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TAXONOMIC DESCRIPTION

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Pseudomonas boanensis sp. nov., a bacterium isolated from river water used for household purposes in Boane District, Mozambique

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Abstract

A Gram-negative rod with a single polar flagellum was isolated from a freshwater reservoir used for household purposes in Boane District, near Maputo, Mozambique, and designated as strain DB1^T. Growth was observed at 30–42 °C (optimum, 30–37 °C) and with 0.5–1.5% NaCl. Whole-genome-, *rpoD*- and 16S rRNA-based phylogenies revealed this isolate to be distant from other *Pseudomonas* species with *Pseudomonas resinovorans*, *Pseudomonas furukawaii* and *Pseudomonas lalkuanensis* being the closest relatives. Phenotypic analyses of strain DB1^T showed marked differences with respect to type strains *P. resinovorans* CCUG 2473^T, *P. lalkuanensis* CCUG 73691^T, *P. furukawaii* CCUG 75672^T and *Pseudomonas otiditis* CCUG 55592^T. Taken together, our results indicate that strain DB1^T is a representative of a novel species within the genus *Pseudomonas* for which the name *Pseudomonas boanensis* is proposed. The type strain is DB1^T (=CCUG 62977^T=CECT 30359^T).

The genus *Pseudomonas* is diverse, comprising bacteria of versatile metabolism and physiology that inhabit a wide variety of environments. Some species like *Pseudomonas aeruginosa* are recognized for their pathogenicity to humans, but the genus also includes important plant pathogens such as *Pseudomonas syringae* and species with biotechnological potential such as *Pseudomonas putida* [1]. The genus was first described by Migula [2] and the taxonomy has experienced numerous changes ever since. At present, the List of Prokaryotic Names with standing in Nomenclature recognizes 297 *Pseudomonas* species with a validly published and correct name (https://lpsn.dsmz.de/genus/pseudomonas, accessed 22 May 2022). A milestone in bacterial taxonomy has been the introduction of indexes for the precise identification of isolates at the specific or even sub-specific level based on whole-genome sequencing (WGS) [3–5], which has redefined the phylogenomic relationships within a plethora of bacterial genera including *Pseudomonas* [6]. Here we describe the identification of an environmental strain, DB1^T, isolated from the Boane District in Mozambique, that constitutes a representative of a new species within the genus *Pseudomonas*. We propose to name the species *Pseudomonas boanensis* sp. nov.

ISOLATION AND ECOLOGY

Strain DB1^T was isolated from river water collected at the Boane Drift on the Umbeluzi River in the Boane District, Maputo Province, Mozambique (26.0552° S; 32.3264° E) in the context of a study investigating the prevalence and persistence of enteropathogens

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Abbreviations: dDDH, digital DNA–DNA hybridization; TYGS, Type Strain Genome Server; WGS, whole-genome sequencing.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAGTIS000000000. The version described in this paper is version JAGTIS010000000.1. The 16S rRNA sequence has been deposited at DDBJ/ENA/GenBank under the accession number MZ358391.1.

One supplementary table is available with the online version of this article.

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Fig. 1. Drift Boane on the Umbeluzi river in Boane District, Maputo Province in Mozambique.

in environmental water sources during spring 2012. The Umbulezi River constitutes the intake water to the water treatment plant and serves as the main water source for Maputo province and Maputo city. The water in the Boane Drift is used mainly for domestic purposes by the inhabitants of the local area (Fig. 1). Water samples were cleared of debris by low-speed centrifugation, and the concentration of inorganic nutrients measured in duplicate supernatant samples using an automatic analyser (Traacs 800, Braun and Luebe) was NH_4^+=38.4±2.3 µmol l^{-1}; NO_2+NO_3<0.1 µmol l^{-1}; SiO_2=23.9±4.2 µmol l^{-1}. For bacterial isolation, the supernatant was incubated at 30 °C for several weeks. The pH in water after this long-term incubation was slightly alkaline (pH 7.5). Water samples were plated on blood agar, and DB1^T colonies were found after 2 days of incubation at 37 °C. DB1^T colonies were preserved as glycerol stocks at −80 °C.

GENOME FEATURES AND PHYLOGENY

Genomic DNA was extracted using DNeasy Blood and Tissue kit (Qiagen), and sequenced on an Illumina MiSeq platform using v3 chemistry, 2×300 bp pair-end sequencing. Quality trimming of the raw data was performed using TrimGalore (www.bioinformatics.babraham.ac.uk/projects/trim_galore). Assembly was performed using SPAdes assembler version 3.9.0 [7], and Prokka version 1.12 [8] was used for genome annotation. The draft genome, consisting of 65 contigs, was deposited in GenBank

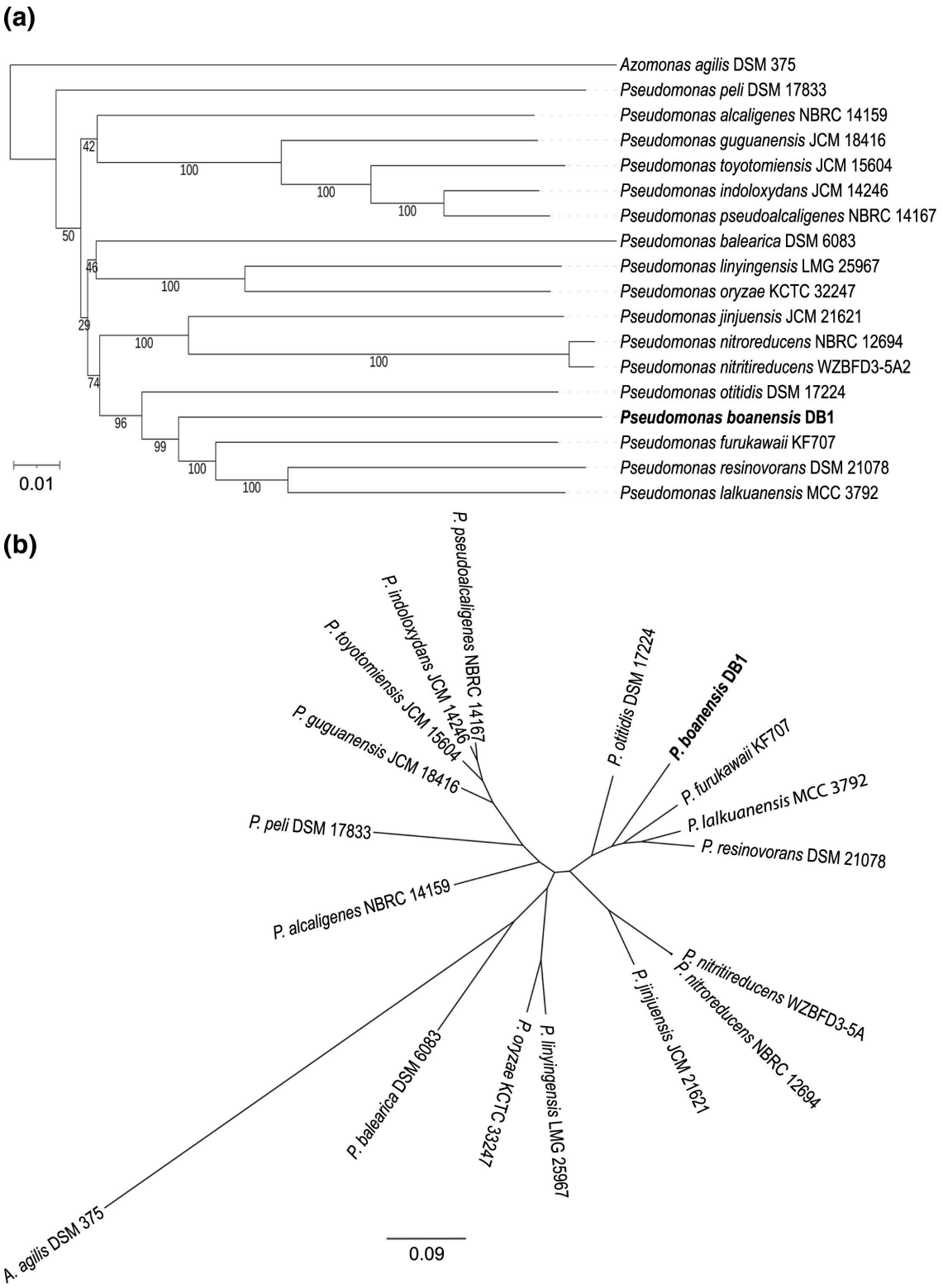

Fig. 2. (a) Whole genome dDDH relatedness was performed using the TYGS database (https://tygs.dsmz.de). Pairwise comparisons among genomes were conducted using genome BLAST distance phylogeny. One hundred distance replications were calculated for each genome The resulting intergenomic distances were used to infer a balanced minimum-evolution tree with branch support via FastME 2.1.6.1 including SPR postprocessing. Branch support was inferred from 100 pseudobootstrap replicates each. (b) Core genome phylogenetics was performed using a core genome alignment from the Roary pan genome pipeline [13] using 70% BLASTp identity cutoff. The core genomes were aligned using MAFFT version 7.477 [14] and the trees were reconstructed with PhyML version 3.1 [15] using default settings and aLRT branch support estimation.

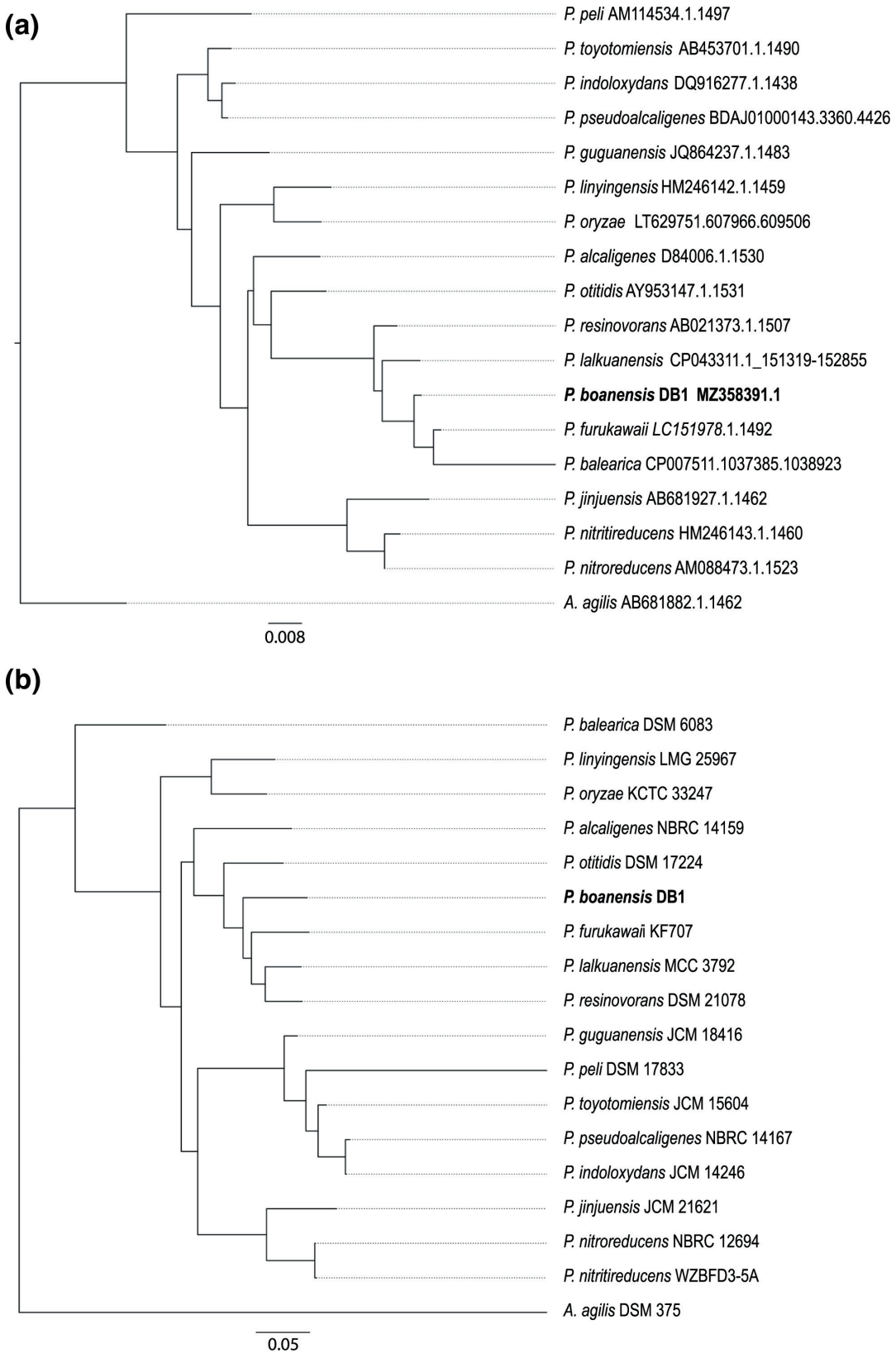

Fig. 3. Maximum-likelihood phylogenetic reconstructions based on (a) 16S rRNA sequence similarity (b) the *rpoD* genomic sequence. The gene sequences were extracted from whole genome sequences. Gene sequences were aligned using MAFFT version 7.477 [14] and the trees were reconstructed with PhyML version 3.1 [15] using default settings and aLRT branch support estimation.

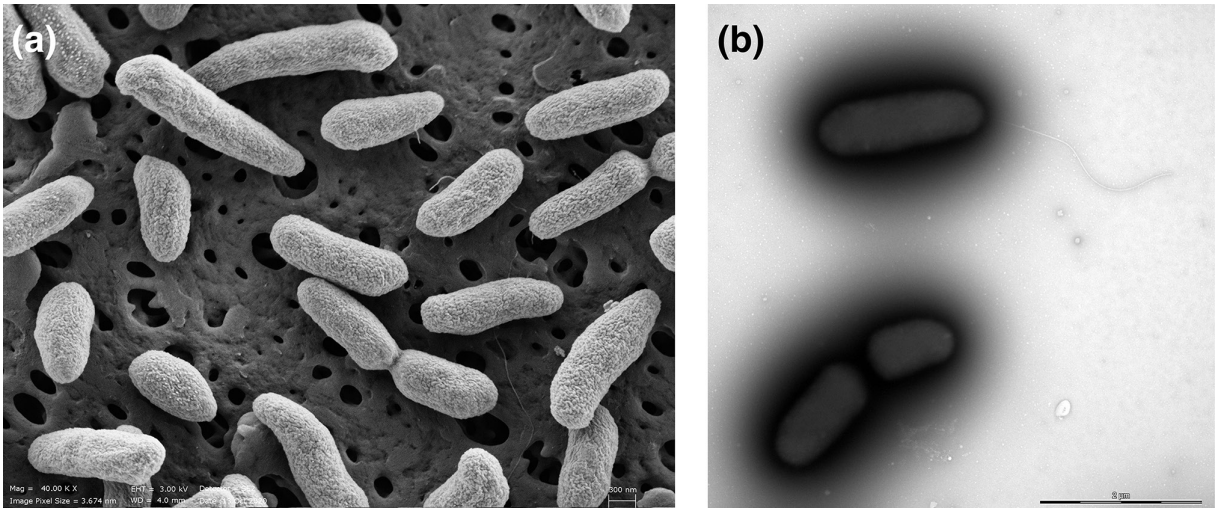

Fig. 4. Images of strain DB1^T by scanning electron microscopy (a) and negative staining (b). Cells were grown in LB medium at 37 °C overnight prior to image acquisition.

under BioProject PRJNA718467, accession number JAGTIS000000000. The genome size of strain DB1^T is 5710896 bp, with a G+C content of 62.33 mol% as determined by the Genome-to-Genome Distance Calculator [9].

To define the phylogeny of the novel species, we reconstructed a WGS-based phylogeny for DB1^T that included 17 reference strains for well-established *Pseudomonas* species using the Type Strain Genome Server (TYGS; www.tygs.dsmz.de) (Fig. 2a). TYGS uses digital DNA–DNA hybridization (dDDH) to delineate species circumscriptions based on a 70% threshold for species definition [10]. The whole genome-based maximum likelihood phylogeny showed that DB1^T was distant from other *Pseudomonas* species, with *Pseudomonas resinovorans*, *Pseudomonas lalkuanensis* [11] and *Pseudomonas furukawaii* [12] being close relatives. The dDDH and average nucleotide identity values towards the closest relatives were 39.5 and 81.93% for *P. lalkuanensis*, 41.5 and 81.85% for *P. resinovorans* and 38.8 and 81.62% for *P. furukawaii*, respectively. This phylogenomic reconstruction supported the positioning of DB1^T as representing a separate taxonomic species within the genus *Pseudomonas*. In addition, we reconstructed a core genome phylogenetic tree for the same strains, confirming the topology of the TYGS tree (Fig. 2b). The 16S rRNA-based maximum-likelihood phylogeny also placed *Pseudomonas balearica* within the group of closely related species but this was not supported by the other analyses (Fig. 3a, Table S1, available in the online version of this article). An additional phylogeny construction using the *rpoD* gene sequence from the genomes of each reference strain and DB1^T corroborated the branch positions observed previously and placed strain DB1^T close to *P. resinovorans*, *P. lalkuanensis* and *P. furukawaii* (Fig. 3b). The core genome tree was based on a core genome alignment from the Roary pan genome pipeline [13] using 70% BLASTp identity cutoff. The core, 16S and *rpoD* genes were all aligned using MAFFT version 7.477 [14] and the trees were reconstructed with PhyML version 3.1 [15] using default settings and aLRT branch support estimation.

Additional comparative analyses with the DB1^T and 17 reference strains bolstered results obtained from the analyses and were made publicly available on Github at: https://github.com/ctmrbio/pseudomonas_boanensis_db1 and in Table S1.

PHYSIOLOGY AND CHEMOTAXONOMY

Cells of DB1^T were Gram-stain negative, rod-shaped with a single polar flagellum (Fig. 4). Phenotypic characteristics of strain DB1^T were determined using the NFX (non fermentative Gram negative bacteria) protocol at Culture Collection University of Gothenburg (CCUG) with appropriate API tests API NH and API Zym (Biomerieux, Marcy l'Etoile, France) and compared to those of the type strains *P. resinovorans* CCUG 2473^T, *P. otiditis* CCUG 55592^T, *P. lalkuanensis* CCUG 73691^T and *P. furukawaii* CCUG 75672^T. (Table 1). Growth of DB1^T was observed in blood agar medium at 30, 37 and 42 °C with an optimal temperature of 37 °C. NaCl tolerance was tested with 0.5, 1.5, 3.0, 4.5 and 6% NaCl; strain DB1^T was unable able to grow at 3.0% or higher salt concentrations. Numerous phenotypic features differentiate DB1^T from its closest relatives *P. resinovorans* CCUG 2473^T, *P. lalkuanensis* CCUG 73691^T, *P. otitidis* CCUG 55592^T and *P. furukawaii* CCUG 75672^T. Thus, compared to *P. resinovorans* CCUG 2473^T, *P. lalkuanensis* CCUG 73691^T and *P. otitidis* CCUG 55592^T, DB1^T was more sensitive to NaCl above 1.5 %; it was able to use D-gluconate as a carbon source but lacked the ability to ferment arginine dihydrolase. DB1^T showed lower fluorescin than *P. resinovorans* CCUG 2473^T and *P. lalkuanensis* CCUG 73691^T but had a similar fluorescin content to *P. otitidis* CCUG 55592^T, in addition, DB1^T was devoid of nitrate reduction similar to *P. resinovorans* CCUG 2473^T and *P. otitidis* CCUG 55592^T, while *P.*

Table 1. Phenotypic tests with features differentiating strain DB1^T from the reference strains of the four closest related species

Strains: 1, DB1^T; 2, *Pseudomonas resinovorans* CCUG 2473^T; 3, *Pseudomonas otitidis* CCUG 55592^T; 4, *Pseudomonas lalkuanensis* CCUG 73691^T; 5, *Pseudomonas furakawii* CCUG 5672^T. ND, Not done; –, negative; un, uncertain results probably negative; +, weak positive; ++, positive; +++, strong positive. All strains were oxidase-positive, catalase-negative and unable to reduce nitrate. All strains were strongly positive for utilization of arginine, glucose, malate, citrate, lactate, caprate, and leucine arylamidase. All strains were catalase, indole, DNase, β-galactosidase, and nitrite-reduction negative and negative for utilization of amylase, lysine, ornithine, urease, esculine hydrolysis, acetamide, trehalose, norleucin, and adipate.

Test panel	Test*	1	2	3	4	5
OX	Drigalski agar: growth	++	++	++	+++	++
	Nutrient agar 42 °C: growth	++	++	+++	+++	+++
	Motility HD/30 °C	+++	+++	+++	+++	ND
	Motility OF	+	++	+++	+++	+++
OF	OF-D-Glucose	ND	+++	++	++	un
	Fluorescin	+	+++	+	+++	–
	Centrimide	++	+++	+++	+++	+++
	Tween 80	+++	+	++	+	+++
DEC	NO_3 reduction	–	–	–	+++	+++
	Gelatine Kohn	–	–	+++	–	–
ESC	NaCl 1.5%: growth	+++	+++	+++	++	+++
	NaCl 3.0%: growth	–	+	+	un	+
ASSIM	Arginine	+++	++	+++	+++	++
	Gluconate	+++	–	–	–	+++
	Caprate	+++	un	+++	+++	++
	Phenylacetate	+++	+++	+++	+++	++
	Sucrose	–	–	–	–	+
API 20NE	Nitrate reduction	–	–	–	+++	+++
	Arg. dihydrol	–	+++	++	+++	un
	Gelatinase	–	–	un	–	–
	L-Arabinose	–	–	+	–	++
	D-Mannose	–	–	–	–	++
	D-Mannitol	–	–	–	–	++
	N-Acetyl-glucosamine	–	–	–	–	+++
	Maltose	–	–	–	–	++
	D-Gluconate	+++	–	–	–	+++
ZYM	Alkaline phosphatase	–	+++	++	un	–
	Esterase (C4)	+++	+	++	++	+++
	Ester lipase (C8)	un	+	++	++	+++
	Lipase (C14)	–	–	++	–	+
	Acid phosphatase	–	+++	–	un	un
	Phosphoamidase	un	+	++	++	+

NFX protocol at CCUG, Sweden, API NH, API Zym (Biomerieux, Marcy-l'Étoile, France).

lalkuanensis CCUG 73691^T and *P. furukawaii* CCUG 75672 ^{T} can reduce nitrate (Table 1), thus supporting taxonomic differentiation based on a polyphasic approach.

DESCRIPTION OF *PSEUDOMONAS BOANENSIS* SP. NOV.

Pseudomonas boanensis (bo.a.nen'sis. N.L. fem. adj. *boanensis* pertaining to the Boane District in Mozambique).

Cells are Gram-negative, rod-shaped, motile by a polar flagellum, oxidase-positive and catalase-negative. Cells are positive for utilization of D-glucose, D-gluconate, caprate, malate, citrate, phenylacetate, arginine, lactate, caprate, leucine arylamidase and esterase C4 activity. Strain DB1^T lacks acid phosphatase, indole, β-galactosidadse, haemolysis and alkaline phosphatase activity, and the ability to reduce nitrite and nitrate, and it is unable to utilize amylase, lysine, ornithine, urease, trehalose, norleucin, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, arginine-dihydrolase sucrose and adipate.

The genome of the type strain, DB1^T (CCUG 62977^T=CECT 30359^T), is 5.71 Mbp with a G+C content of 62.33mol%.

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Author contributions
Conceived and designed experiments: B.H., M.N., L.N., A.M., Å.S. Performed the experiments: M.N., K.T., A.J.M.R., L.N., A.M., H.R., L.S-S. Analyzed and interpreted the data: M.N., A.J.M.R., K.T., S.M.H., L.S-S., Å.S. Contributed reagents, materials, analysis tools or data: K.T., S.M.H., B.H., L.S-S, Å.S. Wrote the paper: M.N., A.J.M.R., K.T., Å.S.

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

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