Mesorhizobium calcicola sp. nov., *Mesorhizobium waitakense* sp. nov., *Mesorhizobium sophorae* sp. nov., *Mesorhizobium newzealandense* sp. nov. and *Mesorhizobium kowhaii* sp. nov. isolated from *Sophora* root nodules in New Zealand

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *glnII*, *recA* and *rpoB* gene sequences reported in this paper are respectively for *Mesorhizobium calcicola* sp. nov. ICMP 19560^T: KC237406, KC237633, KC237686 and KJ450947; *Mesorhizobium waitakense* sp. nov. ICMP 19523^T: KC237413, KC237640, KC237693 and KJ450952; *Mesorhizobium sophorae* sp. nov. ICMP 19535^T: KC237424, KC237651, KC237704 and KJ450963; *Mesorhizobium newzealandense* sp. nov. ICMP 19545^T: KC237410, KC237637, KC237690 and KJ450969; *Mesorhizobium kowhaii* sp. nov. ICMP 19512^T: KC237394, KC237621, KC237674 and KJ450972. Accession numbers for the remaining strains can be found in Table S1.

Abstract

In total, 31 strains of gram-negative, rod-shaped bacteria were isolated from *Sophora* root nodules and authenticated as rhizobia on this hos. Based on the 16S rRNA gene phylogeny, they were shown to belong to the genus *Mesorhizobium*, with the representative strains ICMP 19560^T, ICMP 19523^T, ICMP 19535^T, ICMP 19545^T and ICMP 19512^T being most closely related to *Mesorhizobium sangaii* SCAU7^T (99.9 – 99.6%), *Mesorhizobium cantuariense* ICMP 19515^T (99.7 – 99.6%) and *Mesorhizobium ciceri* UMP-CA7^T (99.7 – 99.5%). Additionally, our strains formed distinct groups in the housekeeping gene analysis and were closely related to *Mesorhizobium waimense* ICMP 19557^T (93.5 – 94.9%, 92.5 – 95.6% and 94.2 – 96.0%), *Mesorhizobium cantuariense* ICMP 19515^T (93.1 – 97.7%, 93.5 – 95.4% and 94.8 – 96.8%) and *Mesorhizobium ciceri* UMP-CA7^T (93.2 – 97.2%, 94.6 – 96.8% and 95.5 – 97.3%) for *glnII*, *recA* and *rpoB* respectively. Chemotaxonomic data, supported the assignment of our strains to the genus *Mesorhizobium*, and DNA-DNA hybridisations, MALDI-TOF MS analysis, ERIC PCR, physiological and biochemical tests allowed genotypic and phenotypic differentiation from their nearest neighbouring species. Therefore, these strains represent five novel species for which the names *Mesorhizobium calcicola* sp. nov. (ICMP 19560^T = LMG 28224^T = HAMBI 3609^T), *Mesorhizobium waitakense* sp. nov. (ICMP 19523^T = LMG 28227^T = HAMBI 3605^T), *Mesorhizobium sophorae* sp. nov. (ICMP 19535^T = LMG 28223^T = HAMBI 3606^T), *Mesorhizobium newzealandense* sp. nov. (ICMP 19545^T = LMG 28226^T = HAMBI 3607^T) and *Mesorhizobium kowhaii* sp. nov. (ICMP 19512^T= LMG 28222^T = HAMBI 3603^T) are proposed.

The main New Zealand (NZ) islands contain four native nodulating legume genera, *Carmichaelia, Clianthus, Montigena* and *Sophora* (Heenan, 2000; Heenan *et al.*, 2001; Heenan *et al.*, 2004; Wagstaff *et al.*, 1999). The genus *Sophora* belongs to the polyphyletic tribe *Sophoreae* (*Fabaceae* family) and is characterized by relatively simple yellow coloured flowers with free stamens and unspecialized pinnate leaves (Heenan *et al.*, 2004). A taxonomic revision of New Zealand *Sophora* recognized eight endemic species, *S. chathamica, S. fulvida, S. godleyi, S. longicarinata, S. microphylla, S. molloyi, S. prostrata* and *S. tetraptera* (Heenan *et al*., 2001). The *Sophora* host species investigated in this study for their root nodule bacteria are *S. longicarinata, S. microphylla* and *S. prostrata*. *S. microphylla* are trees up to 25m with leaves up to 15cm long and 30 to 50 leaflets, whereas *S. prostrata* is a shrub and easily distinguished by its short leaves and fewer leaflets (Heenan *et al.*, 2001). *S. longicarinata* is characterized by numerous and small leaflets that are distant from each other, uniform in size, dark green, glabrous or with a few appressed hairs, and with distinct petiolules (Heenan *et al.*, 2001).

Previous studies investigating the nitrogen fixing symbionts of *Sophora* species revealed the presence of *Mesorhizobium*, *Rhizobium* and *Phyllobacterium* species inside their root nodules (Jiao *et al.*, 2015a; Jiao *et al.*, 2015b; Weir *et al.*, 2004). As part of a continuing study on native New Zealand legumes, and their associated rhizobia, forty-eight strains were isolated from surface sterilized root nodules of *Sophora* species originating from natural ecosystems. Sequence analysis showed that all isolates belonged to the genus *Mesorhizobium* and that they grouped in seven different clusters (Tan *et al.*, 2015). Recently, two clusters were identified as two new *Mesorhizobium* species, *Mesorhizobium waimense* and *Mesorhizobium cantuariense* (De Meyer *et al.*, 2015). In the present study, thirty one strains originating from *S. microphylla, S. prostrata* and *S. longicarinata* root nodules collected from Marlborough, Westland, Otago and Canterbury (Tan *et al.*, 2015) were selected for further investigation using a polyphasic approach. Strains ICMP 19560^{T}, ICMP 19523^{T}, ICMP 19535^{T}, ICMP 19545^{T} and ICMP 19512^{T} have been deposited in the BCCM/LMG bacteria collection (http://www.belspo.be/bccm) and the HAMBI Culture Collection, University of Helsinki, Finland (http://www.helsinki.fi/hambi/). All strains were subcultured on yeast mannitol agar (YMA) medium (Vincent, 1970) at 28°C unless otherwise indicated.

For PCR, genomic DNA of all isolates was prepared using the standard Qiagen-Gentra PUREGENE DNA Purification Kit as described previously (Tan *et al.*, 2015). Nearly full-

length amplicons for the 16S rRNA gene were obtained for all strains using the primers and conditions described previously by Tan *et al*. (2015). The resulting 16S rRNA gene sequences were aligned using the MEGA 5 software package and phylogenetic trees were constructed with the Maximum Likelihood (ML) and Neighbor Joining (NJ) method/ Tamura-3-parameter model with G and I substitutions (Tamura *et al.*, 2011). Bootstrap analysis with 500 replicate data sets was performed to assess the support of the clusters. The overall topologies of the phylogenetic trees obtained with the ML and NJ methods were similar (data not shown). Our strains formed four novel branches within the genus *Mesorhizobium* (Fig. 1), and shared sequence similarities of 99.9 – 99.6% with *M. sangaii* SCAU7^T, 99.7 – 99.6% with *M. cantuariense* ICMP 19515^T, 99.7 – 99.5% with *M. ciceri* UMP-CA7^T and 99.7 – 99.5% with *M. qingshengii* CCBAU 33460^T, as determined with the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/, Kim *et al.*, 2012). G*lnII* [484bp], *recA* [342bp] and *rpoB* [769bp] gene sequence analysis was based on the method described by Tan *et al.* (2015) and the new sequences were deposited in NCBI (Accession numbers in Table S1). Sequences were aligned using the MEGA 5 software package (Tamura *et al.*, 2011) and phylogenetic trees were constructed using the ML method, with the Tamura-Nei model and GI substitutions. Bootstrap analysis with 500 replicates was performed to assess the support of the clusters. The existence of congruence among the different gene sequences was investigated using the partition homogeneity tests (Farris *et al.*, 1994) performed with PAUP software v. 4.0b10 (Swofford, 1991). Congruence (p>0.01) was found among all investigated genes and subsequently concatenation using the software SeaView v. 4.0 was performed (Gouy *et al.*, 2010). The phylogenetic trees based on the concatenated *glnII, recA* and *rpoB* gene sequences of all strains (Fig. 2) revealed five monophyletic clades supported by high bootstrap values (100%). Within the *M. sophorae* group, two closely related subclusters are present. Levels of gene sequence similarity between the novel type strains (ICMP 19560^T, ICMP 19523^T, ICMP 19535^T, ICMP 19545^T and ICMP 19512^T) and their closest neighbours were 93.5 – 94.9% for *glnII,* 92.5 – 95.6% for *recA* and 94.2 – 96.0% for *rpoB* with *M. waimense* ICMP 19557^T, 93.1 – 97.7% for *glnII,* 93.5 – 95.4% for *recA* and 94.8 – 96.8% for *rpoB* with *M. cantuariense* ICMP 19515^T and 93.2 – 97.2% for *glnII,* 94.6 – 96.8% for *recA* and 95.5 – 97.3% for *rpoB* with *M. ciceri* LMG 14989^T (Table S2).

The ERIC-PCR fingerprints were obtained as described previously (Versalovic *et al.*, 1994) and analysed using the Phoretix 1D Pro v12.2 software package (Phoretix Ltd, UK). The similarity among the digitised profiles was calculated using the Dice coefficient (Dice, 1945) and an unweighted pair group using arithmetic averages (UPGMA) dendrogram was derived

from the similarity matrix. The Dice coefficient is used as a general measure of similarity (if two lanes are identical, Distance (D) = 0 and if two lanes are totally different, D =1) but gives more weight to matching bands. Fig. S1 shows the ERIC-PCR fingerprints of all *Mesorhizobium* isolates investigated in this study. The fingerprints suggest that the isolates represent six genetically different clusters and one outlier (ICMP 19537) due to fingerprint quality. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) was performed as described previously (Wieme *et al.*, 2012). All conditions were as previously described except that YMA growth medium was used to culture the strains prior to protein extraction (Wieme *et al.*, 2012). All strains representing these novel species form distinct clusters that could be distinguished from the strains representing the closest neighbour *M. ciceri* (Fig. S2).

Phenotypic analysis was performed on YMA medium at 28°C unless otherwise indicated. Cells were Gram stained (Vincent, 1970). Cell morphology and motility were observed by phase contrast microscopy. Oxidase activity was detected by immersion of cells in 1% N,N,N′,N′-tetramethyl-p-phenylenediamine solution and catalase activity was determined by flooding a colony with 10% H_2O_2 and checking for the presence of bubbles. Biochemical tests were performed by inoculating API 20NE and API 20E strips (BioMérieux) and Biolog GENIII MicroPlates™ (Biolog Inc, CA, USA), according to the manufacturer's instructions. GENIII MicroPlates™ were read using the MicroStation™ ID System reader (Biolog Inc, CA, USA). Growth was tested at 28°C in Yeast Mannitol broth with 1% to 8% NaCl and with pH4 - pH9, buffered with acetic acid/sodium acetate (pH4 - 5), citric acid/Na_2HPO_4 (pH6 - 7), NaH_2PO_4/Na_2HPO_4 (pH8) or Tris/HCl (pH9). Growth on YMA medium was tested at 4, 7, 15, 20, 25, 28, 30 and 37°C. Colonies were visible after 48h growth at 15 - 30°C on YMA medium. The results of the phenotypic and biochemical tests are given in Table 1 and supplementary Table S3. Most notably, weak positive reactions were recorded for Glucuronamide and Lithium Chloride, and negative reactions for N-Acetyl Neuraminic acid, Citric acid, Stachyose and Mucic acid. The antibiotic susceptibility tests were performed on YMA medium using the antibiotic Sensi-disc dispenser system (Oxoid) with bio-discs (Oxoid) containing ampicillin (10µg), chloramphenicol (30µg), erythromycin (15µg), gentamycin (10µg), kanamycin (30µg), and streptomycin (25µg). All strains were grown on YMA for 72h prior to testing. The plates were incubated at 28°C and read between two and seven days. All strains investigated were resistant to ampicillin and sensitive to

streptomycin. Species dependent reactions were also recorded and mentioned in the species description.

The whole-cell fatty acid composition was analysed and the fatty acid methyl esters were extracted according to the MIDI protocol (http://www.microbialid.com/PDF/TechNote_101.pdf). All characteristics such as temperature, medium and physiological age (overlap area of the second and third quadrant from a quadrant streak) were as in the MIDI protocol. The profiles were generated using an Agilent Technologies 6890N gas chromatograph (Santa Clara, CA USA), identified and clustered using the Microbial Identification System software and MIDI TSBA database version 5.0. Fatty acid profiles are listed in Table 2. The most abundant fatty acids for our strains were $C_{18:1}$ ω7c (77.6 – 35.7%), $C_{16:0}$ (33.1 – 15.9%) and 11 methyl $C_{18:1}$ ω7c (16.4 – 0%). All strains lacked $C_{20:3}$ ω6,9,12cis which is characteristic for *Mesorhizobium* species (Tighe *et al.*, 2000). Additionally, there were noticeable differences between the fatty acid profiles of the *Sophora* strains and other *Mesorhizobium* type strains (Table 2).

For DNA-DNA hybridization and for the determination of the DNA G+C content, high-molecular weight DNA was prepared as described by Pitcher *et al*. (1989). DNA-DNA hybridizations were performed using a microplate method and biotinylated probe DNA (Ezaki *et al.*, 1989). As described previously, the DNA hybridisation values ranged from 30.1 – 52.1% between our species and 26.1 – 36.3% similarity to *M. ciceri* LMG 14989^T (Tan *et al.*, 2015). To verify that the two *M. sophorae* subgroups belong to the same species additional hybridisations were performed between ICMP 19535^T and ICMP 19531, which gave an average hybridisation value of 84.2%, indicating they belong to the same group. The G+C content of DNA was determined by HPLC according to the method of Mesbah *et al.* (1989) using a Waters Breeze HPLC system and XBridge Shield RP18 column thermostabilised at 37°C. The solvent was 0.02M $NH_4H_2PO_4$ (pH 4.0) with 1.5% (v/v) acetonitrile. Non-methylated lambda phage (Sigma) and *E. coli* DNA were used as calibration reference and control, respectively. The DNA G+C content of our strains was 62.3 – 62.6 mol% (Table 1), which is within the range reported for *Mesorhizobium* (59 – 64 mol%) (Jarvis *et al.*, 1997).

The nodulation and nitrogen fixation capacity of all strains was previously studied on their original host (*S. microphylla, S. prostrata* or *S. longicarinata*) and a selection of strains was also tested on additional *Sophora* species, *Carmichaelia australis* and *Clianthus puniceus,*

using the sterile jar system described by Tan *et al.* (2015). These results confirmed that they could form effective N_2-fixing symbioses with their original host. Additionally, strains ICMP 14330 and ICMP 19520 formed ineffective nodules on *C. australis* and selected strains could effectively nodulate *Cl. puniceus* (Tan *et al.*, 2015).

The genotypic and phenotypic data presented in this study demonstrate that the 31 strains isolated from New Zealand native *Sophora* root nodules form five novel species in the genus *Mesorhizobium*. Therefore we propose to classify the strains as *Mesorhizobium calcicola* sp. nov. (ICMP 19560^T = LMG 28224^T = HAMBI 3609^T), *Mesorhizobium waitakense* sp. nov. (ICMP 19523^T = LMG 28227^T = HAMBI 3605^T), *Mesorhizobium sophorae* sp. nov. (ICMP 19535^T = LMG 28223^T = HAMBI 3606^T), *Mesorhizobium newzealandense* sp. nov. (ICMP 19545^T = LMG 28226^T = HAMBI 3607^T) and *Mesorhizobium kowhaii* sp. nov. (ICMP 19512^T= LMG 28222^T = HAMBI 3603^T).

Description of *Mesorhizobium calcicola* sp. nov.

Mesorhizobium calcicola (cal.ci'co.la. L. fem. n. calx, calcis chalk; L. suff. -cola (from L. n. incola), an inhabitant; N.L. n. calcicola, referring to the limestone area this species was first isolated from.)

Cells are rod shaped (approx. 1.0 x 2.0 µm), gram-negative, catalase-negative and oxidase positive. Colonies are light cream/white, smooth, round, diameter 0.8 – 1.0 mm and convex with entire margins on YMA medium. Growth occurs on YMA medium between 15°C and 30°C but not at 4, 7 and 37°C. Growth was visible in YMB medium with 1% – 7% NaCl and pH 6 – 9 at 28°C. Detailed phenotypic and biochemical information can be found in Table S3. This species is sensitive to ampicillin, chloramphenicol, gentamycin and streptomycin, resistant to erythromycin and kanamycin. The whole-cell fatty acids profile is given in Table 2. The DNA G+C content of the type strain is 62.6 mol%. The type strain ICMP 19560^T (= LMG 28224^T = HAMBI 3609^T) was isolated from root nodules of *Sophora longicarinata* from alluvial limestone river terrace, Waima/Ure River, Marlborough, New Zealand.

Description of *Mesorhizobium waitakense* sp. nov.

Mesorhizobium waitakense (wai.tak.en'se. N.L. neut. adj. waitakense, of Waitaki river, referring to the vicinity of the river where the nodules were collected and this species was first isolated from.)

Cells are rod shaped (approx. 0.5 – 0.7 x 1.5 – 3 µm), gram-negative, catalase and oxidase positive. Colonies are white, smooth, round, diameter 0.5 – 1.0 mm and convex with entire margins on YMA medium. Growth occurs on YMA medium between 15°C and 30°C but not at 4, 7 and 37°C. Growth was visible in YMB medium with 1% – 7% NaCl and pH 5 – 9 at 28°C. Detailed phenotypic and biochemical information can be found in Table S3. This species is sensitive to ampicillin, gentamycin and streptomycin, resistant to chloramphenicol, erythromycin and kanamycin. The whole-cell fatty acids profile is given in Table 2. The DNA G+C content of the type strain is 62.3 mol%. The type strain ICMP 19523^T (= LMG 28227^T = HAMBI 3605^T) was isolated from root nodules of *Sophora microphylla* from Haast Schist rock outcrop, Waitaki River, Otago, New Zealand.

Description of *Mesorhizobium sophorae* sp. nov.

Mesorhizobium sophorae (so.pho.'rae. N.L. gen. n. *Sophora*, botanical name of a genus of leguminous plants; N.L. gen. n. sophorae, of *Sophora*, referring to the host this species was first isolated from.)

Cells are rod shaped (approx. 1.0 x 2.0 µm), gram-negative, catalase-negative and oxidase positive. Colonies are light cream, smooth, round, diameter 0.2 – 0.7 mm and convex with entire margins on YMA medium. Growth occurs on YMA medium between 15°C and 30°C but not at 4, 7 and 37°C. Growth was visible in YMB medium with 1% – 8% NaCl and pH 4 – 7 at 28°C. Detailed phenotypic and biochemical information can be found in Table S3. This species is sensitive to gentamycin and streptomycin, and resistant to chloramphenicol, erythromycin and kanamycin. The whole-cell fatty acids profile is given in Table 2. The DNA G+C content of the type strain is 62.6 mol%. The type strain ICMP 19535^T (= LMG 28223^T = HAMBI 3606^T) was isolated from root nodules of *Sophora microphylla* from river outwash fan, Pororari River, Westland, New Zealand.

Description of *Mesorhizobium newzealandense* sp. nov.

Mesorhizobium newzealandense (new.zea.land.en'se. N.L. neut. adj. newzealandense pertaining to New Zealand, named after the country it was isolated from).

Cells are rod shaped (approx. 0.6 – 0.7 x 1.5 – 2.5 µm), gram-negative, catalase-negative and oxidase positive. Colonies are white, smooth, round, diameter 0.2 – 0.7 mm and convex with

entire margins on YMA medium. Growth occurs on YMA medium between 15°C and 30°C but not at 4, 7 and 37°C. Growth was visible in YMB medium with 1% – 8% NaCl and pH 4 – 9 at 28°C. Detailed phenotypic and biochemical information can be found in Table S3. This species is sensitive to chloramphenicol, gentamycin and streptomycin, resistant to ampicillin, erythromycin and kanamycin. The whole-cell fatty acids profile is given in Table 2. The DNA G+C content of the type strain is 62.6 mol%. The type strain ICMP 19545^{T} (= LMG 28226^{T} = HAMBI 3607^{T}) was isolated from root nodules of *Sophora prostrata* from alluvial limestone river terrace, Waima/Ure River, Marlborough, New Zealand.

Description of *Mesorhizobium kowhaii* sp. nov.

Mesorhizobium kowhaii (kow.hai'i N.L. gen. n. kowhaii of kowhai, indigenous Maori name referring to *Sophora* the host this species was first isolated from.)

Cells are rod shaped (approx. 1.0 x 1.5 – 2.0 µm), gram-negative, catalase-negative and oxidase positive. Colonies are cream, smooth, round, diameter 0.7 – 1.2 mm and convex with entire margins on YMA medium. Growth occurs on YMA medium between 15°C and 30°C but not at 4, 7 and 37°C. Growth was visible in YMB medium with 1% – 8% NaCl and pH 5 – 9 at 28°C. Detailed phenotypic and biochemical information can be found in Table S3. This species is sensitive to gentamycin and streptomycin, resistant to ampicillin, chloramphenicol, erythromycin and kanamycin. The whole-cell fatty acids profile is given in Table 2. The DNA G+C content of the type strain is 62.4 mol%. The type strain ICMP 19512^{T} (= LMG 28222^{T} = HAMBI 3603^{T}) was isolated from root nodules of *Sophora microphylla* from alluvial Greywacke river terrace, upper Rakaia River, Canterbury, New Zealand.

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References

De Meyer, S. E., Tan, H. W., Heenan, P. B., Andrews, M. & Willems, A. (2015). *Mesorhizobium waimense* sp. nov. isolated from *Sophora longicarinata* root nodules and *Mesorhizobium cantuariense* sp. nov. isolated from *Sophora microphylla* root nodules in New Zealand. *Int J Syst Evol Microbiol*.

Dice, L. R. (1945). Measures of the amount of ecologic association between species. *Ecology* **26**, 297-302.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224-229.

Farris, J. S., Källersjö, M., Kluge, A. G. & Bult, C. (1994). Testing significance of incongruence. *Cladistics* **10**, 315-319.

Gao, J.-L., Turner, S. L., Kan, F. L., Wang, E. T., Tan, Z. Y., Qiu, Y. H., Gu, J., Terefework, Z., Young, J. P. W. & other authors (2004). *Mesorhizobium septentrionale* sp. nov. and *Mesorhizobium temperatum* sp. nov., isolated from *Astragalus adsurgens* growing in the northern regions of China. *Int J Syst Evol Microbiol* **54**, 2003-2012.

Gouy, M., Guindon, S. & Gascuel, O. (2010). SeaView Version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* **27**, 221-224.

Heenan, P. B. (2000). *Clianthus* (*Fabaceae*) in New Zealand: A reappraisal of Colenso's taxonomy. *N Z J Bot* **38**, 361-371.

Heenan, P. B., de Lange, P. J. & Wilton, A. D. (2001). *Sophora* (*Fabaceae*) in New Zealand: Taxonomy, distribution, and biogeography. *N Z J Bot* **39**, 17-53.

Heenan, P. B., Dawson, M. I. & Wagstaff, S. J. (2004). The relationship of *Sophora* sect. *Edwardsia* (*Fabaceae*) to *Sophora tomentosa*, the type species of the genus *Sophora*, observed from DNA sequence data and morphological characters. *Bot J Linn Soc* **146**, 439-446.

Jarvis, B. D. W., Van Berkum, P., Chen, W. X., Nour, S. M., Fernandez, M. P., Cleyet-Marel, J. C. & Gillis, M. (1997). Transfer of *Rhizobium loti, Rhizobium huakuii, Rhizobium ciceri, Rhizobium mediterraneum, Rhizobium tianshanense* to *Mesorhizobium* gen.nov. *Int J Syst Evol Microbiol* **47**, 895-898.

Jiao, Y. S., Yan, H., Ji, Z. J., Liu, Y. H., Sui, X. H., Zhang, X. X., Wang, E. T., Chen, W. X. & Chen, W. F. (2015a). *Phyllobacterium sophorae* sp. nov., a symbiotic bacterium isolated from root nodules of *Sophora flavescens*. *Int J Syst Evol Microbiol* **65**, 399-406.

Jiao, Y. S., Yan, H., Ji, Z. J., Liu, Y. H., Sui, X. H., Wang, E. T., Guo, B. L., Chen, W. X. & Chen, W. F. (2015b). *Rhizobium sophorae* sp. nov. and *Rhizobium sophoriradicis* sp. nov., nitrogen-fixing rhizobial symbionts of the medicinal legume *Sophora flavescens*. *Int J Syst Evol Microbiol* **65**, 497-503.

Kim, O.-S., Cho, Y.-J., Lee, K., Yoon, S.-H., Kim, M., Na, H., Park, S.-C., Jeon, Y. S., Lee, J.-H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716-721.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic-acid by high-preformance liquid-chromatography *Int J Syst Bacteriol* **39**, 159-167.

Nour, S. M., Fernandez, M. P., Normand, P. & Cleyetmarel, J. C. (1994). *Rhizobium ciceri* sp. nov, consisting of strains that nodulate Chickpeas (*Cicer arietinum* L.). *Int J Syst Bacteriol* **44**, 511-522.

Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151-156.

Swofford, D. L. (1991). *PAUP: Phylogenetic Analysis Using Parsimony, version 3.1*. Washington DC: Smithsonian Institution.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using Maximum Likelihood, evolutionary distance, and Maximum Parismony methods. *Mol Biol Evol* **28**, 2731-2739.

Tan, H. W., Heenan, P. B., De Meyer, S. E., Willems, A. & Andrews, M. (2015). Diverse novel mesorhizobia nodulate New Zealand native *Sophora* species. *Syst Appl Microbiol* **38**, 91-98.

Tighe, S. W., de Lajudie, P., Dipietro, K., Lindstrom, K., Nick, G. & Jarvis, B. D. W. (2000). Analysis of cellular fatty acids and phenotypic relationships of *Agrobacterium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* species using the Sherlock Microbial Identification System. *Int J Syst Evol Microbiol* **50**, 787-801.

Versalovic, J., Schneider, M., De Bruijn, F. J. & Lupski, J. R. (1994). Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Meth Mol Cell Biol* **5**, 25-40.

Vincent, J. M. (1970). *A manual for the practical study of the root-nodule bacteria*, International Biological Programme Handbook 15 edn. Oxford, UK Blackwell Scientific Pubilications.

Wagstaff, S. J., Heenan, P. B. & Sanderson, M. J. (1999). Classification, origins, and patterns of diversification in New Zealand *Carmichaelinae* (*Fabaceae*). *Am J Bot* **86**, 1346-1356.

Weir, B. S., Turner, S. J., Silvester, W. B., Park, D. C. & Young, J. A. (2004). Unexpectedly diverse *Mesorhizobium* strains and *Rhizobium leguminosarum* nodulate native legume genera of New Zealand, while introduced legume weeds are nodulated by *Bradyrhizobium* species. *Appl Environ Microbiol* **70**, 5980-5987.

Wieme, A., Cleenwerck, I., Van Landschoot, A. & Vandamme, P. (2012). *Pediococcus lolii* DSM 19927T and JCM 15055T are strains of *Pediococcus acidilactici*. *Int J Syst Evol Microbiol* **62**, 3105-3108.

Legends to figures

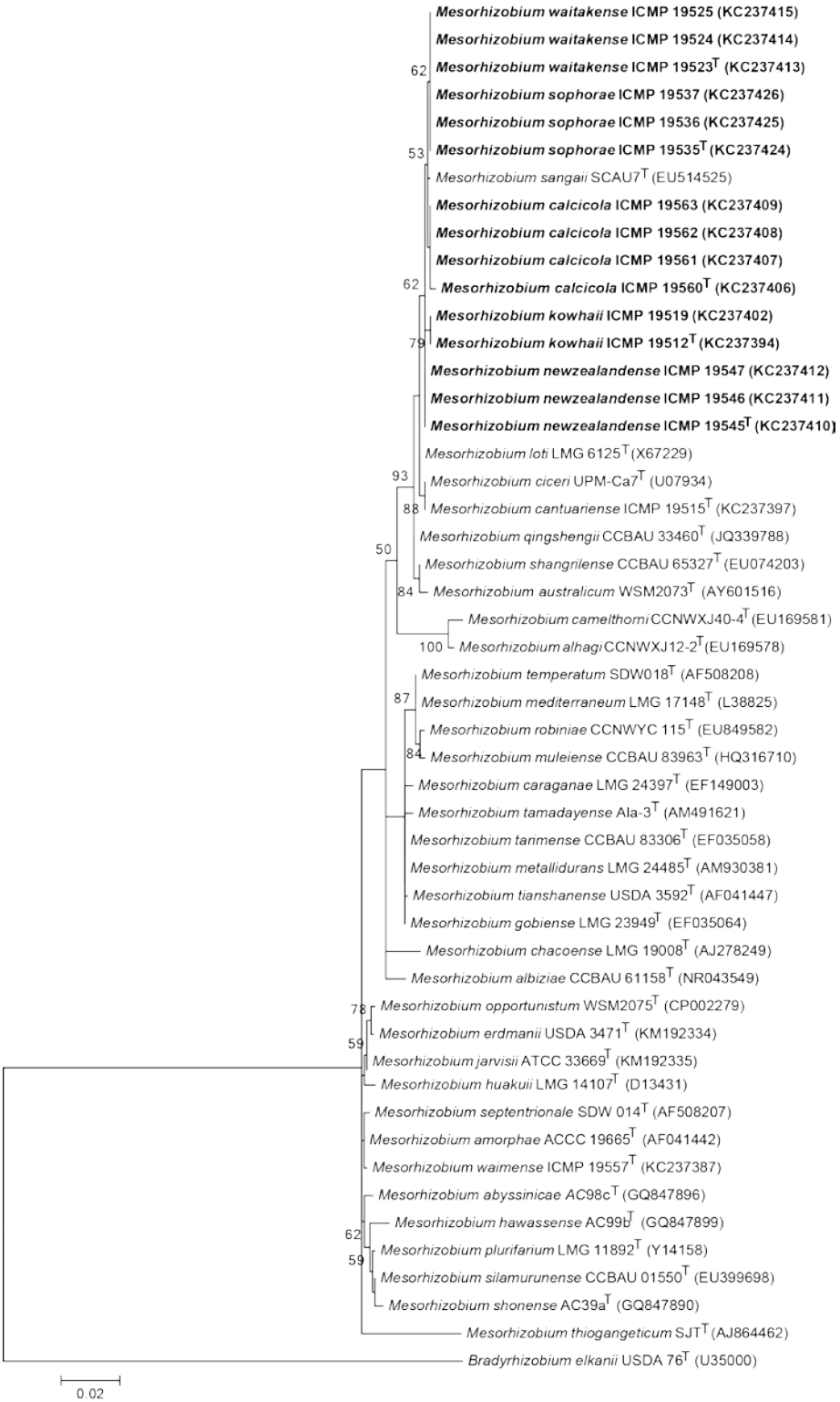

Fig. 1: Maximum likelihood tree based on almost complete 16S rRNA gene sequences (approx. 1272bp) of a selection of the novel *Mesorhizobium* strains and phylogenetically related species. Bootstrap values after 500 replicates are expressed as percentages, values less than 50% are not shown. *Bradyrhizobium elkanii* USDA 76^T is included as outgroup. The scale bar indicates the fraction of substitutions per site.

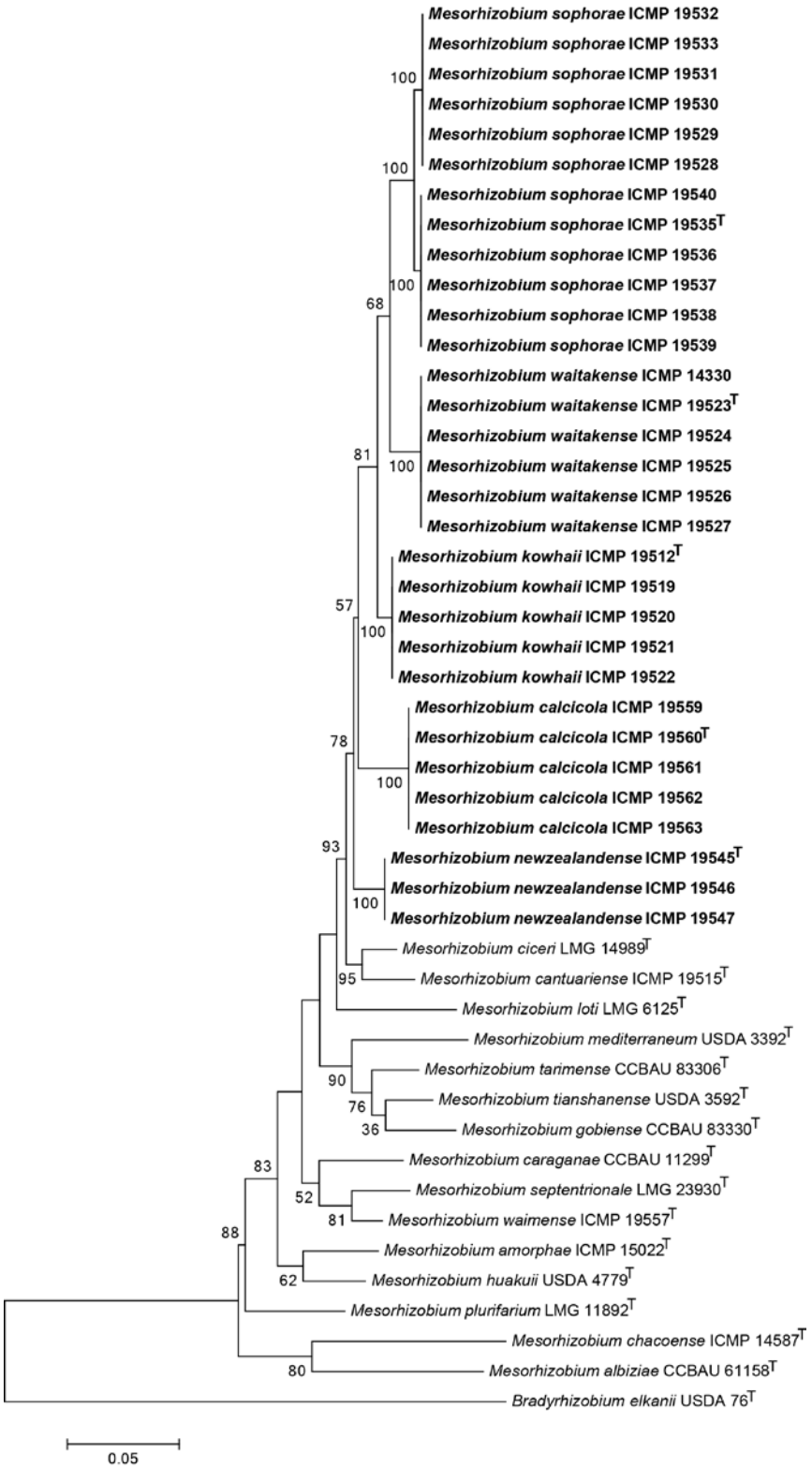

Fig. 2: Maximum likelihood tree based on the concatenated *recA*, *glnII* and *rpoB* gene sequences (approx. 1595bp) of the novel *Mesorhizobium* strains and phylogenetically related species. Bootstrap values after 500 replicates are expressed as percentages, values less than 50% are not shown. *Bradyrhizobium elkanii* USDA 76^{T} is

included as outgroup. The scale bar indicates the fraction of substitutions per site. Gene Accession numbers are shown in table S1.

Table 1. Phenotypic characteristics distinguishing our novel *Mesorhizobium* species from other *Mesorhizobium* species. Strains: 1, *M. calcicola* (n = 5); 2, *M. kowhaii* (n = 5); 3, *M. waitakense* (n = 6); 4, *M. newzealandense* (n = 3); 5, *M. sophorae* (n = 12); 6, *M. waimense* ICMP 19557^{T}; 7, *M. cantuariense* ICMP 19515^{T}; 8, *M. ciceri* LMG 14989^{T}; 9, *M. septentrionale* LMG 23930^{T}. +, Positive reaction; +^{W}, weak reaction; -, negative reaction. Data taken from [#] De Meyer *et al.* (2015), [$]Nour *et al.* (1994) and [§]Gao *et al.* (2004).

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Carbohydrates									
N-Acetyl-Beta-D-Mannosamine	+^{W}	+	-	+^{W}	+^{W}	+	+	+	+^{W}
D-Fructose-6-PO4	-	+^{W}	+^{W}	+^{W}	-	+^{W}	+^{W}	+^{W}	+^{W}
D-Glucose-6-PO4	-	+^{W}	-	+^{W}	-	+^{W}	+^{W}	+^{W}	+^{W}
3-Methyl Glucose	-	+^{W}	-	+^{W}	-	+	+	+	-
Pectine	+^{W}	+^{W}	-	+^{W}	-	+^{W}	+^{W}	+	+
D-Raffinose	+	-	+^{W}	+	+	+	+	+	+^{W}
D-Salicin	+^{W}	+^{W}	-	+^{W}	+^{W}	+^{W}	+^{W}	+	+^{W}
Stachyose	-	-	-	-	-	-	-	+^{W}	-
Carboxylic acids									
N-Acetyl Neuraminic acid	-	-	-	-	-	-	-	+^{W}	-
Citric acid	-	-	-	-	-	+^{W}	+^{W}	+	+^{W}
Formic acid	-	+^{W}	-	+^{W}	-	-	-	+	-
D-Galacturonic acid	-	+^{W}	-	+^{W}	-	+^{W}	+^{W}	+^{W}	+^{W}
D-Gluconic acid	-	+^{W}	-	+^{W}	-	+^{W}	+^{W}	+^{W}	+^{W}
D-Glucuronic acid	-	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}
p-Hydroxy-Phenylacetic acid	+^{W}	+	-	+^{W}	-	-	+^{W}	-	-
Mucic acid	-	-	-	-	-	+^{W}	+^{W}	+^{W}	+^{W}
Propionic acid	+^{W}	+^{W}	-	+	+	+	+	-	+
Quinic acid	-	+^{W}	-	+^{W}	-	+^{W}	+^{W}	+^{W}	+^{W}
D-Saccharic acid	-	-	-	+^{W}	-	+^{W}	+	+^{W}	+^{W}
Amides									
Glucuronamide	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}	+	+^{W}
Amino acids									
D-Aspartic acid	-	-	-	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}	+^{W}
L-Aspartic acid	-	+	-	+	+	+	+	+	+

L-Histidine	-	-	-	+[W]	+[W]	+[W]	+	+	+
Alpha-Hydroxy-Butyric acid	-	-	-	-	-	-	-	+	-
L-Pyroglutamic acid	-	+[W]	-	-	-	+[W]	+[W]	+[W]	+[W]
L-Serine	-	-	-	+	-	-	-	-	+
Antibiotic									
Troleandomycin	I	S	S	R	S	R	R	R	I
Vancomycin	I	I	S	R	I	I	I	R	S
Ester									
Methyl Pyruvate	-	-	-	-	-	+	+[W]	+	+[W]
Brominated chemicals									
Bromo-Succinic acid	-	-	-	+[W]	-	-	+	+	+
Polymers									
Tween 40	-	+[W]	-	+[W]	-	+[W]	+[W]	+[W]	+[W]
Metal chloride									
Lithium Chloride	+[W]	+[W]	+[W]	+[W]	+[W]	-	-	-	-
%GC	62.6	62.4	62.3	62.6	62.6	62.6[#]	62.5[#]	63.0[$]	59.4[§]

389 **Table 2.** Fatty acid composition of the *Mesorhizobium* strains investigated in this study. All values are given as a percentage of the total

390 composition. SF3 = 16:1 ω7c/15 iso 2OH. All strains were grown on YMA medium prior to extraction.

Strain	11 methyl 18:1 ω7c	12:0 3OH	12:0 ISO	12:0 ISO 3OH	13:0 ISO 3OH	15:0 ISO	16:00	17:0 CYCLO	17:0 ISO	17:1 ω8c	18:00	18:1 2OH	18:1 ω7c	19:0 10 methyl	19:0 CYCLO ω8c	SF 3
M. kowhaii																
ICMP 19512^{T}	12.2	<1	0	0	<1	<1	17.0	1.7	5.2	<1	3.2	0	35.7	1.1	17.9	1.5
ICMP 19519	8.7	0	0	0	0	1.5	20.1	2.0	4.8	0	1.9	0	38.2	3.6	13.6	5.7
ICMP 19520	13.0	2.0	0	0	1.8	0	18.2	0	3.7	0	2.1	0	49.5	3.2	2.9	3.7
ICMP 19521	9.4	1.4	0	0	2.3	0	19.8	1.9	5.0	0	2.2	0	39.5	3.1	12.8	2.7
ICMP 19522	7.9	0	0	0	0	0	17.6	0	0	0	2.4	0	57.0	0	11.4	3.8
M. waitakense																
ICMP 14330	5.3	3.4	0	0	7.1	0	15.9	0	4.9	0	0	2.6	48.1	0	5	7.6
ICMP 19523^{T}	7.8	0	0	0	0	0	19.2	0	0	0	0	5.1	61.7	0	6.3	0
ICMP 19524	7.8	0	0	0	<1	0	16.1	0	4.4	1.5	1.8	2.1	54.1	1.9	6.0	3.5
ICMP 19525	6.5	1.5	0	0	3.8	0	17.2	0	4.6	0	0	4.4	52.0	0	5.7	4.2
ICMP 19526	8.9	0	0	2.7	0	0	20.1	0	6.8	0	0	0	61.6	0	0	0
ICMP 19527	0	5.7	4.1	0	5.8	0	22.6	0	0	0	0	0	52.7	0	0	0
M. sophorae																
ICMP 19528	12.3	0	0	0	5.8	0	17.1	0	4.6	0	0	0	47.3	3.0	3.3	6.6
ICMP 19529	10.3	2.6	0	0	7.4	0	20.5	0	2.8	0	0	0	46.9	0	0	9.6
ICMP 19530	10.9	2.4	0	0	3.3	0	19.5	0	4.2	0	0	3.2	50.8	0	0	5.8
ICMP 19531	0	0	0	0	0	0	22.5	0	0	0	0	0	77.6	0	0	0
ICMP 19532	13.3	4.0	0	0	7.8	0	16.7	0	0	0	0	0	51.3	0	0	6.9
ICMP 19533	10.1	2.0	2.0	0	4.5	0	16.2	0	3.5	0	0	3.2	46.0	4.2	3.0	5.4
ICMP 19535^{T}	12.0	0	0	0	0	0	21.4	0	5.3	0	0	0	48.8	0	10.1	2.4
ICMP 19536	13.9	0	0	0	3.0	0	19.1	0	6.1	0	0	0	46.3	0	8.3	3.3
ICMP 19537	16.4	0	0	0	0	0	20.9	0	5.4	0	0	0	45.6	0	11.7	0
ICMP 19538	13.3	0	0	0	0	0	21.1	0	5.9	0	0	0	47.9	0	12.0	0
ICMP 19539	16.3	0	0	0	0	0	20.6	0	5.9	0	0	0	44.7	0	12.5	0

Strain	11 methyl 18:1 ω7c	12:0 3OH	12:0 ISO	12:0 ISO 3OH	13:0 ISO 3OH	15:0 ISO	16:00	17:0 CYCLO	17:0 ISO	17:1 ω8c	18:00	18:1 2OH	18:1 ω7c	19:0 10 methyl	19:0 CYCLO ω8c	SF 3
ICMP 19540	13.7	<1	0	0	0	0	21.4	0	4.4	0	1.8	0	45.7	0	8.9	3.5
M. newzealandense																
ICMP 19545^{T}	14.2	0	0	0	0	0	22.1	0	0	0	0	0	42.5	0	21.2	0
ICMP 19546	12.8	0	0	0	0	0	21.9	0	0	0	0	0	45.6	0	19.6	0
ICMP 19547	12.7	0	0	0	0	0	21.1	0	3.2	0	0	0	43.1	0	20.0	0
M. calcicola																
ICMP 19559																
ICMP 19560^{T}	6.9	4.2	0	0	2.1	0	20.3	0	4.5	0	0	0	39.7	6.6	9.2	5.3
ICMP 19561	0	0	0	0	0	0	33.1	0	0	0	0	0	52.0	0	15.0	0
ICMP 19562	3.9	0	0	0	0	0	16.3	0	8.7	0	0	0	44.0	0	6.0	0
ICMP 19563	10.6	0	0	0	0	0	21.2	0	4.0	0	0	0	50.4	0	13.8	0
M. cantuariense																
ICMP 19515^{T}	10.8	<1	0	0	<1	1.1	18.0	2.6	2.2	<1	1.3	0	37.1	1.1	17.5	4.6
M. ciceri																
LMG 14989^{T}	6.7	0	0	0	0	0	14.7	0	0	0	0	0	47.6	0	15.2	0
M. septentrionale																
LMG 23930^{T}	4.7	0	0	0	0	0	14.6	0	5.3	0	0	0	38.6	0	36.8	0
M. waimense																
ICMP 19557^{T}	2.7	1.9	0	0	2.6	1.3	16.2	1.1	1.2	0	2.0	0	55.3	5.2	5.6	3.8