D-galactose, L-rhamnose, D-arabinose, D-glucose, xylose, sucrose, maltose, lactose, D-cellobiose, D-trehalose, lactulose are assimilated. Capable of hydrolyzing aesculin, arabinogalactan, dextrin, laminarin, locust bean gum, gelatin, starch, xanthan gum and xylan. Amino acids, casamino acids, fructose, L-fucose, D-ribose, L-sorbose, D-raffinose, D-mannitol, sorbitol, glycerol, succinate, malate, citrate, benzoate, fumarate, formate, acetate, pyruvate and inulin are not assimilated. Cannot hydrolyze casein, chitosan, chitin, carboxymethyl cellulose and cellulose. Nitrate is reduced to nitrite. Arginine dihydrolase is positive (API 20NE strip). In the API ZYM strip, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, α-glucosidase and α-galactosidase were detected. The following enzyme activities were not detected: lipase, trypsin, chymotrypsin, phosphohydrolase, N-acetyl-β-glucosaminidase, β-galactosidase, cystine arylamidase, β-glucuronidase, α-fucosidase and α-mannosidase. The major fatty acids are C18:1ω9c, C16:0, and 16:1ω7c. The major polar lipids are diacylglyceryl-(N,N,N)-trimethylhomoserines. The DNA G+C of the type strain is 57.8 mol%. The type strain is strain PX40^T (=KCTC 92369^T = VKM B-3621^T= UQM 41463^T), which was isolated from the boreal lake Morotskoye (Vologda region, European North Russia).

Funding information

This study was supported by the Ministry of Science and Higher Education of the Russian Federation (Research topic No 122041100029-2 and the Grant agreement № 075-15-2021-

1051). J.S.S.D. was supported by the SIAM Gravitation Grant (024.002.002) from the Dutch Ministry of Education, Culture and Science (OCW).

Acknowledgements

The authors thank W. Irene C. Rijpstra (NIOZ Royal Netherlands Institute for Sea Research) for the help with lipid analyses.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Table 1. Relative abundance (%; normalized on their sum) of fatty acids and other lipids including an alkene present in the acid hydrolysate of cell material of strain PX40^T. Only components with a relative abundance ≥0.5% of the total are listed. Major components (>5%) are given in bold type face.

Lipid type	Relative abundance (%)
Fatty acids	
isoC16:0	2.0
C16:1ω7	**5.7**
C16:0	**35.0**
C17:1ω8	1.0
C18:1ω9	**34.9**
C18:1ω7	2.3
C18:0	1.4
C20:1ω9	4.4
C20:1ω7	2.7
Hydroxy fatty acids	
(ω-1)OH-C24:0	1.1
(ω-1)OH-C26:0	0.6
ωOH-C32:1	0.6
ωOH-C32:0	0.8
β,x-diOH- C32:0	1.6
Other lipids	
C31:0-keto-ol	1.0
C33:1-keto-ol	0.8
C31:9 *n*-alkene	4.1
IPLs	
DGTS	+++
Lyso-DGTS	+

Table 2. Major characteristics that distinguish strain PX40^T and '*Anatilimnocola aggregata*' ETA_A8^T (Kallscheuer et al. 2021).

Characteristic	Strain PX40^T	*Anatilimnocola aggregata* ETA_A8^T
Phenotypic features		
Habitat	Eutrophic boreal lake	Municipal duck pond
Cell size (µm)	0.7 ± 0.2× 1.9 ± 0.5	2.0 ± 0.3 ×1.4 ± 0.2
Cell shape	Ellipsoidal to pear shaped	Round grain rice shaped
Type of rosettes	Flower-shaped	Shapeless aggregates
Temperature range (optimum) (°C)	15–30 (22–25)	15–33 (30)
pH growth range (optimum)	5.0–7.5 (6.5–7.0)	5.0–10.0 (8.0)
Flagella	Yes	n.o.
Crateriform structures	Yes	n.o.
Major fatty acids	C18:1ω9c, C16:0, C16:1ω7c	n.d.
Genomic features		
Genome size (Mb)	8.93	9.01
DNA G+C content, mol%	57.8	57.8

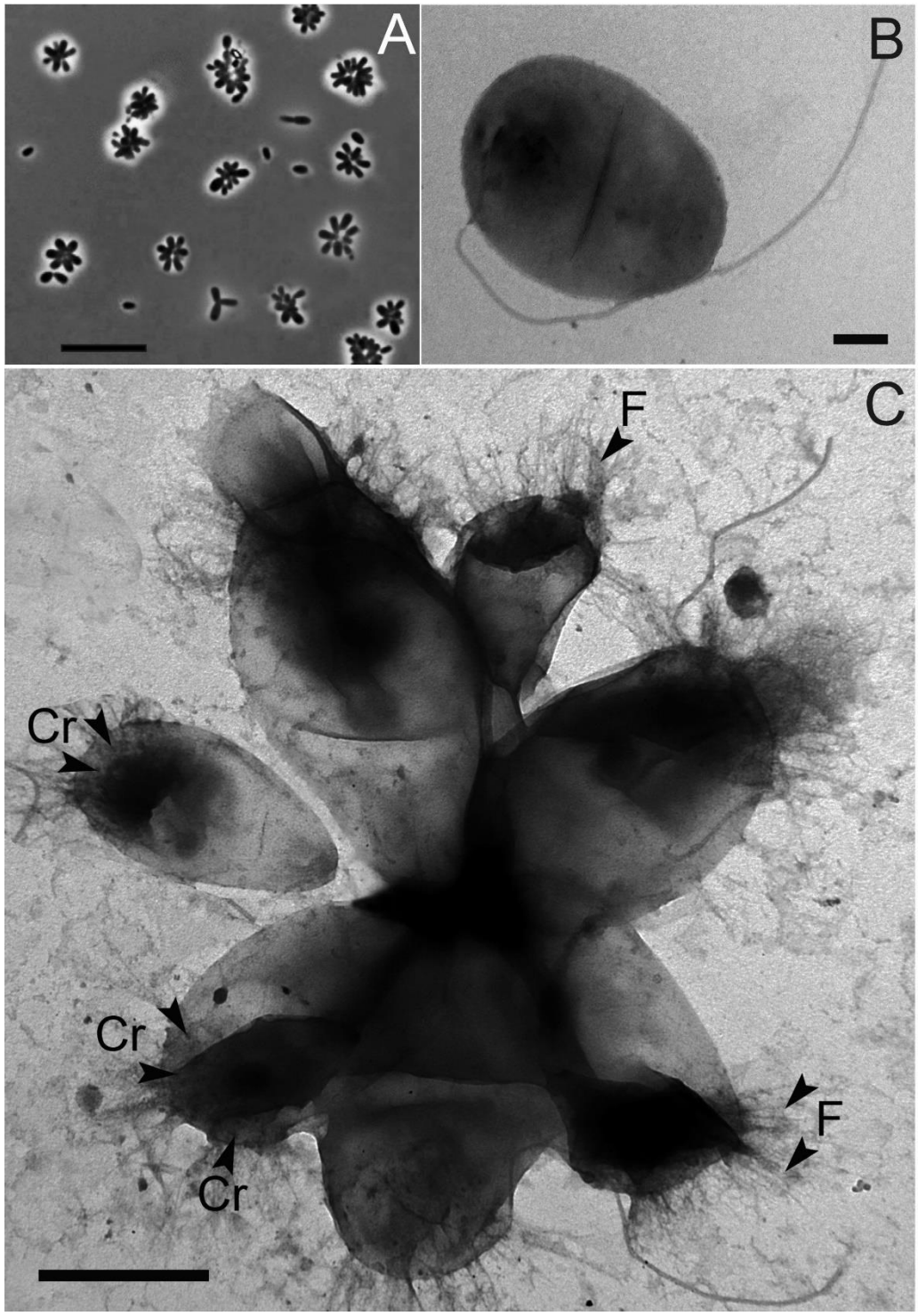
n.d., not determined; n.o., not observed

FIGURE CAPTIONS

Figure 1. (**A**) Phase-contrast image of cells of strain PX40^T grown for 7 days on solid medium MPVGY; bar, 10 μm. (**B**) Electron micrographs of a negatively stained daughter cell with flagellum; bar, 0.2 μm. **(C**) Electron micrograph of negatively stained cells displaying crateriform pits (Cr) and bundles of fimbriae (F) bar, 1μm.

Figure 2. 16S rRNA gene-based maximum-likelihood tree showing the phylogenetic relationship of strain PX40^T to other described *Pirellulaceae* planctomycetes and some environmental 16S rRNA gene clone sequences displaying >95% similarity to those of strain PX40T and *Pirellula staley* DSM 6068. Habitats are coded by colored triangles. The clade defined by the genera *Pirellula* and '*Anatilimnocola*' is depicted with a greenish background. Bootstrap values are shown as circles (70-100%) with the respective legend.

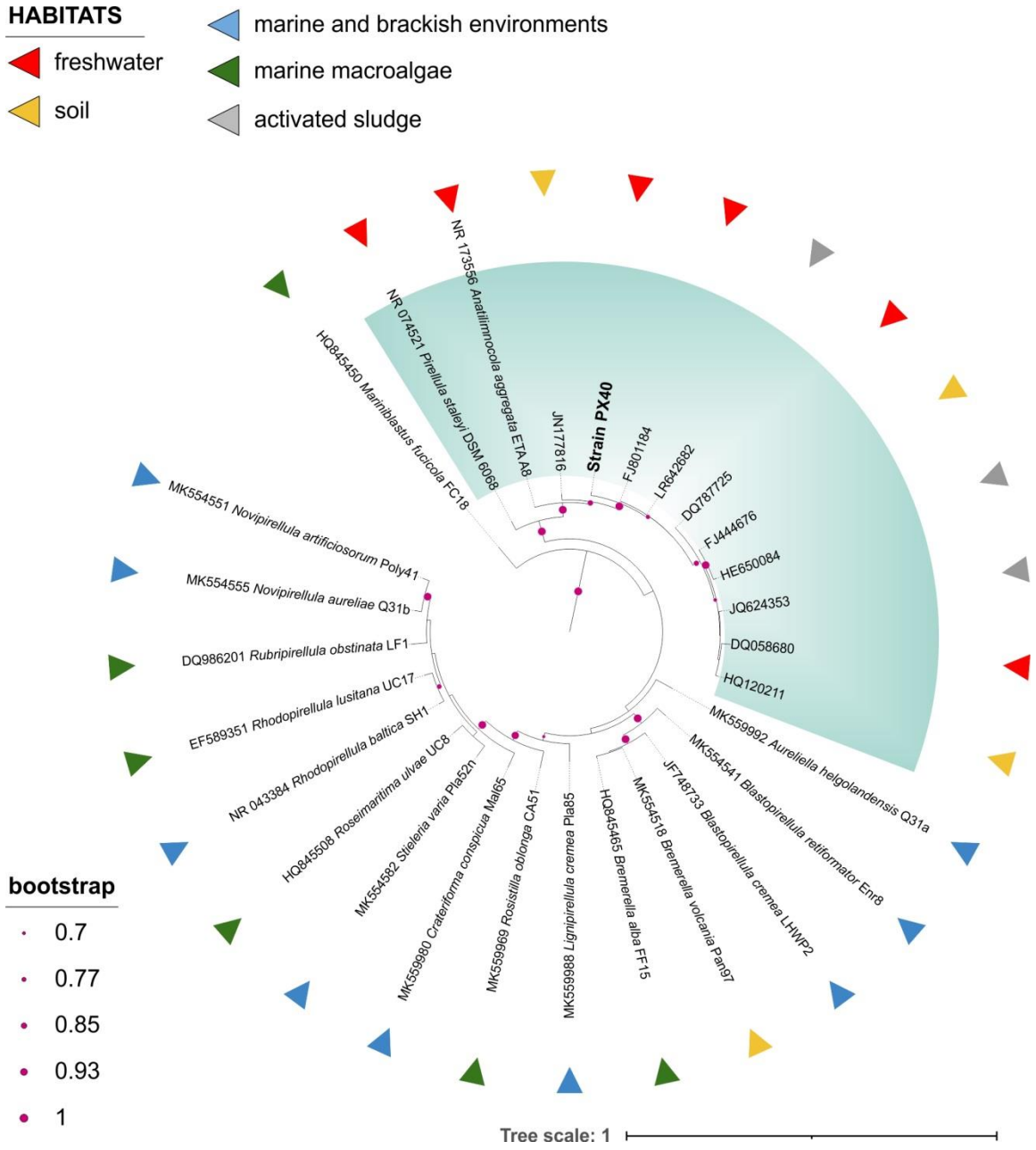
Figure 3. Genome-based phylogeny of the family *Pirellulaceae* defined in the GTDB taxonomy. The tree was reconstructed using the GTDB-Tk v.2.0.0 (Parks et al. 2018). The significance levels of interior branch points obtained in maximum- likelihood analysis were determined by bootstrap analysis (100 data resamplings). The root (not shown) was composed of 22 genomes of members of the family *Scalinduaceae*. Bar, 0.1 substitutions per amino acid position



505

506 **Figure 1.**

507



508

509 **Figure 2.**

510

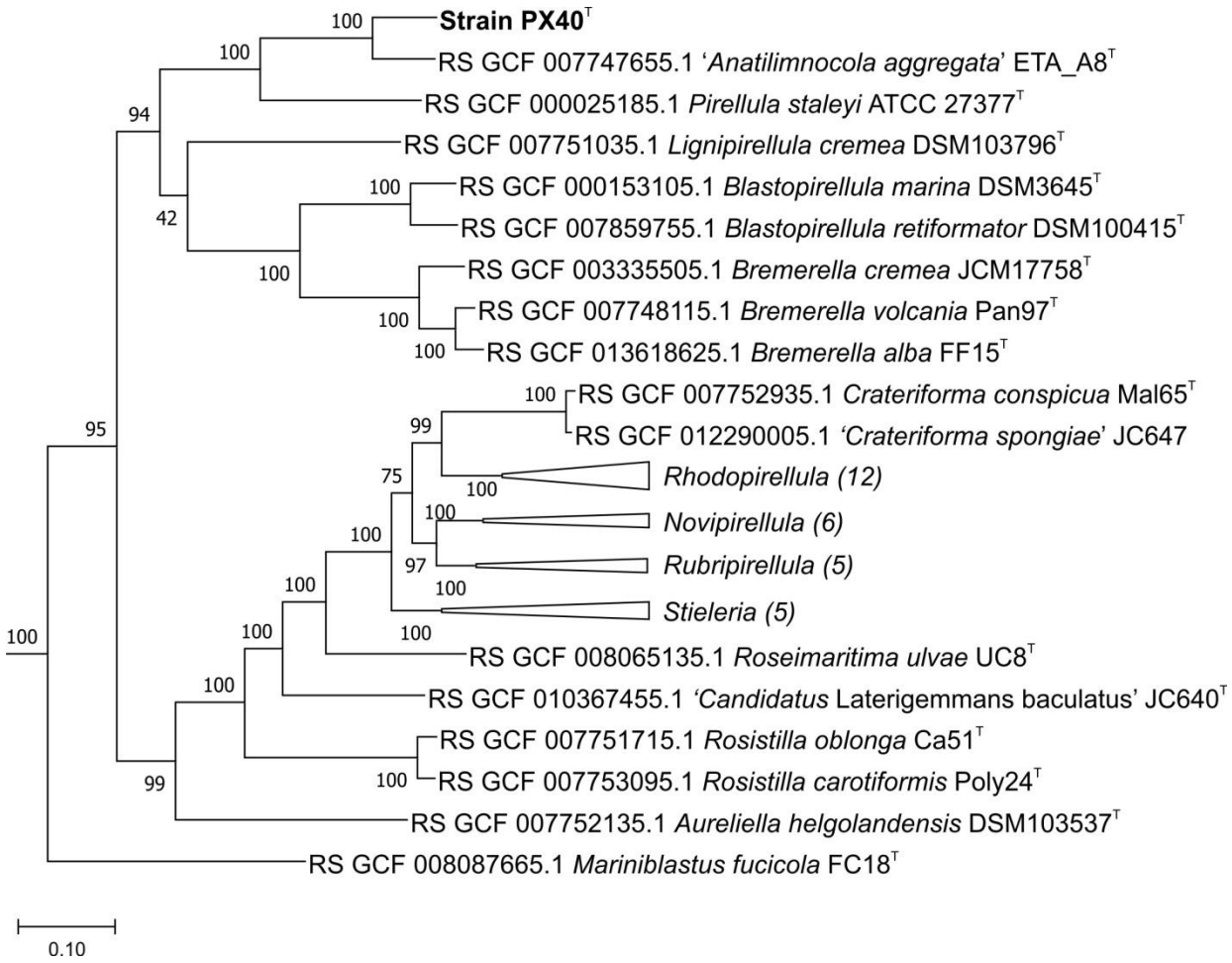

Strain PX40T
RS GCF 007747655.1 'Anatilimnocola aggregata' ETA_A8T
RS GCF 000025185.1 Pirellula staleyi ATCC 27377T
RS GCF 007751035.1 Lignipirellula cremea DSM103796T
RS GCF 000153105.1 Blastopirellula marina DSM3645T
RS GCF 007859755.1 Blastopirellula retiformator DSM100415T
RS GCF 003335505.1 Bremerella cremea JCM17758T
RS GCF 007748115.1 Bremerella volcania Pan97T
RS GCF 013618625.1 Bremerella alba FF15T
RS GCF 007752935.1 Crateriforma conspicua Mal65T
RS GCF 012290005.1 'Crateriforma spongiae' JC647
Rhodopirellula (12)
Novipirellula (6)
Rubripirellula (5)
Stieleria (5)
RS GCF 008065135.1 Roseimaritima ulvae UC8T
RS GCF 010367455.1 'Candidatus Laterigemmans baculatus' JC640T
RS GCF 007751715.1 Rosistilla oblonga Ca51T
RS GCF 007753095.1 Rosistilla carotiformis Poly24T
RS GCF 007752135.1 Aureliella helgolandensis DSM103537T
RS GCF 008087665.1 Mariniblastus fucicola FC18T
0.10

Figure 3.