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TAXONOMIC DESCRIPTION
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Pandoraea fibrosis sp. nov., a novel *Pandoraea* species isolated from clinical respiratory samples

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Abstract

Pandoraea species have been isolated from diverse environmental samples and are emerging important respiratory pathogens, particularly in people with cystic fibrosis (CF). In the present study, two bacterial isolates initially recovered from consecutive sputum samples collected from a CF patient and identified as *Pandoraea pnomenusa* underwent a polyphasic taxonomic analysis. The isolates were found to be Gram-negative, facultative anaerobic motile bacilli and subsequently designated as strains 6399^T (=LMG29626^T=DSM103228^T) and 7641 (=LMG29627=DSM103229), respectively. Phylogenetic analysis based on 16S rRNA and *gyrB* gene sequences revealed that 6399^T and 7641 formed a distinct phylogenetic lineage within the genus *Pandoraea.* Genome sequence comparison analysis indicated that strains 6399^T and 7641 are clonal and share 100 % similarity, however, similarity to other type strains (ANIb 73.2–88.8 %, ANIm 83.5–89.9 % and OrthoANI 83.2–89.3 %) indicates that 6399^T and 7641 do not belong to any of the reported type species. The major cellular fatty acids of 6399^T were $C_{16:0}$ (32.1 %) $C_{17:0}$cyclo (18.7 %) and $C_{18:1}\omega7c$ (14.5 %), while Q-8 was the only respiratory quinone detected. The major polar lipids identified were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The genomic DNA G+C content of 6399^T was 62.9 (mol%). Strain 6399^T can be differentiated from other members of *Pandoraea* by the absence of $C_{19:0}\omega8c$ cyclo and by the presence of $C_{17:0}\omega8c$ cyclo. Together our data show that the bacterial strains 6399^T and 7641 represent a novel species of the genus *Pandoraea,* for which the name *Pandoraea fibrosis* sp. nov. is proposed (type strain 6399^T).

INTRODUCTION

The genus *Pandoraea* was first described [1] to accommodate bacterial isolates that were tentatively identified as *Burkholderia cepacia, Ralstonia pickettii* or *Ralstonia paucula* based on polyphasic taxonomic studies but retained some distinct genotypic and phenotypic characteristics. Bacteria classified as *Pandoraea* are Gram-negative, oxidase-variable and catalase-variable motile bacilli with a single polar flagellum and produce ubiquinone-8 as their major isoprenoid quinone [2]. At the time of writing, the genus *Pandoraea* comprises ten validly recognized species (*Pandoraea apista*, *Pandoraea pulmonicola*, *Pandoraea pnomenusa*, *Pandoraea sputorum*, *Pandoraea norimbergensis*, *Pandoraea oxalativorans*, *Pandoraea faecigallinarum*, *Pandoraea vervacti* and *Pandoraea thiooxydans* with *Pandoraea terrae* as the newest member) and four unnamed genomospecies.

While *Pandoraea* have been isolated from a range of environmental samples, certain of these organisms are emerging

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Keywords: *Pandoraea*; cystic fibrosis.
Abbreviations: ANIb, average nucleotide identity based on BLAST; ANIm, average nucleotide identity based on MUMmer; CF, cystic fibrosis; ERIC-PCR, enterobacterial repetitive intergenic consensus polymerase chain reaction; ML, maximum-likelihood; OrthoANI, orthologous average nucleotide identify.
The GenBank accession numbers for strain 6399^T and 7641 16S rRNA (KX712083, KX712096) and *gyrB* (KX712081, KX712082) genes, respectively. The genome accession numbers for *Pandoraea* species that are used in this study are *Pandoraea fibrosis* 6399^T (JTCR00000000), *P. fibrosis* 7641 (JTCS00000000), *P. pnomenusa* CCUG 38742^T (CP009553.3), *P. apista* CCUG 38412^T (CP013481.2 and CP013482.1), *P. faecigallinarum* KOx^T (CP011807.3, CP011808.2, and CP011809.2), *P. norimbergensis* CCUG 39188^T (CP013480.3), *P. oxalativorans* TA25^T (CP011253.3, CP011518.2, CP011519.2, CP011520.2, and CP011521.2), *P. sputorum* DSM21091^T (CP010431.2), *P. pulmonicola* (CP010310.2), *P. vervacti* NS15^T (CP010897.2 and CP010898.2) and *P. thiooxydans* ATSB16^T (CP014839.1).
Four supplementary figures and three supplementary tables are available with the online version of this article.

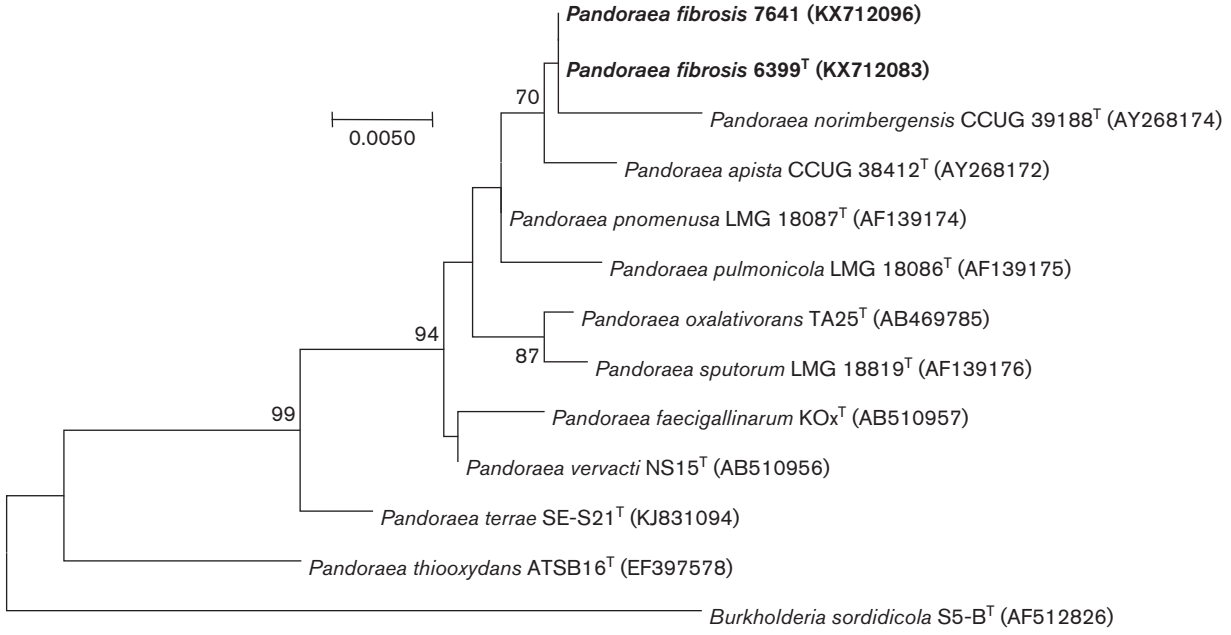

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences indicated the phylogenetic positions of strain 6399^T and 7641 and other *Pandoraea* type species. Bootstrap values (over 50 %) are shown on nodes in percentages of 1000 replicates. *Burkholderia sordidicola* S5-B (AF512826) was used as the outgroup. Bar, 0.005 changes per nucleotide.

as serious respiratory pathogens in people with cystic fibrosis (CF). The role(s) of *Pandoraea* in respiratory disease, however, is currently unclear and under-investigated.

Two *Pandoraea*-like isolates designated as strains 6399^T and 7641, were recovered on *Burkholderia cepacia*-selective medium from consecutive (11 months apart) sputum samples collected from an Australian (Tasmanian) CF patient [2, 3] attending the Royal Hobart Hospital (42° 52′ 55.7″ S 147° 19′ 37.9″ E). Isolates were preserved at −80 °C in Luria–Bertani (LB) broth supplemented with 20 % (v/v) glycerol. Isolates were routinely cultured aerobically on LB agar medium (BD Biosciences, USA) at 37 °C. Colonies of 6399^T and 7641 usually appear small (1–2 mm in diameter), white, circular, and convex after 24 h incubation at 37 °C. Gram-staining of bacterial cells and microscopy was performed as previously described [4] but with the inclusion of 5 μg ml^{-1} of the fluorescent lipid binding dye AM1-43 (Biotium). Bacterial morphology was examined using a Deltavision OMX SR (super resolution) microscope (GE Healthcare Life Sciences) and analysed using FIHI software [5]. Motility was assessed on a Richter Optica U2B light microscope (Richter Optica) using the hanging drop motility assay. Cells were found to be Gram-negative motile rods (1.6±0.3×0.6±0.1 μm, Fig. S1, available in the online version of this article). Here, we also used a polyphasic taxanomic approach to better determine the position of strains 6399^T and 7641 within the genus *Pandoraea*.

The draft genomes of 6399^T and 7641 have been published [3]. The genome of strain 6399^T is 5.5 kbp with a 62.9 % G+C content, while that of strain 7641 is 5.5 kbp with a 62.8 % G+C content. Since the 16S rRNA gene sequence is highly similar between *Pandoraea* species, better differentiation of species is usually achievable by comparing the nucleotide sequence of their *gyrB* genes as well [6]. The complete 16S rRNA and *gyrB* gene sequences (1537 and 2478 bp, respectively) were extracted from the 6399^T and 7641 genomes for phylogenetic analyses and compared to those of validly reported type strains available in EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) [7, 8]. Pairwise similarity analysis of the 16S rRNA gene sequence revealed that strains 6399^T and 7641 were closely related to *P. pnomenusa* CCUG 38742^T (99.7 %), *P. apista* CCUG 38412^T (99.5 %), *P. faecigallinarum* KOx^T (99.4 %), *P. norimbergensis* CCUG 39188^T (99.3 %), *P. oxalativorans* TA25^T (99.1 %), *P. sputorum* DSM21091^T (99.1 %), *P. vervacti* NS15^T (99.0 %) and *P. thiooxydans* ATSB16^T (97.0 %). Phylogenetic analyses of 16S rRNA and *gyrB* gene sequences were subsequently reconstructed using MEGA6 [9], and sequence alignment performed using the MUSCLE alignment tool [10] and default settings for the reconstruction of maximum-likelihood (ML) phylogenetic trees. Phylogenetic analyses of the 16S rRNA gene sequences demonstrated that strains 6399^T and 7641 formed a phyletic lineage within the genus *Pandoraea*, where the closest species appeared to be *P. norimbergensis* (Fig. 1). Phylogenetic analysis using the *gyrB* gene sequence (based on ML algorithms) also showed that these two strains formed a phyletic lineage within the genus *Pandoraea,* where the nearest species were found to be *P. apista* CCUG 38412^T (Fig. 2).

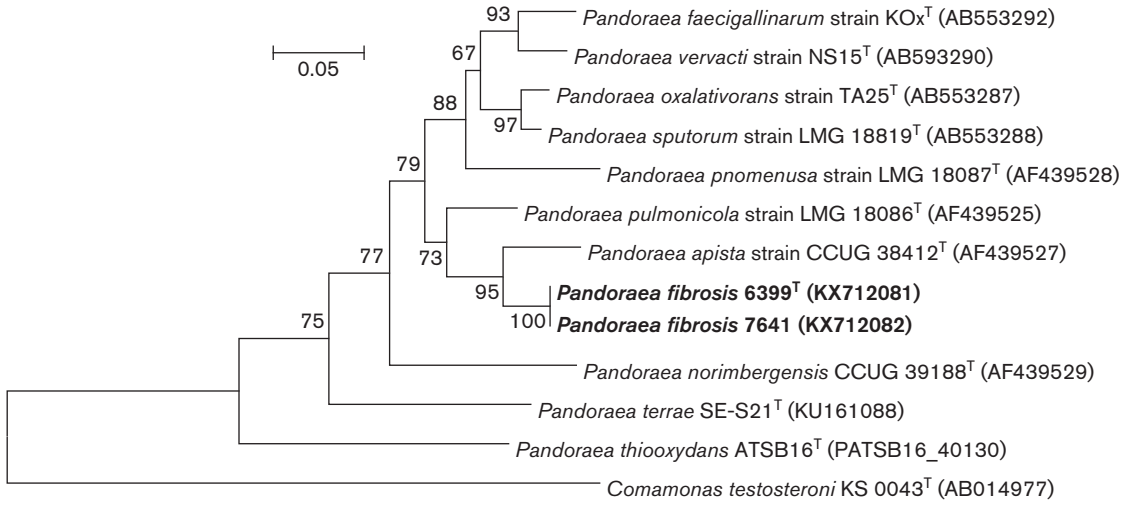

Fig. 2. Maximum-likelihood tree based on *gyrB* gene sequences (439 nucleotides) showing the phylogenetic relationships of strain 6399^T and 7641 and other *Pandoraea* type species. Bootstrap values (over 50 %) are shown on nodes in percentages of 1000 replicates. *Comamonas testosteroni* DSM 50244^T (AB014977) was used as the outgroup. Bar, 0.05 changes per nucleotide.

Genome sequence comparison analyses based on BLAST alignment algorithms (ANIb, the gold standard for prokaryote species definition) and the MUMmer algorithm (ANIm) were performed using JSpecies software [11] and the OrthoANI values were determine using OrthoANI calculator [12]. The species demarcation 95–96 % ANIb similarity [11] was used as a benchmark, and the results indicated that strains 6399^T and 7641 shared 100 % similarity. However, the ANIb value compared to other type species ranged from 73.2 to 88.8 % (Table S1). The ANIm values (ranging from 83.5–89.9 %) also indicated that 6399^T and 7641 do not belong to any of the reported type species (Table S2). OrthoANI analysis also supports the results of ANIb and ANIm, in which the OrthoANI value (ranging from 83.2–89.3 %) of both 6399^T and 7641 to other type strains were below the species circumscriptions threshold (Fig. S2). Thereby, all analyses support the notion that the strains are clonal (100 % similarity for all analyses) and belong to a novel taxon.

To further analyse the genetic variability between strains 6399^T and 7641, the enterobacterial repetitive intergenic consensus (ERIC-PCR) was performed as previously described [13], with the exception that PCRs were carried out using GoTaq Green Master Mix (Promega). The ERIC-PCR analysis showed identical banding patterns between 6399^T and 7641 (Fig. S3), clearly supporting the conclusion that strains 6399^T and 7641 are clonal, as expected given the high relatedness of their genomes (including identical 16S rRNA gene sequences) [3] and the fact that they were isolated from the same host.

Growth of strains 6399^T and 7641 was assessed in brain heart Infusion (BHI) broth over a range of temperatures (28, 37 and 42 °C), pH values (from 4 to 10 in 0.5 step increments) and NaCl concentrations (0.5, 1, 3, 4.5, 6, 8, 10, 12 and 15 %). The pH level of the BHI broth was adjusted by using 0.1 mM sodium acetate (pH 4.0–6.0), 0.1 mM phosphate (pH 7.0–8.0) and 0.1 mM sodium carbonate (pH 8.5–10.0). Both strains were able to grow at 28 and 37 °C but not at 42 °C. Growth of both strains occurred in BHI broth pH 4.5 to 9.5 and NaCl 0.05 to 3 %, at 28 and 37 °C. Interestingly, cultures of both strains incubated at 37 °C seemed to have a slightly higher NaCl tolerance, since growth was possible even in the presence of 4.5 % NaCl.

For assessment of their anaerobic growth, strains 6399^T and 7641 were inoculated on BHI agar plates supplemented with 1 % potassium nitrate and incubated in an anaerobic jar (containing an anaerobic sachet [AN0035; AnaeroGen, Oxoid] and an anaerobic indicator) for 5 days at 37 °C. While growth of both 6399^T and 7641 was scant under these conditions, it was nonetheless reproducible. Similarly, Daneshvar *et al.* [14] reported on the growth of nine *Pandoraea* isolates in a candle jar atmosphere. Collectively, these data would seem to indicate that at least some *Pandoraea* species might well be facultative anaerobes, analogous to the closely related *Burkholderia* (denitrification pathway described, [15]).

The catalase activity of strains 6399^T and 7641 was determined by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. The oxidase activity was determined by oxidation of 1 % (w/v) tetramethyl-p-phenylenediamine (Merck) [16]. Nitrate reduction was assessed using the supplementary test from the API 20NE Identification System (as per the manufacturer's instructions, Biomérieux, France). Strains were catalase- and oxidase-positive and reduced nitrate to nitrite.

Table 1. Phenotypic comparison of strain 6399^T and other *Pandoraea* type species

Strains: 1. strain 6399^T (this study); 2. *P. norimbergensis* DSM 11628^T; 3. *P. apista* KACC 11947^T; 4. *P. pulmonicola* FC330^T; 5. *P. pnomenusa* KACC 15013^T; 6. *P. faecigallinarum* DSM 23572^T; 7. *P. vervacti* 561^T; 8. *P. oxalativorans* DSM 23570^T; 9. *P. sputorum* DSM 21091^T and 10. *P. thiooxydans* KACC 12757^T. All strains were motile, catalase/oxidase variable, nitrate reduction variable, able to grow in 1.5 % NaCl and up to 37 °C. There was no single carbon source able to be oxidized by all strains, and in chemical sensitivity assays, all strains were sensitive to tetrazolium blue. Characteristic data were from [1, 22–24], while carbon utilization and chemical sensitivity data were generated in the present study. +, Denotes utilization of carbon sources or growth in presence of inhibitor and –, denotes no measurable utilization of carbon sources or absence of growth in presence of inhibitor, ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10
Gram stain	–	–	–	–	–	–	–	–	–	–
Motility	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	–
Oxidase	+	+	–	+	–	+	+	+	+	+
Nitrate reduction	+	–	–	–	+	–	–	–	+	+
Growth:										
Up to NaCl (%, w/v)	4.5	1.5	1.5	1.5	3	3	3	3	3	5
Up to (°C)	37	37	42	42	42	42	42	37	42	42
Oxidation of:										
Acetic acid	+	+	+	+	+	+	+	+	+	–
Acetoacetic acid	–	–	+	–	–	–	–	–	–	–
Bromo-succinic acid	–	+	+	+	+	+	+	–	+	–
Citric acid	+	+	+	+	+	+	+	+	+	–
D-Aspartic acid	+	+	+	+	+	+	+	+	+	–
D-Galacturonic acid	–	+	+	+	+	+	+	+	–	–
D-Gluconic acid	+	+	+	+	+	+	+	–	+	–
D-Glucose-6-PO_4	–	–	–	+	–	–	–	–	–	–
D-Glucuronic acid	+	+	+	+	+	+	+	–	+	–
D-Malic acid	+	+	+	+	+	+	+	–	+	–
D-Saccharic acid	–	–	–	+	+	–	–	–	–	–
D-Serine	+	–	+	–	+	+	–	–	+	–
Formic acid	–	–	–	+	+	–	–	–	–	–
Glucuronamide	+	–	+	+	+	+	+	–	+	–
Glycerol	+	–	+	+	+	+	+	–	+	–
Glycyl-L-proline	+	+	+	+	+	+	+	–	+	–
L-Alanine	+	–	+	+	+	+	+	–	+	–
L-Aspartic acid	+	+	+	+	+	+	+	+	+	–
L-Galactonic acid lactone	–	+	+	+	+	+	+	–	–	–
L-Glutamic acid	+	+	+	+	+	+	+	+	+	–
L-Histidine	+	+	+	+	+	+	+	+	+	–
L-Lactic acid	–	–	+	+	+	+	–	–	–	+
L-Malic acid	+	+	+	+	+	+	+	+	+	–
L-Pyroglutamic acid	+	+	+	+	+	+	+	–	+	+
L-Serine	+	–	+	+	+	+	+	+	+	–
Methyl pyruvate	+	–	–	–	+	+	+	–	+	+
Mucic acid	–	–	–	+	+	–	–	–	–	–
p-Hydroxy-phenylacetic acid	+	+	+	+	+	+	+	–	+	–
Propionic acid	+	–	+	+	+	+	+	–	+	–
α-D-Glucose	–	+	–	+	+	–	–	–	–	–
α-Hydroxy-butyric acid	+	–	–	+	+	+	+	–	+	+
α-keto-Butyric acid	+	–	+	+	+	+	+	–	+	+
α-keto-Glutaric acid	+	+	+	+	+	+	+	–	+	–
Chemical sensitivity:										
pH 5	+	+	+	+	+	+	+	+	+	–
Fusidic acid	+	+	+	+	+	+	–	–	+	–
Guanidine HCl	+	+	+	+	+	+	+	+	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10
Lithium chloride	−	−	−	+	−	−	−	−	−	−
Minocycline	+	−	+	+	−	−	−	−	−	−
Nalidixic acid	+	+	+	+	+	+	+	+	+	−
Niaproof 4	−	+	−	−	−	−	−	−	−	−
Potassium tellurite	+	+	+	+	+	+	+	+	+	−
Tetrazolium blue	+	+	+	+	+	+	+	+	+	+
Tetrazolium violet	+	+	+	+	+	+	+	+	+	−
Genome features:										
Genome size (Mb)	5.57	6.17	5.54	5.87	5.39	5.73	5.74	6.5	5.74	4.46
DNA G+C content (mol%)	62.9	63.1	62.7	64.3	64.9	63.7	63.6	63.1	62.8	63.2
No. of genes	4891	5416	4979	5080	4821	5085	5015	5734	5084	4144
No. of coding sequences	4356	5335	4797	4989	4659	5003	4934	5650	5003	4085

The biochemical characterization of the strains was performed using the GEN III MicroPlate (Biolog, per the manufacturer's instructions). Briefly, bacteria were cultivated in LB overnight at 37 °C and the wells of the GEN III MicroPlate inoculated with *Pandoraea* suspended in inoculating fluid A and plates incubated at 37 °C for 2 days. Strain 6399^T oxidized acetic acid, citric acid, D-aspartic acid, D-gluconic acid, D-glucuronic acid, D-malic acid, D-serine, glucuronamide, glycerol, glycyl-L-proline, L-alanine, L-aspartic acid, L-glutamic acid, L-histidine, L-malic acid, L-pyroglutamic acid, L-serine, methyl pyruvate, p-hydroxy-phenylacetic acid, propionic acid, α-hydroxy-butyric acid, α-keto-butyric acid, α-keto-glutaric acid but not acetoacetic acid, bromo-succinic acid, D-galacturonic acid, D-glucose-6-PO_4, D-saccharic acid, formic acid, L-galactonic acid lactone, L-lactic acid, mucic acid or α-D-glucose. Growth was inhibited by lithium chloride and niaproof anionic surfactant 4 (7-ethyl-2-methyl-4-undecyl sulfate sodium salt) but not at pH 5, or by fusidic acid, guanidine HCl, minocycline, nalidixic acid, potassium tellurite, tetrazolium blue or tetrazolium violet. The phenotypic and biochemical characteristics of 6399^T are presented in Table 1 and compared to phenotypic (taken from [1, 17–19]) and biochemical characteristics (this study) of closely related bacterial species. Strain 6399^T could not be differentiated from the other type strains based solely on its growth, biochemical characteristics, or genome features.

Characterization of polar lipids, fatty acids and respiratory quinones was carried out by the Identification Service, DSMZ, Braunschweig, Germany (complete methods available /www.dsmz.de/services/services-microorganisms/identification.html). Briefly, cells were harvested from cultures grown in tryptic soy broth for 16 h at 28 °C (*P. faecigallinarum* DSM 23572^T, *P. vervacti* DSM 23571^T, *P. oxalativorans* DSM 23570^T), 30 °C (*P. thiooxydans* DSM 25325^T) or 37 °C (strain 6399^T, *P. pnomenusa* DSM 16536^T, *P. pulmonicola* DSM 16583^T, *P. sputorum* DSM 21091^T, *P. apista* DSM 16535^T, *P. norimbergensis* DSM 11628^T). Polar lipids and respiratory quinones were extracted according to Tindall [20, 21] and polar lipids analysed as described previously [22]. Respiratory quinones were first separated using thin-layer chromatography (TLC) and subsequently analysed and identified by high performance liquid chromatography (HPLC). Cellular fatty acids were extracted as described [23, 24]. The fatty acid methyl esters were separated and identified and quantified using the Sherlock Microbial Identification System (MIS, MIDI, Microbial ID) and database.

The total polar lipids of strain 6399^T were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminophospholipid, an unidentified aminolipid, and two unidentified lipids. The unidentified aminophospholipid, unidentified aminolipid and two unidentified lipids represent minor polar lipids (Fig. S4). Ubiquinone-8 (Q-8) was the only respiratory lipoquinone able to be detected in extracts derived from 6399^T. The major cellular fatty acids of strain 6399^T (>65 % total) were $C_{16:0}$, $C_{17:0}$ cyclo, and $C_{18:1}\omega7c$. While the fatty acid profiles of strain 6399^T were similar to those of other closely related *Pandoraea* type species (Table S3), there were some important differences. Strain 6399^T can be differentiated from other members of *Pandoraea* by the absence of $C_{19:0}\omega8c$ cyclo and by the presence of $C_{17:0}\omega8c$ cyclo.

On the basis of the phenotypic and genotypic properties described above for strains 6399^T and 7641, it seems that they do represent a novel species of the genus *Pandoraea,* for which the name *Pandoraea fibrosis* sp. nov. is proposed.

DESCRIPTION OF *PANDORAEA FIBROSIS* SP. NOV.

Pandoraea fibrosis (fi.bro′sis. L. dim. n. *fibra*, fibre/fiber; fibrous; Gr. suff. *-osis*, suffix expressing state or condition, in medical terminology denoting a state of disease; N.L. gen. n. *fibrosis*, referring to the organism being isolated from the patient with cystic fibrosis).

Cells are facultative anaerobic, Gram-stain-negative motile rods. Colonies are small white, circular, and convex. Both strains grew in BHI broth up to 37 °C (no growth was observed at 42 °C). Growth occurred at pH 4.5–9.5 and

0.05–4.5 % NaCl. The strains were oxidase- and catalase-positive. Nitrate was reduced to nitrite. The type strain, 6399^T, originally collected from the sputum of a CF patient, oxidized, acetic acid, citric acid, D-aspartic acid, D-gluconic acid,D-glucuronic acid, D-malic acid, D-serine, glucuronamide, glycerol, glycyl-L-proline, L-alanine, L-aspartic acid, L-glutamic acid, L-histidine, L-malic acid, L-pyroglutamic acid, L-serine, methyl pyruvate, p-hydroxy-phenylacetic acid, propionic acid, α-hydroxy-butyric acid, α-keto-butyric acid, α-keto-glutaric acid but not acetoacetic acid, bromo-succinic acid, D-galacturonic acid, D-glucose-6-PO_4, D-saccharic acid, formic acid, L-galactonic acid lactone, L-lactic acid, mucic acid, and α-D-glucose. Growth was inhibited by lithium chloride and niaproof anionic surfactant 4, but not at pH 5 or by fusidic acid, guanidine HCl, minocycline, nalidixic acid, potassium tellurite, tetrazolium blue or tetrazolium violet. The following fatty acids were detectable at >0.5 % of total: $C_{12:0}$, $C_{12:0}$ 2-OH, $C_{16:0}$, $C_{16:0}$ 3-OH, $C_{17:0}$, $C_{17:0}\omega 8c$ cyclo, $C_{17:0}$ cyclo, $C_{18:0}$, $C_{18:0}$ 2-OH, $C_{18:1}\omega 7c$ and summed features 2 (comprising $C_{14:0}$ 3-OH and iso-$C_{16:1}$) and 3 (comprising $C_{16:1}\omega 6c/\omega 7c$). The isoprenoid quinone detected was Q-8 and the major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Two unidentified lipids, an unidentified aminolipid and an unidentified aminophospholipid were also detected. The genomic DNA G+C content of strain 6399^T was 62.9 (mol%).

The type strain (6399^T=LMG29626^T=DSM103228^T), originally isolated from the sputum of a CF patient in Tasmania (Australia) can be differentiated from other *Pandoraea* members by the absence of $C_{19:0}\omega 8c$ cyclo and by the presence of $C_{17:0}\omega 8c$ cyclo fatty acid. The draft genome, 16S rRNA and *gyrB* gene sequences of the type strain have been deposited in GenBank under the accession numbers JTCR00000000, KX712083 and KX712081, respectively.

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

Ethical statement
Isolate collection was approved by the Tasmanian Human Research Ethics Committee (approval number H0012530).

References

1. Coenye T, Falsen E, Hoste B, Ohlén M, Goris J *et al.* Description of *Pandoraea* gen. nov. with *Pandoraea* apista sp. nov., *Pandoraea* pulmonicola sp. nov., *Pandoraea* pnomenusa sp. nov., *Pandoraea* sputorum sp. nov. and *Pandoraea* norimbergensis comb. nov. *Int J Syst Evol Microbiol* 2000;50 Pt 2:887–899.
2. Ambrose M, Malley RC, Warren SJ, Beggs SA, Swallow OF *et al.* *Pandoraea pnomenusa* Isolated from an Australian Patient with Cystic Fibrosis. *Front Microbiol* 2016;7:692.
3. Ee R, Ambrose M, Lazenby J, Williams P, Chan K-G *et al.* Genome sequences of two *Pandoraea pnomenusa* isolates recovered 11 months apart from a cystic fibrosis patient. *Genome Announc* 2015;3.
4. Turnbull L, Whitchurch CB. Motility assay: twitching motility. *Methods Mol Biol* 2014;1149:73–86.
5. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M *et al.* Fiji: an open-source platform for biological-image analysis. *Nat Methods* 2012;9:676–682.
6. Coenye T, Lipuma JJ. Use of the gyrB gene for the identification of *Pandoraea* species. *FEMS Microbiol Lett* 2002;208:15–19.
7. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M *et al.* Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
8. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
9. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
10. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.
11. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
12. Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–1103.
13. Atkinson RM, Lipuma JJ, Rosenbluth DB, Dunne WM. Chronic colonization with *Pandoraea apista* in cystic fibrosis patients determined by repetitive-element-sequence PCR. *J Clin Microbiol* 2006;44:833–836.
14. Daneshvar MI, Hollis DG, Steigerwalt AG, Whitney AM, Spangler L *et al.* Assignment of CDC weak oxidizer group 2 (WO-2) to the genus *Pandoraea* and characterization of three new *Pandoraea* genomospecies. *J Clin Microbiol* 2001;39:1819–1826.
15. Chin CY, Hara Y, Ghazali AK, Yap SJ, Kong C *et al.* Global transcriptional analysis of Burkholderia pseudomallei high and low biofilm producers reveals insights into biofilm production and virulence. *BMC Genomics* 2015;16:471.
16. Smibert RM, Krieg NR. Phenotypic characterization. In: Gerhardt P (editor). *Methods for General and Molecular Bacteriology*. Washington, DC: American society for microbiology; 1994. pp. 607–654.
17. Anandham R, Indiragandhi P, Kwon SW, Sa TM, Jeon CO *et al.* *Pandoraea thiooxydans* sp. nov., a facultatively chemolithotrophic, thiosulfate-oxidizing bacterium isolated from rhizosphere soils of sesame (*Sesamum indicum* L.). *Int J Syst Evol Microbiol* 2010;60:21–26.
18. Jeong SE, Lee HJ, Jia B, Jeon CO. *Pandoraea terrae* sp. nov., isolated from forest soil, and emended description of the genus *Pandoraea Coenye et al.* 2000. *Int J Syst Evol Microbiol* 2016;66:3524–3530.
19. Sahin N, Tani A, Kotan R, Sedlácek I, Kimbara K *et al.* *Pandoraea oxalativorans* sp. nov., *Pandoraea faecigallinarum* sp. nov. and *Pandoraea vervacti* sp. nov., isolated from oxalate-enriched culture. *Int J Syst Evol Microbiol* 2011;61:2247–2253.
20. Tindall BJ. A comparative study of the lipid composition of *Halobacterium* saccharovorum from various sources. *Syst Appl Microbiol* 1990;13:128–130.
21. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.

22. **Tindall BJ, Sikorski J, Smibert RM, Kreig NR.** *Phenotypic Characterization and the Principles of Comparative Systematics*, 3rd ed. Washington, DC, USA: ASM Press; 2007.
23. **Kuykendall LD, Roy MA, O'Neill JJ, Devine TE.** Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 1988; 38:358–361.
24. **Miller LT.** Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* 1982;16:584–586.