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Pseudomonas versuta sp. nov., isolated from Antarctic soil

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Abstract:

In this study we used a polyphasic taxonomy approach to analyse three bacterial strains coded L10.10^T, A4R1.5 and A4R1.12, isolated in the course of a study of quorum-quenching bacteria occurring Antarctic soil. The 16S rRNA gene sequence was identical in the three strains and showed 99.7% pairwise similarity with respect to the closest related species *Pseudomonas weihenstephanensis* WS4993^T, and the next closest related species were *P. deceptionensis* M1^T (99.5%), *P. psychrophila* E-3^T, *P. endophytica* BSTT44^T and *P. fragi* ATCC 4973^T (99.2%). Therefore, the three strains were classified within the genus *Pseudomonas*. Analysis of housekeeping genes (*rpoB*, *rpoD* and *gyrB*) sequences showed similarities of 84-95% with respect to the closest relatives, confirming its phylogenetic affiliation. The whole genome average nucleotide identity (ANI) values were more than 99% similar among the three strains, and less than 86% to the closest related species type strains. The respiratory quinone is Q9. The major fatty acids are C16:0, C16:1 ω7c/ C16:1 ω6c in summed feature 3 and C18:1 ω7c / C18:1 ω6c in summed feature 8. The strains are oxidase- and catalase-positive. The arginine dihydrolase and urease tests are positive. Growth occurs at 4–30 °C with an optimum at 28 °C, and at pH 4.0–10. The DNA G+C content is 58.2-58.3 mol %. The combined genotypic, phenotypic and chemotaxonomic data support the classification of strains L10.10^T, A4R1.5 and A4R1.12 into a novel species of *Pseudomonas*, for which the name *P. versuta* sp. nov. is proposed. The type strain is L10.10^T (LMG 29628^T, DSM 101070^T).

Keywords: *Pseudomonas,* taxonomy, Antarctica, Antarctic soil, quorum quenching, quorum sensing

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The genus *Pseudomonas* was first described by Migula [29] and is one of the most commonly reported bacteria in Antarctica [28]. To date, according to the *List of Prokaryotic Names with Standing in Nomenclature* (http://www.bacterio.net/pseudomonas.html), there are more than 150 species described in this genus. *Pseudomonas* is one of the most diverse and ubiquitous bacterial genera, and representatives have been isolated worldwide including from many extreme environments such as Antarctica [37], given their extraordinarily versatile metabolism. Even though psychrophilic strains of *P. aeruginosa, P. fluorescens, P. putida* and *P. syringae* were reported by Shivaji et al. [47] in Antarctic soil and water samples, entirely novel species of *Pseudomonas* from Antarctica were first described by Reddy et al. in 2004 with the isolation of *P. antarctica, P. proteolytica* and *P. meridiana* from cyanobacterial mat samples [39]. Since then, six further novel species have been isolated from Antarctic or sub-Antarctic regions, including *P. guineae* [5], *P. pelagia* [18], *P. extremaustralis* [27], *P. deceptionensis* [6], *P. prosekii* [23], and the most recently described *P. yamanorum* [4], isolated from the Isla de los Estados (Tierra del Fuego, Argentina).

The production of *N*-acyl-homoserine lactones (AHLs) mediating quorum sensing (QS) is a common property identified in numerous *Pseudomonas* strains for the regulation of a variety of phenotypes in relation to the population density [24, 53, 55]. Certain *Pseudomonas* species were also found to possess AHL acylase activity, an enzyme that can degrade AHLs, allowing the utilization of this compound as nitrogen and energy sources [17, 26, 49]. Degradation of QS signaling is termed as quorum quenching (QQ), and has been suggested as a highly promising alternative approach for infection control since QQ does not affect bacterial viability and could minimize the selective pressure to develop resistance [14]. QQ enzyme production has been widely described in mesophilic bacterial strains [12, 14]. In this context, isolation of novel psychrotolerant QQ bacteria will help to unravel the potential of

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QQ bacteria as biocontrol agents in cold environments at high latitudes or in temperate regions.

During an ecological survey of QQ soil bacteria in various locations in Antarctica (Fig. 1), we isolated three strains from soil samples using QQ bacteria enrichment medium [8]. The soil samples were collected from Lagoon Island and Anchorage Island (Ryder Bay, Adelaide Island) and taken to the laboratory for bacterial isolation. In brief, 1 g of soil sample and 5 ml sterile QQ bacteria enrichment medium containing 100 µg synthetic C_6-HSL as sole carbon source, were mixed in a sterile 50 ml plastic tube and incubated at 4 °C and 150 rpm. After 1 week of incubation, 100 µl of the bacterial suspension were added into new QQ bacteria enrichment medium again including C_6-HSL. This step was repeated three times and finally, 100 µl of bacterial suspension were plated onto Luria-Bertani (LB) agar. The three strains obtained, coded as L10.10^T, A4R1.5 and A4R1.12, were subjected to a polyphasic taxonomic study, and classified into the genus *Pseudomonas* within the Class Gammaproteobacteria according to 16S rRNA and housekeeping gene analyses. The genomic, phylogenetic, chemotaxonomic and phenotypic data obtained showed that they represent a novel species for which the name *Pseudomonas versuta* sp. nov. is proposed.

In order to analyse genetic variability and to assess for clonality, the three strains were subjected to PCR RAPD fingerprinting using the primer M13 (5'- GAGGGTGGCGGTTCT –3') as described previously [41]. The bands present in each profile were coded for input into a database including all the strains studied and Dice similarity coefficient was calculated to construct the distance matrix. A dendrogram was constructed from the distance matrix using the unweighted pair group with arithmetic mean (UPGMA), using the GelCompar II program from Bionumerics platform (Applied Maths NV). The RAPD patterns obtained showed that the three strains L10.10^T, A4R1.5 and A4R1.12 are not clones since they present different

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RAPD profiles. Strains L10.10^T and A4R1.5 show less than 90% similarity between them and less than 80% with respect to the strain A4R1.12 in the Dice dendrogram, presenting a high genetic variability with respect to the closely related *Pseudomonas* species (Fig. S1).

Colony morphologies of the three strains were observed after 48 h of incubation on LB agar. Gram-staining was conducted using a Difco Gram stain set followed by observation under a Leica DM750 microscope (Leica Microsystems). Type of flagellation and cell morphology were documented using a Hitachi TM3030 scanning electron microscope and a scanning transmission electron microscope (STEM, LIBRA 120; Carl Zeiss AG, Germany). Briefly, cells were grown on LB broth overnight at 25 °C, harvested by centrifugation and resuspended in PBS. The overnight suspension cells were then stained using 1% phosphotungstic acid on a Formvar grid and observed at an operating voltage of 80 kV. Strain L10.10^T is Gram negative, rod-shaped (0.6-0.8 x 1.8-2.3µm) and motile by one polar-subpolar flagellum (Fig. S2).

The complete genome of strain L10.10^T has been previously sequenced, enabling the discovery of various genes encoding for plant growth promoting properties and plant disease prevention attributes [45]. The draft genomes of strains A4R1.5 and A4R1.12 were obtained in this study in order to perform whole genome sequence analysis. The genomic DNA was extracted using the MasterPure DNA purification kit (Epicenter, USA) following the manufacturer's protocol. The genomic library was then generated using the Nextera DNA Library prepation kit (Illumina, USA) and subsequently subjected to sequencing using an Illumina MiSeq sequencer. The reads generated were filtered and assembled using CLC genomic benchwork.

In order to establish the phylogenetic affiliation of the novel isolates, the complete 16S rRNA gene sequences of strains L10.10^T, (1466 bp), A4R1.5 (1525 bp) and A4R1.12 (1525 bp)

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were retrieved from the complete genome sequence of L10.10^T and draft genome sequences from A4R1.5 and A4R1.12, and compared with those held in databases using Ez-Taxon-e [19] and BlastN [3]. Pairwise similarity analysis demonstrated that the three strains showed 100% sequence similarity among them, and therefore only strain L10.10^T was included in the 16S rRNA gene phylogenetic analysis. Comparison of strain L10.10^T sequence against the databases mentioned showed that it should be classified into the genus *Pseudomonas*. The closest related species is *P. weihenstephanensis* WS4993^T [54] with 99.7% pairwise similarity, and the following closest related species are *P. deceptionensis* M1^T [6] with 99.5%, *P. psychrophila* E-3^T [57], *P. endophytica* BSTT44^T [38] and *P. fragi* ATCC 4973^T [15] with 99.2%, *P. helleri* WS4917^T [54] with 99.0%, *P. taetrolens* IAM1653^T [16] with 98.9% and *P. lundensis* ATCC 49968^T [30] with 98.6% sequence similarity. For 16S rRNA gene phylogenetic analysis, the type strains of the closely related *Pseudomonas* species showing more than 98% sequence similarity with respect to strain L10.10^T were included. Alignment was performed using the Clustal_X software [52]. The distances were calculated according to Kimura´s two-parameter model [20]. Phylogenetic trees of the 16S rRNA gene were inferred using the neighbour-joining analysis (NJ) [43] and maximum likelihood (ML) [42]. All the analyses were performed using MEGA 6.06 software [51]. As can be seen in the ML phylogenetic tree (Fig. 2), the strain L10.10^T clustered separately in a group that included the closest related species *P. weihenstephanensis* and *P. deceptionensis*, branching in a wider group formed by the closest species mentioned above. The results were congruent with the tree topology obtained after NJ phylogenetic analysis (data not shown).

To establish precisely the phylogenetic affiliation of the novel isolates, in addition to the 16S rRNA gene sequence analysis, we also studied the three housekeeping genes more commonly used in the phylogenetic analysis of *Pseudomonas* species, *rpoD, rpoB* and *gyrB* genes [1, 22, 31, 32, 33, 38, 54]. The phylogenetic analysis of the concatenated *rpoD, rpoB* and *gyrB*

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genes sequences was performed with the same methodology as for the 16S rRNA gene and showed that the strains L10.10^T, A4R1.5 and A4R1.12 clustered in a separate branch related to a group formed by the type strains of the closely related species *P. weihenstephanensis, P. deceptionensis, P. psychrophila* and *P. fragi* (Fig. 3). As can be seen in the ML phylogenetic tree (Fig. 3), all these strains are located in a wider group including also the species *P. lundensis, P. taetrolens, P. helleri* and *P. endophytica,* confirming the results obtained from the phylogenetic analysis of the 16S rRNA gene sequence. This group corresponds to the "*P. fragi*" subgroup within the "*P. fluorescens*" group of *Pseudomonas* species defined by Mulet et al. [33] after the phylogenetic analysis of the concatenated 16S rRNA gene and the three mentioned housekeeping genes. The same topologies were again obtained after NJ phylogenetic analysis (data not shown). The identities of the housekeeping genes sequences of the novel isolates with respect to the closely related species belonging to the '*P. fragi* subgroup' ranged from 84.7-95.6% for *rpoD*, 89.7-95.2% for *rpoB*, and 88.9-93.1% in the case of *gyrB* gene. These values are in the range of those found for other species of the genus *Pseudomonas* [38], and support the classification of the novel isolates within the genus *Pseudomonas*, representing an as yet undescribed species of this genus.

In addition to the 16S rRNA gene sequence and MLSA analyses, in order to provide support for the classification of these Antarctic strains as a novel species, we carried out whole genome sequence analysis considering the same closely related species included in the previous phylogenetic analyses described above. Generally, an average nucleotide identity (ANI) value of 95-96 % has been accepted as the cutoff threshold for bacterial species delineation corresponding to 70 % DNA relatedness [13, 40]. ANI analysis was performed using the orthologous average nucleotide identity tool, OAT [25]. According to the data obtained, the strains L10.10^T, A4R1.5 and A4R1.12 showed more than 99% ANI similarity among them, and less than 86% ANI similarity values with respect to all the closest related

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species (Table S1), confirming that the three strains belong to the same species, which should be classified as novel species of the genus *Pseudomonas* when the range threshold values of 94-96 % ANI similarity proposed by Richter and Rossello-Mora [40] for delineation of bacterial species is considered.

A genomic comparison across the L10.10^T, A4R1.5 and A4R1.12 genomes and their close relatives included in the '*P. fragi* subgroup' at nucleotide level was carried out using the BLAST Ring Image Generator (BRIG) [2]. The genome of strain L10.10^T was used as the reference genome. The genome sequence accession numbers for L10.10^T, A4R1.5 and A4R1.12 are CP012676, MPJC00000000 and MPJD00000000, respectively. The genome sequence accession numbers for the closely related species are *P. weihenstephanensis*, JYLF01000000; *P. taetrolens*, JYLA00000000.; *P. psychrophila*, JYKZ01000000; *P. lundensis*, JYKY00000000; *P. helleri*, JYLD00000000; *P. fragi*, AHZX00000000; *P. deceptionensis*, JYKX00000000; and *P. endophytica*, LLWH00000000. The BRIG analysis indicated that most regions within the analyzed genomes were conserved with at least 70 % or greater similarity (Fig. S3a). However, strains L10.10^T, A4R1.5 and A4R1.12 showed higher similarity with each other, with most regions being conserved (Fig. S3b). The pan- and core-genome analysis were performed using the ultra-fast computational pipeline BPGA (Bacterial Pan Genome Analysis tool) [7]. The analysis indicates that all the genomes considered share 2669 common genes, representing about half of the genes in each genome. In the genome of L10.10^T there are 10 unique genes compared to other genomes tested. A summary of the core- and pan-genome analyses is given in Table S2.

G+C content of strain L10.10^T was 58.2 mol % as determined from the complete genome sequence, and ranged between 58.2-58.3 mol % from the draft genome sequences for the

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strains A4R1.5 and A4R1.12. These values are within the range commonly found in *Pseudomonas* species.

The cellular fatty acids were analyzed by using the Microbial Identification System (MIDI; Microbial ID) Sherlock 6.1 and the library RTSBA6, according to the technical instructions provided by this system [44]. Strain L10.10^{T} and the type strains of the same closely related species clustering in the same phylogenetic '*P. fragi* subgroup' grouping together in the MLSA analysis were grown on TSA plates (Becton Dikinson, BBL) for 24 h at 28°C and harvested in late log growth phase. The major fatty acids of strain L10.10^{T} were 16:0 (29.4%), C16:1 ω7c/ C16:1 ω6c in summed feature 3 (35.8%) and C18:1 ω7c / C18:1 ω6c in summed feature 8 (11.5%). As expected, all the relatives clustering in the same phylogenetic group with strain L10.10^{T} shared similar fatty acid profiles (Table 1). L10.10^{T} had the three fatty acids typically present in the genus *Pseudomonas* according to Palleroni [35], which are C10:0 3OH, C12:0 and C12:0 3OH.

The strain L10.10^{T} was cultivated for 24 h on TSA plates (Becton Dikinson, BBL) at 28°C to obtain the cell mass required for quinone analysis, which was carried out from freeze-dried cells as described by Collins and Jones [11], Tamaoka et al. [50] and Collins [10]. The strain L10.10^{T} contained Q9 as the main respiratory quinone, which is also typical in species of the genus *Pseudomonas* [36].

Physiological and biochemical tests were performed as previously described [37] including for comparison the type strains of the same *Pseudomonas* species chosen for FAME analysis and whole genome comparison analysis. For fluorescent pigment analysis, cells were grown in King B broth and tested for pigment production [21]. Strains L10.10^{T}, A4R1.5 and A4R1.12 did not produce a fluorescent pigment in this medium. Catalase activity was

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assayed using 3% (v/v) H_2O_2 and determined by assessing the production of copious bubbles, and oxidase activity was determined using 1% (w/v) *N*,*N*,*N*',*N*',-tetramethyl 1,4-phenylenediamine (bioMérieux) as described by Smibert & Krieg [48]. Positive reactions were observed in both tests for strains L10.10^T, A4R1.5 and A4R1.12. Additionally API 20NE (bioMérieux) and Biolog GN2 Microplates (Biolog, Inc., Hayward, Calif.) were used following the manufacturer's instructions. The results of API 20NE were recorded after 48 h incubation at 28ºC.

Phenotypic characteristics of the new species are reported below in the species description, and the differences with respect to the closest related *Pseudomonas* species and the type species of the genus, *P. aeruginosa*, are recorded in Table 2. The phenotypic characteristics of strains L10.10^T A4R1.5 and A4R1.12 provide further support for their classification as a novel species of *Pseudomonas*, since they are motile Gram-negative strictly aerobic rods, catalase- and oxidase-positive and motile by a polar flagellum [46]. These three strains can be differentiated from other species of the genus by their 16S rRNA and housekeeping gene sequences, ANI values, genome nucleotide analysis, and their phenotypic and chemotaxonomic characteristics, and therefore they should be classified as a novel species of *Pseudomonas*, for which we propose the name *Pseudomonas versuta* sp. nov.

Description of *Pseudomonas versuta* sp. nov.

Pseudomonas versuta (ver.su'ta. L. fem. adj. versuta, adroit, shrewd, ingenious, referring to the ability for quorum-quenching activity).

Strictly aerobic, Gram-stain-negative, non-spore forming straight rods of 1.8-2.3 µm in length and 0.6-0.8 µm in diameter, motile by a polar flagellum, occurring singly and in pairs.

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Colonies are 1.5–2.0 mm in diameter, raised, circular-shaped, and cream-colored after 48 h incubation at 25°C on LB agar. Growth occurs at 4–30°C with an optimum at 28°C, and at pH 4.0–10.0. Tolerant to 0–7% NaCl (w/v) in LB broth. Strictly aerobic with oxidative metabolism and no fermentation of sugars in peptone media. No diffusible fluorescent pigment is produced on King B medium. The major respiratory ubiquinone is Q9. Major fatty acids are 16:0, 16:1ω7c/ 16:1ω6c in summed feature 3 and 18:1ω7c/18:1ω6c in summed feature 8. Oxidase- and catalase-positive. In API 20 NE system arginine dihydrolase and urease are positive. Indole and ß–galactosidase production as well as nitrate reduction and esculine hydrolysis are negative. Assimilation of glucose, arabinose, mannose, mannitol, gluconate, caprate, malate and citrate is positive. Negative results were obtained for assimilation of maltose, adipate and phenylacetate. Assimilation of N-acetyl-glucosamine is weak. In Biolog GN2 plates the assimilation of tween 40, tween80, L-arabinose, D-arabitol, D-fructose, L-fucose, D-galactose, α-D-glucose, m-inositol, D-mannitol, D-mannose, D-sorbitol, sucrose, D-trehalose, methyl-piruvate, acetate, cis-aconitate, citrate, D-galactonate lactone, D-galacturonate, D-gluconate, D-glucosaminate, D-glucuronate, β-hydroxy-butyrate, α-ketoglutarate, D, L-lactate, propionate, quinate, D-saccharate, bromo-succinate, glucuronamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartate, L-glutamate, glycyl-L-glutamate, L-histidine, L-leucine, L-ornithine, L-proline, L-pyroglutamate, D-serine, L-serine, D,L carnitine, γ-aminobutyrate, urocanate, inosine, uridine, putrescine, 2-aminoethanol and glycerol is positive. Negative results were obtained for α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, adonitol, D-cellobiose, i-erythritol, gentiobiose, α-D-lactose, lactulose, maltose, D-melibiose, □β-methyl-D-glucoside, D-psicose, D-raffinose, L-rhamnose, turanose, xylitol, formate, α-hydroxybutirate, γ-hydroxybutyrate, itaconate, α-ketobutyrate, α-ketovalerate, malonate, sebacate, glycyl-L-aspartate, L-threonine, thymidine, phenylethylamine, 2,3-butanediol and glucose-6-

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phosphate. Finally, assimilation of N-acetyl-D-glucosamine, L-phenylalanine, and D,L- α-glycerolphosphate was weak. Variable results were observed for assimilation of succinate, mono-methyl-succinate, L-alaninamide, succinamate, p-hydroxyphenylacetate, hydroxy-L-proline and glucose-1-phosphate. G+C base composition was 58.2-58.3 mol%. The type strain is L10.10^T (LMG 29628^T, DSM 101070^T), isolated from soil obtained from Lagoon Island, Antarctica. Digital Protologue Taxonumber: TA00067

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Figure legends

Fig 1: The location of Lagoon and Anchorage Islands in Ryder Bay, south-eastern Adelaide Island, off the west coast of the Antarctic Peninsula.

Figure 2. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences (1399 nt) of *Pseudomonas versuta* sp. nov. and the type strains of closely related *Pseudomonas* species. Bootstrap values (only values > 50% expressed as percentages of 1000 replications) are shown at the branching points. Bar, 5 nt substitutions per 100 nt.

Figure 3. Maximum Likelihood phylogenetic tree based on concatenated partial *rpoD, rpoB* and *gyrB* gene sequences (2338 nt) of *Pseudomonas versuta* and the type strains of the closely related *Pseudomonas* species. Bootstrap values (only values > 50% expressed as percentages of 1000 replications) are shown at the branching points. Bar, 5 nt substitutions per 100 nt.

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Table 1. Cellular fatty acid composition (%) of *P. versuta* L10.10^T, its phylogenetically closest related species and the type species of this genus, *P. aeruginosa*.
Taxa: 1, *P. versuta* L10.10^T; 2, *P. weihenstephanensis* DSM 29166^T; 3, *P. deceptionensis* LMG25555^T; 4, *P. endophytica* BSTT44^T; 5, *P. psychrophila* LMG24276^T; 6, *P. fragi* LMG2191^T; 7, *P. helleri* DSM 29165^T; 8, *P. taetrolens* DSM 21104^T; 9, *P. lundensis* LMG13517^T; 10, *P. aeruginosa* ATCC 10145^T. Data were obtained in this study and from [ʃ]Xiao *et al.* [56] using the same conditions. nd: no detected, tr: traces.

Fatty acids	1	2	3	4	5	6	7	8	9	[ʃ]10
12:0	**3.0**	**3.4**	**3.3**	**3.6**	**3.9**	**3.9**	**3.6**	**3.5**	**3.1**	**4.8**
14:0	**0.5**	**0.7**	**1.1**	**0.4**	**0.6**	**0.5**	**0.5**	**0.1**	**0.5**	**1.3**
16:0	**29.4**	**28.0**	**36.0**	**29.4**	**29.7**	**25.1**	**28.8**	**29.2**	**31.1**	**20.5**
17:0	**nd**	**0.2**	0.1	**0.1**	0.1	0.2	**0.2**	**0.2**	0.2	**tr**
18:0	**0.5**	**0.5**	**0.5**	**0.7**	**0.7**	**1.4**	**0.4**	**0.1**	**0.6**	**tr**
10:0 3OH	**4.6**	**5.3**	**4.0**	**3.6**	**4.1**	**4.4**	**7.2**	**3.1**	**3.9**	**3.6**
12:0 2OH	**5.2**	**5.1**	**4.0**	**3.4**	**3.4**	**3.7**	**6.3**	**3.4**	**4.1**	**3.7**
12:0 3OH	**5.6**	**5.4**	**4.5**	**4.2**	**4.3**	**4.9**	**6.7**	**5.1**	**3.9**	**4.5**
17:0 cyclo	**3.4**	**4.6**	**3.9**	**4.5**	**1.3**	**0.7**	**13.9**	**11.8**	**2.6**	**nd**
19:0 cyclo ω8c	**0.5**	**0.8**	**0.1**	**0.2**	**nd**	**0.3**	**2.5**	**2.6**	**0.4**	**nd**
Summed feature 3*	**35.8**	**33.2**	**33.0**	**31.8**	**40.2**	**34.0**	**21.4**	**28.0**	**34.8**	**20.0**
Summed feature 8[¥]	**11.5**	**12.6**	**8.1**	**16.5**	**11.3**	**19.1**	**8.0**	**12.5**	**13.6**	**38.9**

*Summed feature 3: C16:1 ω7c/ C16:1 ω6c.
[¥]Summed feature 8: C18:1 ω7c / C18:1 ω6c.

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Table 2. Differential characteristics of *P. versuta*, its phylogenetically closest related species and the type species of this genus, *P. aeruginosa*. Taxa: 1, *P. versuta* L10.10^T; 2, *P. versuta* A4R1.5; 3, *P. versuta* A4R1.12; 4, *P. weihenstephanensis* DSM 29166^T; 5, *P. deceptionensis* LMG25555^T; 6, *P. endophytica* BSTT44^T; 7, *P. psychrophila* LMG24276^T; 8, *P. fragi* LMG2191^T; 9, *P. helleri* DSM 29165^T; 10, *P. taetrolens* DSM 21104^T; 11, *P. lundensis* LMG13517^T; 12, *P. aeruginosa* ATCC 10145^T. Data were obtained in this study unless specifically indicated. +, positive; -, negative; w, weak; nd, no data.

	1	2	3	4	5	6	7	8	9	10	11	12$^¥$
Fluorescent pigments King B Agar	-	-	-	+	-	+	+	+	+	-	+	+
Growth at:												
33°C	-	-	-	+	+	-	+	+	-	+	-	+
Production of:												
Arginine dihydrolase	+	+	+	+	+	+	+	+	-	w	+	+
Assimilation of:												
D-Mannose	+	+	+	-	+	-	+	-	w	+	-	-
D-Mannitol	+	+	+	-	+	-	+	-	+	+	-	+
Phenylacetate	-	-	-	-	-	-	-	-	+	-	-	-
m-Inositol	+	+	+	+	+	-	-	-	-	+	-	-
D-Trehalose	+	+	+	+	+	-	w	+	+	+	-	-
Mono-methyl-succinate	+	-	w	+	+	-	+	+	-	-	+	+
Acetate	+	+	+	+	+	-	+	+	+	+	+	+
D-Galactonate acid lactone	+	+	+	-	-	+	-	-	+	+	-	-
D-Galacturonate	+	+	+	+	-	+	+	-	+	+	-	+
Itaconate	-	-	-	-	-	-	-	-	+	+	-	+
Succinamate	+	-	-	+	+	+	+	+	+	-	-	+
Glucuronamide	+	+	+	+	+	+	+	-	+	+	-	-
L-Alaninamide	+	-	+	-	-	-	-	-	-	w	-	-
L-Alanyl-glycine	+	+	+	+	+	-	w	+	-	-	+	nd
Hydroxy-L-Proline	+	-	-	-	+	-	+	+	-	+	-	+
D-L Carnitine	+	+	+	+	-	-	-	-	w	+	-	w
Urocanate	+	+	+	+	-	-	+	+	+	+	+	-
Inosine	+	+	+	+	+	-	+	-	-	+	-	+
Uridine	+	+	+	-	-	-	w	-	-	+	-	w
Genome feature:												
Genome size (Mb)	5.15	5.20	5.10	4.79	5.05	4.97	5.33	5.02	5.67	4.92	4.99	6.29
DNA G+C content (mol %)	58.2	58.2	58.3	57.3	58.6	55.2	57.5	59.4	58.1	58.3	58.5	66.6
Number of genes #	4639	4774	4690	4417	4631	4598	4889	4557	5040	4481	4609	5787
Number of coding sequences #	4425	4636	4553	4196	4460	4276	4718	4297	4811	4300	4365	5698

$^¥$Data for *P. aeruginosa* ATCC 10145^T are from Palleroni [34], Clark *et al.* [9] and Xiao *et al.* [56]

Figure 1

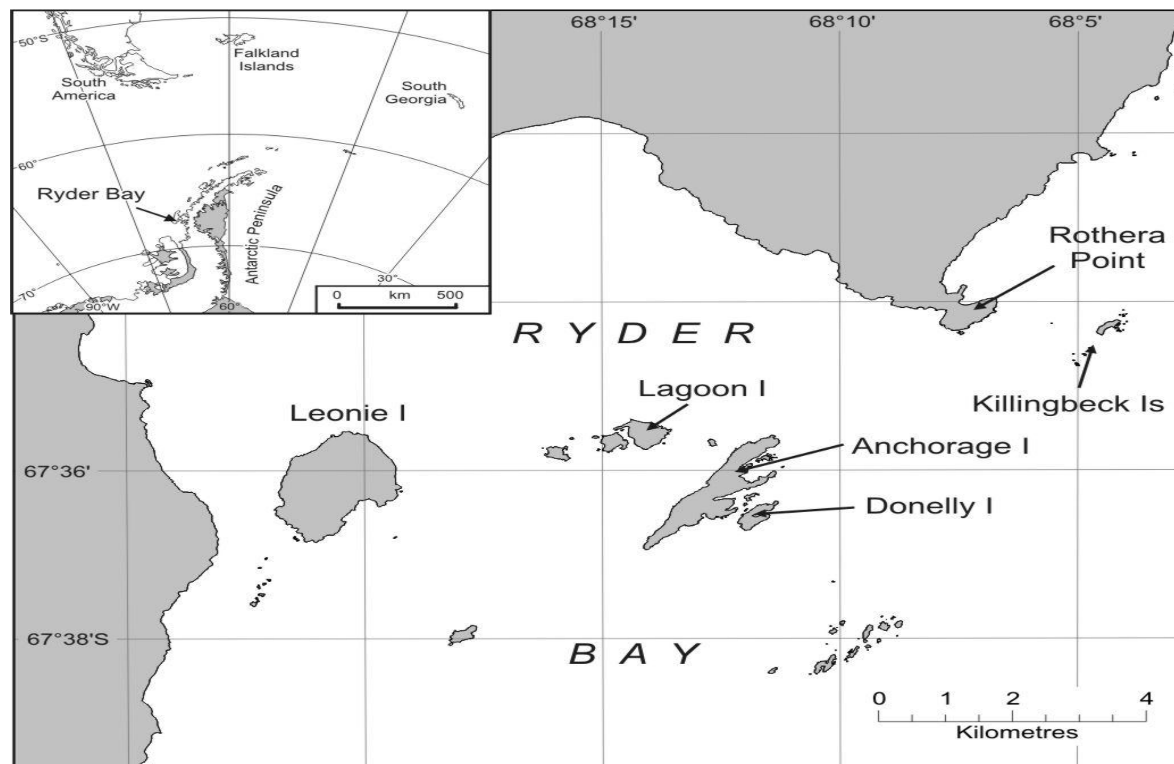

Figure2 R1

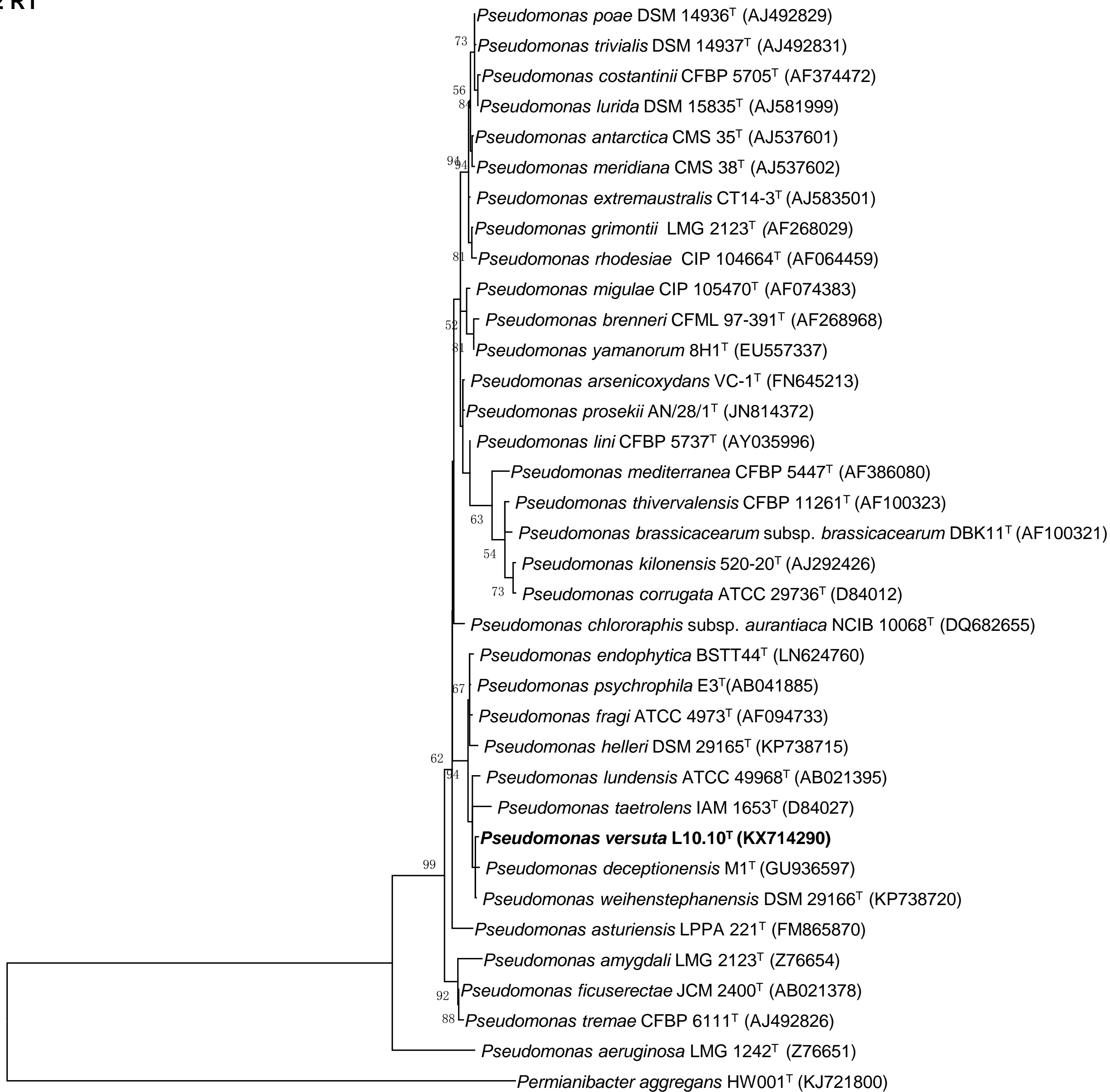

Figure 3