**Paludisphaera borealis** gen. nov., sp. nov., a hydrolytic planctomycete from northern wetlands, and proposal of *Isosphaeraeaceae* fam. nov.

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Two isolates of aerobic, budding, pink-pigmented bacteria, designated strains PX4 T and PT1, were isolated from a boreal *Sphagnum* peat bog and a forested tundra wetland. Cells of these strains were non-motile spheres that occurred singly or in short chains. Novel isolates were capable of growth at pH values between 3.5 and 6.5 (optimum at pH 5.0–5.5) and at temperatures between 6 and 30 °C (optimum at 15–25 °C). Most sugars and a number of polysaccharides including pectin, xylan, lichenin and Phytagel were used as growth substrates. The major fatty acids were C16 : 0, C18 : 1

9 and C18 : 0; the major polar lipids were phosphocholine and trimethylornithine. The quinone was menaquinone-6, and the G + C content of the DNA was 66 mol%. Strains PX4 T and PT1 were members of the order *Planctomycetales* and displayed 93–94 % 16S rRNA gene sequence similarity to *Aquisphaera giovannonii*, 91–92 % to species of the genus *Singulisphaera* and 90–91 % to *Isosphaera pallida*. The two novel strains, however, differed from members of these genera by cell morphology, substrate utilization pattern and a number of physiological characteristics. Based on these data, the novel isolates should be considered as representing a novel genus and species of planctomycetes, for which the name *Paludisphaera borealis* gen. nov., sp. nov., is proposed. The type strain is PX4T (=DSM 28747 T =VKM B-2904 T). We also suggest the establishment of a novel family, *Isosphaeraceae* fam. nov., to accommodate stalk-free planctomycetes with spherical cells, which can be assembled in short chains, long filaments or shapeless aggregates. This family includes the genera *Isosphaera*, *Aquisphaera*, *Singulisphaera* and *Paludisphaera*.

Planctomycetes of the phylogenetic lineage defined by the genus *Isosphaera* colonize a wide range of terrestrial and aquatic environments with diverse conditions. The first taxonomically described member of this lineage, i.e. the filamentous budding bacterium *Isosphaera pallida*, was isolated from hot springs (Giovannoni et al., 1987). This moderately thermophilic and neutrophilic planctomycete displayed a number of unique features, such as gliding motility and phototaxis. The second characterized genus in this phylogenetic group, *Singulisphaera*, was distinctly different from *I. pallida* and was represented by non-filamentous, non-motile, moderately acidophilic and cold-adapted spherical cells that occurred singly, in pairs or in shapeless aggregates (Kulichevskaya et al., 2008, 2012). The strains representing the two described species of this genus, *Singulisphaera acidiphila* and *Singulisphaera rosea*, were isolated from *Sphagnum*-dominated acidic wetlands. The third currently recognized genus in the group of *Isosphaera*-like planctomycetes is *Aquisphaera*. It includes a single species of non-filamentous and neutrophilic planctomycete, which was isolated from a freshwater aquarium, i.e., *Aquisphaera giovannonii* (Bondoso et al., 2011).
Recently, representatives of the *Isosphaera*-like group were recognized as one of the most abundant planctomycete populations in acidic northern wetlands (Ivanova & Dedys, 2012; Serkebaeva *et al*., 2013; Moore *et al*., 2015). Notably, their abundance peaked at the oxic–anoxic interface, where the transition occurs from living vegetation to dead plant material. At the oxic–anoxic interface of the boreal peat bog Obukhovskoye, Yaroslavl region, Russia (58° 14’ N 38° 12’ E), 16S rRNA gene reads from *Isosphaera*-like planctomycetes comprised 53 % of total reads retrieved from the peat horizon (Moore *et al*., 2015). One of the isolates described here, strain PX4, was obtained from just above the oxic–anoxic interface (a depth of 15–20 cm) of this peat bog. The enrichment approach was designed to select for chitin-degrading micro-organisms capable of growth under micro-oxic or anoxic conditions. Freshly collected peat (5 g) was used to inoculate 500 ml flasks containing 200 ml liquid MM1 medium of the following composition (g l−1 distilled water): KH2PO4, 0.1; (NH4)2Cl, 0.2; MgCl2, 0.1; CaCl2 . 2H2O, 0.02; yeast extract, 0.05; chitin from crab shells (Sigma), 500; pH 5.5–5.8. The flasks were then sealed with rubber septa, flushed with N2 for 10 min and incubated in static conditions at 20 °C. After 1 month of incubation, the aliquots of peat suspension from these enrichment cultures were screened by hybridization with two planctomycete-specific Cy3-labelled probes, PLA46 and PLA886 (Neef *et al*., 1998). The probes hybridized to numerous spherical cells that occurred in short chains or in shapeless aggregates, suggesting the presence of *Isosphaera-Singulisphaera*-like planctomycetes. Further isolation involved spread-plating cell suspensions from the enrichment cultures onto the same medium solidified with 10 g Phytagel (Sigma–Aldrich), since this solidifying agent was shown to be highly useful for the isolation of diverse peat-inhabiting bacteria (Dedys, 2011). One portion of plates was placed in anaerobic jars with AnaeroGen anaerobic system envelopes (Oxoid), while another portion was kept in aerobic conditions. Examination of the plates after 1 month of incubation revealed no growth under anoxic conditions. On plates kept under aerobic conditions, however, we noticed development of numerous small (0.5–1 mm in diameter), circular, bright-pink colonies that formed visible depressions in a Phytagel-solidified medium (Fig. 1a). These colonies contained spherical cells, which reproduced by budding and occurred singly, in pairs or in short chains containing up to 10 cells (Fig. 1b).

Another isolate with identical colony and cell morphologies, strain PT1, was obtained from a forested tundra wetland, Nadym, Western Siberia, Russia (65° 37’ N 72° 43’ E). In this case, the cultivation approach was targeted at the isolation of methanotrophic bacteria. Peat suspensions were spread-plated onto the same MM1 medium with Phytagel but without yeast extract and chitin, and were incubated at 20 °C for 1 month in desiccators containing 30 % methane (v/v) in the gas phase. Small bright-pink colonies, which produced depressions in Phytagel, formed on these plates among the colourless colonies produced by methanotrophic bacteria.

The apparent ability of strains PX4 and PT1 to hydrolyse Phytagel (a complex heteropolysaccharide of microbial origin), as indicated by the depressions produced in association with colonies, made them attractive objects for further studies, since the hydrolytic capabilities of planctomycetes remain poorly characterized. Partial sequencing of the 16S rRNA gene fragments (≈500 bp) from these isolates showed that they affiliate with the *Isosphaera*-like lineage of the family Planctomycetaceae but display only a distant relationship (90–94 % 16S rRNA gene similarity) to currently described members of this lineage, i.e. the genera *Isosphaera*, *Singulisphaera* and *Aquisphaera*. This study, therefore, was undertaken to characterize strains PX4 and PT1 and to describe them taxonomically.

Although both strains were isolated on MM1 medium, they grew significantly better on either agar- or Phytagel-solidified medium M31 (modification of medium 31 described by Staley *et al*., 1992) containing (g l−1 distilled water): KH2PO4, 0.1; Hutner’s basal salts, 20 ml; N-acetylglucosamine, 0.5; ampicillin sodium salt, 0.2; yeast extract, 0.1; pH 5.8. Successive restreaking on agar medium M31 was used to purify strains PX4 and PT1, which were then routinely maintained on this medium without ampicillin and were subsampled at 1 month intervals.

Morphological observations and cell measurements were made with a Zeiss Axioplan 2 microscope and Axiovision 4.2 software (Zeiss). Negative staining was performed as described for *Planctomicrobium piriforme* (Kulichevskaya *et al*., 2015), with the only difference that one additional round of staining with 1 % aqueous solution of phosphotungstic acid (pH 7.0) was made. The specimen samples were examined with a JEM-100C transmission electron microscope (JEOL).

Mature cells of strains PX4 and PT1 were spherical and varied in size from 1.5 to 2.5 μm. Cells occurred singly, in pairs or in short chains (Fig. 1b) and reproduced by budding (Fig. 1c, d). Examination of negatively stained cells using electron microscopy showed the presence of crateriform pits on the cell surface (Fig. 1c). Negative staining revealed also that many cells produce some extracellular material of a net-like structure (Fig. 1e). The nature of this excreted material remains unclear. On agar-solidified M31 medium, strains PX4 and PT1 formed small (1–3 mm in diameter), bright-pink-pigmented, round colonies. Notably, no depressions were formed, i.e. these isolates were incapable of hydrolysing agar. Hydrolysis of Phytagel was observed on MM1 medium or on M31 medium without N-acetylglucosamine. Liquid cultures displayed light-pink turbidity.

Physiological tests were performed in liquid medium M31. Growth of strains PX4 and PT1 was monitored by nephelometry at 600 nm in an Eppendorf BioPhotometer for 7–14 days under a variety of conditions, including...
Paludisphaera borealis gen. nov., sp. nov.

Fig. 1. (a) Development of depressions in Phytagel-solidified medium during colony growth of strain PX4°. Bar, 10 mm. (b) Phase-contrast image of cells of strain PX4° in 10-day-old culture. Bar, 10 µm. (c–e) Electron micrographs of negatively stained cells of strain PX4° displaying crateriform pits scattered all over cell surface (c), a newly formed bud [indicated by black arrow in (c) and (d)] and an extracellular material of a net-like structure excreted by the cell (e). Bars, 1 µm (c, e) and 0.2 µm (d).
temperatures of 4–37 °C, pH 3.8–8.0 and NaCl concentrations of 0–3.0 % (w/v). Variations in the pH were achieved by using MES (pH 4.0–6.5) and MOPS (pH 6.5–7.9) buffer systems. The pH range of 3–4 was achieved by adjusting the medium pH with 0.5 M H2SO4. Strain PX4T grew in the temperature range of 10–30 °C, with an optimum at 22–25 °C. Strain PT1 developed in the temperature range of 6–30 °C, with an optimum at 15–25 °C. The pH range for growth was pH 3.5–6.5, with an optimum at pH 5.0–5.5 (Fig. S1, available in the online Supplementary Material). Growth was completely inhibited at NaCl concentrations above 0.5 % (w/v). The doubling time of this bacterium under optimal growth conditions was about 32 h.

Carbon source utilization was determined using mineral medium supplemented with respective carbon sources (0.05 %, w/v). Mineral medium contained (g l−1 distilled water): KH2PO4, 0.1; (NH4)2SO4, 0.1; MgSO4·7H2O, 0.1; yeast extract, 0.05; 1 ml metal salt solution ‘44’ (Staley et al., 1992), the pH being adjusted to pH 5.8. Strains were cultivated in 100 ml flasks containing 10 ml mineral medium and were incubated at 24 °C for 2–3 weeks on a shaker. The capacity to degrade different biopolymers was examined by measuring the rate of CO2 production in tightly closed 120 ml flasks containing 10 ml liquid medium with 0.05 % (w/v) of the corresponding polymer substrate for 1 month at 24 °C. Control incubations were run in parallel under the same conditions but without substrate. Nitrogen sources were tested using liquid mineral medium with 0.05 % (w/v) glucose in which (NH4)2SO4 was replaced with one of the following compounds at a concentration of 0.01 % (w/v): KNO3, KNO2, urea or one of the amino acids listed in the species description. Cultures were tested for growth under anaerobic conditions in anaerobic jars by using AnaeroGen anaerobic system envelopes (Oxoid), which absorb atmospheric oxygen with the simultaneous generation of CO2 (up to 9–13 %, v/v). For cultivation in micro-oxic conditions, medium M31 was boiled for 10 min to remove oxygen. After that, hermetically closed 500 ml flasks were filled with 450 ml medium M31, inoculated with the cultures being examined and incubated under static conditions for 2 weeks. Dissolved O2 concentration was 1.5 mg O2 l−1 (measured in cultivation flasks prior to inoculation using a dissolved oxygen meter; sensION6, Hach).

Strains PX4T and PT1 grew best under aerobic conditions on media with carbohydrates or N-acetylglucosamine. The carbon substrates tested in our study and the results are given in the species description. Most sugars tested, including N-acetylglucosamine, were the preferred growth substrates. With the exception of pyruvate and succinate, organic acids were not utilized. Strains PX4T and PT1 were capable of hydrolysing aesculin, gelatin, lichenin, pectin, xylan and PhytageI, but not casein, chondroitin sulfate, laminarin, starch, pullulan, protein hydrolysate or cellulose. Despite its isolation from the enrichment culture of chitin-degrading micro-organisms, no growth of strain PX4T was observed in chitin-containing mineral medium. The same was true for strain PT1. Ammonia, nitrate, N-acetylg glucosamine, Bacto peptone, Bacto yeast extract, alanine, asparagine and valine were utilized as nitrogen sources. Neither of the two isolates grew under anoxic conditions; they showed stable albeit slow growth (μ=0.01 h−1) under micro-oxic conditions.

Oxidative and fermentative utilization of carbohydrates and gelatin liquefaction were determined using an API 20NE kit (bioMérieux) and as described for the Hugh–Leifson test (Gerhardt, 1981). Enzymic activities were examined using an API ZYM kit (bioMérieux). Catalase was tested using the method described by Gerhardt (1981). Oxidase was tested using a REF 55 635 Oxidase Reagent (bioMérieux). Strains PX4T and PT1 were catalase and cytochrome oxidase positive but urease negative. Dissimilatory nitrate reduction was negative. The following enzymic activities (API ZYM) were detected in strains PX4T and PT1: acid phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, phosphohydrolase, N-acetyl-β-glucosaminidase and β-galactosidase (API ZYM test). The following enzyme activities were not detected: alkaline phosphatase, cystine arylamidase, lipase, trypsin, chymotrypsin, z-galactosidase, β-glucoauronidase, z-fucosidase and z-mannosidase.

Susceptibility of strains PX4T and PT1 to antibiotics was determined on M31 agar plates using discs containing the following antibiotics: ampicillin (10 mg), gentamicin (10 mg), kanamycin (30 mg), neomycin (10 mg), novobiocin (30 mg), streptomycin (10 mg), chloramphenicol (30 mg) and lincomycin (10 mg) (Oxoid). The isolates were resistant to ampicillin, streptomycin, chloramphenicol, lincomycin and novobiocin but sensitive to kanamycin, neomycin and gentamicin.

For the analysis of fatty acids, intact polar lipids, neutral lipids and quinones, cells of the novel isolates were grown on liquid medium M31 and harvested in the late exponential growth phase. Lipids were analysed after acid hydrolysis of whole cells, following the procedure described by Sinninghe Damsté et al. (2011). The major fatty acids detected in strain PX4T and PT1 were nC16 : 0, nC18 : 1ω9 and nC18 : 0 (Table 1). A number of hydroxy fatty acids, including the nC28 : 0 (ω-1)-OH hydroxy fatty acid and the nC31 : 5 hydrocarbon were also detected. The latter seems to be very common in most planctomycetes. As shown in Table 2, the major polar lipids were phosphocholine and trimethylsphingosine. Phosphoglycerol and 1-acyl-glycerol-3-phosphocholine were also present in minor amounts. As reported by Moore et al. (2015), the ratio of trimethylsphingosine to (phosphocholine+phosphoglycerol) in cells grown in micro-oxic conditions was higher than that in fully aerobic conditions, which suggests that trimethylsphingosines could be synthesized as a response to changing redox conditions in the oxic–anoxic interface.

Isoprenoid quinones of strains PX4T and PT1 were analysed as described for Planctomicrobium piriforme
(Kulichevskaya et al., 2015). Like other members of the order Planctomycetales (Ward, 2010), our isolates contained menaquinone-6 as the only isoprenoid quinone. Based on genome sequence analysis, the DNA G+C content of strain PX4T is 66 mol% (Ivanova and others, unpublished), which is higher than that in members of the genera Isosphaera and Singulisphaera but lower than that in members of the genus Aquisphaera (Table 3).

PCR-mediated amplification of the 16S rRNA gene from DNA of strains PX4T and PT1 was performed using primers 9f and 1492r and the reaction conditions described by Weisburg et al. (1991). 16S rRNA gene amplicons were sequenced on an ABI 377A DNA sequencer using BigDye terminator chemistry, as specified by the manufacturer (PE Applied Biosystems). Phylogenetic analysis was carried out using the ARB program (Ludwig et al., 2004). 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). Comparative analysis based on nearly full-length 16S rRNA gene sequences confirmed that strains PX4T and PT1 belong to the order Planctomycetales and are members of the coherent phylogenetic cluster defined by the genus Isosphaera (Fig. 2). The minimum sequence identity within this cluster is about 90 %, which is close to the taxonomic threshold defined for the family level (Yarza et al., 2014). This cluster is strongly supported by all algorithms used for the tree reconstruction and accommodates morphologically similar, stalk-free planctomycetes with spherical cells, which can be assembled in short chains, long filaments or shapeless aggregates. Notably, daughter cells of these budding bacteria are non-motile. The 16S rRNA gene sequence identity between members of the Isosphaera-like cluster and other taxonomically described organisms within the order Planctomycetales is in the range 76.8–81.9 %. Based on this phylogenetic divergence (Fig. 2) and the morphological similarity between members of the Isosphaera-like cluster, the latter should be given the status of a family, i.e. Isosphaeraeae fam. nov. Strains PX4T and PT1 are members of the Isosphaeraeae, but are phylogenetically divergent, morphologically distinct and phenotypically different from other characterized representatives of this family. These non-filamentous, non-motile, acidophilic and cold-tolerant planctomycetes could clearly be differentiated from filamentous, gliding, thermophilic and neutrophilic I. pallida. Abilities to grow at temperatures below 10 °C, pH ≤6.0 as well as to develop under micro-oxic conditions differentiate the novel isolates and A. giovannonii. Finally, strains PX4T and PT1 can be distinguished from members of the genus Singulisphaera by the formation of cell chains, the ability to hydrolyse Phytagel, the absence of C18 : 2 and C fatty acid, and the lower DNA G+C content (Table 3). In addition, cells of novel isolates are smaller than cells of species of the genus Singulisphaera.

Strains PX4T and PT1 displayed 93–94 % 16S rRNA gene sequence similarity to A. giovannonii, 91–92 % to species of the genus Singulisphaera and 90–91 % to I. pallida. The overall similarities between the genome of strain PX4T (Ivanova and others, unpublished) and the genomes of I. pallida IS1BT and S. acidiphila DSM 18658T estimated using formula 2 of the Genome-to-Genome-Distance-Calculator (Auch et al., 2010) are 19.8 ± 2.3 and 20.0 ± 2.3, respectively. These DNA–DNA hybridization values are similar to those calculated for members of different genera (Scheuere et al., 2014). Average nucleotide identity values generated by comparing the genome of strain PX4T with the genomes of I. pallida IS1BT and S. acidiphila DSM 18658T are also very low, i.e. 75 % and 77 %, respectively. We therefore propose to classify strains PX4T and PT1 as representing a novel genus and species, Paludisphaera borealis gen. nov., sp. nov. The characteristics that differentiate the genus Paludisphaera gen. nov. from the genera Isosphaera, Singulisphaera and Aquisphaera are summarized in Table 3.

**Description of the genus Paludisphaera**

Paludisphaera (Pa.lu.di.sphae‘ra. L. n. palus -udas a swamp, marsh; L. fem. n. sphaera a ball, globe sphere; N.L. fem. n. paludisphaera a spherical cell from wetland).

Cells are Gram-stain-negative, non-motile spheres that occur singly, in pairs or in short chains. Reproduce by budding. Daughter cells are non-motile. Crateriform pits are

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**Table 1.** Relative abundance (percentage of total) of lipids extracted after acid hydrolysis of cell material of strain PX4T and PT1 (major components are given in bold type)

<table>
<thead>
<tr>
<th>Lipid Type</th>
<th>PX4T</th>
<th>PT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n16 : 1o9</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>n16 : 1o7</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>n16 : 0</td>
<td>17.4</td>
<td>16.5</td>
</tr>
<tr>
<td>n17 : 1o8</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>n18 : 1o9</td>
<td>47.9</td>
<td>50.9</td>
</tr>
<tr>
<td>n18 : 0</td>
<td>12.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Hydroxy fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n14 : 0 β-OH</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>n18 : 0 β-OH</td>
<td>0.8</td>
<td>2.4</td>
</tr>
<tr>
<td>n25 : 0 α-OH</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>n27 : 1 α-OH</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>n27 : 0 α-OH</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>n29 : 1 α-OH</td>
<td>0.8</td>
<td>1.7</td>
</tr>
<tr>
<td>n29 : 0 α-OH</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>n29 : 0 α(α-1)-OH</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>n28 : 0((α-1))-OH</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>n30 : 1(α-1)-OH</td>
<td>0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>n31 : 0 α(α-1)-OH</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>C29-3-OH hopanoic acid</td>
<td>2.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Other lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nC31 : 9 hydrocarbon</td>
<td>2.4</td>
<td>1.2</td>
</tr>
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</table>
scattered all over the cell surface. Stalk-like structures are absent. Colonies are opaque and pink coloured. Chemo-organotrophic aerobes. Capable of growth under microoxic conditions. Possess hydrolytic capabilities. Dissimilatory nitrate reduction is negative. Moderately acidophilic and mesophilic. Sensitive to NaCl. The major quinone is menaquinone-6. The major fatty acids are \( nC16:0 \), \( nC18:1\) \( \alpha9 \) and \( nC18:0 \). The major polar lipids are phosphocholine and trimethylornithine. The genus is a member of the phylum Planctomycetes, order Planctomycetales, family Isosphaeraceae. The type species is Paludisphaera borealis.

Table 2. Relative abundances and fatty acid composition of intact polar lipids of strains PX4\(^T\) and PT1

<table>
<thead>
<tr>
<th>Intact polar lipid</th>
<th>PX4(^T)</th>
<th>PT1</th>
<th>Fatty acid composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoglycerol</td>
<td>+</td>
<td>+</td>
<td>(C36 : 2, C34 : 1, C32 : 1)</td>
</tr>
<tr>
<td>Phosphocholine</td>
<td>++ +</td>
<td>++ +</td>
<td>(C36 : 2, C34 : 1, C32 : 1)</td>
</tr>
<tr>
<td>Trimethylornithine</td>
<td>+ +</td>
<td>+</td>
<td>(C18 : 1, β-OH C18)</td>
</tr>
<tr>
<td>1-Acyl-glycero-3-phosphocholine</td>
<td>TR</td>
<td>+</td>
<td>(C18 : 1, C16 : 0)</td>
</tr>
</tbody>
</table>

Table 3. Major characteristics that distinguish the genus Paludisphaera gen. nov. from the genera Isosphaera, Singulisphaera and Aquisphaera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrangement of cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>1.5–2.5</td>
<td>2.5–3.0</td>
<td>1.6–3.5</td>
<td>1.6–2.0</td>
</tr>
<tr>
<td>Gliding motility</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Phototaxis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Habitat</td>
<td>Wetlands</td>
<td>Hot springs</td>
<td>Wetlands</td>
<td>Freshwater</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Pink</td>
<td>Pink</td>
<td>Colourless or pink</td>
<td>Pink</td>
</tr>
<tr>
<td>Respiration</td>
<td>Aerobic or microaerophilic</td>
<td>Strictly aerobic</td>
<td>Aerobic or microaerophilic</td>
<td>Strictly aerobic</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytage</td>
<td>+</td>
<td>nd</td>
<td>–</td>
<td>nd</td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>nd</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Xylan</td>
<td>+</td>
<td>nd</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Temperature growth range (°C)</td>
<td>6–30</td>
<td>34–55</td>
<td>4–33</td>
<td>10–35</td>
</tr>
<tr>
<td>Optimal temperature (°C)</td>
<td>15–25</td>
<td>40–50</td>
<td>15–28</td>
<td>30–35</td>
</tr>
<tr>
<td>pH growth range</td>
<td>3.5–6.5</td>
<td>nd</td>
<td>4.2–7.5</td>
<td>6.5–9.5</td>
</tr>
<tr>
<td>pH optimum</td>
<td>5.0–5.5</td>
<td>7.8–8.8</td>
<td>5.0–6.2</td>
<td>7.5–8.5</td>
</tr>
<tr>
<td>Vitamin requirement</td>
<td>None</td>
<td>nd</td>
<td>None</td>
<td>B12</td>
</tr>
<tr>
<td>Presence of C18 : 206c, 12c fatty acids</td>
<td>–</td>
<td>nd</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)*</td>
<td>66</td>
<td>62</td>
<td>62</td>
<td>70</td>
</tr>
</tbody>
</table>

*Values given for Paludisphaera, Isosphaera and Singulisphaera are based on genome sequence analyses.

Description of Paludisphaera borealis sp. nov.

Paludisphaera borealis (bo.re.a’lis. L. fem. adj. borealis pertaining to the north, boreal).

The description is as for the genus but with the following additional traits. Spherical cells with diameter of 1.5–2.5 μm. Carbon sources (0.05%, w/v) include glucose, fructose, galactose, lactose, cellobiose, maltose, mannose, melibiose, rhamnose, ribose, trehalose, sucrose, xylose, N-acetylglucosamine, salicin, pyruvate and succinate. Cannot utilize leucrose, raffinose, sorbose, melezitose, fucose, glycerol, methanol, ethanol, starch, glucuronic
**Fig. 2.** 16S rRNA gene-based neighbour-joining tree (Jukes–Cantor correction) showing the phylogenetic relationship of strains PX4<sup>T</sup> and PT1 to representative members of the order *Planctomycetales*. The bracket on the right indicates boundaries proposed for the family *Isosphaeraceae*. The significance levels of interior branch points obtained in neighbour-joining analyses were determined by bootstrap analysis (1000 data resamplings) using PHYLIP (Felsenstein, 1989). Bootstrap values (1000 data resamplings) of &gt;50% are shown. Filled circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The root (not shown) was composed of five 16S rRNA gene sequences from anammox planctomycetes (AF375993, AF375995, AY254883, AY257181, AY254882). Bar, 0.1 substitutions per nucleotide position.

Isosphaeraceae family nov.

*Isosphaeraeae* (I.so.sphae.rae)ceae. N.L. fem. n. *Isosphaera* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Isosphaeraceae* the *Isosphaera* family).

Gram-stain-negative, budding bacteria with spherical cells, which occur singly or in pairs or are assembled in short chains, long filaments or shapeless aggregates. Crateriform pits are scattered all over the cell surface. Stalk-like structures are absent. Do not form rosettes. Daughter cells are non-motile. Chemo-organotrophic aerobes. Some
representatives are capable of growth in micro-oxic conditions. The family belongs to the class ‘Planctomycetacia’, order Planctomycetales. The type genus is Isosphaera. Other genera in this family are Singulisphaera, Aquisphaera and Paludisphaera.

Acknowledgements

This research was supported by the ‘Molecular and Cell Biology’ programme of the Russian Academy of Sciences and the Russian Fund of Basic Research (project N 15-04-03064) and by the SIAM Gravitation Grant 024.002.002 from the Dutch Ministry of Education, Culture and Science.

References


