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

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

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of *Paludisphaera borealis* strains PX4^T and PT1 are KF467528 and KT372165, respectively.

ABSTRACT

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, lichenan and Phytagel were used as growth substrates. The major fatty acids were $C_{16:0}$, $C_{18:1}\omega9$ and $C_{18:0}$; the major polar lipids were phosphocholine and trimethylornithine. The quinone was MK-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 *Singulisphaera* species 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 PX4^T (= DSM 28747^T = VKM B-2904^T). We also suggest to establish a novel family, the *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*.

Keywords: the phylum *Planctomycetes, Paludisphaera borealis* gen. nov., sp. nov., *Isosphaeraceae* fam. nov.

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 *Isosphaera 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 *Isopshaera*-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 giovannoni* (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, Dedysh, 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^{o} 14’ N, 38^{o} 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^{T}, was obtained from just above the oxic/anoxic interface (depth of 15−20 cm) of this peat bog. The enrichment approach was designed in order to select for chitin-degrading microorganisms capable of growth under micro-oxic or anoxic conditions. Freshly collected peat (5 g) was used to inoculate 500-ml flasks containing 200 ml of liquid medium MM1 of the following composition (g per liter distilled water): KH_2PO_4, 0.1; $(NH_4)Cl$, 0.2; $MgCl_2$, 0.1;

$CaCl_2 \times 2H_2O$, 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 N_2 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 strategy involved spread-plating of cell suspensions from the enrichment cultures onto the same medium solidified with 10 g of Phytagel (Sigma–Aldrich), since this solidifying agent was shown to be highly useful for isolation of diverse peat-inhabiting bacteria (Dedysh, 2011). One portion of plates was placed into anaerobic jars with AnaeroGen anaerobic system envelopes (Oxoid), while another portion of plates 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 ^{o}37’ N, 72 ^{o}43' E). In this case, the cultivation approach was targeted at isolation of methanotrophic bacteria. Peat suspensions were spread-plated onto the same medium MM1 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 colorless colonies produced by methanotrophic bacteria.

The apparent ability of strains PX4^T and PT1 to hydrolyze 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 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 in order to characterize strains PX4^T and PT1 and to describe them taxonomically.

Although both strains were isolated on medium MM1, they grew significantly better on either agar- or Phytagel-solidified medium M31 (modification of medium 31 described by Staley *et al*., 1992) containing (g per liter distilled water): KH_2PO_4, 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 re-streaking on agar medium M31 was used to purify strains PX4^T and PT1, which were then routinely maintained on this medium without ampicillin and were subcultured at 1 month intervals.

Morphological observations and cell-size 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.

Mature cells of strains PX4^T 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^T 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 hydrolyzing agar. Hydrolysis of Phytagel was observed on medium MM1 or on medium M31 without N-acetylglucosamine. Liquid cultures displayed light-pink turbidity.

Physiological tests were performed in liquid medium M31. Growth of strains PX4^T and PT1 was monitored by nephelometry at 600 nm in an Eppendorf BioPhotometer for 7–14 days under a variety of conditions, including temperatures of 4–37 ºC, 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 medium pH with 0.5 M H_2SO_4. Strain PX4^T grew in the temperature range of 10-30ºC, with an optimum at 22-25ºC. Strain PT1 developed in the temperature range of 6-30ºC, with an optimum at 15-25ºC. The pH range for growth was 3.5-6.5, with an optimum at pH 5.0-5.5 (Supplementary Fig. S1). 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 MM supplemented with respective carbon sources (0.05 %, w/v). Medium MM contained (g per liter distilled water): KH_2PO_4, 0.1; $(NH_4)_2SO_4$, 0.1; $MgSO_4 \times 7H_2O$, 0.1; yeast extract, 0.05; 1 ml metal salt solution '44' (Staley *et al*., 1992), the pH being adjusted to 5.8. Cultivation was done in 100 ml flasks containing 10 ml medium. Cultures were incubated at 24^{o}C for 2–3 weeks on a shaker. The capacity to degrade different biopolymers was examined by measuring the rate of CO_2 production in tightly closed 120 ml flasks containing 10 ml liquid medium MM with 0.05% (w/v) of the corresponding polymer substrate for 1 month at 24^{o}C. Control incubations were run in parallel under the same conditions but without substrate. Nitrogen sources were tested using liquid MM medium with 0.05% (w/v) glucose 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 or one of the amino acids listed in 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 CO_2 (up to 9-13%, vol/vol). 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 examined cultures and incubated under static conditions for 2 weeks. Dissolved O_2 concentration was 1.5 mg O_2 L^{-1} (measured in cultivation flasks prior to inoculation by using Dissolved Oxygen Meter sensION6, Hach, USA).

Strains PX4^T 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 (see below). Most sugars tested, including N-acetylglucosamine, were the preferred growth substrates. With the exception of pyruvate and succinate, organic acids were not utilized. Strains PX4^T and PT1 were capable of hydrolyzing aesculin, gelatin, lichenan, pectin, xylan and Phytagel, but not casein, chondroitin sulfate, laminarin, starch, pullulan, protein hydrolysate or cellulose. Despite its isolation from the enrichment culture of chitin-degrading microorganisms, no growth of strain PX4^T was detected in chitin-containing medium MM. The same was true for strain PT1. Ammonia, nitrate, N-acetylglucosamine, Bacto peptone, Bacto yeast extract, alanine, asparagine and valine were utilized as nitrogen sources. Neither of the two isolates grew under anoxic conditions, although 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 by using an API 20NE kit (bioMérieux) and as described for the Hugh–Leifson test (Gerhardt *et al*., 1981). Enzymatic activities were examined by using an API ZYM kit (bioMérieux). Catalase was tested by using the method 1 described by Gerhardt *et al*. (1981). Oxidase was tested using a REF 55 635 Oxidase Reagent (bioMérieux). Strains PX4^T and PT1

were catalase- and cytochrome oxidase -positive, but urease -negative. Dissimilatory nitrate reduction was negative. The following enzymatic activities (API ZYM) were detected in strains PX4^T and PT1: acid phosphatase, esterase, esterase lipase, leucine and valine arylamidases, 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, α-galactosidase, β-glucuronidase, α-fucosidase and α-mannosidase.

Susceptibility of strains PX4^T 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 (IPLs), 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 analyzed after acid hydrolysis of whole cells following the procedure described by Sinninghe Damsté *et al*. (2011). The major fatty acids (FA) detected in strain PX4^T and PT1 were *n*$C_{16:0}$, *n*$C_{18:1}\omega9$ and *n*$C_{18:0}$ (Table 1). A number of hydroxy FA, including the (ω-1)-OH *n*C28:0 hydroxy FA, and the n-C31 : 9 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 (PC) and trimethylornithine (TMO). Phosphoglycerol (PG) and 1-acyl-glycero-3-phosphocholine were also present in minor amounts. As reported by Moore *et al*. (2015), the ratio of TMO/(PC +PG) in cells grown in micro-oxic conditions was higher than that in fully aerobic conditions, which suggests that TMOs could be synthesized as a response to changing redox conditions in the oxic/anoxic interface.

Isoprenoid quinones of strains PX4^T and PT1 were analyzed as described for *Planctomicrobium piriforme* (Kulichevskaya *et al*., 2015). Similar to other members of the order

204 *Planctomycetales* (Ward, 2010), our isolates contained menaquinone-6 (MK-6) as the only
205 isoprenoid quinone. Based on genome sequence analysis, the DNA G+C content of strain PX4^T
206 is 66 mol % (Ivanova *et al*., unpublished data), which is higher than that in *Isosphaera* and
207 *Singulisphaera* but lower than that in *Aquisphaera* (Table 3).
208 PCR-mediated amplification of the 16S rRNA gene from DNA of strains PX4^T and PT1 was
209 performed using primers 9f and 1492r and reaction conditions described by Weisburg *et al.*
210 (1991). 16S rRNA gene amplicons were sequenced on an ABI 377A DNA sequencer using
211 BigDye terminator chemistry, as specified by the manufacturer (PE Applied Biosystems).
212 Phylogenetic analysis was carried out using the ARB program package (Ludwig *et al*., 2004).
213 The significance levels of interior branch points obtained in the neighbour-joining analysis were
214 determined by bootstrap analysis (based on 1000 data resamplings) using PHYLIP (Felsenstein,
215 1989). The comparative analysis based on nearly full-length 16S rRNA gene sequences
216 confirmed that strains PX4^T and PT1 belong to the order *Planctomycetales* and are members of
217 the coherent phylogenetic cluster defined by the genus *Isosphaera* (Fig. 2). The minimum
218 sequence identity within this cluster is about 90%, which is close to the taxonomic threshold
219 defined for the family level (Yarza *et al*., 2014). This cluster is strongly supported by all
220 algorithms used for the tree construction and accommodates morphologically similar, stalk-free
221 planctomycetes with spherical cells, which can be assembled in short chains, long filaments or
222 shapeless aggregates. Notably, daughter cells of these budding bacteria are non-motile. The 16S
223 rRNA gene sequence identity between members of the *Isosphaera*-like cluster and other
224 taxonomically described organisms within the order *Planctomycetales* is in the range of 76.8-
225 81.9%. Based on this phylogenetic divergence (Fig. 2) and the morphological similarity between
226 members of the *Isosphaera*-like cluster, the latter should be given the status of a family, i.e.
227 *Isosphaeraceae* fam. nov. Strains PX4^T and PT1 are members of the *Isosphaeraceae*, but are
228 phylogenetically divergent, morphologically distinct and phenotypically different from other
229 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 *Isosphaera 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 *Aquisphaera giovannonii*. Finally, strains PX4^T and PT1 can be distinguished from members of the genus *Singulisphaera* by the formation of cell chains, the ability to hydrolyze Phytagel, the absence of C18:2ω6c,12c fatty acid, and the lower DNA G+C content (Table 3). In addition, cells of novel isolates are smaller than cells of *Singulisphaera* spp.

Strains PX4^T and PT1 displayed 93-94% 16S rRNA gene sequence similarity to *Aquisphaera giovannonii*, 91-92% to *Singulisphaera* species and 90-91% to *Isosphaera pallida*. The overall similarities between the genome of strain PX4^T (Ivanova *et al*., unpublished data) and the genomes of *Isosphaera pallida* IS1B^T and *Singulisphaera acidiphila* DSM 18658^T estimated using formula 2 of the Genome-to-Genome-Distance-Calculator (Auch *et al*., 2010) is 19.8 ± 2.3 and 20.0 ± 2.3, respectively. These DDH values are similar to those calculated for members of different genera (Scheuner *et al*., 2014). Average nucleotide identity values generated by comparing the genome of strain PX4^T and the genomes of *Isosphaera pallida* IS1B^T and *Singulisphaera acidiphila* DSM 18658^T are also very low, i.e. 75 and 77%, respectively. We, therefore, propose to classify strains PX4^T and PT1 as representing a novel genus and species, *Paludisphaera borealis* gen. nov., sp. nov. The characteristics that differentiate the genus *Paludisphaera* 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 –udis* 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 scattered all over cell

surface. Stalk-like structures are absent. Colonies are opaque and pink colored. Chemo-organotrophic aerobes. Capable of growth under micro-oxic conditions. Possess hydrolytic capabilities. Dissimilatory nitrate reduction is negative. Moderately acidophilic and mesophilic. Sensitive to NaCl. The major quinone is MK-6. The major fatty acids are *n*C16:0, *n*C18:1ω9 and *n*C18:0. 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.*

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 acid, benzoate, caproate, citrate, formate, formaldehyde, fumarate, glutarate, lactate, malate, propionate, mannitol, tartrate, alanine, arginine, asparagine, aspartate, cysteine, cystine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, norleucine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine or valine. Capable of hydrolyzing aesculin, gelatin, lichenan, pectin, xylan and Phytagel. Cannot hydrolyze casein, chondroitin sulfate, laminarin, starch, peptone, pullulan, protein hydrolysate, fucoidan, chitin or cellulose. Catalase-, cytochrome oxidase-positive, but urease-negative. Nitrogen sources (0.05 %, w/v) include ammonia, nitrate, N-acetylglucosamine, Bacto peptone, Bacto yeast extract, alanine, asparagine and valine. Aspartate, arginine, glutamine, glycine, isoleucine, lysine, threonine, tryptophan, phenylalanine, proline and urea are not utilized. Growth factors are required. Possess the following enzyme activities: acid phosphatases, esterase, esterase lipase, leucine arylamidase,

valine arylamidase, phosphohydrolase, N-acetyl-β-glucosaminidase and β-galactosidase (API ZYM test). The following enzyme activities are not present: alkaline phosphatase, cystine arylamidase, lipase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-fucosidase and α-mannosidase. Resistant to ampicillin, streptomycin, chloramphenicol, lincomycin, kanamycin, but sensitive to neomycin, novobiocin and gentamicin. Growth occurs at pH 3.5-6.5 (optimum at pH 5.0-5.5) and at temperatures between 6°C and 30°C (optimum at 15-25°C). Growth is inhibited at NaCl concentrations above 0.5% (w/v). The DNA G+C content is 66 mol%. Wetlands are the main habitat. The type strain, PX4^T (= DSM 28747^T = VKM B-2904^T), was isolated from the peat bog Obukhovskoye, Yaroslavl region, Russia.

Description of *Isosphaeraceae* fam. nov.

Isosphaeraceae (I.so.sphae.ra.ce´ae. 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, in pair or are assembled in short chains, long filaments or shapeless aggregates. Crateriform pits are scattered all over 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*.

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376 **Table 1**. Relative abundance (% of total) of lipids extracted after acid hydrolysis of cell material
377 of strain PX4^{T} and PT1. (Major components are given in bold).
378

	PX4^{T}	**PT1**
Fatty acids		
*n*C14:0	3.4	3.2
*n*C16:1ω9	1.8	1.7
*n*C16:1ω7	0.3	0.6
*n*C16:0	**17.4**	**16.5**
*n*C17:1ω8	0.4	-
*n*C18:1ω9	**47.9**	**50.9**
*n*C18:0	**12.4**	**7.4**
Hydroxy fatty acids		
β-OH *n*C14:0	-	1.0
β-OH *n*C18:0	0.8	2.4
α- OH *n*C25:0	0.4	0.3
α- OH *n*C27:1	-	0.5
α- OH *n*C27:0	4.7	4.4
α- OH *n*C29:1	0.8	1.7
α- OH *n*C29:0	0.5	0.5
α,(ω-1)-diOH *n*C29:0	0.7	0.5
(ω-1)-OH *n*C28:0	1.2	1.3
(ω-1)-OH *n*C30:1	0.6	1.3
α,(ω-1)-diOH *n*C31:0	1.9	1.6
C_{33} α-OH hopanoic acid	2.4	2.9
Other lipids		
*n*C31:9 hydrocarbon	2.4	1.2

379

Table 2. Relative abundances and fatty acid composition of IPLs of strain PX4^T and PT1.

IPL	Strain PX4^T	Strain PT1	Fatty acid composition
PG	+	+	(C36:2, C34:1, C32:1)
PC	+++	+++	(C36:2, C34:1, C32:1)
TMO	++	+	(C18:1, βOH C18)
lyso-PC	tr	+	(C18:1,C16:0)

The abundance is relative to the major peak in the LC/MS base peak chromatogram: +++=base peak, ++=50-100% of base peak, +=10-50% of base peak, tr=<10% of base peak. Note that the mass spectral response factor for different IPL groups can be quite different. The predominant fatty acid composition is reported as the total number of the acyl moieties and the number of double bonds (except for TMO and lyso-PC where individual acyl moieties are given). PG=phosphoglycerol, PC=phosphocholine, TMO= trimethylornithine , lyso-PC=1-acyl-glycero-3-phosphocholine

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

Characteristic	***Paludisphaera***	***Isosphaera****	***Singuli-sphaera***	***Aquisphaera****
Arrangement of cells	Single, in pairs or short chains	Filaments	Single or in pairs	Single or aggregates
Cell size, µm	1.5-2.5	2.5-3.0	1.6-3.5	1.6-2.0
Gliding motility	-	+	-	-
Photo-taxis	-	+	-	-
Habitat	Wetlands	Hot springs	Wetlands	Freshwater
Colony colour	Pink	Pink	Colourless or pink	Pink
Respiration	Aerobic or microaerophilic	Strictly aerobic	Aerobic or microaerophilic	Strictly aerobic
Hydrolysis of Phytagel aesculin starch xylan	 + + - +	 ND ND ND ND	 - + - +	 ND - + -
Temperature growth range (^{o}C) Optimal temperature (^{o} C)	6-30 15-25	34-55 40-50	4-33 15-28	10-35 30-35
pH growth range pH optimum	3.5-6.5 5.0-5.5	ND 7.8-8.8	4.2-7.5 5.0-6.2	6.5-9.5 7.5-8.5
Vitamin requirement	None	ND	None	B_{12}
Presence of C18 : 2ω6c,12c fatty acids	-	ND	+	-
G+C content (mol%)****	66	62	62	70

393 *Data from Giovannoni *et al*. (1987) and Göker *et al*. (2011).

394 **Data from Kulichevskaya *et al*. (2008, 2012) and Scheuner *et al.* (2014).

395 ***Data from Bondoso *et al*. (2011).

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

398 ND, not determined

399

400 **FIGURE CAPTIONS**

401

402 **Figure 1.** (**a**) Development of depressions in Phytagel-solidified medium during colony growth
403 of strain PX4^T. Bar, 10 mm. (**b**) Phase-contrast image of cells of strain PX4^T in 10-day-old
404 culture. Bar, 10μm. (**c -d**) Electron micrographs of negatively stained cells of strain PX4^T
405 displaying crateriform pits scattered all over cell surface (**c**), a newly formed bud (indicated by
406 black arrow in **c** and **d**) and an extracellular material of a net-like structure excreted by the cell
407 (**e**). Bars, 1 μm (**c, e**) and 0.2 μm (**d**).

408

409 **Figure 2**. 16S rRNA gene-based neighbour-joining tree (Jukes-Cantor correction) showing the
410 phylogenetic relationship of strain PX4^T and PT1to representative members of the order
411 *Planctomycetales.* The bracket on the right indicates boundaries proposed for the family
412 *Isosphaeraceae*. The significance levels of interior branch points obtained in neighbor-joining
413 analysis were determined by bootstrap analysis (1000 data re-samplings) using PHYLIP
414 (Felsenstein, 1989). Bootstrap values (1000 data resamplings) of >50% are shown. Black circles
415 indicate that the corresponding nodes were also recovered in the maximum-likelihood and
416 maximum-parsimony trees. The root (not shown) was composed of five 16S rRNA gene
417 sequences from anammox planctomycetes (AF375994, AF375995, AY254883, AY257181,
418 AY254882). Bar, 0.1 substitutions per nucleotide position.

Figure 1

Click here to download Figure Figure 1.jpg

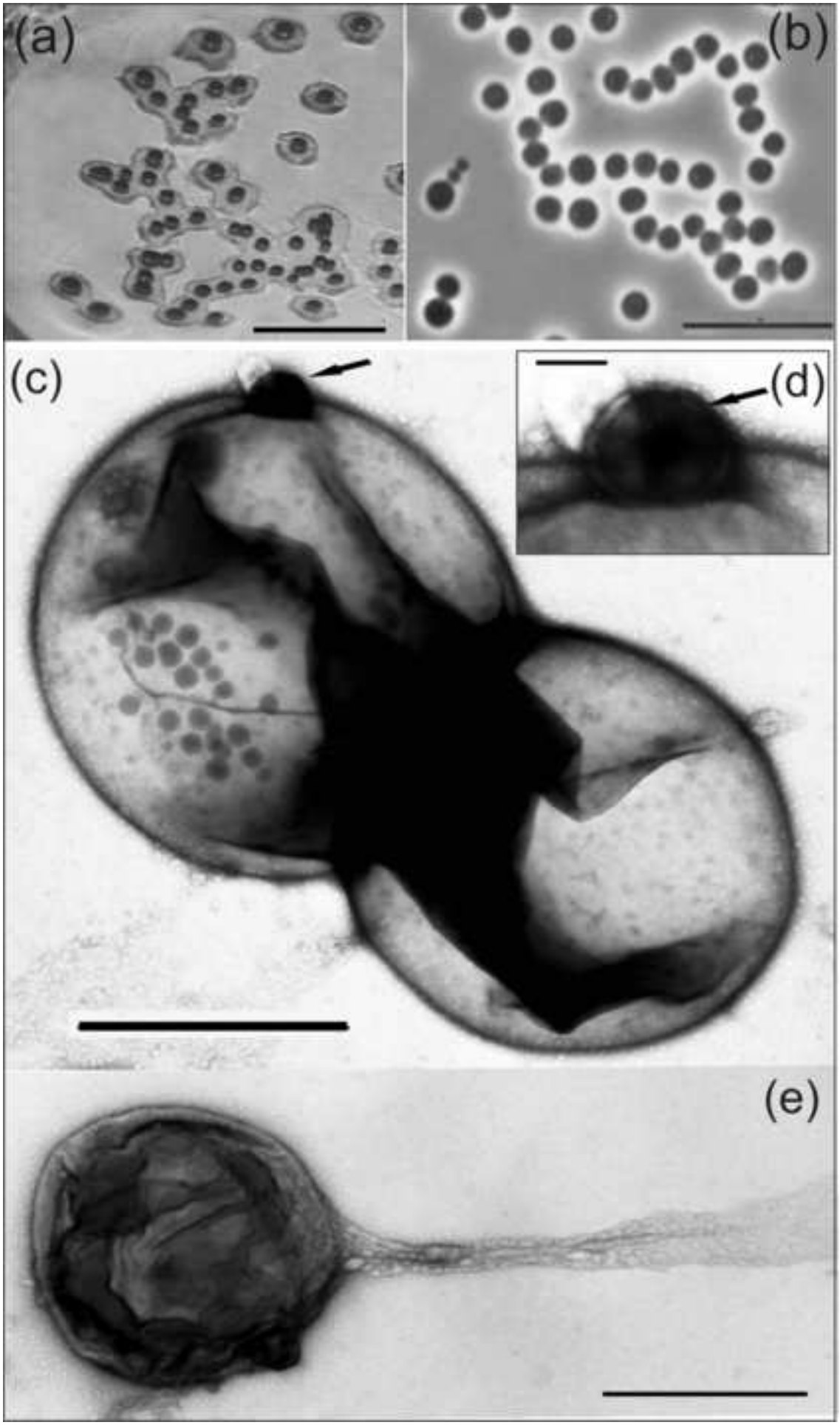

Figure 2

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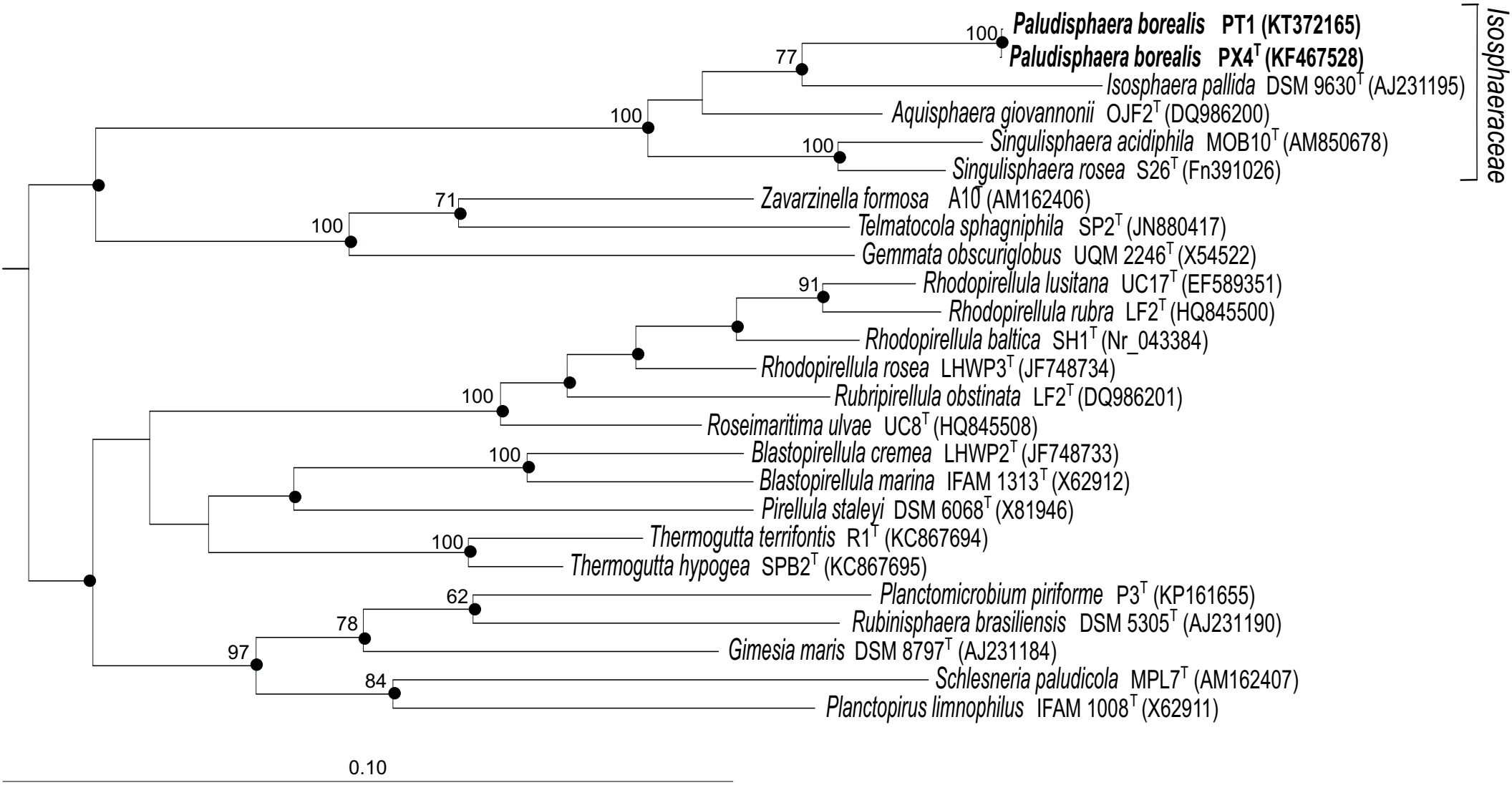

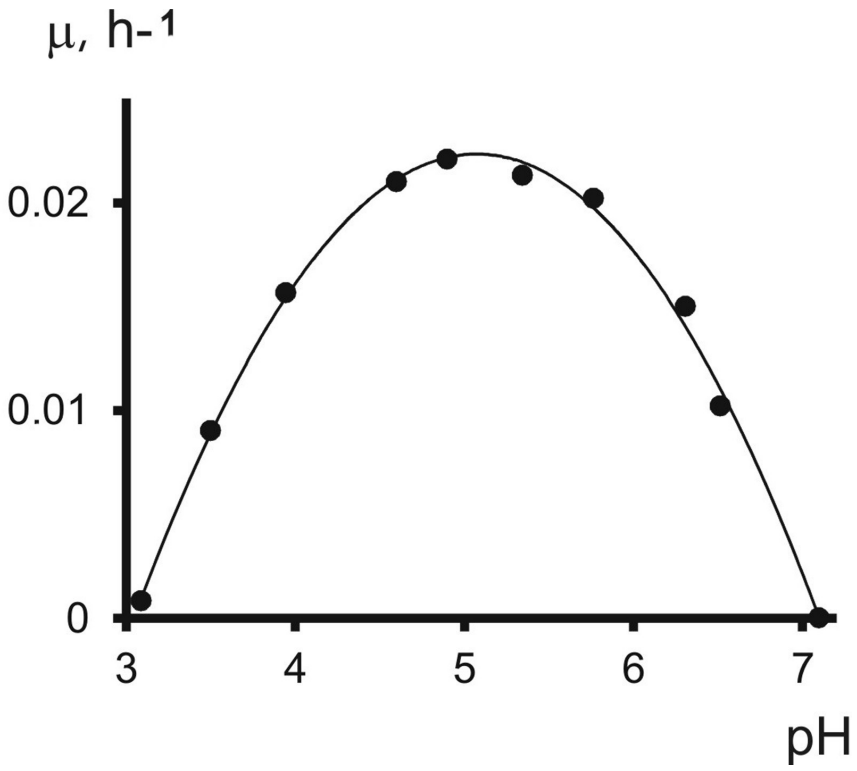

Supplementary Fig. S1. Influence of medium pH on the growth of strain PX4^{T}.