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1 ***Williamsia faeni* sp. nov., a novel actinomycete isolated from a hay meadow**

2

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4

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6

7

8 **Keywords:** *Williamsia faeni*, polyphasic taxonomy, hay meadow

9 **Subject category:** New Taxa: Actinobacteria

10 **Running Title:** *Williamsia faeni* sp. nov.

11

12

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19

20 The GenBank accession number for the 16S rRNA gene sequence of strain N1350<sup>T</sup> is  
21 DQ157929.

22

23

24 The taxonomic status of an actinomycete isolated from soil collected from a hay  
25 meadow was determined using a polyphasic approach. The strain, isolate N1350, had  
26 morphological and chemotaxonomic properties consistent with its classification in the  
27 genus *Williamsia* and formed a distinct phyletic line in the *Williamsia* 16S rRNA gene  
28 tree. It shared its highest phylogenetic similarities with the type strains of *Williamsia*  
29 *marianensis* (98.1%) and *Williamsia muralis* (98.3%) but was readily distinguished  
30 from these and the other type strains of *Williamsia* species using a combination of  
31 phenotypic properties. On the basis of these data the isolate is considered to represent  
32 a new species of the genus *Williamsia*. The name proposed for this taxon is  
33 *Williamsia faeni* sp. nov. with the type strain N1350<sup>T</sup> (= DSM 45372<sup>T</sup> = NCIB 14575<sup>T</sup>  
34 = NRRL B24794<sup>T</sup>).

35

36 The genus *Williamsia* (Kämpfer *et al.*, 1999) has been classified in the family  
37 *Nocardiaceae* together with the genera *Gordonia*, *Millisia*, *Nocardia*, *Rhodococcus*  
38 and *Skermania* (Zhi *et al.*, 2009). At the time of writing the genus encompasses five  
39 validly described species, *Williamsia muralis* (Kämpfer *et al.*, 1999), the type species,  
40 was isolated from indoor building material of a children's day centre in Finland,  
41 *Williamsia deligens* (Yassin & Hupfer, 2006) from human blood, *Williamsia*  
42 *marianensis* (Pathom-aree *et al.*, 2006) from sediment taken from the Mariana Trench  
43 in the Pacific Ocean, *Williamsia maris* (Stach *et al.*, 2004) from sediment collected  
44 from the Sea of Japan, and *Williamsia serinedens* (Yassin *et al.*, 2007) from an oil-  
45 contaminated soil. The type strains of these species form a clade within the  
46 evolutionary radiation occupied by the suborder *Corynebacterineae* (Stackebrandt *et*  
47 *al.*, 1997; Zhi *et al.*, 2009). The genus *Williamsia* can also be distinguished from the  
48 other mycolic acid-containing genera using a combination of chemotaxonomic and

49 morphological properties (Soddell *et al.*, 2006; Adachi *et al.*, 2007). Similarly,  
50 *Williamsia* species can be separated by using a range of phenotypic properties (Yassin  
51 *et al.*, 2007).

52

53 The aim of the present study was to determine the taxonomic position of an  
54 actinomycete, isolate N1350 that had been recovered from a hay meadow soil and  
55 provisionally assigned to the genus *Williamsia*. The isolate was the subject of a  
56 polyphasic taxonomic study which showed that it should be recognised as a new  
57 species of *Williamsia*. The name proposed for this taxon is *Williamsia faeni* sp. nov.

58

59 Strain N1350 was isolated on Gauze's medium 2 supplemented with cycloheximide  
60 ( $50\mu\text{gml}^{-1}$ ), nalidixic acid ( $10\mu\text{gml}^{-1}$ ), novobiocin ( $10\mu\text{gml}^{-1}$ ) and nystatin ( $50\mu\text{gml}^{-1}$ )  
61 following inoculation with a soil suspension and incubation at  $30^{\circ}\text{C}$  for 21 days (Tan  
62 *et al.*, 2006). The soil sample had been collected from underneath the surface of  
63 Palace Leas meadow hay plot 6 (Atalan *et al.*, 2000) at Cockle Park Experimental  
64 Farm, Northumberland, UK (National Grid Reference NZ 200913). The organism was  
65 maintained on glucose-yeast extract agar (GYEA; Gordon & Mihm, 1962) at room  
66 temperature and as glycerol suspensions (20%, v/v) at  $-20^{\circ}\text{C}$ . Biomass for the  
67 chemotaxonomic and 16S rRNA gene sequence analyses was grown in shake flasks of  
68 GYE broth for 5 days at  $28^{\circ}\text{C}$ , checked for purity and harvested by centrifugation.  
69 Cells for the chemosystematic studies were washed twice in distilled water and  
70 freeze-dried; those for the 16S rRNA study in NaCl/EDTA buffer (0.1M EDTA, 0.1M  
71 NaCl, pH 8.0) and stored at  $-20^{\circ}\text{C}$  until required.

72

73 The phylogenetic position of isolate N1350 was determined in a 16S rRNA gene  
74 sequence analysis. Isolation of chromosomal DNA, PCR amplification and direct  
75 sequencing of the purified products were carried out after Kim et al. (1998) The  
76 almost complete 16S rRNA gene sequence (1441 nucleotides [nt]) was aligned  
77 manually with corresponding sequences of representatives of the genera classified in  
78 the suborder *Corynebacterineae*, retrieved from the DDBJ/EMBL/GenBank  
79 databases, using the pairwise alignment option and 16S rRNA secondary structural  
80 information held in the PHYDIT program (available at  
81 <http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using the  
82 least-squares (Fitch & Margoliash, 1967), neighbour-joining (Saitou & Nei, 1987),  
83 maximum-parsimony (Kluge & Farris 1969) and maximum-likelihood (Felsenstein,  
84 1981), tree-making algorithms from the PHYLIP suite of programs (Felsenstein,  
85 1993), and evolution distance matrices prepared after Jukes and Cantor (1969). The  
86 resultant unrooted tree topologies were evaluated in a bootstrap analysis (Felsenstein,  
87 1985) based on 1,000 resamplings of the neighbour-joining dataset using the  
88 CONSENSE and SEQBOOT options from the PHYLIP package.

89

90 It can be seen in Figure 1 that strain N1350 was recovered within the *Williamsia* 16S  
91 rRNA gene clade, an association supported by all of the tree-making algorithms and  
92 by a 100% bootstrap value in the neighbour-joining analysis. The organism showed its  
93 highest similarity with the type strain of *W. muralis*, the two strains shared a 16S  
94 rRNA gene similarity of 98.3%, a value that corresponds to 24 nt differences at 1416  
95 locations. DNA:DNA relatedness studies were not carried out between these strains as  
96 the type strain of *W. marianensis* and *W. muralis*, which form a subclade with isolate  
97 N1350, shared a much higher 16S rRNA similarity value (99.5%) but have a DNA-

98 DNA homology value of only 11% (Pathom-aree *et al.*, 2006), a figure well below the  
99 70% cut-off point recommended for the delineation of bacterial species (Wayne *et al.*,  
100 1987).

101

102 Strain N1350 was examined for key chemotaxonomic markers to establish if it had  
103 chemical properties characteristic of *Williamsia* strains. Standard procedures were  
104 used to determine the diagnostic isomers of diaminopimelic acid (A<sub>2</sub>pm; Staneck &  
105 Roberts, 1974), fatty acids (Sutcliffe, 2000), isoprenoid quinones (Collins, 1994),  
106 muramic acid type (Uchida *et al.*, 1999), mycolic acids (Minnikin *et al.*, 1975), polar  
107 lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Hasegawa *et al.*, 1983).  
108 The organism contained *meso*-A<sub>2</sub>pm, arabinose and galactose in whole-organism  
109 hydrolysates (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), N-glycolyl  
110 muramic acid, dihydrogenated menaquinones with nine isoprene units as the sole  
111 isoprenologue, major proportions of straight chain saturated, unsaturated and  
112 tuberculostearic acids (fatty acid type 1b *sensu* Kroppenstedt, 1985),  
113 phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and  
114 phosphatidylinositol as major polar lipids, and mycolic acids that co-migrated with  
115 those from *W. muralis* DSM 44343<sup>T</sup>. This chemotaxonomic profile is consistent with  
116 the classification of the strain in the genus *Williamsia* (Goodfellow & Maldonado,  
117 2005; Yassin & Hupfer, 2006).

118

119 Strain N1350 was examined for a range of phenotypic properties using a range of  
120 media and methods known to yield data of value for the classification and  
121 identification of mycolic-acid-containing actinomycetes (Jones *et al.*, 2008). The  
122 organism is aerobic, Gram-positive, non-acid-alcohol fast, asporogenous and catalase-

123 positive, and uses a diverse range of compounds as sole carbon sources, properties in  
124 line with its classification in the genus *Williamsia* (Kämpfer *et al.*, 1999; Yassin *et al.*,  
125 2007). It can be seen from Table 1 that the isolate can be readily distinguished from  
126 the type strains of *Williamsia* species using a combination of phenotypic properties. It  
127 can also be distinguished from *W. deligens* DSM 449002<sup>T</sup> and *W. serinedens* DSM  
128 45037<sup>T</sup> by its ability to degrade L-tyrosine (Yassin & Hupfer, 2006; Yassin *et al.*,  
129 2007) and from *W. marianensis* DSM 44944<sup>T</sup> by its capacity to degrade tributyrin, but  
130 not hypoxanthine (Pathom-aree *et al.*, 2006).

131

132 It can be concluded from the genotypic and phenotypic data that isolate N1350 can be  
133 readily distinguished from the validly described *Williamsia* species and hence should  
134 be classified as a representative of a novel species in the genus *Williamsia*. The name  
135 proposed for this taxon is *Williamsia faeni* sp. nov.

136

#### 137 **Description of *Williamsia faeni* sp. nov.**

138 *Williamsia faeni* (fae'ni. L.n. *faenum*, hay; L. gen.n. *faeni* of hay, referring to its  
139 isolation from a hay meadow).

140

141 Forms coccoid elements. Irregular, convex, matt yellow pink pigmented colonies are  
142 produced on glucose-yeast extract agar after incubation for 5 days at 28°C. Grows  
143 between 10 and 30°C, but not at 37°C. Hydrolyses allantoin and urea, but not arbutin.  
144 DNA, RNA, starch and uric acid are degraded, but not adenine, chitin, elastin,  
145 xanthine or xylan. D(-)-amygdalin, D(-)-arabinose, D(+)-arabitol, arbutin, D(-)-  
146 fructose, D(-)-fucose, D(-)-glucose, inulin, D(+)-lactose, D(+)-mannose, D(+)-  
147 melibiose,  $\alpha$ -methyl-D-glucoside, D(-)-ribose, and D(+)-turanose are used as sole

148 carbon sources for energy and growth, but not dulcitol or salicin (all at 1%, w/v);  
149 butan-1,3-diol, butan-1,4-diol, butan-1-ol, butan-2,3-diol, ethanol, propan-1-ol and  
150 propan-2-ol are also used as sole carbon sources (all at 1% v/v); with *iso*-amyl  
151 alcohol, benzoic acid, fumaric acid, glycerol, glycogen, L+lactic acid, L-malic acid,  
152 oleic acid, propanoic acid, pyruvic acid, sodium acetate, sodium *n*-butyrate, L+tartaric  
153 acid, valeric acid and xylitol used as sole carbon sources (all at 0.1%, w/v), but not  
154 adipic acid, citric acid, glutaric acid, malonic acid, D-mandelic acid, oxalic acid,  
155 sebacic acid, suberic acid or succinic acid. Acetamide, L-alanine, L-aminobutyl, L-  
156 arginine, L- gelatin, D-gluconic acid, L-glycine, histidine, L-leucine, DL-methionine,  
157 mononethanolamine, DL-*nor*leucine, L-*nor*valine, DL-phenylalanine, L-proline,  
158 serine, uric acid, urea and L-valine are used as sole carbon and nitrogen sources (all at  
159 0.1%, w/v), but not L-cysteine, L-glutamic acid, L-*isoleucine*, L-ornithine, Additional  
160 phenotypic properties are cited either in the text or in Table 1. Exhibits  
161 chemotaxonomic markers characteristic of the genus *Williamsia*. The fatty acid profile  
162 includes major amounts of hexadecanoic (C<sub>16:0</sub>, 21% of total), monosaturated  
163 octadecanoic (C<sub>18:1</sub>, 15%), tridecanoic (C<sub>13:0</sub>, 11%), tuberculostearic (10-methyl  
164 octadecanoate, 8%) and octadecanoic (C<sub>18:0</sub>, 7%) acids; minor components include,  
165 tetradecanoic (C<sub>14:0</sub>); pentadecanoic (C<sub>15:0</sub>); *iso*-hexadecanoic (iC<sub>16:0</sub>) and eicosanoic  
166 acids (C<sub>20:0</sub>).

167

168 The type strain, N1350<sup>T</sup> (= DSM 45372<sup>T</sup> = NCIB 14575<sup>T</sup> = NRRL B24794<sup>T</sup>), was  
169 isolated from a hay meadow plot at Cockle Park Experimental Farm, Northumberland,  
170 UK.

171

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173

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175 with the fatty acid analysis of the *Williamsia faeni* strain.

176

177 **References**

178 **Adachi, K., Katsuta, A., Matsuda, S., Peng, X., Misawa, N., Shizuri, Y.,**  
179 **Kroppenstedt, R.M., Yokota, A. & Kasai, H. (2007).** *Smaragdicoccus niigatensis*  
180 gen. nov., sp. nov., a novel member of the suborder *Corynebacterineae*. *Int J Syst*  
181 *Evol Microbiol* **57**, 297-301.

182

183 **Atalan, E., Manfio, G.P., Ward, A.C., Kroppenstedt, R.M. & Goodfellow, M.**  
184 **(2000).** Biosystematic studies on novel streptomycetes from soil. *Antonie van*  
185 *Leeuwenhoek* **77**, 337-353.

186

187 **Collins, M.D. (1994).** Isoprenoid quinones. In *Chemical Methods in Prokaryotic*  
188 *Systematics*, pp. 265-309. Edited by M. Goodfellow and A. G. O'Donnell. Chichester,  
189 John Wiley & Sons.

190

191 **Felsenstein, J. (1981).** Evolutionary trees from DNA sequences: a maximum  
192 likelihood approach. *J Mol Evol* **17**, 368-376.

193

194 **Felsenstein, J. (1985).** Confidence-limits on phylogenies - an approach using the  
195 bootstrap. *Evolution* **39**, 783-791.

196

197 **Felsenstein, J. (1993).** PHYLIP (Phylogenetic Inference Package), version 3.5c.  
198 Department of Genetics, University of Washington, Seattle, USA.  
199

200 **Fitch, W.M. & Margoliash, E. (1967).** Construction of phylogenetic trees. *Science*  
201 **155**, 279-284.  
202

203 **Goodfellow, M. & Maldonado, L.A. (2005).** The families *Dietziaceae*,  
204 *Gordoniaceae*, *Nocardiaceae* and *Tsukamurellaceae*. In *The Prokaryotes, Volume 3*,  
205 *Archaea, Bacteria, Firmicutes, Actinomycetes*, pp. 843-888. Edited by F. Dworkin, S.  
206 Falkow, K. H. Schleifer and E. Stackebrandt. New York, Berlin and Heidelberg,  
207 Springer.  
208

209 **Gordon, R.E. & Mihm, J.M. (1962).** Identification of *Nocardia caviae* (Erikson)  
210 nov. comb. *Ann NY Acad Sci* **98**, 628-636.  
211

212 **Hasegawa, T., Takizawa, M. & Tanida, S. (1983).** A rapid analysis for chemical  
213 grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319-322.  
214

215 **Jones, A.L., Koerner, R.J., Natarajan, S., Perry, J.D. & Goodfellow, M. (2008).**  
216 *Dietzia papillomatosis* sp. nov., a novel actinomycete isolated from the skin of an  
217 immunocompetent patient with confluent and reticulated papillomatosis. *Int J Syst*  
218 *Evol Microbiol* **58**, 68-72.  
219

220 **Jukes, T.H. & Cantor, C.R. (1969).** Evolution of protein molecules. In *Mammalian*  
221 *Protein Metabolism*, pp. 21-132. Edited by H. N. Munro. New York, Academic Press.

222

223 **Kämpfer, P., Andersson, M.A., Rainey, F.A., Kroppenstedt, R.M. & Salkinoja-**  
224 **Salonen, M. (1999).** *Williamsia muralis* gen. nov., sp. nov., isolated from the indoor  
225 environment of a children's day care centre. *Int J Syst Bacteriol* **49**, 681-687.

226

227 **Kim, S.B., Falconer, C., Williams, E. & Goodfellow, M. (1998).** *Streptomyces*  
228 *thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two  
229 moderately thermophilic carboxydotrophic species from soil. *Int J Syst Bacteriol* **48**,  
230 59-68.

231

232 **Kluge, A.G. & Farris, F.S. (1969).** Quantitative phyletics and the evolution of  
233 anurans. *Syst Zool* **18**, 1-32.

234

235 **Kroppenstedt, R.M. (1985).** Fatty acid and menaquinone analysis of actinomycetes  
236 and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173-199.  
237 Edited by M. Goodfellow and D. E. Minnikin. London., Academic Press.

238

239 **Lechevalier, M.P. & Lechevalier, H. (1970).** Chemical composition as a criterion in  
240 the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435-443.

241

242 **Minnikin, D.E., Alshamaony, L. & Goodfellow, M. (1975).** Differentiation of  
243 *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of  
244 whole-organism methanolysates. *J Gen Microbiol* **88**, 200-204.

245

246 **Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M.,**  
247 **Schaal, A. & Parlett, J.H. (1984).** An integrated procedure for the extraction of  
248 bacterial isoprenoid quinones and polar lipids. *J Microbiol Meth* **2**, 233-241.  
249  
250 **Pathom-aree, W., Nogi, Y., Sutcliffe, I.C., Ward, A.C., Horikoshi, K., Bull, A.T.**  
251 **& Goodfellow, M. (2006).** *Williamsia marianensis* sp. nov., a novel actinomycete  
252 isolated from the Mariana Trench. *Int J Syst Evol Microbiol* **56**, 1123-1126.  
253  
254 **Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for  
255 reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.  
256  
257 **Soddell, J.A., Stainsby, F.M., Eales, K.L., Kroppenstedt, R.M., Seviour, R.J. &**  
258 **Goodfellow, M. (2006).** *Millisia brevis* gen. nov., sp. nov., an actinomycete isolated  
259 from activated sludge foam. *Int J Syst Evol Microbiol* **56**, 739-744.  
260  
261 **Stach, J.E., Maldonado, L.A., Ward, A.C., Bull, A.T. & Goodfellow, M. (2004).**  
262 *Williamsia maris* sp. nov., a novel actinomycete isolated from the Sea of Japan. *Int J*  
263 *Syst Evol Microbiol* **54**, 191-194.  
264  
265 **Stackebrandt, E., Rainey, F.A. & Ward-Rainey, N.L. (1997).** Proposal for a new  
266 hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**,  
267 479-491.  
268  
269 **Staneck, J.L. & Roberts, G.D. (1974).** Simplified approach to identification of  
270 aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226-231.

271

272 **Sutcliffe, I.C. (2000).** Characterisation of a lipomannan lipoglycan from the mycolic  
273 acid containing actinomycete *Dietzia maris*. *Antonie van Leeuwenhoek* **78**, 195-201.

274

275 **Tan, G.Y., Ward, A.C. & Goodfellow, M. (2006).** Exploration of *Amycolatopsis*  
276 diversity in soil using genus-specific primers and novel selective media. *Syst Appl*  
277 *Microbiol* **29**, 557-569.

278

279 **Uchida, K., Kudo, T., Suzuki, K.I. & Nakase, T. (1999).** A new rapid method of  
280 glycolate test by diethyl ether extraction, which is applicable to a small amount of  
281 bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49-56.

282

283 **Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O.,**  
284 **Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt,**  
285 **E., Starr, M.P. & Trüper, H.G. (1987).** Report of the ad hoc committee on  
286 reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463-464.

287

288 **Yassin, A.F. & Hupfer, H. (2006).** *Williamsia deligens* sp. nov., isolated from  
289 human blood. *Int J Syst Evol Microbiol* **56**, 193-197.

290

291 **Yassin, A.F., Young, C.C., Lai, W.A., Hupfer, H., Arun, A.B., Shen, F.T., Rekha,**  
292 **P.D. & Ho, M.J. (2007).** *Williamsia serinedens* sp. nov., isolated from an oil-  
293 contaminated soil. *Int J Syst Evol Microbiol* **57**, 558-561.

294

295 **Zhi, X.Y., Li, W.J. & Stackebrandt, E. (2009).** An update of the structure and 16S  
296 rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*,  
297 with the proposal of two new suborders and four new families and emended  
298 descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* **59**, 589-608.  
299

**Table 1. Phenotypic properties that distinguish strain N1350<sup>T</sup> from the type strains of *Williamsia* species**

Reference strains: 1, Isolate N1350<sup>T</sup>; 2, *W. deligens* DSM 44902<sup>T</sup>; 3, *W. marianensis* DSM 44944<sup>T</sup> (data from Pathom-aree *et al.*, 2006); 4, *W. maris* DSM 44693<sup>T</sup>; 5, *W. muralis* DSM 44343<sup>T</sup>; 6, *W. serinedens* DSM 45037<sup>T</sup>.

+, positive; -, negative; ND, not determined

Characteristics	1	2	3	4	5	6
Aesculin hydrolysis	+	-	-	ND	-	-
Growth on sole carbon sources at 1% (w/v):						
Adonitol	+	-	-	-	+	+
L(-)-Arabinose	+	-	+	-	-	+
D(+)-Cellobiose	+	-	-	-	-	-
D(-)-Galactose	+	-	-	-	-	+
<i>meso</i> -Inositol	+	-	-	-	-	-
D(+)-Maltose	+	+	-	-	-	+
D(-)-Mannitol	+	+	+	-	+	+
D(+)-Melibiose	+	-	-	-	+	+
D(+)-Raffinose	+	-	-	-	-	-
$\alpha$ -L-Rhamnose	+	-	+	+	+	-
D(-)-Sorbitol	+	+	+	-	+	+
D(+)-Sucrose	+	+	+	-	+	+
D(+)-Trehalose	+	+	+	+	-	+
D(+)-Xylose	+	+	-	+	-	+
Growth on sole carbon sources at 0.1% (w/v):						
<i>m</i> -Hydroxybenzoic acid	-	-	-	-	+	+
<i>p</i> -Hydroxybenzoic acid	+	-	-	-	-	-
Growth on:						
1,2-Propanediol (1% v/v)	+	-	-	-	-	+
Growth at:						
4°C	+	-	+	-	-	-
10°C	+	-	+	+	+	+
37°C	-	+	-	+	+	-
45°C	-	-	-	-	+	-

## Legend for Figure

Fig.1. Maximum likelihood tree (Felsenstein, 1981) based on a nearly complete 16S rRNA gene sequence of strain N1350<sup>T</sup> showing its position in the *Williamsia* clade. Asterisks indicate branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms; the symbols F, P and N indicate branches recovered using the least-squares, maximum-parsimony and neighbour-joining methods, respectively. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 re-sampled datasets; only values above 50% are given. The scale bar indicates 10 substitutions per nucleotide position. <sup>T</sup>, type strain.