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Burkholderia dipogonis sp. nov., isolated from root nodules of Dipogon lignosus in New Zealand and Western Australia

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Abstract:	Seven strains, ICMP 19430T, ICMP 19429, ICMP 19431, WSM4637, WSM4638, WSM4639 and WSM4640 were isolated from nitrogen-fixing nodules on the roots of the invasive South African legume Dipogon lignosus (subfamily Papilionoideae, tribe Phaseoleae) in New Zealand and Western Australia, and their taxonomic positions were investigated by a polyphasic approach. All seven strains grew at 10-37 °C (optimum, 25-30 °C), at pH 4.0-9.0 (optimum, pH 6.0-7.0) and with 0-2 % (w/v) NaCl (optimum, 0 % [w/v]). On the basis of 16S rRNA gene sequence analysis, the strains showed 99.0-99.5 % sequence similarity to the closest species Burkholderia phytofirmans PsJNT and 98.4-99.7 % sequence similarity to Burkholderia caledonica LMG 19076T. The predominant fatty acids were C18:1 7c (21.0 %), C16:0 (19.1 %), C17:0 cyclo (18.9 %), summed feature 3 (comprising C16:1 7c and/or C16:1 6c; 10.7 %) and C19:0 cyclo 8c (7.5 %). The polar lipid profile consisted of a mixture of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and several uncharacterized aminophospholipids and phospholipids. The major isoprenoid quinone was Q-8 and the DNA G+C content was 63.2 mol%. The DNA-DNA relatedness of the novel strains with respect to the closest neighbouring Burkholderia species was 55 % or less. On the basis of 16S rRNA and recA gene sequence similarities, and on chemotaxonomic and phenotypic data, these strains represent a novel symbiotic species in the genus Burkholderia, for which the name Burkholderia dipogonis sp. nov. is proposed with the type strain ICMP 19430T (= LMG 28415T = HAMBI 3637T).

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1 # *Burkholderia dipogonis* sp. nov., isolated from root nodules of
2 *Dipogon lignosus* in New Zealand and Western Australia

3

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18

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27

28 The expanded phylogenetic tree based on 16S rRNA gene sequences (Fig. S1),
29 *recA*-based neighbour-joining tree (Fig. S2), dendrogram based on numerical
30 analysis of the whole-cell protein profiles (Fig. S3), two-dimensional TLC of polar
31 lipids (Fig. S4), GN2 microplate (Biolog) oxidation data (Table S1) and DNA-DNA
32 hybridization values (Table S2) are available as supplementary material with the
33 online version of this paper.

34 The GenBank/EMBL/DDBJ accession numbers of strains ICMP 19430^T, ICMP
35 19429, ICMP 19431, WSM4637, WSM4638, WSM4639 and WSM4640 are
36 JX009148, JX009147, JX009149, KM067130, KM067131, KM067132 and
37 KM067133 for 16S rRNA and JX009159, JX009158, JX009160, KR071790,
38 KR071791, KR071792 and KR071793 for *recA*.

39

Seven strains, ICMP 19430^T, ICMP 19429, ICMP 19431, WSM4637, WSM4638, WSM4639 and WSM4640 were isolated from nitrogen-fixing nodules on the roots of the invasive South African legume *Dipogon lignosus* (subfamily *Papilionoideae*, tribe Phaseoleae) in New Zealand and Western Australia, and their taxonomic positions were investigated by a polyphasic approach. All seven strains grew at 10-37 °C (optimum, 25-30 °C), at pH 4.0-9.0 (optimum, pH 6.0-7.0) and with 0-2 % (w/v) NaCl (optimum, 0 % [w/v]). On the basis of 16S rRNA gene sequence analysis, the strains showed 99.0-99.5 % sequence similarity to the closest species *Burkholderia phytofirmans* PsJN^T and 98.4-99.7 % sequence similarity to *Burkholderia caledonica* LMG 19076^T. The predominant fatty acids were $C_{18:1}$ $\omega 7c$ (21.0 %), $C_{16:0}$ (19.1 %), $C_{17:0}$ cyclo (18.9 %), summed feature 3 (comprising $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; 10.7 %) and $C_{19:0}$ cyclo $\omega 8c$ (7.5 %). The polar lipid profile consisted of a mixture of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and several uncharacterized aminophospholipids and phospholipids. The major isoprenoid quinone was Q-8 and the DNA G+C content was 63.2 mol%. The DNA-DNA relatedness of the novel strains with respect to the closest neighbouring *Burkholderia* species was 55 % or less. On the basis of 16S rRNA and *recA* gene sequence similarities, and on chemotaxonomic and phenotypic data, these strains represent a novel symbiotic species in the genus *Burkholderia*, for which the name *Burkholderia dipogonis* sp. nov. is proposed with the type strain ICMP 19430^T (= LMG 28415^T = HAMBI 3637^T).

62 The genus *Burkholderia*, belonging to the family *Burkholderiaceae* of the
63 *Betaproteobacteria*, was proposed by Yabuuchi *et al*. (1992). At the time of writing,
64 it comprises 87 validly published species
65 (http://www.bacterio.net/burkholderia.html). Members of the genus *Burkholderia* are
66 characterized as Gram-stain-negative, aerobic, non-spore-forming, non-fermentative,
67 straight rod-shaped, and catalase-positive bacteria, and most species are motile by
68 using a single polar flagellum or a tuft of polar flagella. They possess high metabolic
69 versatility, have $C_{16:0}$ 3-OH as the major cellular hydroxyl fatty acid and a DNA G+C
70 content between 59.0 and 69.5 mol% (Gillis *et al*., 1995; Palleroni, 2005).
71 *Burkholderia* species have been isolated from humans (particularly cystic fibrosis
72 patients), from rhizosphere soil, animals, plants, water and hospital equipment
73 (Coenye *et al*., 2001; Sessitsch *et al*., 2005; Kim *et al*., 2006; Vandamme *et al.*, 2007;
74 Suárez-Moreno *et al.*, 2012). Over the last 12 years, however, there has been an
75 increasing number of *Burkholderia* species described as N_2-fixing symbionts of
76 legumes (see Gyaneshwar *et al.,* 2011 for a review). These can be broadly divided
77 into two groups: those that have been isolated from *Mimosa* spp. (subfamily
78 *Mimosoideae*, tribe Mimoseae) native to the Neotropics (Chen *et al.,* 2006, 2007,
79 2008; Sheu *et al.,* 2012, 2013), and those that have been isolated from various
80 papilionoid legumes native to South Africa (Elliott *et al.,* 2007; Garau *et al.,* 2009;
81 De Meyer *et al*., 2013a, b; 2014; Lemaire *et al.,* 2015).

82 Recently, 10 nodule isolates from the invasive South African legume *Dipogon*
83 *lignosus* (tribe Phaseoleae) growing in New Zealand were sampled and surveyed for
84 their symbiotic diversity. Sequences of their 16S rRNA, *recA*, *nifH*, *nodA* and *nodC*
85 genes showed that seven of these isolates belonged to the genus *Burkholderia*. (Liu *et*
86 *al*., 2014). Three isolates were deposited in the International Collection of

87 Microorganisms from Plants (ICMP), Landcare Research, Auckland, NZ as ICMP
88 19430^T, ICMP 19429 and ICMP 19431. Additionally, ICMP 19430^T was deposited in
89 the BCCM/LMG bacteria collection (http://www.belspo.be/bccm) and the HAMBI
90 Culture Collection, University of Helsinki, Finland (http://www.helsinki.fi/hambi/), as
91 LMG 28415^T and HAMBI 3637^T, respectively. ICMP 19429, ICMP 19430^T and
92 ICMP 19431 nodulated and fixed nitrogen with their original host *D. lignosus* and
93 with *Phaseolus vulgaris* (also in tribe Phaseoleae), but were unable to nodulate
94 *Mimosa pudica* (Liu *et al*., 2014). In addition, ICMP 19430^T was shown to produce
95 N_2-fixing nodules on the South African native legumes *Cyclopia subternata*,
96 *Hypocalyptus sophoroides*, *Podalyria calyptrata* and *Virgilia oroboides* (Liu *et al*.,
97 2014).

98 Four isolates were obtained from four nitrogen-fixing nodules of *D. lignosus*
99 growing in sandy soil in the Dugalup Brook vegetation reserve in the coastal town of
100 Dunsborough, southwestern Australia (latitude: 33 36' 55" S, longitude: 115 6' 13" E),
101 and were deposited in the Western Australian Soil Microbiology (WSM) culture
102 collection at the Centre for Rhizobium Studies, Murdoch University, Western
103 Australia (WA) as WSM4637, WSM4638, WSM4639 and WSM4640. All isolates
104 were tested and confirmed as able to form N_2-fixing nodules on *D. lignosus*, using the
105 axenic sand-culture system described previously (Howieson *et al.,* 1995). The
106 enterobacterial repetitive intergenic consensus (ERIC) PCR (Versalovic *et al.,* 1991)
107 banding patterns obtained for WSM4637, WSM4638, WSM4639 and WSM4640
108 indicated that they were very closely related (data not shown). Preliminary 16S rRNA
109 sequence data showed that these four isolates belonged to the genus *Burkholderia* and
110 were most closely related to the New Zealand *D. lignosus* isolates. Subsequent
111 analysis indicated that these four isolates along with the three isolates from *D.*

112 *lignosus* growing in New Zealand constituted seven separate strains (supported by
113 supplementary data, this paper).

114 As the study by Liu *et al.* (2014) suggested that ICMP 19429, ICMP 19430^T and
115 ICMP 19431 formed a novel taxonomic group, these strains together with the four
116 Western Australian *D. lignosus* strains were subjected to a polyphasic taxonomic
117 approach. All strains were grown on yeast extract-mannitol (YEM) agar plates
118 (Vincent, 1970) and incubated at 25 °C, unless otherwise indicated. Subculturing was
119 performed on YEM agar at 25 °C for 2 days. The strains were stored at -80 °C in
120 YEM broth with 20 % (v/v) glycerol or by lyophilization. *Burkholderia phytofirmans*
121 LMG 22146^T, *Burkholderia caledonica* LMG 19076^T, *Burkholderia phenoliruptrix*
122 LMG 22037^T, *Burkholderia fungorum* LMG 16225^T, *Burkholderia xenovorans* LMG
123 21463^T and *Burkholderia rhynchosiae* LMG 27174^T were obtained from the
124 BCCM/LMG Bacteria Collection, Belgium (LMG) and *Burkholderia ginsengisoli*
125 NBRC 100965^T was obtained from the NITE Biological Research Center (NBRC),
126 Japan. All type strains were used as references for phenotypic and genotypic tests.

127 Bacterial cells grown on YEM agar at 25 °C for 2 days were observed by
128 phase-contrast microscopy (DM2000; Leica). Flagellar motility was tested using the
129 hanging drop method, and the Spot Test flagella stain (BD Difco) was used for
130 flagellum staining (Beveridge *et al*., 2007). The Gram Stain Set S (BD Difco) kit and
131 the Ryu non-staining KOH method (Powers, 1995) were used for testing the Gram
132 reaction. The presence of a capsule was assessed using the Hiss staining method
133 (Beveridge *et al*., 2007). Poly-β-hydroxybutyrate granule accumulation was
134 examined under light microscopy after staining the cells with Sudan black (Schlegel
135 *et al.,* 1970) and visualized by UV illumination after growing bacteria on plates
136 containing Nile red at 25 °C for 2 days (Spiekermann *et al.,* 1999). Colony

137 morphology was observed on YEM agar using a stereoscopic microscope (SMZ 800;
138 Nikon).

139 The pH range for growth was determined for all investigated strains by
140 measuring the optical densities (wavelength 600 nm) of YEM broth. The medium
141 was adjusted prior to sterilization to pH 4.0-9.0 (at intervals of 0.5 pH units) using
142 the following biological buffers (Breznak & Costilow, 2007): citrate/Na_2HPO_4 (pH
143 4.0-5.5); phosphate (pH 6.0-7.5); and Tris (pH 8.0-9.0). The NaCl requirement was
144 determined using YEM broth containing 0, 0.5 and 1.0-8.0 % (w/v) NaCl (at 1.0 %
145 intervals). Growth at various temperatures (4-50 °C) was examined in YEM broth.
146 Cellular growth in the different conditions mentioned above was determined by
147 measuring the turbidity (OD_{600}) of the cultures. Anaerobic growth was determined
148 after incubating the strains on YEM agar in the Oxoid AnaeroGen system (Miller *et*
149 *al.,* 1995).

150 All investigated strains were examined for a broad range of phenotypic
151 properties. Activities of catalase, oxidase, DNase, urease and lipase (corn oil), and
152 hydrolysis of starch, casein and Tweens 20, 40, 60 and 80 were determined using
153 standard methods (Tindall *et al*., 2007). Hydrolysis of alginate (1 % w/v sodium
154 alginate) was examined on YEM agar. Chitin hydrolysis was assessed on
155 chitinase-detection agar (Wen *et al*., 2002) and visualized by the formation of clear
156 zones around the colonies. Hydrolysis of carboxymethylcellulose (CM-cellulose) was
157 tested as described by Bowman (2000) using YEM agar as the basal medium.
158 Additional biochemical tests were performed using the API 20NE and API ZYM kits
159 (bioMérieux) and carbon source utilization was evaluated using the GN2 microplate
160 (Biolog). All commercial phenotypic tests were performed according to the
161 manufacturer's recommendations.

162 The antibiotic sensitivities of the strains and the reference strains were analyzed
163 by the diffusion method after spreading cell suspensions (0.5 McFarland) on YEM
164 agar. The following antibiotic discs (Oxoid) were used: ampicillin (10 μg),
165 chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30
166 μg), novobiocin (30 μg), rifampicin (5 μg), penicillin G (10 U), streptomycin (10 μg),
167 sulfamethoxazole (23.75 μg) plus trimethoprim (1.25 μg), and tetracycline (30 μg).
168 The effect of antibiotics on cell growth was assessed after 2 days at 25 °C. Strains
169 were considered susceptible or resistant as described by Nokhal & Schlegel (1983).
170 Detailed results of the biochemical characterization and antibiotic sensitivity tests are
171 given in the species description, Table 1 and Supplementary Table S1. Our strains
172 can be distinguished from closely related *Burkholderia* type strains by using a
173 combination of phenotypic attributes, especially the activity of urease, assimilation
174 of phenyl-acetate, and the utilisation of putrescine, α-D-glucose-1-phosphate and
175 D-alanine as sole carbon sources (Table S1).

176 The 16S rRNA and *recA* gene sequences of strains ICMP 19430^T, ICMP 19429
177 and ICMP 19431 were obtained previously by Liu *et al.* (2014). For strains
178 WSM4637, WSM4638, WSM4639 and WSM4640, 16S rRNA sequences were
179 obtained as reported by Ardley *et al.* (2012) and *recA* gene sequences were obtained
180 as reported by Liu *et al.* (2014). The 16S rRNA gene sequences were compared to
181 those available in EzTaxon-e (Kim *et al.*, 2012), the Ribosomal Database Project
182 (Cole *et al.*, 2009) and the GenBank database
183 (http://blast.ncbi.nlm.nih.gov/Blast.cgi.). Sequence analyses were performed using
184 the software packages BioEdit (Hall, 1999) and MEGA 5 (Tamura *et al.*, 2011), after
185 multiple alignments of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances
186 were calculated using the Kimura's two-parameter model (Kimura, 1983) and

187 clustering was performed with the neighbour-joining method (Saitou & Nei, 1987).
188 The maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge &
189 Farris, 1969) trees were generated using the treeing algorithms contained in the
190 PHYLIP software package (Felsenstein, 1993). In each case, bootstrap values were
191 calculated based on 1000 replications.

192 The phylogenetic tree based on 16S rRNA gene sequence comparison (Fig. 1
193 and Supplementary Fig. S1) showed that the strains formed a separate phylogenetic
194 branch within the genus *Burkholderia*. The overall topologies of the phylogenetic
195 trees obtained with the neighbour-joining, maximum-likelihood and
196 maximum-parsimony methods were similar (data not shown). The 16S rRNA gene
197 sequences of the strains showed high similarity (more than 99.5 %) to each other and
198 were closely related to *B. phytofirmans* PsJN^T (99.0-99.5 %), *B. caledonica* W50D^T
199 (98.1-99.1 %), *B. phenoliruptrix* AC1100^T (98.1-98.5 %), *B. ginsengisoli* KMY03^T
200 (97.2-97.5 %), *B. fungorum* P763-2^T (98.1-99.1 %), *B. xenovorans* LB400^T
201 (98.0-98.8 %) and *B. rhynchosiae* WSM3937^T (98.0-98.6 %). Lower sequence
202 similarities (<97.0 %) were found with the type strains of all other species listed in
203 Fig.1.

204 According to pairwise *recA* gene sequence comparisons, the similarity of the
205 investigated strains ranged from 99.9 to 100 % with each other. Strain ICMP 19430^T
206 showed the highest similarity value (97.8 %) with *B. phytofirmans* PsJN^T, and the
207 levels of the *recA* gene sequence similarity with other validly published
208 *Burkholderia* species were below 94.5 %. Phylogenetic analyses of the partial *recA*
209 sequences were performed using MEGA6. Neighbour-joining, maximum-likelihood
210 and maximum-parsimony trees were generated and bootstrap values were calculated
211 based on 1000 replications. The overall topologies of the phylogenetic trees were

212 similar and showed that all strains (ICMP 19430^T, ICMP 19429, ICMP 19431 WSM4637, WSM4638, WSM4639 and WSM4640) formed a separate monophyletic cluster within the genus *Burkholderia* (Supplementary Fig. S2).

215 Whole genome DNA-DNA hybridization experiments were performed at 50 °C with photobiotin-labelled probes as described by Ezaki *et al*. (1989). DNA-DNA hybridization experiments were performed with all investigated strains, and their phylogenetically closest neighbours within the genus *Burkholderia*, *B. phytofirmans* LMG 22146^T, *B. caledonica* LMG 19076^T, *B. phenoliruptrix* LMG 22037^T, *B. fungorum* LMG 16225^T, *B. xenovorans* LMG 21463^T, *B. rhynchosiae* LMG 27174^T and *B. ginsengisoli* NBRC 100965^T. The degree of DNA-DNA relatedness was calculated from triplicate measurements and the DNA-DNA binding values of all strains examined are shown in Supplementary Table S2. The values for DNA-DNA relatedness between our strains were 85-99 %, indicating that all seven are members of the same genomic species (Wayne *et al.*, 1987). In addition, the values for DNA-DNA relatedness between our strains and their seven closest neighbours were in the range of 17-55 %. Since the recommended DNA-DNA relatedness threshold for the definition of a species is 70 % (Wayne *et al.,* 1987), these results indicate that strains ICMP 19430^T, ICMP 19429, ICMP 19431, WSM4637, WSM4638, WSM4639 and WSM4640 do not belong to any known species of the genus *Burkholderia*.

232 Preparation of whole-cell proteins and SDS-PAGE were performed as described by Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of the protein profiles and numerical analysis using Pearson's product-moment correlation coefficient were performed using the GelCompar 4.2 software package (Applied Maths). Whole-cell protein extracts were prepared from

237 all investigated strains and compared with closely related species. Our strains formed
238 a single cluster with similarities of >93 %, in comparison with similarities of less
239 than 86 % to other *Burkholderia* species (see Supplementary Fig. S3).

240 The fatty acid profiles of strains ICMP 19430^T, *B. phytofirmans* LMG 22146^T,
241 *B. caledonica* LMG 19076^T, *B. phenoliruptrix* LMG 22037^T, *B. fungorum* LMG
242 16225^T, *B. xenovorans* LMG 21463^T, *B. rhynchosiae* LMG 27174^T and *B.*
243 *ginsengisoli* NBRC 100965^T were determined using cells grown on YEM agar at 25
244 °C for 2 days. The physiological age of the different bacterial cultures at the time of
245 harvest was standardized by selecting a sector from a quadrant streak on YEM agar
246 plates according to the MIDI protocol
247 (http://www.microbialid.com/PDF/TechNote_101.pdf). In this study, the different
248 *Burkholderia* species exhibited very similar growth rates on YEM agar. Fatty acid
249 methyl esters were prepared and separated according to the standard protocol of
250 MIDI (Sherlock Microbial Identification System, version 6.0), analyzed by GC
251 (Hewlett-Packard 5890 Series II) and identified by using the RTSBA6.00 database of
252 the microbial identification system (Sasser, 1990). The overall fatty acid profile of
253 strain ICMP 19430^T was similar to the reference *Burkholderia* strain profiles,
254 although there were differences in the proportions of certain components (Table 2).
255 The major fatty acids (>5 %) of strain ICMP 19430^T were $C_{18:1}$ $\omega7c$ (21.0 %), $C_{16:0}$
256 (19.1 %), $C_{17:0}$ cyclo (18.9 %), summed feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$
257 $\omega6c$; 10.7 %) and $C_{19:0}$ cyclo $\omega8c$ (7.5 %).

258 Isoprenoid quinones were extracted and purified according to the method of
259 Collins (1994) and analyzed by HPLC, which revealed Q-8 as the main respiratory
260 quinone for strain ICMP 19430^T. The DNA G+C content of strain ICMP 19430^T, as
261 determined by HPLC (Mesbah *et al.*, 1989), was 63.2 mol%, which is within the

262 range previously reported for *Burkholderia* species (59-69.5 mol%) (Garrity *et al.*,
263 2005; Gillis *et al.*, 1995; Yabuuchi *et al.*, 1992).

264 Polar lipids were extracted and analyzed by two-dimensional TLC according to
265 Embley & Wait (1994). Molybdophosphoric acid was used for the detection of the
266 total polar lipids, ninhydrin for amino lipids, the Zinzadze reagent for phospholipids,
267 the Dragendorff reagent for choline-containing lipids and the α-naphthol reagent for
268 glycolipids. Strain ICMP 19430^T exhibited a complex polar lipid profile consisting of
269 phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol
270 (DPG), two uncharacterized aminophospholipids (APL1-APL2) and several
271 uncharacterized phospholipids (PLs) (see Supplementary Fig. S4). Moreover, the
272 polar lipid profile of strain ICMP 19430^T was very similar to that of its closest
273 relatives, *B. phytofirmans* LMG 22146^T, *B. caledonica* LMG 19076^T, *B.*
274 *phenoliruptrix* LMG 22037^T, *B. fungorum* LMG 16225^T, *B. xenovorans* LMG
275 21463^T, *B. rhynchosiae* LMG 27174^T and *B. ginsengisoli* NBRC 100965^T; with PE,
276 PG, DPG and APL1 as major polar lipids. However, there were differences in the
277 uncharacterized PLs.

278 Based on the phenotypic and genotypic data obtained in this study, the strains
279 investigated constitute a novel species within the genus *Burkholderia*, for which the
280 name *Burkholderia dipogonis* sp. nov. is proposed.

281

282 **Description of *Burkholderia dipogonis* sp. nov.**

283 *Burkholderia dipogonis* (di.po.go'nis. N.L. gen. n. *dipogonis* of *Dipogon lignosus*,
284 from where the strains were first isolated).

285 Cells are Gram-stain-negative, motile, aerobic, non-spore-forming rods surrounded
286 by a thick capsule. Poly-β-hydroxybutyrate accumulation is observed and the strains

287 are catalase and oxidase positive. After 24 h growth on YEM agar at 25 °C, the mean
288 cell size is 0.6-0.8 μm in diameter and 1.8-2.8 μm in length. Colonies on YEM agar
289 are pale yellow pigmented, circular, smooth and convex with entire edges. The
290 colony size is approximately 1.2-1.8 mm in diameter on YEM agar after 48 h
291 incubation at 25 °C. Growth occurs at 10-37 °C (optimum, 25-30 °C), at pH 4.0-9.0
292 (optimum, pH 6.0-7.0) and with 0-2 % (w/v) NaCl (optimum, 0 % [w/v]). Positive
293 reactions were recorded for the hydrolysis of Tween 40 and 60, weakly positive for
294 hydrolysis of CM-cellulose, and negative for hydrolysis of DNA, starch, chitin,
295 casein, corn oil, alginate and Tween 20 and 80. Positive for urease, alkaline
296 phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, valine arylamidase,
297 cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase
298 activity and assimilation of glucose, arabinose, mannose, mannitol,
299 *N*-acetyl-glucosamine, gluconate, caprate, adipate and malate; negative for nitrate
300 reduction, indole production, glucose fermentation, arginine dihydrolase activity,
301 aesculin and gelatin hydrolysis, C14 lipase, trypsin, α-chymotrypsin, α-galactosidase,
302 β-galactosidase, β-glucouronidase, α-glucosidase, β-glucosidase,
303 *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities and
304 assimilation of maltose. Additional chemotaxonomic information can be found in
305 Tables 1 and S1. All strains are sensitive to chloramphenicol, rifampicin, gentamicin,
306 kanamycin, penicillin G, ampicillin, novobiocin, tetracycline, streptomycin,
307 sulfamethoxazole plus trimethoprim and nalidixic acid. The major fatty acids are
308 $C_{18:1}$ *ω7c*, $C_{16:0}$, $C_{17:0}$ cyclo, summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or $C_{16:1}$
309 *ω6c*) and $C_{19:0}$ cyclo *ω8c*. The major respiratory quinone is Q-8. The polar lipid
310 profile consisted of a mixture of phosphatidylethanolamine, phosphatidylglycerol,
311 diphosphatidylglycerol, two uncharacterized aminophospholipids and several

312 uncharacterized phospholipids.

313 The type strain is ICMP 19430^T (= LMG 28415^T = HAMBI 3637^T), which was
314 isolated from root nodules of *Dipogon lignosus* in New Zealand. The DNA G+C
315 content of the type strain is 63.2 mol%.

316

317 **References**

318 **Ardley, J.K., Parker, M.A., De Meyer, S.E., Trengove, R.D., O'Hara, G.W.,**
319 **Reeve, W.G., Yates, R.J., Dilworth, M.J., Willems, A. & Howieson, J.G.**
320 **(2012).** *Microvirga lupini* sp. nov., *Microvirga lotononidis* sp. nov. and
321 *Microvirga zambiensis* sp. nov. are alphaproteobacterial root-nodule bacteria
322 that specifically nodulate and fix nitrogen with geographically and
323 taxonomically separate legume hosts. *Int J Syst Evol Microbiol* **62,** 2579-2588.

324 **Beveridge, T. J., Lawrence, J. R. & Murray, R. G. E. (2007).** Sampling and
325 staining for light microscopy. In *Methods for General and Molecular*
326 *Bacteriology,* 3rd pp. 19-33. Edited by T. J. Beveridge, J. A. Breznak, G. A.
327 Marzluf, T. M. Schmidt & L. R. Snyder. American Society for Microbiology,
328 Washington, D.C.

329 **Bowman, J. P. (2000).** Description of *Cellulophaga algicola* sp. nov., isolated from
330 the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa*
331 (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa*
332 comb. nov. *Int J Syst Evol Microbiol* **50**, 1861-1868.

333 **Breznak, J. A. & Costilow, R. N. (2007).** Physicochemical factors in growth. In
334 *Methods for General and Molecular Bacteriology,* 3rd pp. 309-329. Edited by
335 T. J. Beveridge, J. A. Breznak, G. A. Marzluf, T. M. Schmidt & L. R. Snyder.
336 American Society for Microbiology, Washington, D.C.

337 **Chen, W. M., James, E. K., Coenye, T., Chou, J. H., Barrios, E., de Faria, S. M.,**
338 **Elliott, G. N., Sheu, S. Y., Sprent, J. I. & Vandamme, P. (2006).**

339 *Burkholderia mimosarum* sp. nov., isolated from root nodules of *Mimosa* spp.
340 from Taiwan and South America. *Int J Syst Evol Microbiol* **56,** 1847-1851.
341 **Chen, W. M., de Faria, S. M., James, E. K., Elliott, G. N., Lin, K. Y., Chou, J. H.,**
342 **Sheu, S. Y., Cnockaert, M., Sprent, J. I. & Vandamme, P. (2007).**
343 *Burkholderia nodosa* sp. nov., isolated from root nodules of the woody
344 Brazilian legumes *Mimosa bimucronata* and *Mimosa scabrella*. *Int J Syst Evol*
345 *Microbiol* **57,** 1055-1059.
346 **Chen, W. M., de Faria, S. M., Chou, J. H., James, E. K., Elliott, G. N., Sprent, J.**
347 **I., Bontemps, C., Young, J. P. W. & Vandamme, P. (2008).** *Burkholderia*
348 *sabiae* sp. nov., isolated from root nodules of *Mimosa caesalpiniifolia*. *Int J*
349 *Syst Evol Microbiol* **58,** 2174-2179.
350 **Coenye, T., Laevens, S., Willems, A., Ohlen, M., Hannant, W., Govan, J. R. W.,**
351 **Falsen, E. & Vandamme, P. (2001).** *Burkholderia fungorum* sp. nov. and
352 *Burkholderia caledonica* sp. nov., two new species isolated from the
353 environment, animals and human clinical samples. *Int J Syst Evol Microbiol*
354 **51**, 1099-1107.
355 **Coenye, T., Henry, D., Speert, D. P. & Vandamme, P. (2004).** *Burkholderia*
356 *phenoliruptrix* sp. nov., to accommodate the 2,4,5-trichlorophenoxyacetic
357 acid and halophenol-degrading strain AC1100. *Syst Appl Microbiol* **27**,
358 623-627.

359 **Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J.,**
360 **Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Marsh, T., Garrity, G.**
361 **M. & Tiedje, J. M. (2009).** The Ribosomal Database Project: improved
362 alignments and new tools for rRNA analysis. *Nucleic Acids Res* **37**, Database
363 issue, D141-D145.

364 **Collins, M. D. (1994).** Isoprenoid quinones. In *Chemical Methods in Prokaryotic*
365 *Systematics*, pp. 265-309. Edited by M. Goodfellow & A. G. O'Donnell.
366 Chichester: Wiley.
367 **De Meyer, S. E., Cnockaert, M., Ardley, J. K., Trengove, R. D., Garau, G.,**
368 **Howieson, J. G. & Vandamme, P. (2013a).** *Burkholderia rhynchosiae* sp.
369 nov., isolated from *Rhynchosia ferulifolia* root nodules. *Int J Syst Evol*
370 *Microbiol* **63**, 3944-3949.
371 **De Meyer, S. E., Cnockaert, M., Ardley, J. K., Maker, G., Yates, R., Howieson,**
372 **J.G. & Vandamme, P. (2013b)** *Burkholderia sprentiae* sp. nov. isolated from
373 *Lebeckia ambigua* root nodules from South Africa. *Int. J Syst Evol Microbiol*
374 **63,** 3950-3957.
375 **De Meyer, S. E., Cnockaert, M., Ardley, J. K., van Wyk, B-E., Vandamme, P.A.**
376 **& Howieson, J.G. (2014)**. *Burkholderia dilworthii* sp. nov. isolated from
377 *Lebeckia ambigua* root nodules from South Africa. *Int J Syst Evol Microbiol*
378 **64,** 1090-1095.
379 **Elliott, G.N., Chen, W-M., Bontemps, C., Chou, J-H., Young, J.P.W., Sprent, J.I.**
380 **& James, E.K. (2007).** Nodulation of *Cyclopia* spp. (Leguminosae,
381 Papilionoideae) by *Burkholderia tuberum*. *Ann Bot* **100,** 1403-1411.
382 **Embley, T. M. & Wait, R. (1994).** Structural lipids of eubacteria. In Chemical
383 Methods in Prokaryotic Systematics, pp. 121-161. Edited by Goodfellow M.
384 & O'Donnell, A. G. John Wiley & Sons Ltd, England.
385 **Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989).** Fluorometric DNA-DNA
386 hybridization in microdilution wells as an alternative to membrane filter
387 hybridization in which radioisotopes are used to determine genetic
388 relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224-229.

389 **Felsenstein, J. (1981).** Evolutionary trees from DNA sequences: a maximum
390 likelihood approach. *J Mol Evol* **17**, 368-376.
391 **Felsenstein, J. (1993).** PHYLIP (phylogeny inference package), version 3.5c.
392 Distributed by the author. Department of Genome Sciences, University of
393 Washington, Seattle, USA.
394 **Garau, G., Yates, R. J., Deiana, P., Howieson, J. G. (2009).** Novel strains of
395 nodulating *Burkholderia* have a role in nitrogen fixation with papilionoid
396 herbaceous legumes adapted to acid, infertile soils. *Soil. Biol. Biochem.* **41**,
397 125-134.
398 **Garrity, G. M., Bell, J. A. & Liburn, T.** (2005). Family I. *Burkholderiaceae*. In
399 Bergey's Manual of Systematic Bacteriology, pp. 438-475. Edited by D. J.
400 Brenner, N. R. Krieg and J. T. Staley. New York: Springer.
401 **Gillis, M., Van, T.V., Bardin, R., Goor, M., Hebbar, P., Willems, A., Segers, P.,**
402 **Kersters, K., Heulin, T. & Fernandez, M. P. (1995).** Polyphasic taxonomy
403 in the genus *Burkholderia* leading to an emended description of the genus and
404 proposition of *Burkholderia vietnamiensis* sp. nov. for N_2-fixing isolates from
405 rice in Vietnam. *Int J Syst Bacteriol* **45**, 274-289.
406 **Goris, J., De Vos, P., Caballero-Mellado, J., Park, J., Falsen, E., Quensen, J. F.,**
407 **III, Tiedje, J. M. & Vandamme, P. (2004).** Classification of the biphenyl-
408 and polychlorinated biphenyl-degrading strain LB400^T and relatives as
409 *Burkholderia xenovorans* sp. nov. *Int J Syst Evol Microbiol* **54**, 1677-1681.
410 **Gyaneshwar, P., Hirsch, A. M., Moulin, L., Chen, W. M., Elliott, G. N., Bontemps,**
411 **C., Estrada-de los Santos, P., Gross, E., dos Reis Junior, F. B., & other**
412 **authors (2011).** Legume-nodulating betaproteobacteria: diversity, host range
413 and future prospects. *Mol Plant-Microbe Interact* **24,** 1276-1288.

414 **Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and
415 analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95-98.
416 **Howieson, J. G., Loi, A. & Carr, S. J. (1995).** *Biserrula pelecinus* L. - a legume
417 pasture species with potential for acid, duplex soils which is nodulated by
418 unique root- nodule bacteria. *Aust J of Agric Res* 46, 997-1009.

419 **Kim, H.-B., Park, M.-J., Yang, H.-C., An, D.-S., Jin, H.-Z. & Yang, D.-C. (2006).**
420 *Burkholderia ginsengisoli* sp. nov., a β-glucosidase-producing bacterium
421 isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **56**, 2529-2533.
422 **Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon,**
423 **Y. S., Lee, J. H., Yi, H., Won, S. & Chun, J. (2012).** Introducing EzTaxon-e:
424 a prokaryotic 16S rRNA gene sequence database with phylotypes that
425 represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716-721.
426 **Kimura, M. (1983).** *The Neutral Theory of Molecular Evolution.* Cambridge:
427 Cambridge University Press.
428 **Kluge, A. G. & Farris, F. S. (1969).** Quantitative phyletics and the evolution of
429 anurans. *Syst Zool* **18**, 1-32.
430 **Lemaire, B., Dlodlo, O., Chimphango, S., Stirton, C., Schrire, B., Boatwright, J.**
431 **S., Honnay, O., Smets, E., Sprent, J. I., James, E. K. & Muasya, A. M.**
432 **(2015).** Symbiotic diversity, specificity and distribution of rhizobia in native
433 legumes of the Core Cape Subregion (South Africa). *FEMS Microbiol Ecol* **17,**
434 doi: http://dx.doi.org/10.1093/femsec/fiu024.
435 **Liu, W. Y. Y., Ridgway, H. J., James, T. K., James, E. K., Chen, W.-M., Sprent,**
436 **J. I., Young, J. P. W. & Andrews, M. (2014).** *Burkholderia* sp. induces
437 functional nodules on the South African invasive legume *Dipogon lignosus*
438 (Phaseoleae) in New Zealand soils. *Microb Ecol* **68,** 542-555.

439 **Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement
440 of the G+C content of deoxyribonucleic acid by high-performance liquid
441 chromatography. *Int J Syst Bacteriol* **39**, 159-167.
442 **Miller, P. H., Wiggs, L. S. & Miller, J. M. (1995).** Evaluation of Anaero*Gen* system
443 for growth of anaerobic bacteria. *J Clin Microbiol* **33**, 2388-2391.
444 **Nokhal, T. H. & Schlegel, H. G. (1983).** Taxonomic study of *Paracoccus*
445 *denitrificans. Int J Syst Bacteriol* **33**, 26-37.
446 **Palleroni NJ** (2005) Genus I. *Burkholderia.* In *Bergey's Manual of Systematic*
447 *Bacteriology*. Volume 2. Second edition. Edited by Garrity GM, Brenner DJ,
448 Krieg NR, Staley JT. New York: Springer – Verlag.
449 **Pot, B., Vandamme, P. & Kersters, K. (1994).** Analysis of electrophoretic
450 whole-organism protein fingerprints. In *Modern Microbial*
451 *Methods* (*Chemical Methods Prokaryotic Systematics Series*), pp. 493-521.
452 Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
453 **Powers, E. M. (1995).** Efficacy of the Ryu nonstaining KOH technique for rapidly
454 determining gram reactions of food-borne and waterborne bacteria and yeasts.
455 *Appl Environ Microbiol* **61**, 3756-3758.
456 **Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for
457 reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.
458 **Sasser, M. (1990).** Identification of bacteria by gas chromatography of cellular fatty
459 acids. Newark, DE: MIDI Inc.
460 **Schlegel, H. G., Lafferty, R. & Krauss, I. (1970).** The isolation of mutants not
461 accumulating poly-β-hydroxybutyric acid. *Arch Mikrobiol* **70**, 283-294.
462 **Sessitsch, A., Coenye, T., Sturz, A. V. & 9 other authors (2005).** *Burkholderia*
463 *phytofirmans* sp. nov., a novel plant-associated bacterium with

464 plant-beneficial properties. *Int J Syst Evol Microbiol* **55**, 1187-1192.

465 **Sheu, S. Y., Chou, J. H., Bontemps, C., Elliott, G. N., Gross, E., James, E. K.,**
466 **Sprent, J. I, Young, J. P. W., & Chen, W. M. (2012).** *Burkholderia*
467 *symbiotica* sp. nov., isolated from root nodules of *Mimosa* spp. native to
468 North East Brazil. *Int J Syst Evol Microbiol* **62,** 2272-2278.

469 **Sheu, S. Y., Chou, J. H., Bontemps, C., Elliott, G.N., Gross, E., dos Reis Junior, F.**
470 **B., Melkonian, R., Moulin, L., James, E. K., Sprent, J. I, Young, J. P. W.,**
471 **& Chen, W. M. (2013).** *Burkholderia diazotrophica* sp. nov., isolated from
472 root nodules of *Mimosa* spp. *Int J Syst Evol Microbiol* **63,** 435-441.

473 **Spiekermann, P., Rehm, B. H. A., Kalscheuer, R., Baumeister, D. & Steinbuchel,**
474 **A. (1999).** A sensitive, viable-colony staining method using nile red for direct
475 screening of bacteria that accumulate polyhydroxyalkanoic acids and other
476 lipid storage compounds. *Arch Microbiol* **171**, 73-80.

477 **Suárez-Moreno, Z. R., Caballero-Mellado, J., Coutinho, B., Mendonça-Previato,**
478 **L., James E. K. & Venturi V. (2012).** Common features of environmental
479 and potentially beneficial plant-associated *Burkholderia*. *Microb Ecol* **63**,
480 249-266.

481 **Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011).**
482 MEGA5: Molecular evolutionary genetics analysis using maximum
483 likelihood, evolutionary distance, and maximum parsimony methods. *Mol*
484 *Biol Evol* **28**, 2731-2739.

485 **Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G.**
486 **(1997).** The Clustal_X windows interface: flexible strategies for multiple
487 sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**,
488 4876-4882.

489 **Tindall, B. J., Sikorski, J., Smibert, R. A. & Krieg, N. R. (2007).** Phenotypic
490 characterization and the principles of comparative systematics. In *Methods*
491 *for General and Molecular Bacteriology,* 3rd pp. 330-393. Edited by T. J.
492 Beveridge, J. A. Breznak, G. A. Marzluf, T. M. Schmidt & L. R. Snyder.
493 American Society for Microbiology, Washington, D.C.

494 **Vandamme, P. A. R., Govan, J. R. W. & LiPuma, J. J. (2007).** Diversity and role
495 of *Burkholderia* spp. In *Burkholderia* Molecular Microbiology and Genomics,
496 vol. 1, pp. 1-28. Edited by Coenye, T. & Vandamme, P. Wymondham, UK:
497 Horizon Bioscience.

498 **Versalovic, J., Koeuth, T. & Lupski, J. R. (1991).** Distribution of repetitive DNA
499 sequences in eubacteria and application to fingerprinting of bacterial genomes.
500 *Nucleic Acids Res* **19,** 6823-6831.

501 **Vincent, J. M. (1970).** A Manual for the Practical Study of the Root-Nodule
502 Bacteria. Oxford: Blackwell Scientific.

503 **Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O.,**
504 **Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. &**
505 **other authors (1987).** International Committee on Systematic Bacteriology.
506 Report of the ad hoc committee on reconciliation of approaches to bacterial
507 systematics. *Int J Syst Bacteriol* **37**, 463-464.

508 **Wen, C. M., Tseng, C. S., Cheng, C. Y. & Li, Y. K. (2002).** Purification,
509 characterization and cloning of a chitinase from *Bacillus* sp. NCTU2.
510 *Biotechnol Appl Biochem* **35**, 213-219.

511 **Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki,**
512 **T. & Arakawa, M. (1992).** Proposal of *Burkholderia* gen. nov. and transfer

513 of seven species of the genus *Pseudomonas* homology group II to the new
514 genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes
515 1981) comb. nov. *Microbiol Immunol* **36**, 1251-1275.

516

517 **Table 1. Phenotypic characteristics distinguishing *Burkholderia dipogonis* sp.**

518 **nov. from other species of the genus *Burkholderia***

519 Strains: 1, *B. dipogonis* sp. nov. (n = 7); 2, *B. phytofirmans* LMG 22146^T; 3, *B.*

520 *caledonica* LMG 19076^T; 4, *B. phenoliruptrix* LMG 22037^T; 5, *B. fungorum* LMG

521 16225^T; 6, *B. xenovorans* LMG 21463^T; 7, *B. rhynchosiae* LMG 27174^T; 8, *B.*

522 *ginsengisoli* NBRC 100965^T. All data were obtained from this study except the DNA

523 G+C content of *B. phytofirmans* LMG 22146^T (Sessitsch *et al*., 2005), *B. caledonica*

524 LMG 19076^T and *B. fungorum* LMG 16225^T (Coenye *et al*., 2001), *B. phenoliruptrix*

525 LMG 22037^T (Coenye *et al*., 2004), *B. xenovorans* LMG 21463^T (Goris *et al*., 2004),

526 *B. rhynchosiae* LMG 27174^T (De Meyer *et al*., 2013a) and *B. ginsengisoli* NBRC

527 100965^T (Kim *et al*., 2006). +, Positive reaction; -, negative reaction; w, weakly

528 positive reaction; v, result is strain dependent; S, sensitive; R, resistant.

529 All strains are Gram-stain-negative, motile, non-spore-forming, rod-shaped, positive

530 for catalase, alkaline phosphatase, C8 esterase lipase, leucine arylamidase and acid

531 phosphatase activities, and assimilation of glucose, mannose, mannitol,

532 *N*-acetyl-glucosamine, gluconate and malate, negative for C14 lipase, trypsin,

533 α-chymotrypsin, α-galactosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and

534 α-fucosidase activities, indole production, assimilation of maltose, and for hydrolysis

535 of gelatin, DNA, starch, chitin, casein, alginate and Tween 80. All strains were

536 sensitive to chloramphenicol, gentamicin, kanamycin, penicillin G, tetracycline,

537 streptomycin, and nalidixic acid.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Isolation source	Root nodule	Onion roots	Rhizosphere	Chemostat	White-rot fungus	Soil	Root nodule	Soil
Growth at 37°C	+	-	-	+	+	-	+	+
Growth in the presence of 1.5% NaCl	+	+	-	+	+	-	+	+
Acid produced aerobically from glucose	-	-	-	+	-	-	-	-
Nitrate reduction	-	-	-	-	+	-	w	-
Enzymatic activities:								
Oxidase	+	+	-	+	+	+	+	-
Arginine dihydrolase	-	-	-	-	-	-	w	+
Urease	+	-	-	-	-	-	-	+
Lipase (corn oil)	-	-	-	-	+	-	-	-
C4 esterase	+	-	-	+	+	+	+	-
Valine arylamidase	+	-	-	+	-	+	+	-
Cystine arylamidase	+	-	-	+	+	+	+	-
α-Glucosidase	-	-	-	+	-	-	-	-
β-Galactosidase	v	-	-	-	-	-	-	+
β-Glucuronidase	-	-	-	-	-	-	-	+
β-Glucosidase	-	-	-	-	-	-	+	+
Hydrolysis of:								
Tween 20	-	-	+	+	-	+	-	-
Tween 40	+	+	+	+	+	+	-	+

Tween 60	+	+	-	+	-	+	-	+
CM-cellulose	w	-	-	+	w	+	+	-
Aesculin	-	-	-	-	-	-	-	+
Assimilation of (API 20 NE):								
Arabinose	+	+	+	+	+	-	+	+
Caprate	+	-	-	-	+	+	w	-
Adipate	+	-	-	+	+	+	-	+
Citrate	v	-	-	+	+	+	w	-
Phenyl-acetate	v	+	+	-	+	+	+	+
Susceptibility to:								
Ampicillin	S	S	S	S	S	R	R	R
Novobiocin	S	S	S	R	S	S	S	S
Rifampicin	S	S	S	S	R	R	S	S
Sulphamethoxazole/trimethoprim	S	S	S	S	S	S	R	S
DNA G+C content (mol%)	63.2	61.0	62.0	62.6	62.0	62.6	61.2	61.6

Table 2. Cellular fatty acid compositions of *B. dipogonis* and related species of the genus *Burkholderia*

Strains: 1, *B. dipogonis* sp. nov. ICMP 19430^T; 2, *B. phytofirmans* LMG 22146^T; 3, *B. caledonica* LMG 19076^T; 4, *B. phenoliruptrix* LMG 22037^T; 5, *B. fungorum* LMG 16225^T; 6, *B. xenovorans* LMG 21463^T; 7, *B. rhynchosiae* LMG 27174^T; 8, *B. ginsengisoli* NBRC 100965^{T}2, *B. phytofirmans* LMG 22146^T; 3, *B. caledonica* LMG 19076^T; 4, *B. phenoliruptrix* LMG 22037^T; 5, *B. fungorum* LMG 16225^T; 6, *B. xenovorans* LMG 21463^T; 7, *B. rhynchosiae* LMG 27174^T; 8, *B. ginsengisoli* NBRC 100965^T. All strains were grown on YEM agar at 25 ºC for 2 days. Values are percentages of the total fatty acids; fatty acids that make up <1 % of the total are not shown or indicated by "-".

Fatty acid	1	2	3	4	5	6	7	8
$C_{14:0}$	4.3	3.7	3.6	4.0	3.7	3.7	3.4	3.9
$C_{16:0}$	19.1	20.9	16.7	18.4	20.1	22.9	13.0	18.9
$C_{16:0}$ 3-OH	3.6	4.0	3.5	3.8	3.9	3.6	3.5	3.8
$C_{16:1}$ 2-OH	1.2	-	-	2.3	-	1.2	1.8	-
$C_{17:0}$ cyclo	18.9	-	4.6	9.8	2.3	1.1	1.8	7.9
$C_{18:0}$	1.6	1.4	1.4	1.1	9.2	2.9	1.6	3.3
$C_{19:0}$ cyclo $\omega 8c$	7.5	-	2.1	3.7	1.2	1.1	1.2	2.8
$C_{18:1}\omega 7c$	21.0	37.3	36.6	23.3	35.2	30.4	44.0	31.4
Summed feature 2*	4.3	5.4	4.9	3.8	5.3	4.9	5.3	5.0
Summed feature 3*	10.7	23.6	23.3	16.9	16.6	21.2	20.8	19.5

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 2 comprises $C_{14:0}$ 3-OH, $C_{16:1}$ iso I. Summed feature 3 comprises $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$.

Figure 1. Neighbour-joining phylogenetic tree of the novel strains (*Burkholderia dipogonis* sp. nov.) and related bacteria, based on 16S rRNA gene sequence comparisons. Numbers at nodes are bootstrap percentages >70 % based on the neighbour-joining (above nodes) and maximum-parsimony (below nodes) algorithms. Filled circles indicate branches of the tree that were also recovered using the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. *Cupriavidus taiwanensis* LMG 19424^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position. The full tree from which Fig. 1 was taken is available as Supplementary Fig. S1.

Figure
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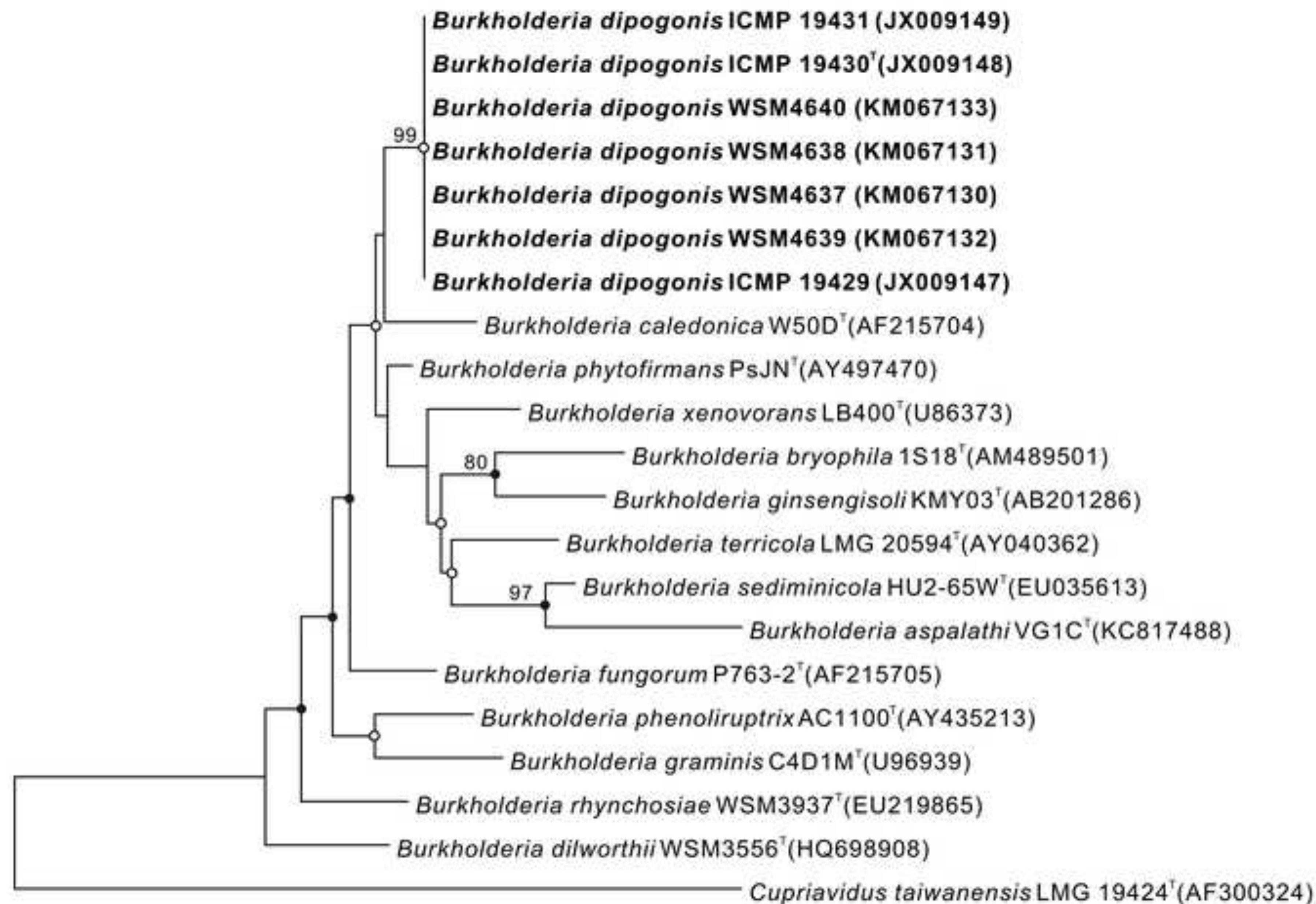

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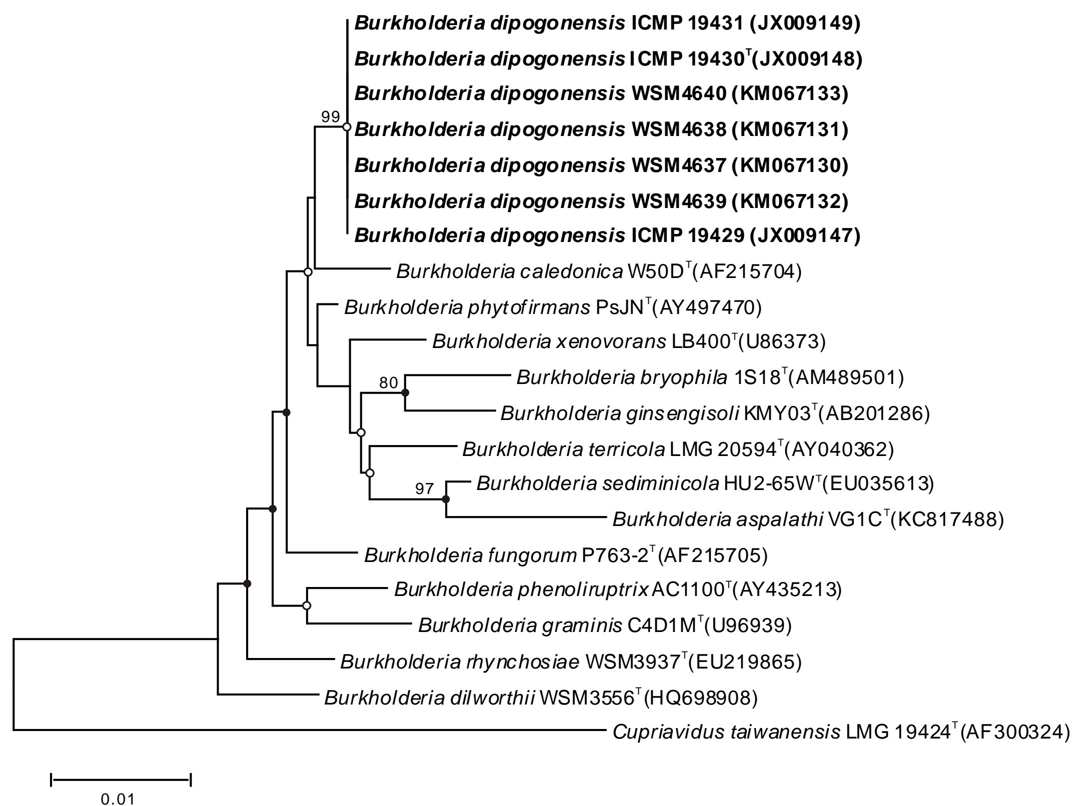

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Burkholderia dipogonis sp. nov., isolated from root nodules of *Dipogon lignosus* in New Zealand and Western Australia

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Supplementary Table S1. DNA-DNA hybridization values amongst strains belonging to the novel species *Burkholderia dipogonis* sp. nov. and the closest neighbours within the genus *Burkholderia*

	DNA-DNA binding value (%) with:						
	ICMP 19430^T	ICMP 19429	ICMP 19431	WSM4637	WSM4638	WSM4639	WSM4640
ICMP 19430^T	100	-	-	-	-	-	-
ICMP 19429	98	100	-	-	-	-	-
ICMP 19431	95	92	100	-	-	-	-
WSM4637	89	88	90	100	-	-	-
WSM4638	90	87	86	95	100	-	-
WSM4639	88	85	88	90	95	100	-
WSM4640	90	86	85	97	99	98	100
B. phytofirmans LMG 22146^T	45	50	51	48	37	26	50
B. caledonica LMG 19076^T	46	46	39	52	44	32	33
B. phenoliruptrix LMG 22037^T	43	28	26	27	38	35	46
B. fungorum LMG 16225^T	38	44	39	33	46	28	37
B. xenovorans LMG 21463^T	27	37	34	28	40	49	35
B. rhynchosiae LMG 27174^T	49	55	44	22	33	36	50
B. ginsengisoli NBRC 100965^T	17	20	25	18	47	37	22

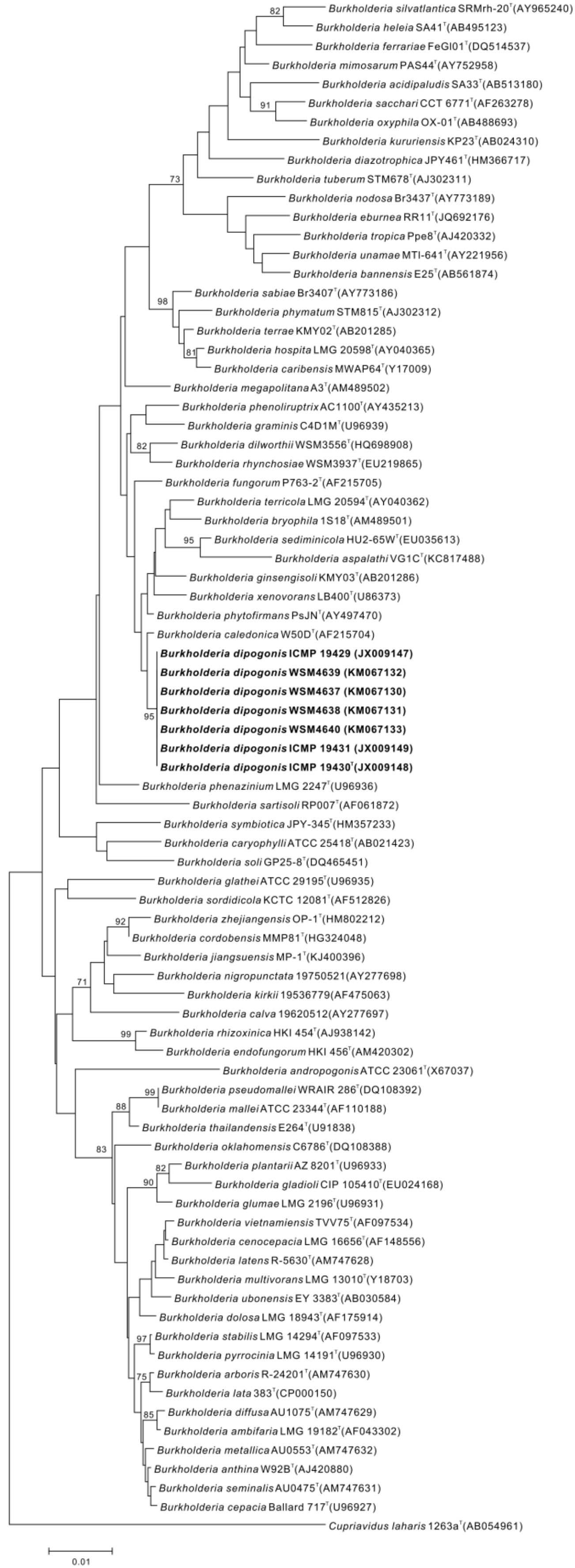
Supplementary Table S2. GN2 microplate oxidation data that can be used to distinguish *B. dipogonis* sp. nov. from seven type strains of species of the genus *Burkholderia*. Strains: 1, *B. dipogonensis* sp. nov. (7 strains studied); 2, *B. phytofirmans* LMG 22146^T; 3, *B. caledonica* LMG 19076^T; 4, *B. phenoliruptrix* LMG 22037^T; 5, *B. fungorum* LMG 16225^T; 6, *B. xenovorans* LMG 21463^T; 7, *B. rhynchosiae* LMG 27174^T; 8, *B. ginsengisoli* NBRC 100965^T. +, Positive reaction; -, negative reaction; w, weakly positive reaction; v, result is strain dependent.

Substrate	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dextrin	-	-	-	+	-	+	w	+
Glycogen	-	-	-	+	-	+	-	-
L-Arabinose	+	+	+	+	+	-	+	+
D-Cellobiose	v	-	+	+	+	+	+	+
Gentiobiose	+	+	+	-	+	+	+	+
Lactulose	+	+	-	+	-	-	+	+
β-Methyl-D-glucoside	v	-	-	-	+	+	+	-
D-Psicose	-	-	+	+	-	+	-	+
D-Raffinose	-	-	-	-	-	w	+	-
Sucrose	-	-	-	-	-	-	+	-
D-Trehalose	+	-	+	+	-	-	+	+
Xylitol	+	+	+	+	+	-	-	-

Cis-aconitic acid	+	+	+	+	+	+	+	-
γ-Hydroxybutyric acid	v	w	+	-	+	-	+	+
Itaconic acid	+	+	-	-	-	+	-	+
α-Keto butyric acid	+	+	+	-	+	+	w	+
α-Keto glutaric acid	+	+	+	+	-	+	-	+
α-Keto valeric acid	v	-	-	-	+	w	-	-
Malonic acid	+	+	+	+	-	-	+	+
Glucuronamide	+	+	+	+	+	-	+	-
L-Alaninamide	+	+	+	+	+	+	-	+
D-Alanine	v	+	+	+	+	+	+	+
Glycyl-L-aspartic acid	+	-	-	+	-	-	-	-
Glycyl-L-glutamic acid	+	+	+	+	+	+	-	-
L-Leucine	+	+	-	w	+	+	+	+
L-Ornithine	+	+	+	-	+	+	+	+
D-Serine	-	-	+	+	-	-	+	+
D, L-Carnitine	+	+	+	+	+	+	+	-
Urocanic acid	+	+	+	+	+	-	+	+
Inosine	+	+	+	+	+	-	+	+

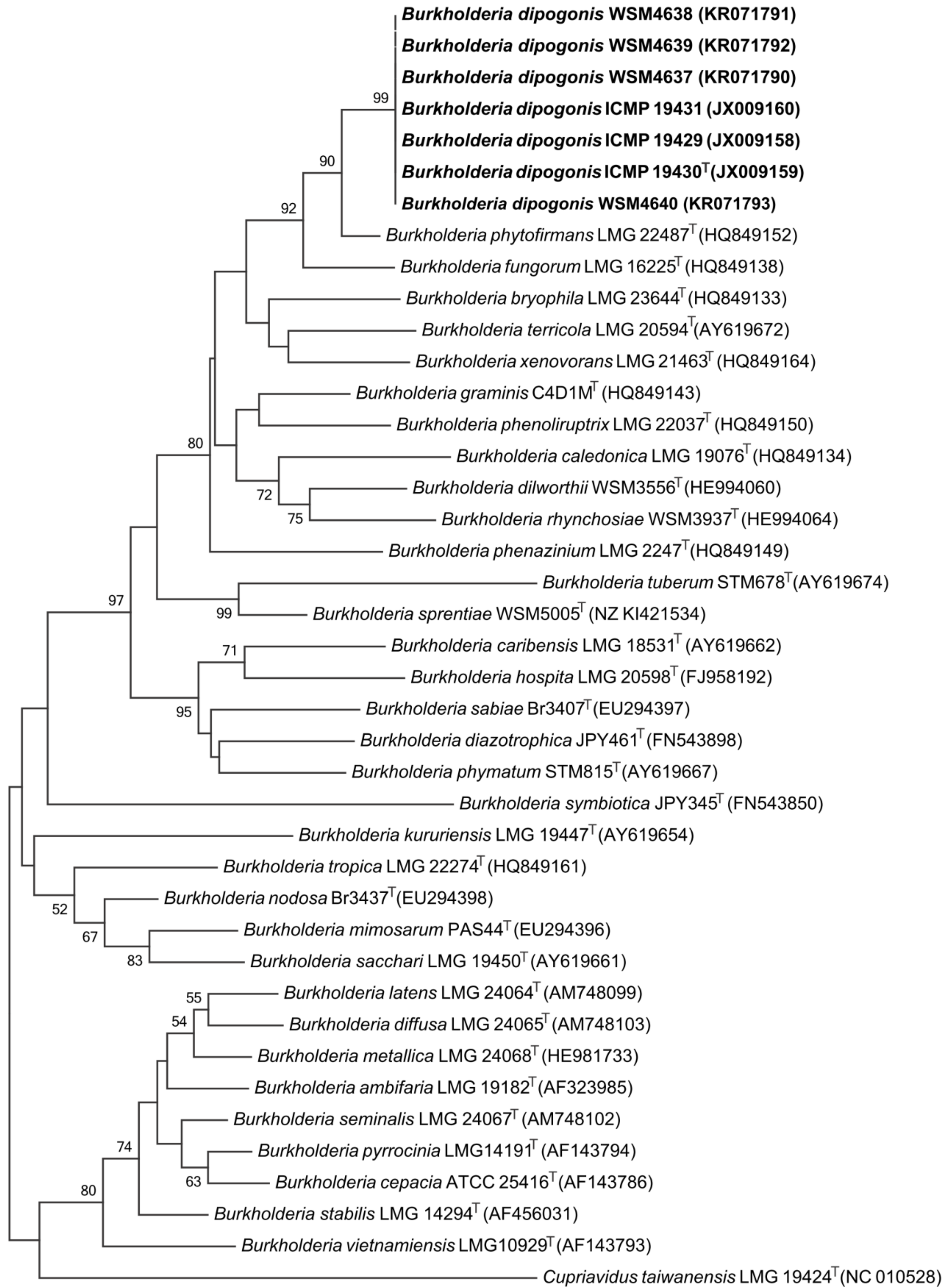
Uridine	+	+	-	+	+	+	-	-
Phenylethylamine	+	+	-	+	+	-	-	-
Putrescine	+	-	-	-	-	-	-	-
D, L-α-Glycerol phosphate	+	-	+	+	-	+	+	+
α-D-Glucose-1-phosphate	v	-	-	-	-	-	-	-

Supplementary Fig. S1. Expanded neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *Burkholderia dipogonis* strains and closely related species of the genus *Burkholderia*. Numbers at nodes are bootstrap percentages >70 % based on the neighbour-joining. *Cupriavidus laharis* 1263a^{T} was used as an outgroup. Accession numbers are given in parentheses. Bar, 0.01 substitutions per nucleotide position.



Burkholderia silvatlantica SRMrh-20^{T}(AY965240)
Burkholderia heleia SA41^{T}(AB495123)
Burkholderia ferrariae FeGl01^{T}(DQ514537)
Burkholderia mimosarum PAS44^{T}(AY752958)
Burkholderia acidipaludis SA33^{T}(AB513180)
Burkholderia sacchari CCT 6771^{T}(AF263278)
Burkholderia oxyphila OX-01^{T}(AB488693)
Burkholderia kururiensis KP23^{T}(AB024310)
Burkholderia diazotrophica JPY461^{T}(HM366717)
Burkholderia tuberum STM678^{T}(AJ302311)
Burkholderia nodosa Br3437^{T}(AY773189)
Burkholderia eburnea RR11^{T}(JQ692176)
Burkholderia tropica Ppe8^{T}(AJ420332)
Burkholderia unamae MTI-641^{T}(AY221956)
Burkholderia bannensis E25^{T}(AB561874)
Burkholderia sabiae Br3407^{T}(AY773186)
Burkholderia phymatum STM815^{T}(AJ302312)
Burkholderia terrae KMY02^{T}(AB201285)
Burkholderia hospita LMG 20598^{T}(AY040365)
Burkholderia caribensis MWAP64^{T}(Y17009)
Burkholderia megapolitana A3^{T}(AM489502)
Burkholderia phenoliruptrix AC1100^{T}(AY435213)
Burkholderia graminis C4D1M^{T}(U96939)
Burkholderia dilworthii WSM3556^{T}(HQ698908)
Burkholderia rhynchosiae WSM3937^{T}(EU219865)
Burkholderia fungorum P763-2^{T}(AF215705)
Burkholderia terricola LMG 20594^{T}(AY040362)
Burkholderia bryophila 1S18^{T}(AM489501)
Burkholderia sediminicola HU2-65W^{T}(EU035613)
Burkholderia aspalathi VG1C^{T}(KC817488)
Burkholderia ginsengisoli KMY03^{T}(AB201286)
Burkholderia xenovorans LB400^{T}(U86373)
Burkholderia phytofirmans PsJN^{T}(AY497470)
Burkholderia caledonica W50D^{T}(AF215704)
Burkholderia dipogonis ICMP 19429 (JX009147)
Burkholderia dipogonis WSM4639 (KM067132)
Burkholderia dipogonis WSM4637 (KM067130)
Burkholderia dipogonis WSM4638 (KM067131)
Burkholderia dipogonis WSM4640 (KM067133)
Burkholderia dipogonis ICMP 19431 (JX009149)
Burkholderia dipogonis ICMP 19430^{T}(JX009148)
Burkholderia phenazinium LMG 2247^{T}(U96936)
Burkholderia sartisoli RP007^{T}(AF061872)
Burkholderia symbiotica JPY-345^{T}(HM357233)
Burkholderia caryophylli ATCC 25418^{T}(AB021423)
Burkholderia soli GP25-8^{T}(DQ465451)
Burkholderia glathei ATCC 29195^{T}(U96935)
Burkholderia sordidicola KCTC 12081^{T}(AF512826)
Burkholderia zhejiangensis OP-1^{T}(HM802212)
Burkholderia cordobensis MMP81^{T}(HG324048)
Burkholderia jiangsuensis MP-1^{T}(KJ400396)
Burkholderia nigropunctata 19750521(AY277698)
Burkholderia kirkii 19536779(AF475063)
Burkholderia calva 19620512(AY277697)
Burkholderia rhizoxinica HKI 454^{T}(AJ938142)
Burkholderia endofungorum HKI 456^{T}(AM420302)
Burkholderia andropogonis ATCC 23061^{T}(X67037)
Burkholderia pseudomallei WRAIR 286^{T}(DQ108392)
Burkholderia mallei ATCC 23344^{T}(AF110188)
Burkholderia thailandensis E264^{T}(U91838)
Burkholderia oklahomensis C6786^{T}(DQ108388)
Burkholderia plantarii AZ 8201^{T}(U96933)
Burkholderia gladioli CIP 105410^{T}(EU024168)
Burkholderia glumae LMG 2196^{T}(U96931)
Burkholderia vietnamiensis TVV75^{T}(AF097534)
Burkholderia cenocepacia LMG 16656^{T}(AF148556)
Burkholderia latens R-5630^{T}(AM747628)
Burkholderia multivorans LMG 13010^{T}(Y18703)
Burkholderia ubonensis EY 3383^{T}(AB030584)
Burkholderia dolosa LMG 18943^{T}(AF175914)
Burkholderia stabilis LMG 14294^{T}(AF097533)
Burkholderia pyrrocinia LMG 14191^{T}(U96930)
Burkholderia arboris R-24201^{T}(AM747630)
Burkholderia lata 383^{T}(CP000150)
Burkholderia diffusa AU1075^{T}(AM747629)
Burkholderia ambifaria LMG 19182^{T}(AF043302)
Burkholderia metallica AU0553^{T}(AM747632)
Burkholderia anthina W92B^{T}(AJ420880)
Burkholderia seminalis AU0475^{T}(AM747631)
Burkholderia cepacia Ballard 717^{T}(U96927)
Cupriavidus laharis 1263a^{T}(AB054961)
0.01

Supplementary Fig. S2. Neighbour-joining tree based on a 406 bp alignment of partial *recA* sequences of members of the genus *Burkholderia*. The phylogenetic tree was rooted using the *Cupriavidus taiwanensis* LMG 19424^{T} *recA* gene as the outgroup sequence. Numbers at nodes are bootstrap percentages >50% based on the neighbour-joining. Accession numbers are given in parentheses. Bar, 0.01 substitutions per nucleotide position.



Burkholderia dipogonis WSM4638 (KR071791)
Burkholderia dipogonis WSM4639 (KR071792)
Burkholderia dipogonis WSM4637 (KR071790)
Burkholderia dipogonis ICMP 19431 (JX009160)
Burkholderia dipogonis ICMP 19429 (JX009158)
Burkholderia dipogonis ICMP 19430T (JX009159)
Burkholderia dipogonis WSM4640 (KR071793)
Burkholderia phytofirmans LMG 22487T (HQ849152)
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Burkholderia bryophila LMG 23644T (HQ849133)
Burkholderia terricola LMG 20594T (AY619672)
Burkholderia xenovorans LMG 21463T (HQ849164)
Burkholderia graminis C4D1MT (HQ849143)
Burkholderia phenoliruptrix LMG 22037T (HQ849150)
Burkholderia caledonica LMG 19076T (HQ849134)
Burkholderia dilworthii WSM3556T (HE994060)
Burkholderia rhynchosiae WSM3937T (HE994064)
Burkholderia phenazinium LMG 2247T (HQ849149)
Burkholderia tuberum STM678T (AY619674)
Burkholderia sprentiae WSM5005T (NZ KI421534)
Burkholderia caribensis LMG 18531T (AY619662)
Burkholderia hospita LMG 20598T (FJ958192)
Burkholderia sabiae Br3407T (EU294397)
Burkholderia diazotrophica JPY461T (FN543898)
Burkholderia phymatum STM815T (AY619667)
Burkholderia symbiotica JPY345T (FN543850)
Burkholderia kururiensis LMG 19447T (AY619654)
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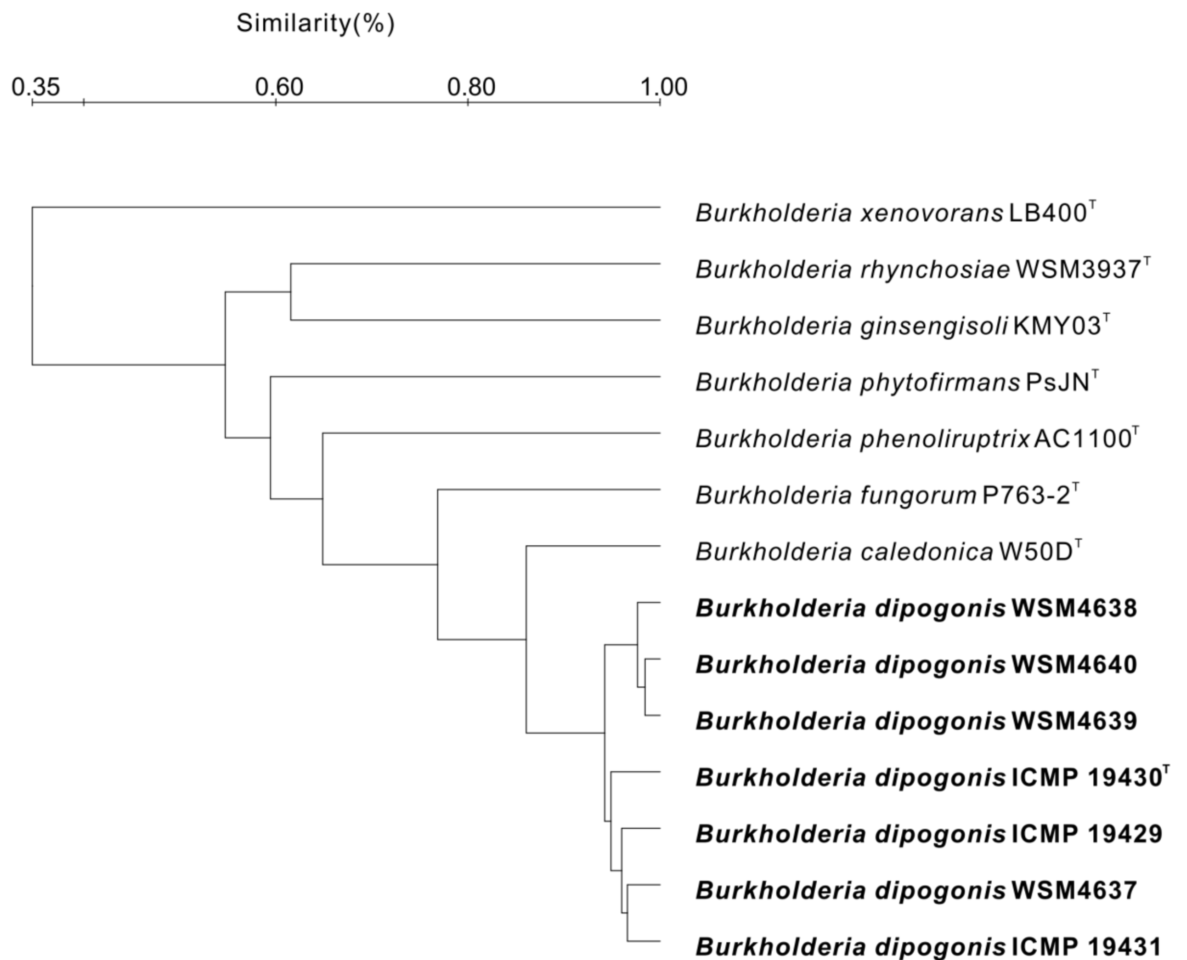
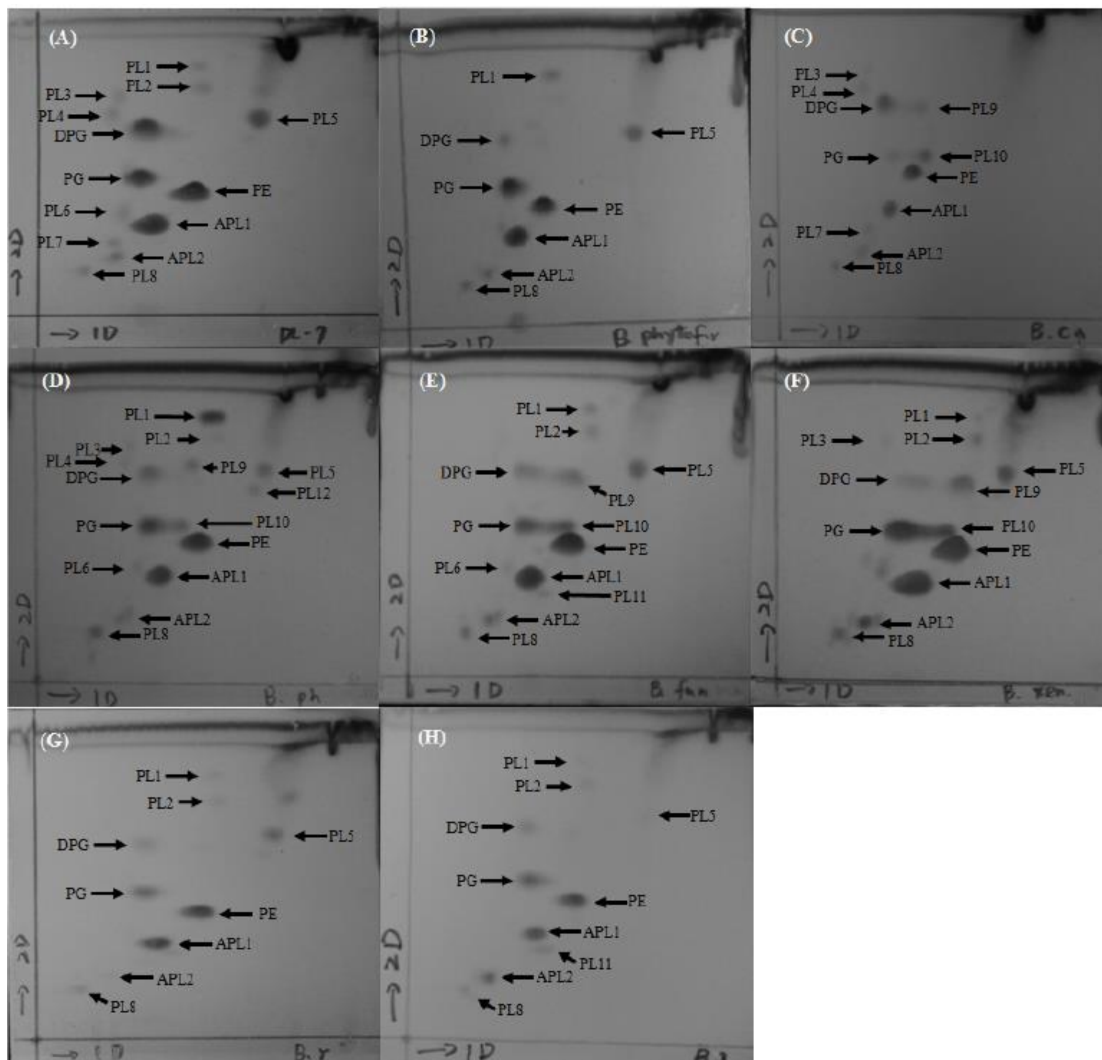
Burkholderia mimosarum PAS44T (EU294396)
Burkholderia sacchari LMG 19450T (AY619661)
Burkholderia latens LMG 24064T (AM748099)
Burkholderia diffusa LMG 24065T (AM748103)
Burkholderia metallica LMG 24068T (HE981733)
Burkholderia ambifaria LMG 19182T (AF323985)
Burkholderia seminalis LMG 24067T (AM748102)
Burkholderia pyrrocinia LMG14191T (AF143794)
Burkholderia cepacia ATCC 25416T (AF143786)
Burkholderia stabilis LMG 14294T (AF456031)
Burkholderia vietnamiensis LMG10929T (AF143793)
Cupriavidus taiwanensis LMG 19424T (NC 010528)
99
90
92
80
72
75
97
99
71
95
52
67
83
55
54
74
63
80
0.01

Supplementary Fig. S3. Dendrogram based on numerical analysis of the whole-cell protein profiles of *Dipogon lignosus* isolates and type strains of closely related *Burkholderia* species.



Supplementary Fig. S4. Two-dimensional thin-layer chromatography of polar lipids of (A) *B. dipogonis* ICMP 19430^{T}, (B) *B. phytofirmans* LMG 22146^{T}, (C) *B. caledonica* LMG 19076^{T}, (D) *B. phenoliruptrix* LMG 22037^{T}, (E) *B. fungorum* LMG 16225^{T}, (F) *B. xenovorans* LMG 21463^{T}, (G) *B. rhynchosiae* LMG 27174^{T} and (H) *B. ginsengisoli* NBRC 100965^{T}. Abbreviations: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PL1-PL11, uncharacterized phospholipids; APL1-APL2, uncharacterized aminophospholipids.



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