# *Brenneria goodwinii* sp. nov., a novel species associated with Acute Oak Decline in Britain

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Running title: *Brenneria goodwinii*, sp. nov. on *Quercus* spp.

**Note:** The GenBank/EMBL accession numbers for the sequences determined in this study are: JN544202 – JN544204 (16S rRNA), JN544205 – JN544213 (*atpD*), JN544214 – JN544222 (*gyrB*), JN544223 – JN544231 (*infB*) and JN544232 – JN544240 (*rpoB*).

**ABSTRACT**

A group of nine Gram-negative staining, facultatively anaerobic bacterial strains isolated from native oak trees displaying symptoms of Acute Oak Decline (AOD) in Britain were investigated using a polyphasic approach. 16S rRNA gene sequencing and phylogenetic analysis revealed that these isolates form a distinct lineage within the genus *Brenneria*, family *Enterobacteriaceae*, and are most closely related to *Brenneria rubrifaciens* (97.6 % sequence similarity). MLSA based on four housekeeping genes (*gyrB*, *rpoB*, *infB* and *atpD*) confirmed their position within the genus *Brenneria*, while DNA-DNA hybridization indicated that the isolates belong to a single taxon. The isolates can be differentiated phenotypically from their closest phylogenetic neighbours. The phylogenetic and phenotypic data demonstrate that these isolates from oak with symptoms of AOD represent a novel species in the genus *Brenneria*. The name *Brenneria goodwinii* sp. nov. (type strain = FRB $141^T$ = R-$43656^T$ = BCC $845^T$ = LMG $26270^T$ = NCPPB $4484^T$) is proposed.

An episode of Acute Oak Decline (AOD) has recently been identified in Britain by Denman & Webber (2009) and has a rapid effect on tree health. Mortalities are reported to occur within three to five years of the onset of symptom development (Denman *et al*., 2010). Affected trees are identified by stem bleeding or oozing of a dark sticky fluid from small (5 – 10 cm) vertical cracks formed between bark plates on tree trunks. Tissues underlying the stem bleed (i.e. periderm, phloem, cambium and in some cases part of the sapwood) are stained and/or necrotic (particularly the phloem tissue). Frequently, but not always, larval galleries of the bark boring buprestid *Agrilus biguttatus* are in close proximity or traverse the necrotic patches. Mature and even veteran oak trees native to Britain, *viz. Quercus robur* (pedunculate oak) and *Q. petraea* (sessile oak), appear to be most affected in the Midlands

(particularly in East Anglia), but there are an increasing number of reports from the south and south-east regions in England. Recent reports of a similar condition have been documented in Spain (Biosca *et al*., 2003; Poza-Carrión *et al*., 2008) and Belgium (Vansteenkiste *et al*., 2004).

During 2008 – 2010 numerous cream-coloured, Gram-negative bacterial strains were isolated from necrotic lesions, fluid exudates and occasionally from larval galleries in symptomatic oak at a number of sites in Britain. The majority of these isolates were identified as belonging to a novel genus and species *Gibbsiella quercinecans* (Brady *et al*., 2010), while a second group of these isolates was identified as a novel subspecies of *Lonsdalea quercina* (formerly *Brenneria quercina*) (Brady *et al*., 2011). A third group of these isolates was tentatively identified as a novel species belonging to the genus *Brenneria* based on partial 16S rRNA gene - and *gyrB*-gene sequencing. In the present study the taxonomic position of these *Brenneria* isolates is further investigated using a polyphasic approach based on multilocus sequence analysis (MLSA), DNA-DNA hybridization, phenotypic assays and fatty acid analyses. Further studies to elucidate whether or not these taxa play a role in the current episode of AOD are underway.

The oak isolates and reference strains investigated in this study are listed in Suppl. Table 1. Genomic DNA for sequencing was extracted using an alkali extraction method (Niemann *et al*., 1997*)* and stored at -20 °C. Almost complete (1346 bp) 16S rRNA gene sequences were determined for three oak isolates (LMG 26270$^{T}$, LMG 26271 and LMG 26272) using the primers and conditions determined by Coenye *et al.* (1999). Sequences for the closest phylogenetic neighbours were downloaded from GenBank, aligned with the oak sequences using the ClustalW application in BioEdit v 7.0.9.0 (Hall, 1999) and the overhangs were

trimmed. MODELTEST 3.7 (Posada & Crandall, 1998) was applied to the data set to determine the best-fit evolutionary model. Maximum likelihood and neighbour joining trees were constructed using PhyML (Guindon & Gascuel, 2003) and PAUP 4.0b10 (Swofford, 2000), respectively by applying the models and parameters set by MODELTEST (Tamura-Nei model including proportion of invariable sites and gamma distribution). Bootstrap analysis with 1000 replicates was performed on the tree to assess the reliability of the clusters generated. As the topology of both trees was similar, only a maximum likelihood tree is shown. The three oak isolates demonstrate > 99.5 % 16S rRNA gene sequence pairwise similarity to each other and > 97.0 % to the type strains of *Brenneria rubrifaciens* (97.6 %) and *Lonsdalea quercina* (97.1 %). They form a single cluster with 100 % bootstrap support on a separate branch in the 16S rRNA gene phylogenetic tree (Suppl. Fig. 1), and demonstrate a close phylogenetic relationship to *B. rubrifaciens* and *B. salicis* (the type species of *Brenneria*), although there is no significant bootstrap support for this clade. The oak isolates are far removed from members of *Lonsdalea*, despite sequence similarities above 97 %. The remaining two *Brenneria* species, *B. nigrifluens* and *B. alni*, cluster with *Samsonia erythrinae* separately from the type species of *Brenneria*. Like many genera in the *Enterobacteriaceae*, *Brenneria* is polyphyletic when analysis is based on 16S rRNA gene sequencing. It is possible that a degree of homoplasy exists in the hypervariable regions of this gene, as a result of tolerance to mutation; or horizontal gene transfer could have taken place in these regions without affecting gene function, but disrupting the phylogenetic signal (Naum *et al*., 2008). However as the investigated oak isolates are closely associated with the type species of the genus *Brenneria*, it is probable that they constitute a single novel species in this genus.

MLSA based on partial gene sequencing of *gyrB, rpoB, infB* and *atpD* was recently used to evaluate the phylogenetic position of species belonging to the genus *Brenneria* within the *Enterobacteriaceae* (Brady *et al*., 2011). The MLSA scheme was proven to be very useful for this purpose, and therefore the same four housekeeping genes were sequenced for nine *Brenneria* isolates from oak. Amplification and sequencing of the above genes was carried out as previously described (Brady *et al*., 2008). Additional sequences for the closest phylogenetic neighbours were downloaded from GenBank, and are listed in Suppl. Table 1. Sequence analysis and tree construction (applying the general time reversible model including proportion of invariable sites and gamma distribution) were performed as for 16S rRNA gene sequencing. MLSA revealed a high degree of sequence similarity between the oak isolates for all four housekeeping genes, with < 0.9 % *gyrB*, < 0.7 % *infB*, < 0.4 % *rpoB* and < 0.5 % *atpD* sequence variation. The oak isolates form a well-supported cluster within the *Brenneria* clade, far removed from *Lonsdalea*, in the phylogenetic tree based on the concatenated sequences of the four housekeeping genes (Fig. 1). This confirms the identity of the isolates as members of the genus *Brenneria* and also indicates that they possibly represent a single novel species in this genus.

High quality DNA for DNA-DNA hybridizations was extracted from four oak isolates (LMG 26270$^{T}$, LMG 26271, LMG 26272 and R-43657) and the type strains of *B. salicis* LMG 2698$^{T}$ and *B. rubrifaciens* LMG 2709$^{T}$, using a modified method (Cleenwerck *et al*., 2002) of Wilson (1987). DNA-DNA hybridizations were performed using the microtitre plate method (Ezaki *et al*., 1989) with minor modifications (Cleenwerck *et al*., 2002). The hybridization temperature was 43 °C. Reciprocal reactions (A x B and B x A) were performed for each possible DNA pair and the variation observed was within the limits of this method (Goris *et al*., 1998). Values presented in Table 1 are based on a minimum of four replicates. When

hybridized against each other, the four oak isolates exhibited high levels of DNA-DNA relatedness, ranging from 90 to 100 %. This confirms that these isolates belong to a single species. By contrast, low levels of DNA-DNA relatedness (28 - 34 %) were observed following hybridization of LMG 26270$^{T}$ and LMG 26271 to *B. salicis* LMG 2698$^{T}$ and *B. rubrifaciens* LMG 2709$^{T}$, confirming that the isolates belong to a novel species. The DNA G + C content of the oak isolates, LMG 26270$^{T}$, LMG 26271, LMG 26272 and R-43657, measured using HPLC (Mesbah *et al*., 1989) was 52.5 mol %, 52.6 mol %, 52.7 mol % and 52.3 mol % respectively. This is within the DNA G + C content range of 50.1 – 56.1 mol % generally observed for the recognized *Brenneria* species (Hauben & Swings, 2005, Brady *et al*., 2011).

Biochemical and physiological tests were performed on all nine oak isolates listed in Suppl. Table 1 using API 20E and API 50CHB/E (bioMérieux). The results were compared to those of reference strains of each recognized *Brenneria* species and *Lonsdalea quercina*, generated under identical conditions (Brady *et al*., 2011). Additionally, GN2 MicroPlate (Biolog) tests were carried out on the same nine oak isolates to determine carbon source utilization. The tests were performed according to the manufacturer's instructions and incubated for 24h (API 20E, Biolog) or 48h (API 50CHB/E). Results are listed in Table 2 and in the species descriptions below. The oak isolates can be distinguished from the recognized *Brenneria* species by various features such as their ability to produce acid from inositol (differentiation from *B. salicis*, *B. alni* and *B. rubrifaciens*), amygdalin (differentiation from *B. salicis*, *B. nigrifluens* and *B. rubrifaciens*), D-galactose (differentiation from *B. salicis*, *B. nigrifluens* and *B. rubrifaciens*) and D-raffinose (differentiation from *B. alni* and *B. rubrifaciens*). Additionally, these oak isolates differ from *Lonsdalea quercina* by their inability to utilize

citrate, their ability to produce acid from L-arabinose and several additional characteristics listed in Table 2.

Whole-cell fatty acid composition was determined for four oak isolates (LMG 26270$^T$, LMG 26271, LMG 26272 and R-43657) using an Agilent Technologies 6890N gas chromatograph (Santa Clara, CA, USA). Cultivation of the isolates, extraction and analysis of the fatty acid methyl esters were performed according to the recommendations of the Microbial Identification System, Sherlock version 3.10 (MIDI). Cells were harvested from cultures grown on trypticase soy agar (BBL 11768) for 24 h at 28 °C. The peaks of the profiles were identified using the TSBA50 identification library version 5.0. Profiles obtained for the oak isolates were compared with profiles of phylogenetically related strains, generated under the same conditions (Brady *et al*., 2011), and were found to be similar to those of the recognized species of the genus *Brenneria* (Hauben *et al*., 1998, Surico *et al*., 1996). The major fatty acid components, contributing 95 % to the whole-cell fatty acid composition, include $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ cyclo, $C_{18:1}\omega7c$, summed feature 2 (iso-$C_{16:1}$ and /or $C_{14:0}$ 3-OH) and summed feature 3 ($C_{16:1}$ ω7*c* and /or iso-$C_{15:0}$ 2-OH). The fatty acid profiles of the oak isolates differ from those of recognized *Brenneria* species with regards to the amounts of $C_{17:0}$ cyclo and summed feature 3 ($C_{16:1}$ ω7*c* and /or iso-$C_{15:0}$ 2-OH) and also from *Lonsdalea quercina* strains with regards to the amounts of $C_{12:0}$ and $C_{14:0}$. The percentages of peak areas for the fatty acids are presented in Suppl. Table 2.

Hypersensitivity reaction (HR) tests were conducted in duplicate on eight wild tobacco seedlings (*Nicotiana sylvestris*) following the method described by Lelliot and Stead (1987). Bacterial suspensions ($10^9$ CFU/ml) were injected into four intercellular spaces per leaf with a fine needle and syringe. The seedlings were incubated at 26 °C and assessed after 48h, and

again after 72h. Of the eight isolates tested, only two elicited a hypersensitivity response (data not shown). However, pathogenicity tests are currently underway to determine if these isolates contribute to lesion formation in the phloem tissue of oak with symptoms of AOD in Britain.

The oak isolates investigated in this study form a single novel taxon in the genus *Brenneria* that can be differentiated from the existing species, based on both gene sequencing and DNA-DNA hybridization values. Furthermore, the novel species conforms to the genus description of *Brenneria* and shares the phenotypic features that are characteristic of *Brenneria* species (Hauben & Swings, 2005, Brady *et al*., 2011) but can also be differentiated from its closest phylogenetic neighbours by several traits. Therefore we propose to classify these oak isolates as *Brenneria goodwinii* sp. nov. (type strain FRB $141^T$ = LMG $26270^T$ = NCPPB $4484^T$). *Brenneria goodwinii* sp. nov. is the second novel bacterial species associated with AOD on native oak in England, the first being *G. quercinecans* (Brady *et al*., 2010). Whether or not these bacteria play a role in AOD has yet to be determined.

**Description of *Brenneria goodwinii* sp. nov.**

*Brenneria goodwinii* (good.win'i.i N.L. masc. gen. n. *goodwinii*, of Goodwin, named in honour of Peter John Goodwin for his major contribution to promoting the health and prosperity of oak in Britain).

Gram-negative short rods (0.8 x 1 – 1.3 μm), facultatively anaerobic, oxidase negative and catalase positive. Cells occur singly and are motile by means of peritrichous flagella (determined by TEM). Colonies are pale cream on nutrient agar, round, convex and smooth with entire margins. Strains can grow at temperatures between 10 and 40 °C. Positive for β-galactosidase and acetoin, but negative for arginine dihydrolase, lysine decarboxylase,

ornithine decarboxylase, citrate, $H_2S$, urease, tryptophan deaminase, indole and gelatinase production. Nitrate is not reduced to nitrite and cells have a weak $N_2$ reaction. Acid is produced from: glycerol, L-arabinose, D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, D-mannitol, D-sorbitol, *N*-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, D-lactose, melibiose, D-saccharose, D-trehalose, D-raffinose, gentiobiose, D-turanose and potassium 5-ketogluconate (API 50CHB/E). Reactions to D-xylose (type strain is positive), methyl-α-D-glucopyranoside (type strain is negative), D-cellobiose (type strain is negative) and potassium gluconate (type strain is negative) are variable. The following carbon sources are utilized at 28 °C: *N*-acetyl-D-glucosamine, L-arabinose, D-fructose, D-galactose, gentiobiose, α-D-glucose, inositol, α-D-lactose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-psicose, D-raffinose, D-sorbitol, sucrose, D-trehalose, turanose, pyruvic acid methyl ester, succinic acid mono-methyl ester, formic acid, D-gluconic acid, succinic acid, bromosuccinic acid, L-aspargine, L-aspartic acid, L-serine, glycerol, D,L,α-glycerol phosphate and α-D-glucose-6-phosphate (Biolog). The G + C content of the type strain is 52.5 mol %. The type strain is FRB $141^T$ (= LMG $26270^T$ = NCPPB $4484^T$), isolated from *Quercus robur* in Outwood, Loughborough, Leicestershire, England. Strains of this species have been isolated from English and sessile oak exhibiting symptoms of Acute Oak Decline.

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**Table 1:** DNA-DNA relatedness values amongst the type strains of *Brenneria salicis*, *Brenneria rubrifaciens* and strains belonging to *Brenneria goodwinii* sp. nov.

Values are expressed as percentages (± difference between reciprocal values/2).

| | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| *Brenneria goodwinii* | | | | | | |
| 1. LMG 26270$^T$ | 100 | | | | | |
| 2. LMG 26271 | 93 (± 0.5) | 100 | | | | |
| 3. R-43657 | 95 (± 2.0) | 94 (± 4.5) | 100 | | | |
| 4. LMG 26272 | 92 (± 2.5) | 101 (± 2.5) | 90 (± 2.5) | 100 | | |
| 5. *Brenneria salicis* LMG 2698$^T$ | 29 (± 1.5) | 34 (± 2.0) | | | 100 | |
| 6. *Brenneria rubrifaciens* LMG 2709$^T$ | 28 (± 1.5) | 34 (± 1.0) | | | 47 (± 4.0) | 100 |

**Table 2:** Phenotypic characteristics distinguishing *Brenneria goodwinii* sp. nov. from the recognized *Brenneria* and *Lonsdalea* species. *n* = number of strains

1 = *Brenneria goodwinii* (*n*=9), 2 = *Brenneria salicis* (*n*=3), 3 = *Brenneria alni* (*n*=5), 4 = *Brenneria nigrifluens* (*n*=5), 5 = *Brenneria rubrifaciens* (*n*=5), 6 = *Lonsdalea quercina* (*n*=17)

All data were generated under the same conditions using API tests (bioMérieux). Data for *Brenneria* and *Lonsdalea quercina* were taken from Brady *et al*., 2011.

+, 90-100 % strains positive in 1-2 days; -, negative; d, 11-89 % strains positive in 1-4 days

All *Brenneria* strains were positive for acid production from: D-ribose, D-glucose, D-fructose, D-mannose, D-mannitol, *N*-acetylglucosamine and D-saccharose. All *Brenneria* strains were negative for acid production from: erythritol, L-xylose, D-adonitol, methyl-D-xylopyranoside, L-sorbose, dulcitol, inulin, D-melezitose, amidon, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol.

| **Characteristic** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| Acid from: | | | | | | |
| L-arabinose | + | - | + | + | + | - |
| amygdalin | + | - | + | - | - | - |
| D-galactose | + | - | + | - | - | d |
| gentiobiose | + | - | - | + | - | - |
| inositol | + | - | - | + | - | - |
| melibiose | + | - | - | + | - | - |
| potassium gluconate | d | + | - | - | - | d |
| D-raffinose | + | + | - | + | - | - |
| D-sorbitol | + | - | - | + | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| D-trehalose | + | - | + | + | - | d |
| D-turanose | + | - | + | - | - | + |
| D-xylose | d | - | + | + | - | - |

**Supplementary Table 1:** *Brenneria*, *Dickeya*, *Erwinia*, *Lonsdalea* and *Pectobacterium* accession numbers for sequences used in this study

CCM, Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic; CFBP, Collection Française de Bactéries Phytopathogènes, Beaucouzé, France; FRB, Forest Research Bacteria Collection, Forest Research, UK, ICMP, International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand; LMG, BCCM/LMG Bacteria Collection, Ghent University, Belgium; NCPPB, National Collection of Plant Pathogenic Bacteria, York, UK, R, Research Collection, Ghent University, Belgium

* GenBank accession numbers

| **Species name** | **Strain** | **Source** | **Location** | ***gyrB**** | ***rpoB**** | ***infB**** | ***atpD**** |
|---|---|---|---|---|---|---|---|
| *Brenneria goodwinii* sp. nov. | FRB 141$^{T}$ = LMG 26270$^{T}$ = NCPPB 4484$^{T}$ | *Quercus robur*, root | Outwood, UK | JN544220 | JN544238 | JN544229 | JN544211 |
| | FRB 184 = LMG 26271 = NCPPB 4485 | *Quercus robur*, inner bark | Gorse Covert, UK | JN544216 | JN544234 | JN544225 | JN544207 |
| | FRB 193 = LMG 26272 = NCPPB 4486 | *Quercus robur*, outer bark | Gorse Covert, UK | JN544222 | JN544240 | JN544231 | JN544213 |
| | FRB 135 = R-43657 | *Quercus robur*, inner bark | Outwood, UK | JN544221 | JN544239 | JN544230 | JN544212 |
| | FRB 171 = R-43655 | *Quercus robur*, outer bark | Gorse Covert, UK | JN544219 | JN544237 | JN544228 | JN544210 |
| | FRB 173 = R-43654 | *Quercus robur*, outer bark | Gorse Covert, UK | JN544218 | JN544236 | JN544227 | JN544209 |
| | FRB 177 = R-43479 | *Quercus robur*, outer bark | Gorse Covert, UK | JN544217 | JN544235 | JN544226 | JN544208 |
| | FRB 182 = R-43477 | *Quercus robur*, inner bark | Gorse Covert, UK | JN544215 | JN544233 | JN544224 | JN544206 |
| | FRB 186 = R-43476 | *Quercus robur*, inner bark | Gorse Covert, UK | JN544214 | JN544232 | JN544223 | JN544205 |
| *Brenneria salicis* | LMG 2698$^{T}$ | *Salix alba* | UK | JF311622 | JF311847 | JF311735 | JF311509 |
| | LMG 2700 | *Salix* sp. | UK | JF311623 | JF311848 | JF311736 | JF311510 |
| | LMG 2706 | *S. caprea* | UK | JF311624 | JF311849 | JF311737 | JF311511 |
| | LMG 5119 | *S. alba* | UK | JF311625 | JF311850 | JF311738 | JF311512 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | LMG 18278 | *Salix* sp. | Belgium | JF311626 | JF311851 | JF311739 | JF311513 |
| *Brenneria alni* | NCPPB 3934$^{T}$ | *Alnus cordata* | Italy | JF311627 | JF311852 | JF311740 | JF311514 |
| | NCPPB 3833 | *A. glutinosa* | Italy | JF311630 | JF311855 | JF311743 | JF311517 |
| | NCPPB 3835 | *A. cordata* | Italy | JF311631 | JF311856 | JF311744 | JF311518 |
| | NCPPB 3935 | *Alnus* sp. | Unknown | JF311628 | JF311853 | JF311741 | JF311515 |
| | NCPPB 3936 | *Alnus* sp. | Unknown | JF311629 | JF311854 | JF311742 | JF311516 |
| *Brenneria nigrifluens* | LMG 2694$^{T}$ | *Juglans regia* | USA | JF311612 | JF311837 | JF311725 | JF311499 |
| | LMG 2696 | *Juglans regia* | USA | JF311613 | JF311838 | JF311726 | JF311500 |
| | LMG 5107 | *Juglans regia* | USA | JF311614 | JF311839 | JF311727 | JF311501 |
| | LMG 5953 | *Juglans regia* | USA | JF311615 | JF311840 | JF311728 | JF311502 |
| | LMG 5956 | *Juglans regia* | USA | JF311616 | JF311841 | JF311729 | JF311503 |
| *Brenneria rubrifaciens* | LMG 2709$^{T}$ | *Juglans regia* | USA | JF311617 | JF311842 | JF311730 | JF311504 |
| | LMG 2711 | *Juglans regia* | USA | JF311618 | JF311843 | JF311731 | JF311505 |
| | LMG 5109 | *Juglans regia* | USA | JF311619 | JF311844 | JF311732 | JF311506 |
| | LMG 5116 | *Juglans regia* | USA | JF311620 | JF311845 | JF311733 | JF311507 |
| | LMG 5118 | *Juglans regia* | USA | JF311621 | JF311846 | JF311734 | JF311508 |
| *Dickeya chrysanthemi* | LMG 2804$^{T}$ | *Dianthus caryophyllus* | UK | JF311636 | JF311861 | JF311749 | JF311523 |
| | LMG 2490 | *Chrysanthemum maximum* | Italy | JF311637 | JF311862 | JF311750 | JF311524 |
| *Dickeya dadantii* ssp. *dadantii* | LMG 25991$^{T}$ | *Pelargonium capitatum* | Comoros | JF311644 | JF311869 | JF311757 | JF311531 |
| | PRI-2122 | *Ipomea batatas* | Cuba | JF311645 | JF311870 | JF311758 | JF311532 |
| *Dickya dadantii* ssp. *dieffenbachiae* | LMG 25992$^{T}$ | *Dieffenbachia* sp. | USA | JF311652 | JF311877 | JF311765 | JF311539 |
| | LMG 2475 | *D. maculata* | USA | JF311653 | JF311878 | JF311766 | JF311540 |
| *Dickeya dianthicola* | LMG 2485$^{T}$ | *Dianthus caryophyllus* | UK | JF311648 | JF311873 | JF311761 | JF311535 |
| | NCPPB 1385 = LMG 25729 | *Dahlia* sp. | Romania | JF311649 | JF311874 | JF311762 | JF311536 |
| *Dickeya paradisiaca* | LMG 2542$^{T}$ | *Musa paradisiaca* | Colombia | JF311640 | JF311865 | JF311753 | JF311527 |
| | LMG 2544 | *Musa paradisiaca* | Colombia | JF311641 | JF311866 | JF311754 | JF311528 |
| *Dickeya zeae* | LMG 2505$^{T}$ | *Zea mays* | USA | JF311632 | JF311857 | JF311745 | JF311519 |
| | LMG 2497 | *Zea mays* | USA | JF311633 | JF311858 | JF311746 | JF311520 |
| *Lonsdalea quercina* ssp. *quercina* | LMG 2724$^{T}$ | *Quercus* sp. | USA | JF311656 | JF311881 | JF311769 | JF311543 |
| | LMG 5277 | *Quercus* sp. | USA | JF311658 | JF311883 | JF311771 | JF311545 |
| *Lonsdalea quercina* ssp. *iberica* | LMG 26264$^{T}$ = NCPPB 4490$^{T}$ = 1915-14 | *Q. ilex* | Madrid, Spain | JF311665 | JF311890 | JF311778 | JF311552 |
| | LMG 26265 = NCPPB 4489 = 1625-1 | *Q. pyrenaica* | Madrid, Spain | JF311662 | JF311887 | JF311775 | JF311549 |
| *Lonsdalea quercina* ssp. *britannica* | LMG 26267$^{T}$ = NCPPB 4481$^{T}$ = FRB 18 | *Q. robur* | Booth Wood, UK | JF311666 | JF311891 | JF311779 | JF311553 |
| | LMG 26269 = NCPPB 4483 = FRB 188 | *Q. robur* | Gorse Covert, UK | JF311669 | JF311894 | JF311782 | JF311556 |

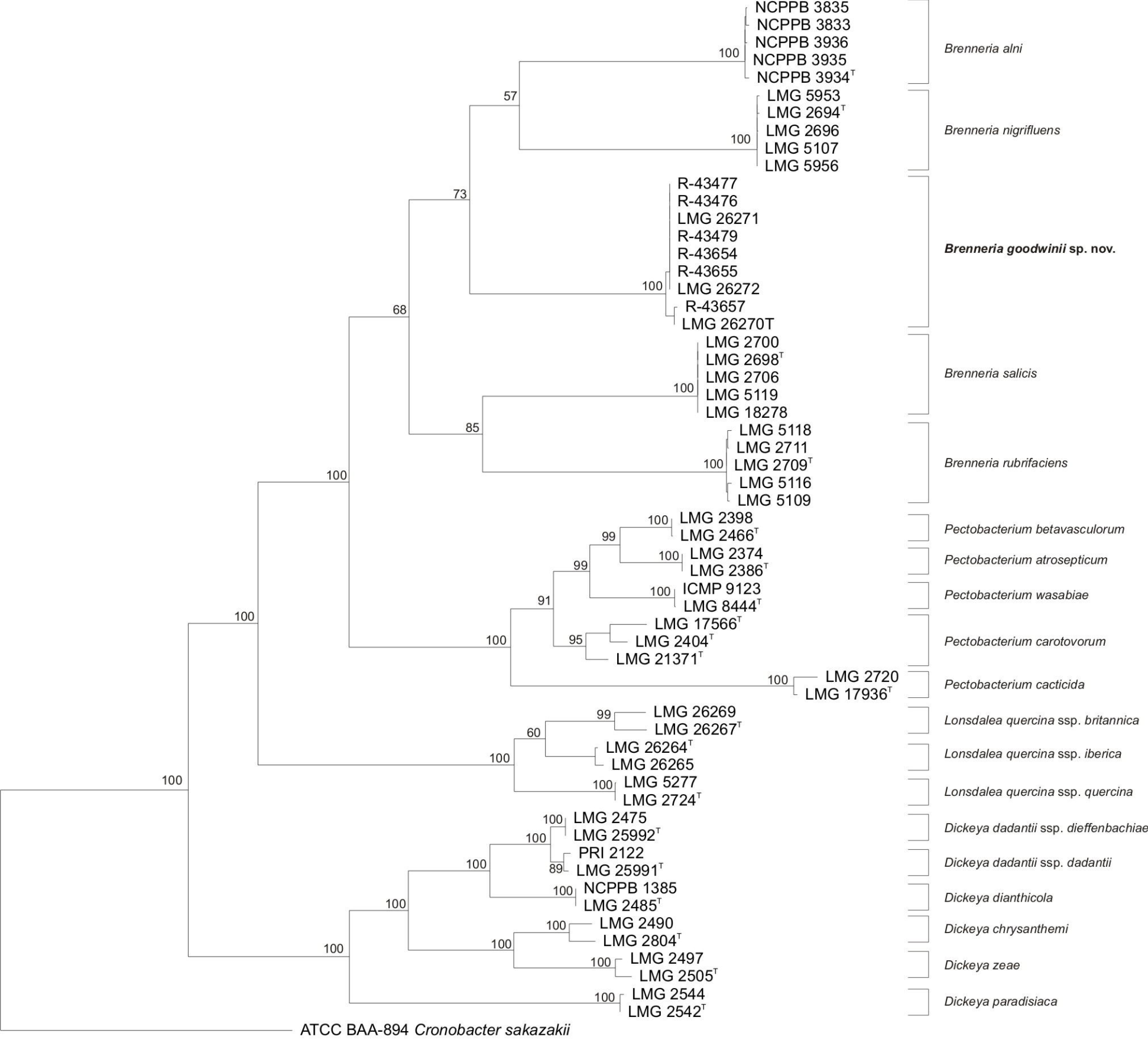
**Supplementary Table 2:** Fatty acid composition (percentage of peak areas) of *Brenneria goodwinii* sp. nov. and selected type strains of *Brenneria* and *Lonsdalea*. *n* = number of strains

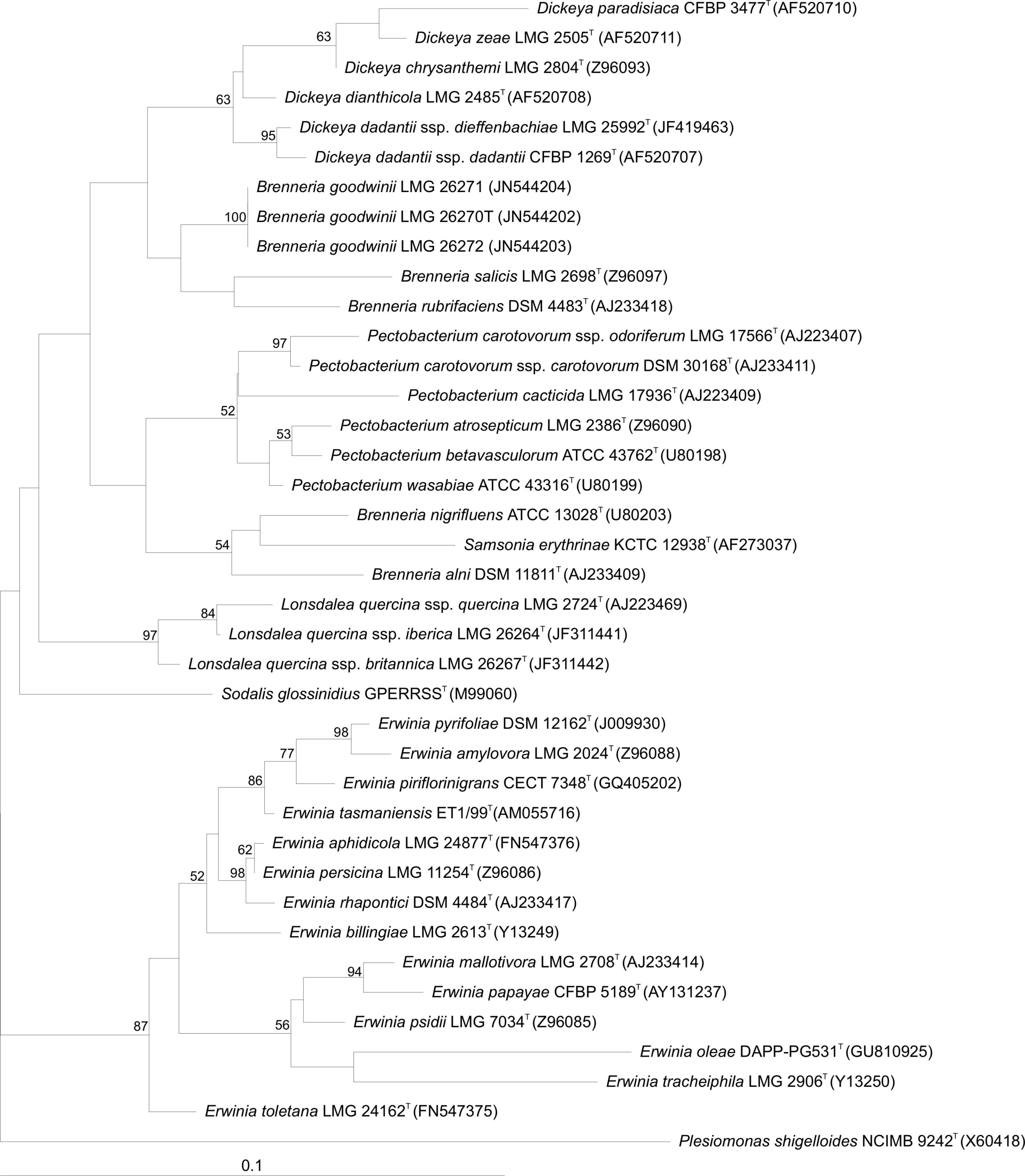
1, *B. goodwinii* (*n*=4), 2, *B. salicis* (LMG 2698$^T$), 3, *B. rubrifaciens* (LMG 2709$^T$), 4, *B. nigrifluens* (LMG 2694$^T$), 5, *L. quercina* (LMG 2724$^T$, LMG 26264$^T$, LMG 26267$^T$).

| **Fatty acid** | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| Saturated fatty acids | | | | | |
| $C_{12:0}$ | 5.24 | 4.03 | 3.14 | 3.55 | 0.83 |
| $C_{14:0}$ | 4.73 | 5.35 | 5.35 | 6.27 | 12.2 |
| $C_{16:0}$ | 33.07 | 29.04 | 32.45 | 35.09 | 32.50 |
| Unsaturated fatty acids | | | | | |
| $C_{18:1}\omega7c$ | 12.70 | 15.32 | 16.53 | 9.95 | 9.92 |
| Cyclopropane fatty acids | | | | | |
| $C_{17:0}$ | 18.64 | 9.58 | 5.84 | 15.05 | 9.36 |
| $C_{19:0}$ | 2.56 | 0.53 | 0.0 | 2.36 | 1.52 |
| Summed features | | | | | |
| 2: iso-$C_{16:1}$ and/or $C_{14:0}$ 3-OH | 10.96 | 11.13 | 10.71 | 11.11 | 12.11 |
| 3: $C_{16:1}$ $\omega7c$ and /or iso-$C_{15:0}$ 2-OH | 9.70 | 23.21 | 24.75 | 15.37 | 21.73 |

**Figure 1:** Maximum likelihood tree based on concatenated housekeeping gene sequences of *Brenneria* species, *Brenneria goodwinii* sp. nov. and phylogenetically related species of the *Enterobacteriaceae*. Bootstrap values after 1000 replicates are expressed as percentages. *Cronobacter sakazakii* ATCC BAA-894 was included as an outgroup. Gene sequences for *C. sakazakii* were obtained from http://www.ncbi.nlm.nih.gov. The scale bar indicates the fraction of substitutions per site.

**Suppl. Figure 1:** Maximum likelihood tree based on almost complete 16S rRNA gene sequences of members of the genus *Brenneria*, *Brenneria goodwinii* sp. nov and phylogenetically related species of the *Enterobacteriaceae*. Bootstrap values after 1000 replicates are expressed as percentages. *Plesiomonas shigelloides* is included as an outgroup. The scale bar indicates the fraction of substitutions per site.


NCPPB 3835
NCPPB 3833
NCPPB 3936
NCPPB 3935
NCPPB 3934T
Brenneria alni
LMG 5953
LMG 2694T
LMG 2696
LMG 5107
LMG 5956
Brenneria nigrifluens
R-43477
R-43476
LMG 26271
R-43479
R-43654
R-43655
LMG 26272
R-43657
LMG 26270T
Brenneria goodwinii sp. nov.
LMG 2700
LMG 2698T
LMG 2706
LMG 5119
LMG 18278
Brenneria salicis
LMG 5118
LMG 2711
LMG 2709T
LMG 5116
LMG 5109
Brenneria rubrifaciens
LMG 2398
LMG 2466T
Pectobacterium betavasculorum
LMG 2374
LMG 2386T
Pectobacterium atrosepticum
ICMP 9123
LMG 8444T
Pectobacterium wasabiae
LMG 17566T
LMG 2404T
LMG 21371T
Pectobacterium carotovorum
LMG 2720
LMG 17936T
Pectobacterium cacticida
LMG 26269
LMG 26267T
Lonsdalea quercina ssp. britannica
LMG 26264T
LMG 26265
Lonsdalea quercina ssp. iberica
LMG 5277
LMG 2724T
Lonsdalea quercina ssp. quercina
LMG 2475
LMG 25992T
Dickeya dadantii ssp. dieffenbachiae
PRI 2122
LMG 25991T
Dickeya dadantii ssp. dadantii
NCPPB 1385
LMG 2485T
Dickeya dianthicola
LMG 2490
LMG 2804T
Dickeya chrysanthemi
LMG 2497
LMG 2505T
Dickeya zeae
LMG 2544
LMG 2542T
Dickeya paradisiaca
ATCC BAA-894 Cronobacter sakazakii
0.1


Dickeya paradisiaca CFBP 3477T (AF520710)
63
Dickeya zeae LMG 2505T (AF520711)
Dickeya chrysanthemi LMG 2804T (Z96093)
63
Dickeya dianthicola LMG 2485T (AF520708)
95
Dickeya dadantii ssp. dieffenbachiae LMG 25992T (JF419463)
Dickeya dadantii ssp. dadantii CFBP 1269T (AF520707)
Brenneria goodwinii LMG 26271 (JN544204)
100
Brenneria goodwinii LMG 26270T (JN544202)
Brenneria goodwinii LMG 26272 (JN544203)
Brenneria salicis LMG 2698T (Z96097)
Brenneria rubrifaciens DSM 4483T (AJ233418)
Pectobacterium carotovorum ssp. odoriferum LMG 17566T (AJ223407)
97
Pectobacterium carotovorum ssp. carotovorum DSM 30168T (AJ233411)
Pectobacterium cacticida LMG 17936T (AJ223409)
52
Pectobacterium atrosepticum LMG 2386T (Z96090)
53
Pectobacterium betavasculorum ATCC 43762T (U80198)
Pectobacterium wasabiae ATCC 43316T (U80199)
Brenneria nigrifluens ATCC 13028T (U80203)
54
Samsonia erythrinae KCTC 12938T (AF273037)
Brenneria alni DSM 11811T (AJ233409)
Lonsdalea quercina ssp. quercina LMG 2724T (AJ223469)
84
97
Lonsdalea quercina ssp. iberica LMG 26264T (JF311441)
Lonsdalea quercina ssp. britannica LMG 26267T (JF311442)
Sodalis glossinidius GPERRSST (M99060)
Erwinia pyrifoliae DSM 12162T (J009930)
98
77
Erwinia amylovora LMG 2024T (Z96088)
86
Erwinia piriflorinigrans CECT 7348T (GQ405202)
Erwinia tasmaniensis ET1/99T (AM055716)
Erwinia aphidicola LMG 24877T (FN547376)
62
52
98
Erwinia persicina LMG 11254T (Z96086)
Erwinia rhapontici DSM 4484T (AJ233417)
Erwinia billingiae LMG 2613T (Y13249)
Erwinia mallotivora LMG 2708T (AJ233414)
94
Erwinia papayae CFBP 5189T (AY131237)
87
56
Erwinia psidii LMG 7034T (Z96085)
Erwinia oleae DAPP-PG531T (GU810925)
Erwinia tracheiphila LMG 2906T (Y13250)
Erwinia toletana LMG 24162T (FN547375)
Plesiomonas shigelloides NCIMB 9242T (X60418)
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