**Pedobacter jamesrossensis** sp. nov., *Pedobacter lithocola* sp. nov., *Pedobacter mendelii* sp. nov. and *Pedobacter petrophilus* sp. nov., isolated from the Antarctic environment

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**Abstract**

A taxonomic study performed on 17 Gram-stain-negative rod-shaped bacterial strains originating from the Antarctic environment is described. Initial phylogenetic analysis using 16S rRNA gene sequencing differentiated the strains into four groups belonging to the genus *Pedobacter* but they were separated from all hitherto described *Pedobacter* species. Group I (n=8) was closest to *Pedobacter aquatilis* (97.8% 16S rRNA gene sequence similarity). Group II (n=2) and group III (n=4) were closely related (98.8% 16S rRNA gene sequence similarity) and had *Pedobacter jejuni* as their common nearest neighbour. Group IV (n=3) was distantly delineated from the remaining *Pedobacter* species. Differentiation of the analysed strains into four clusters was further confirmed by repetitive sequence-based PCR fingerprinting, ribotyping, DNA–DNA hybridization and phenotypic traits. Common to representative strains for the four groups were the presence of major menaquinone MK-7, sym-homospermidine as the major polyamine, phosphatidylethanolamine, two unidentified lipids (L2, L5) and an unidentified aminolipid (AL2) as the major polar lipids, presence of an alkali-stable lipid, and C16:1ω7c/C16:1ω6c (summed feature 3), iso-C15:0 and iso-C17:0 3-ΩH as the major fatty acids, which corresponded to characteristics of the genus *Pedobacter*. The obtained results showed that the strains analysed represent four novel species of the genus *Pedobacter*, for which the names *Pedobacter jamesrossensis* sp. nov. (type strain CCM 8689T=LMG 29686T), *Pedobacter lithocola* sp. nov. (CCM 8691T=LMG 29685T), *Pedobacter mendelii* sp. nov. (CCM 8685T=LMG 29688T) and *Pedobacter petrophilus* sp. nov. (CCM 8687T=LMG 29686T) are proposed.

The genus *Pedobacter* [1] represents strictly aerobic, Gram-stain-negative, rod-shaped bacteria. Phylogenetically, they belong to the family *Sphingobacteriaceae* within the phylum *Bacteroidetes*. Members of the genus *Pedobacter* exhibit catalase, oxidase and phosphatase activity but they typically do not reduce nitrate or exhibit urease activity. The presence of MK-7 as the major respiratory menaquinone, phosphatidylethanolamine as the major polar lipid, sym-homospermidine as the major polyamine and the presence of sphingolipids is typical for the genus [1, 2]. *Pedobacter* species have been isolated from different environmental, mainly terrestrial and aquatic, habitats worldwide, including Arctic and Antarctic regions (e.g. [3–5]).

The present study describes the taxonomic investigation of 17 *Pedobacter* strains isolated in the frame of a project dealing with the investigation of cultivable bacteria inhabiting the Antarctic environment and performed at the Johann Gregor Mendel Antarctic station situated on James Ross Island; Antarctica.

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**Keywords:** Pedobacter jamesrossensis sp. nov.; Pedobacter lithocola sp. nov.; Pedobacter mendelii sp. nov.; Pedobacter petrophilus sp. nov.; James Ross Island; Antarctica.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences obtained in this study are KX611467–KX611483; *Pedobacter jamesrossensis* sp. nov. strain CCM 8689T, accession number KX611467; CCM 8690, KX611474; P3164, KX611468; P3658, KX611469; P3679, KX611471; P3802, KX611471; P3824, KX611472; P4838, KX611473; Pedobacter lithocola sp. nov. CCM 8691T, KX611475; P3926, KX611476; Pedobacter mendelii sp. nov. CCM 8685T, KX611477; CCM 8686, KX611478; P2490, KX611479; P2749, KX611480; Pedobacter petrophilus sp. nov. CCM 8687T, KX611481; CCM 8688, KX611482; P4106, KX611483.

Six supplementary figures and three supplementary tables are available with the online Supplementary Material.
Island near the Antarctic Peninsula. All strains were isolated from environmental materials sampled at James Ross Island (Table 1). Individual samples were suspended in sterile saline solution and 200 µl of the suspension was spread on R2A agar plates and cultivated at 15 ºC for 5–7 days. Individual colonies were picked up, purified and pure cultures were maintained at R2A agar slant agar until transported and analysed.

Reference type strains Pedobacter agri CCM 8572<sup>T</sup>, Pedobacter alluvionis CCM 8562<sup>T</sup>, Pedobacter aquatilis CCM 7347<sup>T</sup>, Pedobacter borealis CCM 8563<sup>T</sup>, Pedobacter ginsengidimutans CCM 8564<sup>T</sup> and Pedobacter jejuensis CCM 8565<sup>T</sup> were obtained from the Czech Collection of Microorganisms and Pedobacter sandarakinus KCTC 12559<sup>T</sup> were obtained from the Korean Collection for Type Cultures.

Genomic DNAs for 16S rRNA gene sequence analysis were extracted using a FastPrep Lysing Matrix type B and FastPrep Homogenizer (MP Biomedicals) and purified by using a High Pure PCR Template Preparation Kit (Roche Diagnostics). A fragment of the 16S rDNA gene corresponding to coordinates 8–1542 used for Escherichia coli was amplified by PCR with FastStart PCR Master (Roche Diagnostics) and conserved primers pA (AGAGTTTATCCTGCTCAAGG) and pH (AAGGAGGTGATCCTGGCACG) described by Edwards et al. [6], and purified using a QIAquick PCR Purification Kit (Qiagen). Sequencing was performed using PCR primers and custom primers F1 (GTGGGGAKCRAARAC), F2 (CGTCARGTCMTCATGGCCCTT), R1 (ATTACCGGCGTGTGCTGAC) and R2 (CACATSMCCCCTRATTGT) in the Eurofins MWG Operon sequencing facility. Initial identification of the 16S rRNA gene sequences using the EzTaxon database [7] showed that all investigated strains are members of the genus Pedobacter. Strain CCM 8689<sup>T</sup> (group I) was closest to P. jejuensis THG-DR3<sup>T</sup> (98.6 % 16S rRNA gene sequence similarity) and P. aquatilis AR107<sup>T</sup> (97.9 %); strain CCM 8691<sup>T</sup> (group II) was closest to P. jejuensis THG-DR3<sup>T</sup> (98.8 %), P. aquatilis AR107<sup>T</sup> (97.6 %) and P. alluvionis NWER-III11<sup>T</sup> (97.3 %); strain CCM 8685<sup>T</sup> (group III) was closest to P. aquatilis AR107<sup>T</sup> (97.8 %) and P. jejuensis THG-DR3<sup>T</sup> (97.5 %); and strain CCM 8687<sup>T</sup> (group IV) was closest to P. agri PB92<sup>T</sup> (97.7 %), P. borealis DSM 19625<sup>T</sup> (97.6 %), P. alluvionis NWER-III11<sup>T</sup> (97.4 %), P. ginsenosidimutans THG-45<sup>T</sup> (97.3 %) and P. sandarakinus DS-27<sup>T</sup> (97.1 %).

Except the similarity value between strain CCM 8691<sup>T</sup> (group II) and P. jejuensis THG-DR3<sup>T</sup> (98.8 %) all 16S rRNA gene sequence similarities were below the 98.7 % similarity threshold suggested by Stackebrandt and Ebers [8], who showed that two strain pairs with 16S rRNA gene sequence similarity of less than 98.7 % have DNA–DNA reassociation values of less than 70 %. Phylogenetic comparison of the obtained sequences with 16S rRNA gene sequences of validly named Pedobacter species retrieved from the GenBank database was performed using MEGA 6 software [9]. Genetic distances were corrected using Kimura’s two-parameter model and the evolutionary history was inferred using the neighbour-joining, maximum-likelihood and maximum-parisimony methods. Sequence similarities between individual strains were calculated using BioNumerics 7.6 software (Applied Maths). Neighbour-joining clustering (Fig. 1).

Table 1. Origin of the analysed Pedobacter strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample</th>
<th>Locality</th>
<th>Year</th>
<th>GPS</th>
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<td>sp. nov. (group I)</td>
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<td>CCM 8689&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Stone fragments</td>
<td>Devils Rocks</td>
<td>2009</td>
<td>63° 51’ 41” S 57° 49’ 10” W</td>
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<tr>
<td>CCM 8690</td>
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<td>Lachman Crags</td>
<td>2012</td>
<td>63° 49’ 38” S 57° 50’ 7.4” W</td>
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<td>2009</td>
<td>63° 51’ 20” S 57° 49’ 52” W</td>
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<tr>
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<tr>
<td>P3679</td>
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<td>2010</td>
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<tr>
<td>P3802</td>
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<td>2011</td>
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<tr>
<td>P3824</td>
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<td>Halozetes Valley</td>
<td>2011</td>
<td>63° 49’ 15” S 57° 48’ 30” W</td>
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<td>P4838</td>
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<td>Big Lachman Lake</td>
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<td>63° 48’ 52” S 57° 50’ 36” W</td>
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<td>Lachman Crags</td>
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<tr>
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<td>Crame Col</td>
<td>2007</td>
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<td>sp. nov. (group IV)</td>
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<td>Devils Rocks</td>
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<td>63° 51’ 41” S 57° 49’ 10” W</td>
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<td>CCM 8688</td>
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<td>P4106</td>
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<td>Devils Rocks</td>
<td>2011</td>
<td>63° 49’ 52” S 57° 50’ 27.6” W</td>
</tr>
</tbody>
</table>
Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of groups I–IV strains within the genus Pedobacter. The evolutionary history was inferred by using the Kimura two-parameter model. Bootstrap probability values (percentages of 1000 tree replications) greater than 50% are indicated at branch points. All positions with less than 95% site coverage were eliminated. Flavobacterium aquatile DSM 1132T (AM230485) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
matched the tree topology obtained by the maximum-likelihood (Fig. S1, available in the online Supplementary Material) and maximum-parsimony analysis (Fig. S2) and separated the strains into four groups (I, II, III and IV) that were differentiated from established Pedobacter species. Group I consisted of eight strains sharing 99.9–100 % 16S rRNA gene sequence similarity. Isolates assigned to groups II, III and IV showed 100 % 16S rRNA gene sequence similarity within individual clusters. Phylogenetically, group I was closest to P. aquatilis AR107T. Groups II and III were closely related to each other (98.8 % 16S rRNA gene sequence similarity) and had P. jejuensis THG-DR3T as their closest phylogenetic neighbour. Group IV was most similar to, but only distantly related to, the P. agri–P. borealis–P. ginsenosidimutans cluster.

Repetitive sequence-based PCR (rep-PCR) fingerprinting using the (GTG)5 primer and automated ribotyping with the EcoRI restriction enzyme were performed to assess the genetic variability between individual strains assigned to groups I–IV. rep-PCR fingerprinting with the (GTG)5 primer was performed as described by Švec et al. [10]. The automatic ribotyping was performed using the RiboPrinter Microbial Characterization System (DuPont Qualicon) in accordance with the manufacturer’s instructions. Numerical analysis and dendrogram construction was done using the BioNumerics 7.6 software (Applied Maths). The ribotype patterns were imported into the BioNumerics software using the load samples import script provided by the manufacturer. Numerical analysis of rep-PCR fingerprints showed a high genetic homogeneity within strains assigned to groups I–IV. Individual strains revealed visually similar fingerprints which clearly separated these groups from each other (Fig. S3). Heterogeneity was revealed only among group I strains due to a distinct fingerprint revealed by strain CCM 8690. In contrast, automated ribotyping using the EcoRI restriction enzyme showed closely similar ribotype patterns within all strains in group I and also confirmed the genetic homogeneity within groups II and IV. This method, however, separated group III strains into two subclusters (Fig. S4). Both DNA fingerprinting techniques also clearly differentiated groups I–IV from the type strains representing the phylogenetically closest Pedobacter species.

Total high-molar-mass genomic DNA extraction for the G+C content analysis and DNA–DNA hybridization experiments was performed as described by Cleenwerck et al. [11]. The G+C DNA content was determined using the HPLC method described by Mesbah and Whitman [12]. Strains CCM 8689T (group I), CCM 8691T (group II), CCM 8685T (group III) and CCM 8687T (group IV) revealed 36.2, 35.6, 35.5 and 38.8 mol% G+C genomic DNA content, respectively. DNA–DNA hybridization was done to confirm the differentiation of groups I, II and III from the phylogenetically nearest Pedobacter species and to confirm that the phylogenetically neighbouring groups II and III are representatives of different species. Group IV, which had a distinct phylogenetic position, was not included in DNA–DNA hybridization experiments. The method was performed using the microplate technique described by Ezaki et al. [13], according to the protocols described previously [11, 14]. DNA–DNA relatedness percentages were calculated as means based on at least three independent hybridizations. Reciprocal reactions were performed and also considered as independent experiments. The standard deviation between reciprocal reactions was approximately 7 %, as reported by Goris et al. [14]. The level of DNA–DNA hybridization obtained between strain CCM 8689T (group I) and P. aquatilis CCM 7347T and P. jejuensis CCM 8565T was 21 and 15 %, respectively. The hybridization values between strains CCM 8691T (group II) and CCM 8685T (group III) and their phylogenetically nearest neighbours P. jejuensis CCM 8565T and P. aquatilis CCM 7347T were in the range from 15 to 20 %. DNA–DNA hybridization also showed that group II and group III strains represent different species because the hybridization value between strains CCM 8691T and CCM 8685T was 29 %. All DNA–DNA hybridization values obtained were well below the 70 % threshold [15], which confirmed that each of the strains analysed represented a novel species.

Phenotypic characteristics of all 17 strains and of the type strains of nearest neighbouring species P. aquatilis CCM 7347T, P. ginsengiterrae CCM 8571T and P. jejuensis CCM 8565T were assessed by a set of key tests relevant for Gram-negative rod-shaped bacteria. Oxidase (OXItest; Erba-Lachema) and catalase (ID colour Catalase; bioMérieux) activity was verified according to the manufacturers’ instructions. Cellular morphology was investigated by Gram staining [16] and transmission electron microscopy using a Morgagni 268D Philips (FEI Company) electron microscope (Fig. S5). Further tests for the following were done: oxidation-fermentation (OF) [17], urease [18], arginine dihydrolase, ornithine and lysine decarboxylase [19], hydrolysis of aesculin, starch [20], gelatin, Tween 80 [21], casein, tyrosine [22] and DNA (CM321; Oxoid), egg-yolk reaction [23], ONPG [24], nitrate and nitrite reduction, indol production, growth on Simon’s citrate agar [20], and utilization of acetamide [25] and sodium malonate [26]. Motility was observed in a glucose oxidation tube. Screening for flexirubin-type pigment production was done using a 20 % KOH test as described by Bernardet et al. [27]. Growth at different temperatures (5–35 °C in increments of 5.0 °C) and NaCl concentrations (0, 1, 2 and 3 %, w/v) was tested on R2A agar (Oxoid) adjusted accordingly. The pH range for growth was tested on R2A agar adjusted to pH 5.0–12.0 (in increments of 1 pH unit) by using the following buffer systems: pH 5.0–8.0, 0.1 M KH2PO4/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO3/0.1 M Na2CO3; pH 11.0–12.0, 0.05 M Na2HPO4/0.1 M NaOH. The pH of the R2A agar was confirmed after autoclaving. Aerobic growth was tested on brain heart infusion agar (Oxoid), Columbia blood agar (Oxoid), MacConkey agar (Oxoid), nutrient agar (Oxoid), plate count agar (Oxoid), R2A agar (Oxoid) and tryptone soya agar (Oxoid) and anaerobic growth was tested on R2A agar using the Anaerocult A system (Merck). The aforementioned
biochemical and physiological tests were assessed using cells grown on R2A agar at 20 °C and read daily for up to 7 days with incubation at 20 °C. Phenotype screening showed that all strains were Gram-stain-negative, aerobic, non-fermenting, oxidase- and catalase-positive rods. Group I, II and III strains produced small red colonies and group IV strains produced pink colonies when cultivated on R2A agar plates at 20 °C. Further characterization using the Biolog system with the Gram-negative identification test panel GEN III MicroPlate (Biolog) and the API ZYM microtest system (bioMérieux) was done to obtain additional phenotypic data. Antibiotic resistance testing was done by the disc diffusion method on R2A agar (Oxoid). Sixteen antibiotic discs (Oxoid) relevant for Gram-negative rods [28, 29] were tested: ampicillin (10 µg), aztreonam (30 µg), carbenicillin (100 µg), cefixim (5 µg), cefotaxim (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), chloramphenicol (30 µg), imipenem (10 µg), kanamycin (30 µg), cotrimoxazol (25 µg), piperacillin (30 µg), polymyxin B (300 U), streptomycin (10 µg) and tetracycline (30 µg). CLSI/EUCAST standards were followed for cultivation and inhibition zone diameter reading [28, 29]. The biochemical and physiological characteristics of group I–IV strains are listed in the species descriptions below. The tests distinguishing group I–IV strains and enabling their straightforward differentiation from their nearest neighbouring species are shown in Table 2. Test results obtained with the GEN III MicroPlate test panel (Biolog) are listed in Table S1 and antibiotic susceptibility profiles of group I–IV strains are given in Table S2.

Chemotaxonomic analyses of the representative strains CCM 8689 T (group I), CCM 8691 T (group II), CCM 8685 T (group III) and CCM 8687 T (group IV) were performed to further characterize the analysed groups.

Analysis of fatty acid methyl esters was performed using an Agilent 7890B gas chromatograph according to the standard protocol of the Sherlock MIDI Identification System (MIDI Sherlock version 6.2, MIDI database RTSBA 6.21). Bacterial cells were grown on R2A agar (Oxoid) at 20±2 °C for 48 h, where the bacterial communities reached the late-exponential stage of growth according to the four quadrants streak method [30]. The predominant fatty acids of strains CCM 8689 T, CCM 8691 T, CCM 8685 T and CCM 8687 T were summed feature 3 (C16:1ω7c/C16:1ω6c), iso-C15:0 3-OH and iso-C17:0 3-OH, which corresponded to the fatty acids typically found in other Pedobacter species [2]. The complete cellular fatty acid compositions of strains CCM 8689 T, CCM 8691 T, CCM 8685 T and CCM 8687 T and the type strains representing the nearest neighbour species are shown in Table S3. Freeze-dried biomass for the following chemotaxonomic analyses was prepared from bacterial cells grown on R2A agar plates cultivated at 20 °C for 72 h. Quinones and polar lipids were extracted from freeze-dried biomass and analysed as described previously [31–34]. Analysis of all four strains revealed the presence of the major menaquinone MK-7 and minor amounts of MK-6 (CCM 8691 T: 90.9 % MK-7, 9.1 % MK-6; CCM 8687 T: 77.9 % MK-7, 22.1 % MK-6; CCM 8689 T: 91.4 % MK-7, 8.6 % MK-6; CCM 8685 T: 97.7 % MK-7, 2.3 % MK-8). In the polar lipid profiles, common to all four strains were the major polar lipids phosphatidylethanolamine, the unidentified lipids L2 and L5 and the unidentified aminolipid AL2 (Fig. S6). The presence of the unidentified aminophospholipid APL1, the aminoglycolipid AG1, the aminoglycophospholipid AGPL1, the glycolipid GL2, the aminolipid AL3 and the lipids L3 and L7 distinguished strain CCM 8687 T from the other three strains. Strain CCM 8689 T was distinguishable from the other three strains by the presence of lipids L8 and L9. The presence of glycolipid GL1 distinguished strain CCM 8685 T from strain CCM 8691 T and both strains showed a less complex polar lipid profile than the other two strains. The presence of alkali-stable lipids characteristic for sphingolipids in the analysed strains was investigated according to Kato et al. [35]. All four strains produced one alkali-stable lipid that was detected after staining with ninhydrin, but none of these was positive for the presence of a phosphate group after staining with molybdenum blue. Hence, the alkali-stable lipids detected are not sphingophospholipids as reported in members of the neighbouring genus Sphingobacterium [36]. Polyamines were extracted from freeze-dried biomass cultivated in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at 20 °C for 72 h as described previously [37]. HPLC conditions applied

Table 2. Biochemical differentiation of Pedobacter jamesrossensis sp. nov., Pedobacter lithocola sp. nov., Pedobacter mendeli sp. nov. and Pedobacter petrophilus sp. nov. from phylogenetically related Pedobacter species

<table>
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<th>Taxa: 1, P. jamesrossensis sp. nov. (group I); 2, P. lithocola sp. nov. (group II); 3, P. mendeli sp. nov. (group III); 4, P. petrophilus sp. nov. (group IV); 5, P. aquatilis CCM 7347 T; 6, P. ginsengiterrae CCM 8571 T; 7, P. jeunesis CCM 8565 T.</th>
<th>Characteristic</th>
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<td>−</td>
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*Two positive out of eight strains.
†Two positive out of four strains.
for analyses of the polyamine patterns were as described by Busse et al. [38] and the HPLC apparatus was as described by Stolz et al. [32]. sym-Homospermidine was the major polyamine in all four strains, which is in accordance with the genus description [1]. Strain CCM 8689T contained (per gram dry weight) 11.8 µmol sym-homospermidine, 0.1 µmol cadaverine and traces of putrescine and spermidine (<0.1 µmol); strain CCM 8691T contained 14.9 µmol sym-homospermidine, 0.1 µmol spermidine and traces of putrescine, cadaverine and spermine (<0.1 µmol); strain CCM 8685T contained 3.1 µmol sym-homospermidine and traces of cadaverine and spermidine (<0.1 µmol); strain CCM 8687T contained 17.8 µmol sym-homospermidine, 0.2 µmol spermidine and traces of cadaverine and spermine (<0.1 µmol). The aforementioned chemotaxonomic characteristics of strains CCM 8689T, CCM 8691T, CCM 8685T and CCM 8687T corresponded with their assignment to the genus Pedobacter [1, 2].

All results obtained in the present study demonstrated that each of the groups I, II, III and IV represents a distinct novel species within the genus Pedobacter, for which the names Pedobacter jamesrossensis sp. nov., Pedobacter lithocola sp. nov., Pedobacter mendelii sp. nov. and Pedobacter petrolitus sp. nov., respectively, are proposed.

In each of the species descriptions, the numbers given in parentheses in strain-dependent test results show the number of strains revealing a positive reaction. Test results obtained using the Biolog GEN III MicroPlate test panel are given in Table S1. Antibiotic susceptibility profiles are given in Table S2.

PEDOBACTER JAMESROSSSENSIS SP. NOV.

Pedobacter jamesrossensis (ja.mes.ross.en’sis. N.L. masc. adj. jamesrossensis pertaining to James Ross Island where the type strain was isolated).

Cells are Gram-stain-negative, short rods, 1–1.2×0.5 μm, occurring predominantly in pairs or in irregular clusters, non-motile and non-spore-forming. Colonies on R2A agar are reddish, circular, slightly convex, smooth and glistening with whole margins, and reach about 1–2 mm in diameter when cultivated at 20°C for 5 days. Flexirubin-type pigments are absent. The species is aerobic; no anaerobic growth on R2A agar is detected. Aerobic but non-fermenting in the OF test. Grows at 1–30°C, but not at 35°C. Grows in the presence of up to 1% (w/v) NaCl; growth in 2% NaCl is strain dependent (4) and is inhibited in 3% NaCl. Grows at pH 6–10; growth at pH 11 is strain dependent (3) and growth is not detected at pH 5 or 12. Most abundant growth is observed on R2A agar without NaCl, at pH 8.0 and at 20°C. Fluorescein pigment is not produced on King B medium. Grows on plate count agar, tryptone soya agar, R2A agar, brain heart infusion agar and nutrient agar, but not on MacConkey agar. Positive reactions for catalase and oxidase, ONPG test and hydrolysis of starch and DNA. Aesculin hydrolysis is strain dependent (2). Negative for Simon’s citrate, sodium malonate, acetamide, hydrolysis of Tween 80, gelatin, casein, tyrosine and lecithin (egg-yolk reaction), production of indol, urease, arginine dihydrolase, lysine and ornithine decarboxylase and reduction of nitrate and nitrite. Enzymatic reactions tested by the API ZYM kit revealed positive test results for alkaline phosphatase, acid phosphatase, leucine arylamidase, valine arylamidase, trypsin, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase, and weakly positive for esterase lipase (C8); negative test results for lipase (C14), chymotrypsin, α-galactosidase, β-glucuronidase; and strain-dependent test results for esterase (C4) (1), cystine arylamidase (6), napthol-AS-BI-phosphohydrolase (7) and α-fucosidase (7). Contains an alkali-stable lipid. The quinone system consists of the major menaquinone MK-7 and small amounts of MK-6. The polar lipid profile is composed of the major lipids phosphatidylethanolamine, the unidentified lipids L2 and L5 and the unidentified aminolipid AL2, and moderate to minor amounts of the unidentified lipids L1, L6, L8 and L9, glycolipid GL1 and aminolipid AL1. Sym-Homospermidine is the major polyamine. iso-C15:0 iso-C17:0 3-0H and summed feature 3 (C16:1ω7c/C16:1ω6c) are the major cellular fatty acids.

The type strain, CCM 8689T (=LMG 29684T), was isolated from stone fragments sampled at Devils Rocks locality at James Ross Island (Antarctica). The genomic DNA G+C content of the type strain is 36.2 mol%. Most characteristics of the type strain are in agreement with the general species description. The strain-dependent characteristics of the type strain are as follows: no hydrolysis of aesculin, no growth in 2% (w/v) NaCl and at pH 11, no production of esterase (C4), cystine arylamidase or α-fucosidase, and weak production of napthol-AS-BI-phosphohydrolase.

PEDOBACTER LITHOCOLA SP. NOV.

Pedobacter lithocola [li.tho.co.la. Gr. n. lithos stone, L. suff. -cola (from L. n. incola) inhabitant; N.L. masc. n. lithocola a dweller of stones].

Cells are Gram-stain-negative, short rods, 0.9–1.4×0.4–0.5 μm, occurring predominantly in pairs or in irregular clusters, non-motile and non-spore-forming. Colonies on R2A agar are reddish, circular, slightly convex, smooth and glistening with whole margins, and reach about 1–2 mm in diameter when cultivated at 20°C for 5 days. Flexirubin-type pigments are absent. The species is aerobic; no anaerobic growth on R2A agar is detected. Aerobic but non-fermenting in the OF test. Grows at 1–30°C, but not at 35°C. Grows in the presence of up to 1% (w/v) NaCl; growth is inhibited with ≥2% NaCl. Grows at pH 6–8; growth at pH 9 is strain dependent and growth is not detected at pH 5 or pH ≥10. Most abundant growth is observed on R2A agar without NaCl, at pH 8.0 and at 20°C. Fluorescein pigment is not produced on King B medium. Grows on plate count agar, tryptone soya agar, R2A agar, brain heart infusion agar and nutrient agar, but not on MacConkey agar. Positive reactions for catalase and oxidase, ONPG test and hydrolysis of starch, aesculin and DNA. Negative for...
Simmon’s citrate, sodium malonate, acetamide, hydrolysis of Tween 80, gelatin, casein, tyrosine and lecinthin (egg-yolk reaction), production of indol, urease, arginine dihydrolase, lysole and ornithine decarboxylylase and reduction of nitrate and nitrite. Enzymatic reactions tested by the API ZYM kit revealed positive results for alkaline phosphatase, acid phosphatase, leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase, and weakly positive for esterase lipase (C8) and cystine arylamidase. Negative test results for lipase (C14), chymotrypsin and β-glucuronidase; and strain-dependent test results are detected for esterase (C4) and α-fucosidase. Contains an alkali-stable lipid. The quinone system consists of the major menaquinone MK-7 and small amounts of MK-6. The polar lipid profile is composed of the major lipids phosphatidylyethanolamine, the unidentified lipids L2 and L5 and the unidentified aminolipid AL2, and minor amounts of unidentified aminolipid and the unidentified aminolipid AL1. _sym-Homospermidine_ is the major polyamine. iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c/C_{16:1}ω6c) are the major cellular fatty acids.

The type strain, CCM 8691^T (=LMG 29685^T), was isolated from stone fragments sampled at Panorama Pass locality at James Ross Island (Antarctica). The genomic DNA G+C content of the type strain is 35.6 mol%. Most characteristics of the type strain are in agreement with the general species description. The strain-dependent characteristics of the type strain are as follows: growth at pH 9, and no production of esterase (C4) or α-fucosidase.

**PEDOBACTER MENDELI SP. NOV.**

_Pedobacter medelii_ (men.de’li.i. N.L. gen. n. mendelii of Mendel, named in honour of Johann Gregor Mendel, a pioneer of genetics, for his contribution to general genetics; the name also reflects the fact that the species was isolated near the Johann Gregor Mendel Antarctic station).

Cells are Gram-stain-negative, short rods, 1–1.3×0.5 µm, occurring predominantly in pairs or in irregular clusters, non-motile and non-spore-forming. Colonies on R2A agar are reddish, circular, slightly convex, smooth and glistening with whole margins, and reach about 1–2 mm in diameter when cultivated at 20 °C for 5 days. Flexirubin-type pigments are absent. The species is aerobic; no anaerobic growth on R2A agar is detected. Aerobic but non-fermenting in the OF test. Grows at 1–30 °C, but not at 35 °C. Grows in the presence of up to 2% (w/v) NaCl; growth is inhibited in 3% NaCl. Grows at pH 6–8; growth at pH 9 is strain dependent (1) and growth is not detected at pH 5 or pH ≥10. Most abundant growth is observed on R2A agar without NaCl, at pH 8.0 and at 20 °C. Fluorescein pigment is not produced on King B medium. Grows on plate count agar, trypolide soya agar, R2A agar, brain heart infusion agar and nutrient agar, but not on MacConkey agar. Positive reactions for catalase and oxidase, ONPG test and hydrolysis of gelatin, aesculin, starch, casein and DNA. Hydrolysis of Tween 80 is strain dependent (2). Negative for Simmon’s citrate, sodium malonate, acetamide, hydrolysis of tyrosine and lecinthin (egg-yolk reaction), production of indol, urease, arginine dihydrolase, lysole and ornithine decarboxylylase and reduction of nitrate and nitrite. Enzymatic reactions tested by the API ZYM kit reveal positive test results for alkaline phosphatase, acid phosphatase, leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase, and weakly positive for esterase lipase (C8); negative test results for esterase (C4), lipase (C14), cystine arylamidase, chymotrypsin and β-glucuronidase. Contains an alkali-stable lipid. The quinone system consists of the major menaquinone MK-7 and small amounts of MK-6. The polar lipid profile is composed of the major lipids phosphatidylyethanolamine, the unidentified lipids L2 and L5 and the unidentified aminolipid AL2, and moderate to minor amounts of the unidentified aminolipid AL1. _sym-Homo-spermidine_ is the major polyamine. iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c/C_{16:1}ω6c) are major cellular fatty acids.

The type strain, CCM 8685^T (=LMG 29688^T), was isolated from stone fragments sampled at Lachman Crag locality at James Ross Island (Antarctica). The genomic DNA G+C content of the type strain is 35.5 mol%. Most characteristics of the type strain are in agreement with the general species description. The strain-dependent characteristics of the type strain are as follows: no growth at pH 9 and no Tween 80 hydrolysis.

**PEDOBACTER PETROPHILUS SP. NOV.**

_Pedobacter petrophilus_ [pe.tro’phi.lus. Gr. n. petra rock; N.L. masc. adj. philus (from Gr. masc. adj. philos) friend, loving; N.L. masc. adj. petrophilus, rock loving].

Cells are Gram-stain-negative, short rods, 1.2–1.6×0.6 µm, occurring predominantly in pairs or in irregular clusters, non-motile and non-spore-forming. Colonies on R2A agar are pink, circular, slightly convex, smooth and glistening with whole margins, and reach about 1–2 mm in diameter when cultivated at 20 °C for 5 days. Flexirubin-type pigments are absent. The species is aerobic; no anaerobic growth on R2A agar is detected. Aerobic but non-fermenting in the OF test. Grows at 1–30 °C, but not at 35 °C. Grows in the presence of up to 2% (w/v) NaCl; growth is inhibited with 3% NaCl. Grows at a pH 6–9; growth at pH 10 is strain dependent (1) and growth is not detected at pH 5 or ≥11. Most abundant growth is observed on R2A agar without NaCl, at pH 8.0 and at 20 °C. Fluorescein pigment is not produced on King B medium. Grows on plate count agar, trypolide soya agar, R2A agar, brain heart infusion agar and nutrient agar, but not on MacConkey agar. Positive reactions for catalase and oxidase, ONPG test and hydrolysis of Tween 80, gelatin, aesculin and casein. Negative for Simmon’s citrate, sodium malonate, acetamide, hydrolysis of starch, DNA, tyrosine and lecinthin (egg-yolk reaction), production of indol, urease, arginine dihydrolase, lysole and ornithine decarboxylylase and reduction of nitrate and nitrite. Enzymatic reactions tested by the API ZYM kit reveal positive test results for alkaline phosphatase, acid phosphatase, leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase and α-fucosidase and weakly positive for esterase lipase (C8); negative test results for esterase (C4), lipase (C14), cystine arylamidase, chymotrypsin and β-glucuronidase. Contains an alkali-stable lipid. The quinone system consists of the major menaquinone MK-7 and small amounts of MK-6. The polar lipid profile is composed of the major lipids phosphatidylyethanolamine, the unidentified lipids L2 and L5 and the unidentified aminolipid AL2, and moderate to minor amounts of the unidentified lipid L6, aminolipid AL1 and glycolipid GL1. _sym-Homo-spermidine_ is the major polyamine. iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (C_{16:1}ω7c/C_{16:1}ω6c) are major cellular fatty acids.
reaction), production of indol, urease, arginine dihydrolase, lysine and ornithine decarboxylase and reduction of nitrate and nitrite. Enzymatic reactions tested by the API ZYM kit revealed positive results for alkaline phosphatase, acid phosphatase, leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase and weakly positive for esterase lipase (C8); negative test results for esterase (C4), lipase (C14), cystine arylamidase, chymotrypsin, β-glucuronidase and α-fucosidase. Contains an alkali-stable lipid. The quinone system consists of the major menaquinone MK-7 and small amounts of MK-6. The polar lipid profile is composed of the major lipids phosphatidylethanolamine, the unidentified lipids L2 and L5 and the unidentified aminolipid AL2, and moderate to minor amounts of 3-OH and summed feature 3 (C₁₆:1ω7c/C₁₆:1ω6c) are major cellular fatty acids.

The type strain, CCM 8687ᵀ (=LMG 29686ᵀ), was isolated from stone fragments sampled at Devils Rocks locality at James Ross Island (Antarctica). The genomic DNA G+C content of the type strain is 38.8 mol%. Most characteristics of the type strain are in agreement with the general species description. The strain-dependent characteristics of the type strain are as follows: weak growth at pH 10.

Funding information
We are grateful to the scientific infrastructure of the J. G. Mendel Czech Antarctic Station as a part of the Czech Polar Research Infrastructure (CzechPolar2) supported by the Ministry of Education, Youth and Sports of the Czech Republic (LM2015078). S. K. is a holder of Brno PhD Talent financial aid.

Acknowledgements
Dr Daniel Krsek (National Reference Laboratory for Diagnostic Electron Microscopy of Infectious Agents, National Institute of Public Health, Prague, Czech Republic) is acknowledged for transmission electron microscopy and Jana Bajerová (Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic) is acknowledged for excellent technical assistance.

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

References

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