

***Mesorhizobium olivaresii* sp. nov. isolated from *Lotus corniculatus* nodules**

1  
2  
3 María J. Lorite<sup>1</sup>, José David Flores-Félix<sup>2</sup>, Álvaro Peix<sup>3,4</sup>, Juan Sanjuán<sup>1</sup>, Encarna  
4 Velazquez<sup>2,3\*</sup>  
5  
6  
7

8  
9 1. Departamento de Microbiología del Suelo y Sistemas Simbióticos. Estación  
10 Experimental del Zaidin. CSIC. Granada. Spain.  
11

12 2 Departamento de Microbiología y Genética. Universidad de Salamanca. Salamanca.  
13 Spain  
14

15 3 Unidad Asociada Universidad de Salamanca-CSIC “Interacciones Planta-  
16 Microorganismo”.  
17

18 4 IRNASA-CSIC. Salamanca. Spain.  
19  
20  
21  
22  
23  
24

25 **Running title:** *Mesorhizobium olivaresii* sp. nov.

26 **Journal’s content category:** New Taxa-Proteobacteria

27 **Keywords:** *Mesorhizobium*, *Lotus corniculatus*, Spain  
28  
29  
30  
31

32 **Accession numbers for type strain of *Mesorhizobium olivaresii*:**

33 16S rRNA gene: FM203302

34 *recA* gene: FN556460

35 *glnII* gene: LN681554

36 *atpD* gene: FM203309

37 *rpoB* gene: KX712097

38 *nodC* gene: FM203320  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Abstract

1  
2  
3  
4 In this study four *Mesorhizobium* strains isolated from *Lotus corniculatus* nodules in  
5 Granada (Spain) were characterized. Their 16S rRNA gene sequences were closely  
6 related to those of *M. albiziae* LMG 23507<sup>T</sup> and *M. chacoense* Pr5<sup>T</sup> showing 99.4 and  
7 99.2% similarity values, respectively. The analysis of concatenated *rpoB*, *recA*, *atpD*  
8 and *glnII* genes showed they formed a cluster with internal similarities higher than 97%.  
9 The closest species also were *M. albiziae* LMG 23507<sup>T</sup> and *M. chacoense* Pr5<sup>T</sup> showing  
10 similarity values lower than 92% in *rpoB*, *recA* and *glnII* genes and lower than 96.5% in  
11 the *atpD* gene. These results indicated that the *L. corniculatus* strains belong to a new  
12 species of genus *Mesorhizobium* which was confirmed by DNA-DNA hybridization and  
13 phenotypic characterization. Therefore a new species with the name *Mesorhizobium*  
14 *olivaresii* sp. nov. is proposed, and the type strain is CPS13<sup>T</sup> (LMG 29295<sup>T</sup> = CECT  
15 9099<sup>T</sup>).  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 The genus *Mesorhizobium* was proposed by Jarvis *et al.* [7] to accommodate several  
2 species phylogenetically divergent to those from genus *Rhizobium* and currently  
3 contains more than 30 species with *Mesorhizobium loti* as the type species of the genus  
4 (<http://www.bacterio.net/mesorhizobium.html>). The type strain of this species was  
5 deposited in several collections and recently it has been reported that those conserved in  
6 ATCC and USDA collections belonged to different species which have been named  
7 *Mesorhizobium erdmanii* and *Mesorhizobium jarvisii* [14]. All these species are  
8 endosymbionts of *Lotus corniculatus*, a legume worldwide distributed that establishes  
9 symbiosis with strains from genus *Mesorhizobium* in America [2, 16, 23], Europe [1, 4,  
10 6, 10, 12], Asia [7, 21] and Oceania [17, 24].

11 Some strains isolated in different continents from *L. corniculatus* nodules belong to  
12 groups phylogenetically divergent to the currently described species of genus  
13 *Mesorhizobium* as was showed by Marcos *et al.* [12]. One of these groups contained  
14 some strains isolated in Granada (Spain) from *L. corniculatus* during a wide study of  
15 *Lotus* spp. endosymbionts [10]. The objective of the present work was to perform a  
16 polyphasic characterization of these strains and the proposal of a novel species named  
17 *Mesorhizobium olivaresii* sp. nov.

18 In this work we obtained for the strains CPS13<sup>T</sup>, CPS1, CGS20 and CGS22 the 16S  
19 rRNA, *atpD*, *recA*, *glnII* and *nodC* gene sequences not previously obtained according  
20 to the methodologies of Lorite *et al.* [10] and Turner and Young [27]. The amplification  
21 and sequencing of the *rpoB* gene was performed according to Martens *et al.* [13]. All  
22 these sequences were aligned with those of the *Mesorhizobium* species using the Clustal  
23 W program [26]. The distances were calculated according to Kimura's two-parameter  
24 model [8]. The phylogenetic trees were inferred using the neighbour joining (NJ) and  
25 maximum likelihood (ML) models [19, 20]. MEGA5.0 [25] was used for all analyses.

26 The 16S rRNA gene sequences of strains CPS13<sup>T</sup>, CPS1, CGS20 and CGS22 are  
27 identical and then only that of the strain CPS13<sup>T</sup> was included in the NJ and ML  
28 phylogenetic analyses (Fig. 1). The results of these analyses showed that the strain  
29 CPS13<sup>T</sup> groups with *M. albiziae* CCBAU 61158<sup>T</sup> and *M. chacoense* Pr5<sup>T</sup>. These strains  
30 presented similarity values of 99.4% and 99.2%, respectively, with respect to the strain  
31 CPS13<sup>T</sup>. These high similarity values in the 16S rRNA gene sequences is a common  
32 finding among species of genus *Mesorhizobium* that are distinguishable by the analysis  
33 of housekeeping genes, from which *rpoB*, *recA*, *atpD* and *glnII* genes are available for  
34 most species of genus *Mesorhizobium*.

1 The NJ and ML analyses of the concatenated *rpoB*, *recA*, *atpD* and *glnII* genes showed  
2 that the strains CPS13<sup>T</sup>, CPS1, CGS20 and CGS22 formed a cluster (Fig. 2), with  
3 internal similarities higher than 97% in the analysed genes. This cluster was related to  
4 *M. chacoense* Pr5<sup>T</sup> (LMG 19008<sup>T</sup>, ICMP14587<sup>T</sup>) and *M. albiziae* LMG 23507<sup>T</sup> with  
5 similarity values lower than 92% in *rpoB*, *recA* and *glnII* genes and lower than 96.5% in  
6 the *atpD* gene. The results of the phylogenetic analyses indicated that the strains  
7 CPS13<sup>T</sup>, CPS1, CGS20 and CGS22 belong to a new species of genus *Mesorhizobium*  
8 since the distances found between the strains of this species and the remaining ones of  
9 this genus are higher than those found among most of the currently described  
10 *Mesorhizobium* species (Fig. 2).  
11

12 This was confirmed by DNA-DNA hybridization experiments carried out following the  
13 method of Ezaki *et al.* [5] with the recommendations of Willems *et al.* [29]. The strain  
14 CPS13<sup>T</sup> was hybridized with *M. albiziae* LMG 23507<sup>T</sup> and *M. chacoense* Pr5<sup>T</sup> showing  
15 50% ( $\pm$  9%) and 54% ( $\pm$  6%) DNA-DNA relatedness, respectively. Both values are  
16 lower than the threshold value of 70% DNA-DNA similarity for definition of bacterial  
17 species [28] supporting that the *L. corniculatus* strains isolated in Granada belong to a  
18 new species of genus *Mesorhizobium*.  
19

20 DNA for analysis of DNA base composition was prepared according to Chun and  
21 Goodfellow [3]. The mol % G+C content of DNA was determined using the thermal  
22 denaturation method [11]. The G+C content of strain CPS13<sup>T</sup> was 62.7 mol %.  
23

24 The cellular fatty acids were analysed by using the Microbial Identification System  
25 (MIDI; Microbial ID) Sherlock 6.1 and the library RTSBA6 according to the technical  
26 instructions provided by this system [22]. The strains were cultured aerobically on TY  
27 plates at 28°C and cells were collected after 48h incubation. The major fatty acids of  
28 strain CPS13<sup>T</sup> are summed feature 8 (C<sub>18:1</sub>ω7c/ C<sub>18:1</sub>ω6c) and C<sub>18:1</sub>ω7c 11-methyl as in  
29 their closest related *Mesorhizobium* species (Table 1).  
30

31 The phenotypic characterization was performed using API 20NE and API ID32GN  
32 galleries inoculated according to the manufacturer's instructions and adding sterile  
33 MgSO<sub>4</sub>.7H<sub>2</sub>O to the supplied medium up to a concentration of 0.2g l<sup>-1</sup> with the aid of a  
34 disposable Pasteur pipette. The results were read after 7 days incubation. Growth  
35 temperature range was determined by incubating cultures in Yeast Mannitol Agar  
36 (YMA) medium at 4, 15, 28, 37 and 45°C. Growth pH range was determined in the  
37 same medium with final pH 4.0, 6, 7, 8, 9 and 10. Salt tolerance was tested in the same  
38 medium containing 0.5, 1, 1.5, 2 and 2.5% (w/v) NaCl. To test the natural antibiotic  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

resistance, the disc diffusion method on YMA medium was used. The discs contained the following antibiotics: ampicillin (2 µg), erythromycin (2 µg), ciprofloxacin (5 µg), penicillin (10 IU), polymyxin (300 IU), cloxacillin (1 µg), oxytetracycline (30 µg), gentamycin (10 µg), cefuroxime (30 µg), netilmicin (30 µg) and neomycin (5 µg), (Becton Dickinson, BBL). The type strains of *M. albiziae* LMG 23507<sup>T</sup> and *M. chacoense* Pr5<sup>T</sup> were included in the phenotypic study as reference. Phenotypic characteristics of the new species are reported below in the species description and the differences with respect to the closest species of *Mesorhizobium* are recorded in Table 2.

Despite symbiotic genes do not offer taxonomic information because they are located in easily interchangeable elements (plasmids or symbiotic islands), the analysis of the *nodC* gene sequences allowed the identification of strains at symbiovar level [15, 18]. Rhizobial symbiovars are constituted by different symbiotic groups within a single species [18] that in the case of genus *Mesorhizobium* have been described on the basis of the *nodC* gene phylogenetic analyses [9]. In the previous work of Lorite *et al.* [10] the *nodC* gene of the type strain CPS13<sup>T</sup> was analysed showing that it belongs to the same symbiovar that the type strain of *M. loti* LMG 6125<sup>T</sup> (NZP 2213<sup>T</sup>). In this work we analysed the other strains from the new species *M. olivaresii* CPS1, CGS20 and CGS22 showing that they were phylogenetically related to the strain CPS13<sup>T</sup> after the *nodC* gene NJ and ML phylogenetic analyses (Fig. 3). These results confirmed that the strains isolated in Granada from *L. corniculatus* belong to the symbiovar *loti*, although they belong to a cluster phylogenetically divergent to those formed by the type strains of other *Mesorhizobium* species nodulating this host, particularly *M. jarvisii* (Fig. 3).

The results from the phylogenetic analyses of core genes, DNA-DNA hybridization experiments and phenotypic and chemotaxonomic characterization showed that the strains isolated from *L. corniculatus* nodules in Granada (Spain) represent a novel species for which we propose the name *Mesorhizobium olivaresii* sp. nov.

#### **Description of *Mesorhizobium olivaresii* sp. nov.**

*Mesorhizobium olivaresii* (o.li.va.res'i.i N.L. masc. gen. n. olivaresii to honour José Olivares, Spanish microbiologist, for his valuable contributions in rhizobial research).

Gram-negative, aerobic rods as for the other species of the genus. Colonies on YMA are white, circular and convex with diameter of 1-2 mm within 4-5 days at 28°C. It grows

1 from 15°C to 37°C and optimally at 28°C. The pH range for growth is 6.5 to 8 with  
2 optimum growth at pH 7. They grow up to 1.5% NaCl. Nitrate reduction, arginine  
3 dehydrolase and gelatinase were negative and urease and  $\beta$ -galactosidase were positive.  
4 Esculin hydrolysis was positive. Assimilation of glucose, L-arabinose, L-rhamnose, D-  
5 ribose, L-fucose, D-mannose, mannitol, inositol, D-sorbitol, maltose, sucrose,  
6 melibiose, valerate, 3-hydroxi-butyrate, L-histidine and L-proline was positive.  
7 Assimilation of salicin, gluconate, caprate, adipate, citrate, phenylacetate itaconate,  
8 suberate, malonate, 2 keto-gluconate, glycogen, 3 and 4 hydroxi-benzoate and L-serine  
9 was negative. Assimilation of N-acetyl-glucosamine, malate and D,L-lactate was  
10 variable. Acetate, L-alanine, 5 keto-gluconate and propionate were weakly assimilated.  
11 Sensitive to neomycin, gentamycin, netilmycin and tetracyclin and resistant to  
12 ampicillin, cefuroxime, cloxacillin, penicillin, and erythromycin. Variable results were  
13 found in the case of cyprofloxacin and polymyxin B. G+C content was 62.7 mol %. The  
14 type strain CPS13<sup>T</sup> (=LMG 29295<sup>T</sup> = CECT 9099<sup>T</sup>) was isolated from root nodules of  
15 *Lotus corniculatus*.  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26

## 27 28 29 **Acknowledgements** 30

31  
32 This work was supported by the EU-INCO project LOTASSA (J.S.) and Junta de  
33 Andalucía (Spain). JDF is recipient of a predoctoral fellowship from Universidad de  
34 Salamanca.  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## References

- 1  
2  
3  
4 [1] Ampomah, O.Y., Huss-Danell, K. (2011) Genetic diversity of root nodule bacteria  
5 nodulating *Lotus corniculatus* and *Anthyllis vulneraria* in Sweden. Syst. Appl.  
6 Microbiol. 34, 267-275.  
7  
8  
9  
10  
11 [2] Binde, D.R., Menna, P., Bangel, E.V., Barcellos, F.G., Hungria, M. (2009) Rep-PCR  
12 fingerprinting and taxonomy based on the sequencing of the 16S rRNA gene of 54 elite  
13 commercial rhizobial strains. Appl. Microbiol. Biotechnol. 83, 897-908.  
14  
15  
16  
17  
18 [3] Chun, J., Goodfellow, M. (1995) A phylogenetic analysis of the genus *Nocardia*  
19 with 16S rRNA sequences. Int. J. Syst. Bacteriol. 45, 240-245.  
20  
21  
22  
23 [4] De Meyer, S.E., Van Hoorde, K., Vekeman, B., Braeckman, T., Willems A. (2011)  
24 Genetic diversity of rhizobia associated with indigenous legumes in different regions of  
25 Flanders (Belgium). Soil Biol. Biochem. 43, 2384-2396.  
26  
27  
28  
29  
30  
31 [5] Ezaki, T., Hashimoto, Y., Yabuchi, E. (1989) Fluorometric deoxyribonucleic acid-  
32 deoxyribonucleic acid hybridization in microdilution wells as an alternative to  
33 membrane filter hybridization in which radioisotopes are used to determine genetic  
34 relatedness among bacterial strains. Int. J. Syst. Bacteriol. 39, 224-229.  
35  
36  
37  
38  
39  
40 [6] Gossmann, J.A., Markmann, K., Brachmann, A., Rose, L.R., Parniske, M. (2012)  
41 Polymorphic infection and organogenesis patterns induced by a *Rhizobium*  
42 *leguminosarum* isolate from *Lotus* root nodules are determined by the host genotype.  
43 New Phytologist. 196, 561-573.  
44  
45  
46  
47  
48  
49 [7] Jarvis, B.D.W., van Berkum, P., Chen, W.X., Nour, S.M., Fernandez, M.P., Cleyet-  
50 Marel, J.C., Gillis, M. (1997) Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium*  
51 *ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen.  
52 nov. Int. J. Syst. Bacteriol. 47, 895-898.  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 [8] Kimura, M. (1980) A simple method for estimating evolutionary rates of base  
2 substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16,  
3 111-120.  
4

5  
6  
7 [9] Laranjo, M., Alexandre, A., Oliveira, S. (2014) Legume growth-promoting rhizobia:  
8 an overview on the *Mesorhizobium* genus. Microbiol. Res. 169:2-17.  
9

10  
11  
12 [10] Lorite, M.J., Muñoz, S., Olivares, J., Soto, M.J., Sanjuán, J. (2010)  
13 Characterization of strains unlike *Mesorhizobium loti* that nodulate *Lotus* spp. in saline  
14 soils of Granada, Spain. Appl. Environ. Microbiol. 76, 4019-4026.  
15  
16  
17

18  
19  
20 [11] Mandel, M., Mamur, J. (1968) Use of ultraviolet absorbance temperature profile  
21 for determining the guanine plus cytosine content of DNA. Methods Enzymol. 12B,  
22 195-206.  
23  
24  
25

26  
27 [12] Marcos-García, M., Menéndez, E., Cruz-González, X., Velázquez, E., Mateos,  
28 P.F., Rivas, R. (2015) The high diversity of *Lotus corniculatus* endosymbionts in soils  
29 of Northwest Spain. Symbiosis 67, 11-20.  
30  
31  
32

33  
34 [13] Martens, M., Dawyndt, P., Coopman, R., Gillis, M., De Vos, P., Willems, A.  
35 (2008) Advantages of multilocus sequence analysis for taxonomic studies: a case study  
36 using 10 housekeeping genes in the genus *Ensifer* (including former *Sinorhizobium*).  
37 Int. J. Syst. Evol. Microbiol. 58: 200-214.  
38  
39  
40  
41

42  
43 [14] Martínez-Hidalgo, P., Ramírez-Bahena, M.H., Flores-Félix, J.D., Rivas, R., Igual,  
44 J.M., Mateos, P.F., Martínez-Molina, E., León-Barrios, M., Peix, Á., Velázquez, E.  
45 (2015) Revision of the taxonomic status of type strains of *Mesorhizobium loti* and  
46 reclassification of strain USDA 3471<sup>T</sup> as the type strain of *Mesorhizobiumerdmanii* sp.  
47 nov. and ATCC 33669<sup>T</sup> as the type strain of *Mesorhizobiumjarvisii* sp. nov. Int. J. Syst.  
48 Evol. Microbiol. 65:1703-1708.  
49  
50  
51  
52

53  
54 [15] Peix, A., Ramírez-Bahena, M.H., Velázquez, E., Bedmar, E.J. (2015) Bacterial  
55 associations with legumes. Crit. Rev. Plant Sci. 34, 17-42.  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



1 [16] Qian, J., Parker, M.A. (2002) Contrasting *nifD* and ribosomal gene relationships  
2 among *Mesorhizobium* from *Lotus oroboides* in northern Mexico. Syst. Appl.  
3 Microbiol. 25, 68-73.  
4

5  
6  
7 [17] Reeve, W., Sullivan, J., Ronson, C., Tian, R., Bräu, L., Davenport, K., Goodwin,  
8 L., Chain, P., Woyke, T., Lobos, E., Huntemann, M., Pati, A., Mavromatis, K.,  
9 Markowitz, V., Ivanova, N., Kyrpides, N. (2014) Genome sequence of the *Lotus*  
10 *corniculatus* microsymbiont *Mesorhizobium loti* strain R88B. Stand. Genomic Sci. 9, 3.  
11  
12  
13

14  
15  
16 [18] Rogel, M.A., Ormeño-Orrillo, E., Martínez-Romero, E. (2011) Symbiovars in  
17 rhizobia reflect bacterial adaptation to legumes. Syst. Appl. Microbiol. 34, 96-104.  
18  
19

20  
21 [19] Rogers, J.S., Swofford, D.L. (1998) A fast method for approximating maximum  
22 likelihoods of phylogenetic trees from nucleotide sequences. Syst. Biol. 47, 77-89.  
23  
24

25  
26  
27 [20] Saitou, N., Nei, M. (1987) A neighbour-joining method: a new method for  
28 reconstructing phylogenetics trees. Mol. Biol. Evol. 44, 406-425.  
29  
30

31  
32 [21] Saeki, K., Kouchi H. (2000) The *Lotus* symbiont, *Mesorhizobium loti*: molecular  
33 genetic techniques and application. J. Plant Res. 113, 457-465.  
34  
35  
36

37  
38 [22] Sasser, M. (1990) Identification of bacteria by gas chromatography of cellular fatty  
39 acids, MIDI Technical Note 101. Newark, DE: MIDI Inc.  
40  
41

42  
43 [23] Sotelo, M., Irisarri, P., Lorite, M.J., Casaretto, E., Rebuffo, M., Sanjuán, J., Monza,  
44 J. (2011) Diversity of rhizobia nodulating *Lotus corniculatus* grown in northern and  
45 southern regions of Uruguay. Appl. Soil Ecol. 49,197-207.  
46  
47  
48

49  
50  
51 [24] Sullivan, J.T., Patrick, H.N., Lowther, W.L., Scott, D.B., Ronson, C.W. (1995)  
52 Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer  
53 in the environment. Proc. Natl. Acad. Sci. USA 92, 8985-8989.  
54  
55  
56

57  
58 [25] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011)  
59 MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood,  
60  
61  
62

1 Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. 28, 2731-  
2 2739.  
3  
4

5 [26] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G. (1997)  
6 The CLUSTAL\_X Windows interface: flexible strategies for multiple sequence  
7 alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876-4882.  
8  
9

10  
11  
12 [27] Turner, S.L., Young, J.P.W. (2000) The glutamine synthetases of rhizobia:  
13 phylogenetics and evolutionary implications. Mol. Biol. Evol. 17, 309-319.  
14  
15  
16

17  
18 [28] Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O.,  
19 Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E.,  
20 Starr, M.P., Trüper, H.G. (1987) Report of the ad hoc committee on reconciliation of  
21 approaches to bacterial systematics. Int. J. Syst. Bacteriol. 37, 463-464.  
22  
23  
24  
25  
26

27 [29] Willems, A., Munive, A., de Lajudie, P., Gillis, M. (2003) In most *Bradyrhizobium*  
28 groups sequence comparison of 16S-23S rDNA internal transcribed spacer regions  
29 corroborates DNA-DNA hybridizations. Syst. Appl. Microbiol. 26, 203-210.  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Figure legends

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Figure 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1270 nucleotides) showing the position of *Mesorhizobium olivaresii* CPS13<sup>T</sup> within genus *Mesorhizobium*. Bootstrap values calculated for 1000 replications are indicated. Bar, 1 nt substitution per 100 nt. The nodes marked with filled circles were also obtained with the maximum likelihood algorithm.

Figure 2. Neighbour-joining phylogenetic tree based on concatenated *recA* and *glnII* gene sequences (2000 nucleotides) showing the position of *Mesorhizobium olivaresii* strains within genus *Mesorhizobium*. Bootstrap values calculated for 1000 replications are indicated. Bar, 1 nt substitution per 100 nt. The nodes marked with filled circles were also obtained with the maximum likelihood algorithm.

Figure 3. Neighbour-joining phylogenetic tree based on *nodC* gene sequences (390 positions) showing the position of *Mesorhizobium olivaresii* strains within genus *Mesorhizobium*. Bootstrap values calculated for 1000 replications are indicated. Bar, 2 nt substitution per 100 nt. The nodes marked with filled circles were also obtained with maximum the likelihood algorithm.

**Table 1.** Cellular fatty acid composition of *M. olivaresii* CPS13<sup>T</sup> and its most closely related species *M. albiziae* LMG 23507<sup>T</sup> and *M. chacoense* Pr5<sup>T</sup>, and the type strain of the type species of the genus *Mesorhizobium*, *M. loti* NZP 2213<sup>T</sup>.

Strains: 1, *M. olivaresii* sp. nov. CPS13<sup>T</sup>; 2, *M. albiziae* LMG 23507<sup>T</sup>; 3, *M. chacoense* Pr5<sup>T</sup>; 4, *M. loti* NZP 2213<sup>T</sup>. Fatty acids present in amounts lower than 1% are not shown. nd, not detected. Data are from this study.

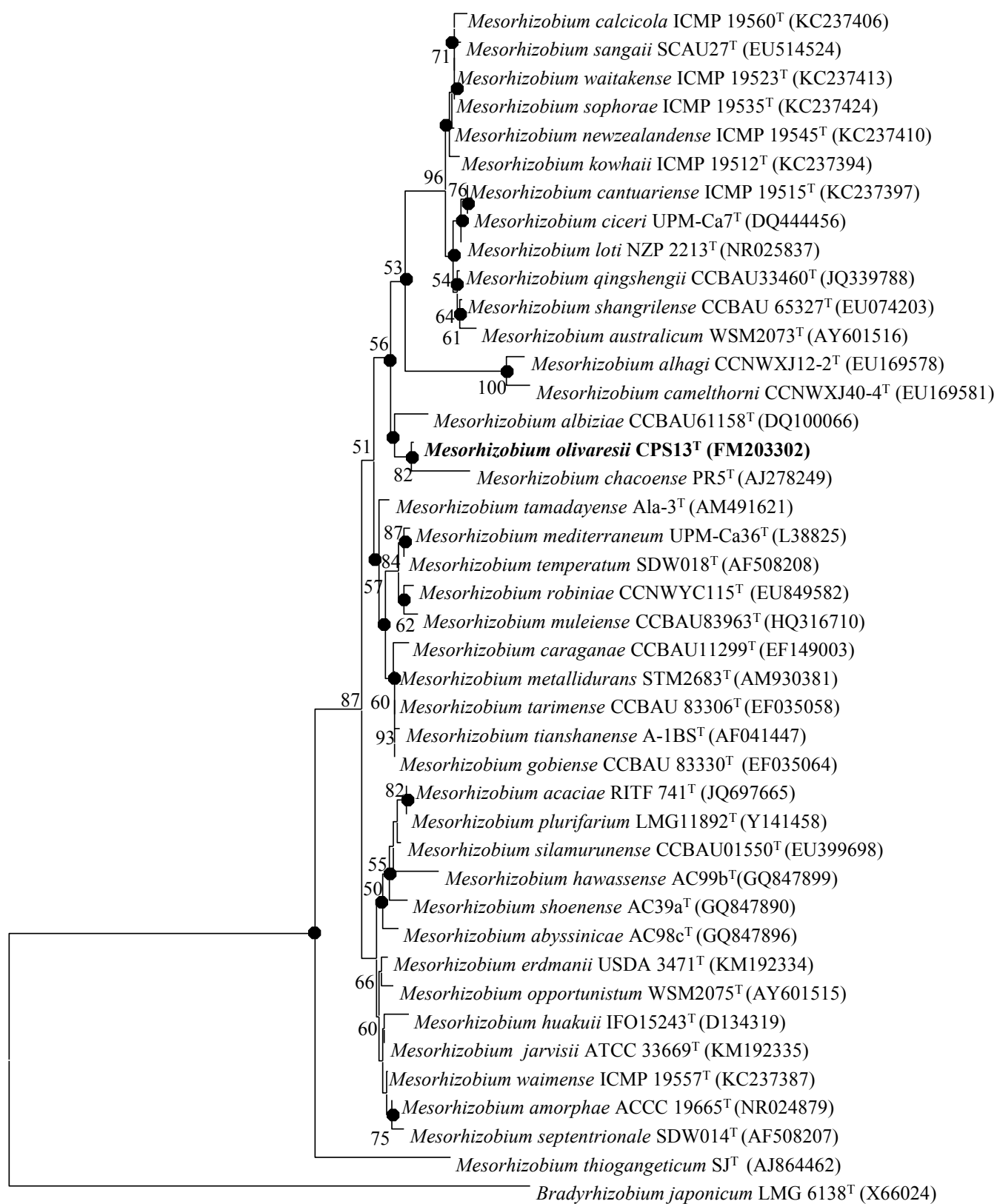
	1	2	3	4
<b>Characteristics</b>				
C <sub>16:0</sub>	3.4	5.1	5.3	12.1
C <sub>17:0</sub>	3.3	0.8	2.1	1.4
C <sub>18:0</sub>	2.7	3.1	4.7	5.6
C <sub>15:0</sub> iso	2.9	6.4	2.9	nd
C <sub>15:0</sub> iso 3 OH	3.3	2.2	4.6	nd
C <sub>17:0</sub> iso	5.7	5.1	8.2	4.6
C <sub>17:1</sub> ω8c	3.4	0.4	1.2	nd
summed feature 3 (C <sub>16:1</sub> ω7c/ C <sub>16:1</sub> ω6c)	2.3	1.5	1.1	nd
summed feature 8 (C <sub>18:1</sub> ω7c/ C <sub>18:1</sub> ω6c)	37.6	62.8	40.3	43.6
C <sub>18:1</sub> ω7c 11-methyl	24.0	11.1	19.9	16.0
C <sub>19:0</sub> cyclo ω8c	8.8	0.4	8.5	16.3

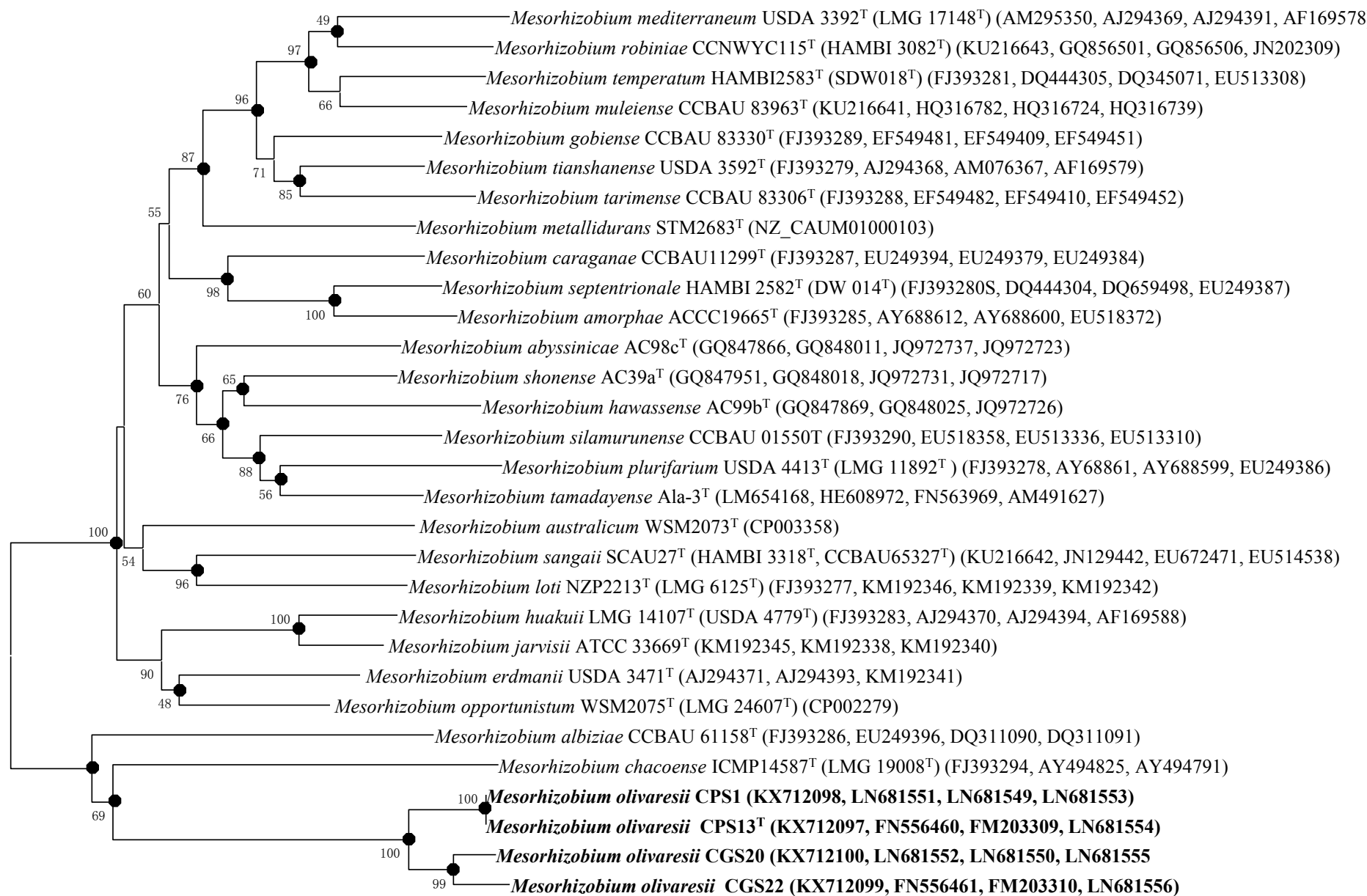
**Table 2.** Phenotypic differences between the new species *M. olivaresii* and its most closely related species *M. albiziae* LMG 23507<sup>T</sup> and *M. chacoense* Pr5<sup>T</sup>, and the type strain of the type species of the genus *Mesorhizobium*, *M. loti* NZP 2213<sup>T</sup>.

Strains: 1, *M. olivaresii* sp. nov. CPS13<sup>T</sup>; 2, *M. olivaresii* sp. nov. CPS1; 3, *M. olivaresii* sp. nov. CGS20; 4, *M. olivaresii* sp. nov. CGS22; 5, *M. albiziae* LMG 23507<sup>T</sup>; 6, *M. chacoense* Pr5<sup>T</sup>; 7, *M. loti* NZP 2213<sup>T</sup>. +: positive, -: negative, w: weak. Data are from this study.

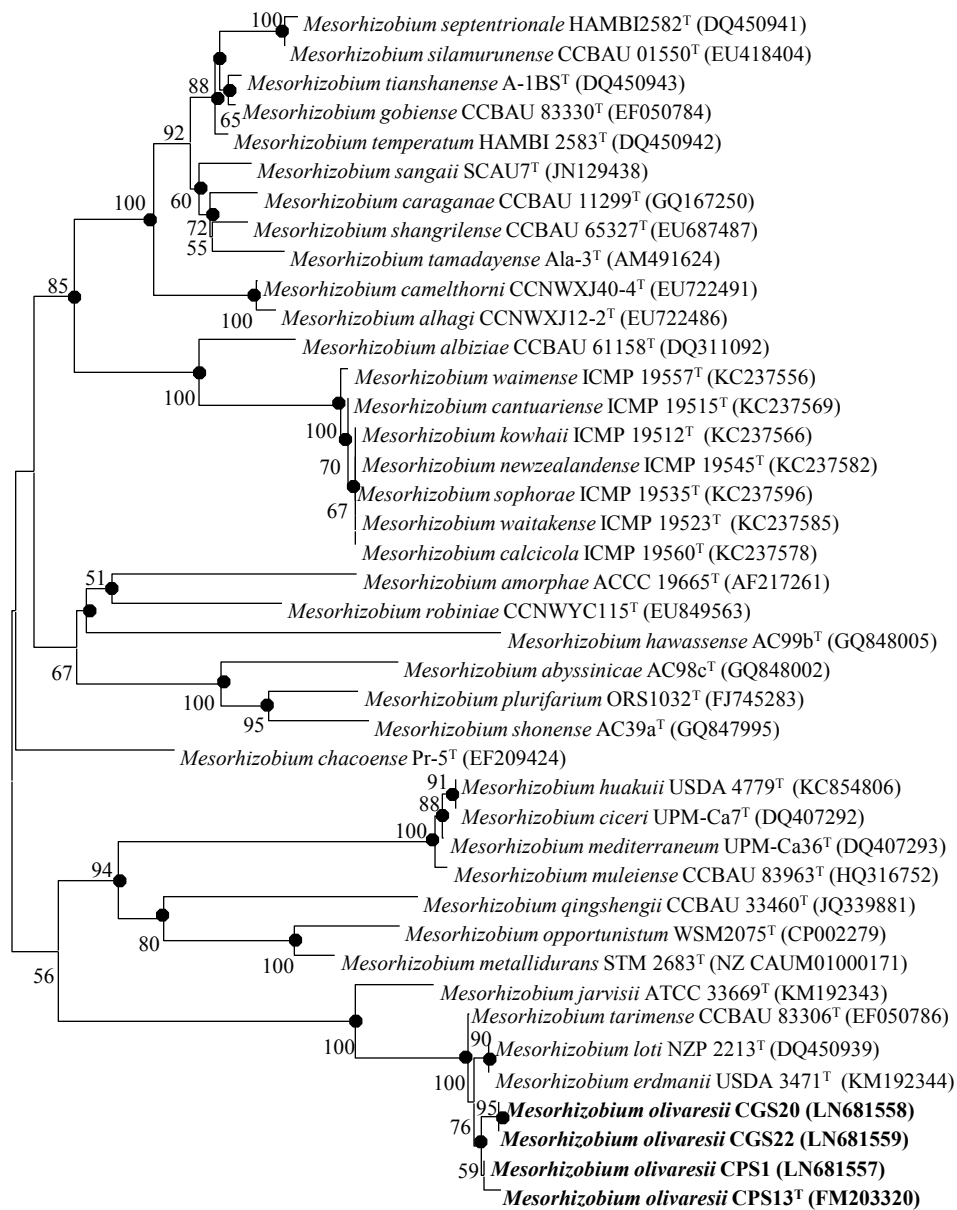
	1	2	3	4	5	6	7
<b>Characteristics</b>							
Growth at 37°C	+	+	+	+	+	-	-
Growth in presence 2% NaCl	-	-	-	-	+	+	-
Hydrolysis of:							
PNP-β-L-arabinopyranoside	-	-	-	-	+	+	+
PNP-α-D-maltopyranoside	-	-	-	-	+	+	-
PNP-α-D-mannopyranoside	-	-	-	-	-	+	-
PNP-β-D-mannopyranoside	-	-	-	-	-	+	-
Assimilation of (API ID32GN):							
Malate	-	-	w	+	+	+	-
N-acetyl-glucosamine	-	+	+	+	+	+	+
Assimilation of (API ID32GN):							
Valerate	+	+	+	+	+	-	w
D,L-lactate	+	-	+	+	+	+	w
Alanine	-	-	-	-	+	-	-
Melibiose	+	+	+	+	+	-	+
Resistance to:							
Netilmicin	-	-	-	-	+	-	+
Penicillin	+	+	+	+	+	-	+

Figure 1



**Figure 2**

0.01

**Figure 3**

0.02