Rhizobium calliandrae sp. nov., *Rhizobium mayense* sp. nov. and *Rhizobium jaguaris* sp. nov., rhizobial species nodulating the medicinal legume *Calliandra grandiflora*

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Calliandra grandiflora has been used as a medicinal plant for thousands of years in Mexico. Rhizobial strains were obtained from root nodules of *C. grandiflora* collected from different geographical regions in Chiapas and characterized by BOX-PCR, amplified rDNA restriction analysis (ARDRA) and 16S rRNA gene sequence analysis. Most isolates corresponded to members of the genus *Rhizobium* and those not related to species with validly published names were further characterized by *recA*, *atpD*, *rpoB* and *nifH* gene phylogenies, phenotypic and DNA–DNA hybridization analyses. Three novel related species of the genus *Rhizobium* within the '*Rhizobium tropici* group' share the same symbiovar that may be named sv. *calliandrae*. The names proposed for the three novel species are *Rhizobium calliandrae* sp. nov. (type strain, CCGE524^T=ATCC BAA-2435^T=CIP 110456^T=LBP2-1^T), *Rhizobium mayense* sp. nov. (type strain, CCGE526^T=ATCC BAA-2446^T=CIP 110454^T=NSJP1-1^T) and *Rhizobium jaguaris* sp. nov. (type strain, CCGE525^T=ATCC BAA-2445^T=CIP 110453^T=SJP1-2^T).

Legumes are outstanding for their symbiotic relationships with nitrogen-fixing bacteria belonging to different genera collectively called rhizobia. Besides their role as human nutrients, some legumes have value as medicinal plants. Rhizobia from nodules of medicinal plants in the Himalayas have been studied (Pandey *et al.*, 2004) and the effect of rhizobial inoculation on rhizosphere communities of medicinal legumes has been reported (Nimnoi *et al.*, 2011). In Mexico native cultures had an important knowledge of medicinal plants. Among them, the legume plant *Calliandra grandiflora* is recognized for healing wounds, as an effective agent against malaria or different infections and also as an antihaemorrhagic and antipyretic (Díaz, 1976; Meckes *et al.*, 1995). This plant has been used by native Amerindians Tzetzales and Tzotziles for a long time. Knowledge of the symbiotic rhizobia associated with *C. grandiflora* may aid in programs for its sustainable exploitation. In this study we identified three novel species of the genus *Rhizobium* from *C. grandiflora* nitrogen-fixing nodules from forests in Chiapas, Mexico.

Bacteria were isolated from *C. grandiflora* nodules collected in the field or obtained in trap plants grown in the laboratory using soils from five different sites in an altitude gradient in Chiapas that included native undisturbed tropical rain forests and high mountains with pines (Table S1 available in IJSEM Online). After surface disinfection and maceration of nodules, rhizobia were grown in YM (Vincent, 1970) and PY medium (Toledo *et al.*, 2003). A total of 159 isolates were obtained and grouped by BOX-PCR (Versalovic *et al.*, 1994) into 42 genomic fingerprints (Table S2 and Fig. S1 for some isolates). Strains representing each different BOX-PCR fingerprint were tested for nodulation ability and effects on plants. BOX-PCR was used to confirm the identity of bacteria from nodules. Evaluations after 40 days of inoculation (d.o.i.) showed that all tested strains were able to form nitrogen-fixing nodules in *C. grandiflora*.

Abbreviations: ANI, average nucleotide identity; ARDRA, amplified rDNA restriction analysis; DDH, DNA–DNA hybridization; d.o.i., days of inoculation; sv., symbiovar.

The GenBank/EMBL/DDBJ accession numbers of the 74 sequences of the *rrs*, *recA*, *atpD*, *rpoB* and *nifH* genes from the isolates of species of the genus *Rhizobium* examined in this study are JX855162–JX855197, JX855199–JX855207, JX855209–JX855217, JX 855219–JX855227 and JX855239–JX855249 and these are detailed in Table S2 available in IJSEM Online.

Three supplementary figures and four supplementary tables are available with the online version of this paper.

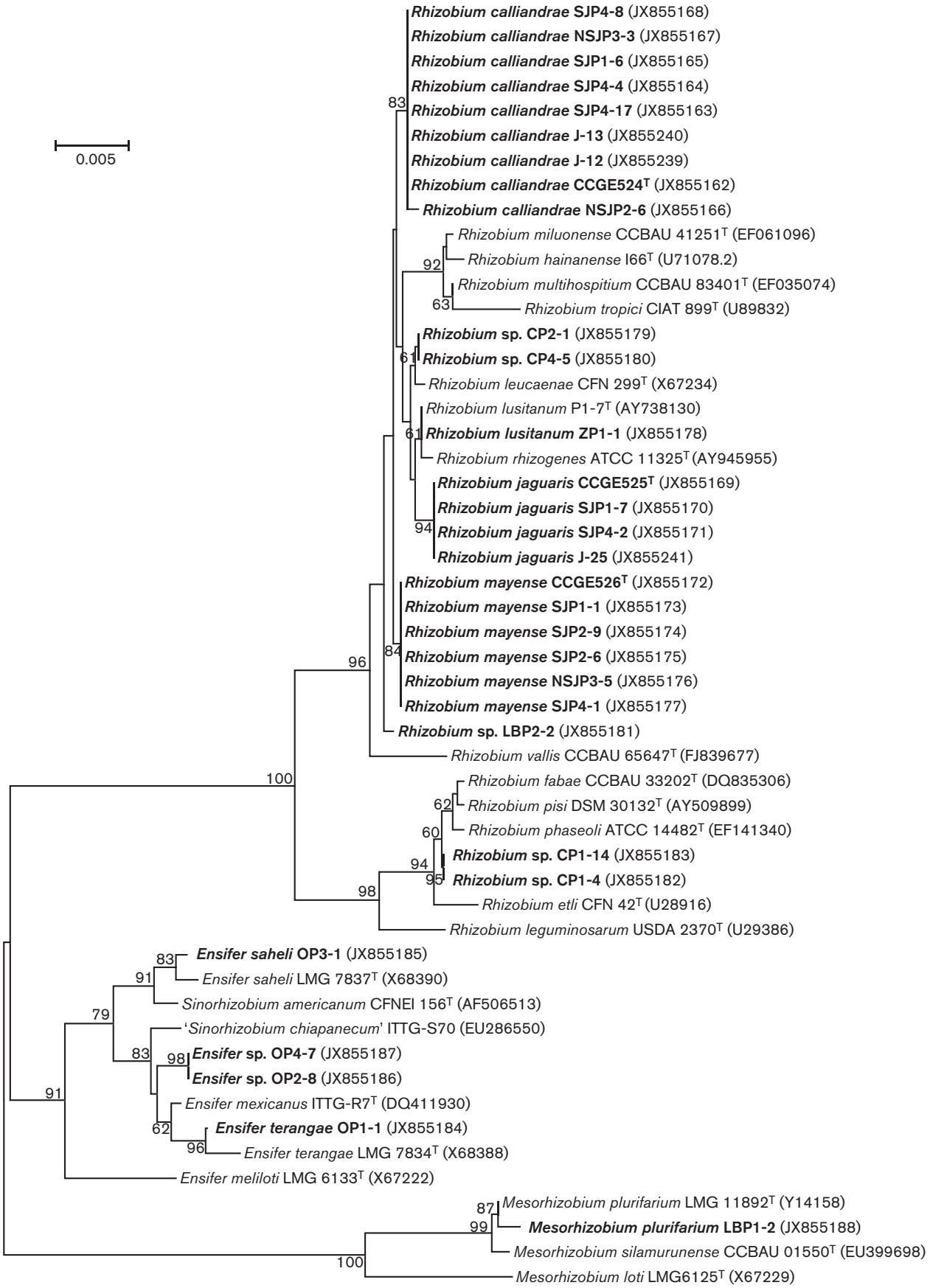
0.005
Rhizobium calliandrae SJP4-8 (JX855168)
Rhizobium calliandrae NSJP3-3 (JX855167)
Rhizobium calliandrae SJP1-6 (JX855165)
Rhizobium calliandrae SJP4-4 (JX855164)
Rhizobium calliandrae SJP4-17 (JX855163)
Rhizobium calliandrae J-13 (JX855240)
Rhizobium calliandrae J-12 (JX855239)
Rhizobium calliandrae CCGE524T (JX855162)
Rhizobium calliandrae NSJP2-6 (JX855166)
Rhizobium miluonense CCBAU 41251T (EF061096)
Rhizobium hainanense I66T (U71078.2)
Rhizobium multihospitium CCBAU 83401T (EF035074)
Rhizobium tropici CIAT 899T (U89832)
Rhizobium sp. CP2-1 (JX855179)
Rhizobium sp. CP4-5 (JX855180)
Rhizobium leucaenae CFN 299T (X67234)
Rhizobium lusitanum P1-7T (AY738130)
Rhizobium lusitanum ZP1-1 (JX855178)
Rhizobium rhizogenes ATCC 11325T (AY945955)
Rhizobium jaguaris CCGE525T (JX855169)
Rhizobium jaguaris SJP1-7 (JX855170)
Rhizobium jaguaris SJP4-2 (JX855171)
Rhizobium jaguaris J-25 (JX855241)
Rhizobium mayense CCGE526T (JX855172)
Rhizobium mayense SJP1-1 (JX855173)
Rhizobium mayense SJP2-9 (JX855174)
Rhizobium mayense SJP2-6 (JX855175)
Rhizobium mayense NSJP3-5 (JX855176)
Rhizobium mayense SJP4-1 (JX855177)
Rhizobium sp. LBP2-2 (JX855181)
Rhizobium vallis CCBAU 65647T (FJ839677)
Rhizobium fabae CCBAU 33202T (DQ835306)
Rhizobium pisi DSM 30132T (AY509899)
Rhizobium phaseoli ATCC 14482T (EF141340)
Rhizobium sp. CP1-14 (JX855183)
Rhizobium sp. CP1-4 (JX855182)
Rhizobium etli CFN 42T (U28916)
Rhizobium leguminosarum USDA 2370T (U29386)
Ensifer saheli OP3-1 (JX855185)
Ensifer saheli LMG 7837T (X68390)
Sinorhizobium americanum CFNEI 156T (AF506513)
'Sinorhizobium chiapanecum' ITTG-S70 (EU286550)
Ensifer sp. OP4-7 (JX855187)
Ensifer sp. OP2-8 (JX855186)
Ensifer mexicanus ITTG-R7T (DQ411930)
Ensifer terangae OP1-1 (JX855184)
Ensifer terangae LMG 7834T (X68388)
Ensifer meliloti LMG 6133T (X67222)
Mesorhizobium plurifarium LMG 11892T (Y14158)
Mesorhizobium plurifarium LBP1-2 (JX855188)
Mesorhizobium silamurunense CCBAU 01550T (EU399698)
Mesorhizobium loti LMG6125T (X67229)
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Fig. 1. Neighbour-joining phylogenetic tree based on16S rRNA gene sequences (1305 nt) showing the relationships of *Calliandra grandiflora* rhizobia isolated in this work and species of related taxa. The four novel lineages of the genus *Rhizobium* found in this work are indicated at the right with Roman numerals. Bootstrap support values ⩾60 % are indicated at nodes. Bar, 0.005 expected changes per site.

The 42 *C. grandiflora* rhizobial isolates selected based upon the BOX-PCR patterns were further characterized by rRNA gene restriction analysis (ARDRA) using the 16S rRNA gene amplified with primers fD1 and rD1 (Weisburg *et al.*, 1991) and restricted with five enzymes (De Baere *et al.*, 2002). Eleven different ARDRA profiles were obtained. Thirty strains covering all ARDRA profiles were chosen for phylogenetic analysis of the 16S rRNA gene (Table S2). PCR products were Sanger sequenced and multiple sequence alignments were obtained with MUSCLE (Edgar, 2004). Neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) phylogenies were inferred with MEGA software (Tamura *et al.*, 2011) using the Tamura–Nei model (Tamura & Nei, 1993). Both methods produced trees with similar topologies, thus, only neighbour-joining phylograms are presented. Tree node support was evaluated with bootstrap analysis with 500 pseudoreplicates.

Based on 16S rRNA gene sequences *C. grandiflora* nodule isolates were classified into three rhizobial genera, *Rhizobium*, *Ensifer* and *Mesorhizobium* (Fig. 1), but the majority of them corresponded to the genus *Rhizobium*. *Ensifer* isolates with 16S rRNA gene sequence similarities over 99.6 % to the type strains of *Ensifer mexicanus* (Lloret *et al.*, 2007), *Ensifer terangae* or *Ensifer saheli* (Delajudie *et al.*, 1994) were obtained only from a single site (Ocuilan de Juárez) with alkaline soils (pH 8) while all other nodule isolates were from acid soils (Table S1). A sequence affiliated with members of the genus *Mesorhizobium* with 99.8 % sequence similarity to *Mesorhizobium plurifarium* LMG 11892^T (de Lajudie *et al.*, 1998) was obtained from Laguna Bélgica.

All strains representing members of the genus *Rhizobium* showed ⩾97.8 % 16S rRNA gene sequence similarity to *Rhizobium leguminosarum* USDA 2370^T, the type strain of the type species of the genus *Rhizobium*. Two strains representing two BOX patterns with a total of seven isolates, obtained from a single site (Chenalhó), grouped within the *Rhizobium phaseoli–Rhizobium etli* clade, while all the other novel strains of the genus *Rhizobium* clustered within the 'tropici' group of species of the genus *Rhizobium* (Ribeiro *et al.*, 2011) and corresponded to six phylogenetic lineages (Fig. 1). Strain ZP1-1 (representing eight isolates with the same BOX pattern) had 100 % 16S rRNA gene sequence similarity with *Rhizobium lusitanum* P1-7^T (Valverde *et al.*, 2006), while strains CP2-1 and CP4-5 (representing two BOX patterns with a total of 15 isolates) showed 99.7 % similarity with *Rhizobium leucaenae* CFN 299^T but lacked the 72 bp insertion typical of this species (Ribeiro *et al.*, 2011). The remaining 20 sequenced strains classified as members of the genus *Rhizobium*, representing 18 BOX patterns with a total of 76 isolates, constituted four novel lineages of the genus *Rhizobium* (marked in Fig. 1) which were obtained from ecological reserves in Laguna Bélgica (so well preserved that jaguars still live there) or Nuevo San Juan Chamula. The highest 16S rRNA gene sequence similarities of novel lineages I, II, III and IV of the genus *Rhizobium* with type strains of the 'tropici' clade were 99.6 %, 99.7 %, 99.8 % and 99.6 %, respectively. Within each of these novel lineages 16S rRNA gene sequence similarity range was 99.8–100 % and between them 99.4–99.8 %.

Lineages I, II and III were chosen for further characterization. A multilocus sequence analysis with partial sequences of *recA*, *atpD* and *rpoB* genes, amplified with primers and conditions described by Vinuesa *et al.* (2005), Gaunt *et al.* (2001) and Khamis *et al.* (2003), respectively, was performed. Phylogenies were inferred as described above for the 16S rRNA gene. Single-gene (not shown) and concatenated (Fig. S2) phylogenies placed strains of each novel lineage in different but related clades which were clearly different from species within the 'tropici' group of the genus *Rhizobium* with validly published names.

Probability ranges (P=0.01) of gene identities within and between species (interspecies and intraspecies variability) were estimated to discriminate between species, as described previously (Lloret *et al.*, 2007; Martínez-Romero *et al.* 2010). Intraspecies 99 % confidence intervals for the three new lineages and the three genes considered (*atpD*, *recA* and *rpoB*) showed that the variability of each gene is significantly smaller in a single lineage (intraspecies) than among closely related species (Fig. 2).

An average nucleotide identity (ANI) level of 96 %, calculated only with conserved core genes, corresponds to 70 % DNA–DNA hybridization (DDH) (Konstantinidis *et al.*, 2006). Nucleotide identities for the concatenated dataset of *recA*, *atpD* and *rpoB* genes used here were calculated as an approximation to ANI. The identity range between described species was 85.2–95.8 %, indicating that these values may be good estimates of ANI. The identity ranges within the novel lineages I, II and III were 98.1–99.8 %, 99.6–100 %, 98.4–100 %, respectively, confirming that these novel lineages are cohesive groups that may be equated to species. The maximum identity between the three novel lineages and between them and species with validly published names was 95 % and 93.6 %, respectively, indicating that lineages I, II and III are distinct from each other and from previously described rhizobia.

Total genomic DNA conservation within each of lineages I, II and III and between them and the type strains of related taxa was estimated by DDH experiments performed by a membrane hybridization method (Martínez-Romero *et al.*,

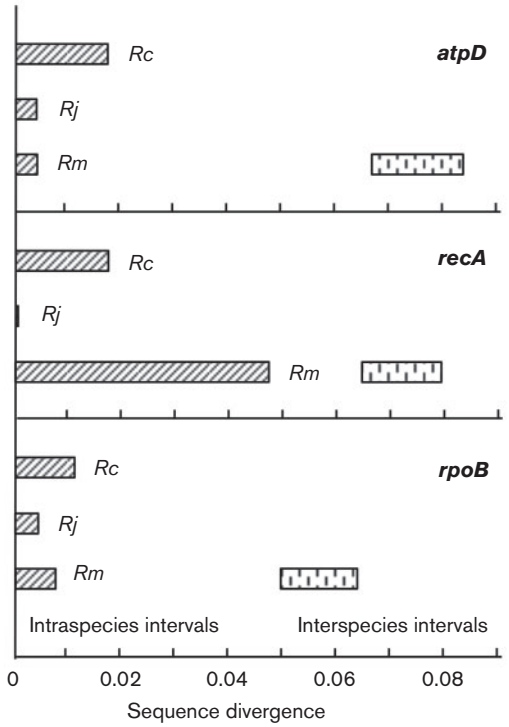

Fig. 2. Confidence intervals (P=0.01) for intraspecies and interspecies variability of *atpD*, *recA* and *rpoB* gene sequences for *R. calliandrae* sp. nov. (*Rc*), *R. jaguaris* sp. nov. (*Rj*) and *R. mayense* sp. nov. (*Rm*). Interspecies variability was estimated also including *R. tropici* LMG 9503^T, *R. leucaenae* USDA 9039^T, *R. rhizogenes* LMG 150^T, *R. lusitanum* P1-7^T, *Rhizobium multihospitium* CCBAU 83401^T, *Rhizobium miluonense* CCBAU 41251^T, *Rhizobium hainanense* CCBAU 57015^T, *Rhizobium vallis* CCBAU 65647^T and *R. leguminosarum* USDA 2370^T.

1991). Hybridization was carried out at 65 °C overnight, membranes were washed with 2 × SSC and 0.1 % SDS at 65 °C for 30 min and with 1 × SSC for 15 min at the same temperature. Strains CCGE524^T, CCGE525^T and CCGE526^T, representing lineages I, II and III, respectively, had DDH values lower than 45 % to all type strains of related taxa clearly indicating that they do not belong to any described species. Among all species compared, they seemed more related to *R. leucaenae*, *Rhizobium rhizogenes* and *R. lusitanum*. Strains recognized as belonging to the same novel lineage (from the ARDRA analysis or the 16S rRNA gene phylogeny) showed DDH values above 70 %, fully supporting their affiliation to a single species. DDH values between the three novel lineages ranged from 43 % to –60 %, larger than that observed with the reference strains examined (Table S3) but still lower than the limit to distinguish species (Stackebrandt & Goebel, 1994).

Phenotypic characteristics of strains from novel lineages I, II and III and type strains of related taxa were determined. Colony morphology, generation times, growth at 37 °C and 40 °C, in the presence of 0.25, 0.5 and 1 M NaCl, or at pH 4, 4.5 and 5 were evaluated in PY medium at 28 °C (except for temperature tests) as previously described (Martínez-Romero *et al.*, 1991). Growth in Luria–Bertani (LB) medium or PY without calcium and acid production in YM medium supplemented with 25 μg bromothymol blue ml^{-1} were also determined. Utilization of different compounds as sole carbon and nitrogen sources at 0.1 % was evaluated in liquid minimal medium (Vincent, 1970) supplemented with 0.1 % KNO_3 or glucose, respectively. Antibiotic resistance was evaluated in PY medium using a standard disc diffusion assay. All strains from the three novel lineages produced acid in YM medium and grew in acid medium at pH 5 and 4.5 but not at pH 4. They were not tolerant to 0.25 M NaCl and they did not grow in LB medium, not in PY without Ca, except for strain SJP1-7. They did not grow at temperatures of 37 °C or over. Distinctive phenotypic characteristics of lineages I, II and III in comparison with related taxa are shown in Table 1. The novel lineages showed similar reactions in most tests performed which is consistent with their close relationship revealed by sequence analyses and DDH, nevertheless, they can be distinguished by swarming behaviour, growth with sucrose, sodium citrate and L-proline as carbon sources and resistance levels to cefalotin, chloramphenicol, nitrofurantoin, pefloxacin, trimethoprim-sulfamethoxazole, piperocillin–tazobactam, cefepime and cefalozin (Table 1). Lineages I, II and III seemed to be less resistant to antibiotics than other species in the 'tropici' group except for *R. leucaenae*. Additionally, the novel lineages could be distinguished from each other by different relative amounts of several membrane fatty acids (Table S4) which were determined with the MIDI system using the TSBA5 database after growing the strains for 24 h on YM agar plates.

Based on all described genotypic and phenotypic data showing the distinctiveness of the three lineages characterized in this work in relation to other species of the genus *Rhizobium* with validly published names, we propose three novel species of the genus *Rhizobium,* with the names *Rhizobium calliandrae* sp. nov., *Rhizobium jaguaris* sp. nov. and *Rhizobium mayense* sp. nov. to accommodate strains belonging to lineages I, II and III, respectively.

Phylogenetic analysis of the nitrogenase reductase (*nifH*) gene (Fig. S3), amplified with primers nifH1 and nifH2 (Eardly *et al.*, 1995), showed that sequences from strains isolated from *C. grandiflora* formed a clade that was related to the *nifH* genes reported from *Rhizobium tropici*-like strains isolated from *Acaciella angustissima* nodule bacteria in Mexico (Rincón-Rosales *et al.*, 2009). This may indicate that the three novel species reported here share the same symbiovar (symbiotic variant, Rogel *et al.*, 2011) that has not been previously described and that may be named sv. *calliandrae*. *R. calliandrae* sp. nov. strains CCGE524^T, J-12 and NSJP2-6, *R. mayense* sp. nov. strains CCGE526^T, SJP1-1 and SJP2-9 and *R. jaguaris* sp. nov. strains CCGE525^T and SJP1-7 formed nitrogen-fixing nodules on *Phaseolus vulgaris* 'Negro Xamapa' when evaluated at 21 d.o.i. by

Table 1. Phenotypic characteristics that differentiate *R. calliandrae* sp. nov., *R. mayense* sp. nov., *R. jaguaris* sp. nov. and related species of the genus *Rhizobium*

Taxa: 1, *R. calliandrae* sp. nov.; 2, *R. mayense* sp. nov.; 3, *R. jaguaris* sp. nov.; 4, *R. miluonense* CCBAU 41251^T; 5, *R. tropici* CIAT 899^T; 6, *R. multihospitium* CCBAU 83401^T; 7, *R. lusitanum* P1-7^T; 8, *R. leucaenae* CFN 299^T; 9, *R. rhizogenes* ATCC 11325^T; 10, *R. hainanense* I66^T. +, Positive, −, negative; w, weakly positive.

Characteristic	1	2	3	4	5	6	7	8	9	10
Growth on PY at/with:										
pH 4.0	−	−	−	−	+	+	+	−	+	−
pH 4.5	+	+	+	+	+	+	+	+	+	−
37 °C	−	−	−	−	+	−	−	+	+	+
42 °C	−	−	−	−	+	−	−	−	+	+
0.25 M NaCl	−	−	−	w	+	+	−	−	+	+
0.5 M NaCl	−	−	−	−	−	−	−	−	−	w
Without Ca	−	−	−	+	+	+	+	−	+	+
Growth on LB medium	−	−	−	−	+	+	−	−	+	+
Swarming motility on agar surface	+	−	−	−	+	+	−	−	+	+
Utilization of sole carbon sources:										
Sucrose	w	w	+	+	+	w	+	−	w	−
Sodium citrate	−	w	w	w	−	−	w	w	−	w
D-Sodium gluconate	−	−	−	+	+	−	+	−	w	−
L-Proline	+	−	−	+	−	−	+	−	−	−
L-Aspartic acid	−	−	−	+	−	−	−	−	−	−
Utilization of sole nitrogen sources:										
L-Arginine	+	+	+	+	w	−	+	+	w	−
L-Aspartic acid	−	−	−	+	−	−	−	−	−	−
Tolerance of (μg ml^{-1}):										
Cefalotin (30)	w	−	−	+	+	+	+	−	+	+
Cefotaxime (30)	−	−	−	w	+	+	+	−	+	+
Ceftriaxone (30)	w	w	w	w	+	+	+	−	+	w
Chloramphenicol (30)	w	−	−	−	+	+	−	−	w	+
Nitrofurantoin (300)	−	w	−	−	+	+	+	w	w	+
Pefloxacin (5)	+	w	w	+	+	+	w	w	w	w
Trimethoprim–sulfamethoxazole (25)	−	+	w	+	+	+	+	+	+	+
Piperocillin–tazobactam (100/10)	−	w	−	+	+	+	+	−	+	−
Cefotetan (30)	−	−	−	−	w	+	+	−	−	−
Cefepime (30)	w	+	+	+	+	+	+	w	+	+
Cefaclor (30)	−	−	−	−	+	+	+	−	−	+
Cefoperazone (75)	−	−	−	−	+	+	+	−	w	w
Cefazolin (30)	w	w	−	+	+	+	+	w	+	+
Ampicilin–sulbactam (20)	−	−	−	−	+	+	+	−	+	−

previously described methods (Lloret *et al.*, 2007). *R. tropici* CIAT 899 also fixed nitrogen in *C. grandiflora*, supporting the notion that CIAT 899 is a broad-host-range species of the genus *Rhizobium* (Hernandez-Lucas *et al.* 1995).

Plasmid patterns, visualized with a modified Eckhard procedure (Hynes & McGregor 1990), were different among different strains even within the same novel species (data not shown) and this further confirmed that the strains within each novel species were not siblings. All strains tested showed megaplasmids of ~1700 bp (not shown), as do other strains from the 'tropici' group (Geniaux *et al.*, 1995).

Description of *Rhizobium calliandrae* sp. nov. *Rhizobium calliandrae* (cal.li.an′drae. N.L. gen. n. *calliandrae* of *Calliandra*, the genus of the medicinal plant *C. grandiflora* from which bacteria were isolated).

Cells are Gram-negative, aerobic, motile, non-spore-forming rods (1.58 × 0.50 μm). Colonies on PY are circular, convex, white–yellow, semi-translucent and shiny, with smooth edges, opalescent and 2.5 mm in diameter after 3 days incubation at 28 °C. The optimum temperature for growth is 28–30 °C; does not grow at temperatures at or above 37 °C. Can grow at pH 4.5 and 5 but not at pH 4. No growth in Luria–Bertani (LB) medium. Produces polysaccharides and acid in YM medium. Uses sucrose and L-proline as sole carbon sources and L-arginine, but not L-aspartic acid, as a sole nitrogen source. Sensitive to 0.25 M NaCl, to cefotaxime (30 μg ml^{-1}),

cefotetan (30 μg ml^{-1}), cefaclor (30 μg ml^{-1}), nitrofurantoin (300 μg ml^{-1}), trimethoprim–sulfamethoxazole (25 μg ml^{-1}), piperocillin–tazobactam (100/10 μg ml^{-1}), cefoperazone (75 μg ml^{-1}) and ampicillin–sulbactam (20 μg ml^{-1}). Weakly resistant to cefalotin (30 μg ml^{-1}), ceftriaxone (30 μg ml^{-1}), chloramphenicol (30 μg ml^{-1}), cefepime (30 μg ml^{-1}) and cefazolin (30 μg ml^{-1}) and resistant to pefloxacin (5 μg ml^{-1}).

The type strain is CCGE524^T (=ATCC BAA-2435^T=CIP 110456^T=LBP2-1^T) isolated from an effective nodule of *Calliandra grandiflora*. This strain has been successfully used as inoculant in field assays (unpublished).

Description of *Rhizobium mayense* sp. nov. *Rhizobium mayense* (ma.yen′se. N.L. neut. adj. *mayense* of or belonging to the Mayas, native people of South-east Mexico that inhabited rainforests).

Cells are Gram-negative, aerobic, non-motile, non-spore-forming-rods (1.08 × 0.53 μm). Colonies on PY are circular, convex, white–yellow, semi-translucent, shiny, smooth-edged, opalescent and usually 2.5–3 mm in diameter after 3 days incubation at 28 °C. The optimum temperature for growth is 28–30 °C; cannot grow at temperatures above 37 °C. Can grow at pH 4.5 and 5 but not at pH 4. No growth in Luria–Bertani (LB) medium. Produces polysaccharides on YM and acid is produced. Utilizes sucrose and sodium citrate as sole carbon sources, but not D-sodium gluconate, L-proline or L-aspartic acid. Uses L-arginine, but not L-aspartic acid, as a sole nitrogen source. Sensitive to 0.25, 0.5 and 1 M NaCl, to cefalotin (30 μg ml^{-1}), cefotaxime (30 μg ml^{-1}), chloramphenicol (30 μg ml^{-1}), cefotetan (30 μg ml^{-1}), cefaclor (30 μg ml^{-1}), cefoperazone (75 μg ml^{-1}) and ampicilin–sulbactam (20 μg ml^{-1}); weakly resistant to ceftriaxone (30 μg ml^{-1}), cefepime (30 μg ml^{-1}), cefazolin (30 μg ml^{-1}), nitrofurantoin (300 μg ml^{-1}), pefloxacin (5 μg ml^{-1}) and piperocillin–tazobactam (100/10 μg ml^{-1}) and resistant to trimethoprim–sulfamethoxazole (25 μg ml^{-1}).

The type strain is CCGE526^T (=ATCC BAA-2446^T=CIP 110454^T=NSJP1-1^T) isolated from an effective nodule of *Calliandra grandiflora*.

Description of *Rhizobium jaguaris* sp. nov. *Rhizobium jaguaris* (ja.gu.a′ris. N.L. n. *jaguar -aris* jaguar; N.L. gen. n. *jaguaris* of a jaguar, feline mammal present in the Mexican rainforests from which the strains were isolated).

Cells are Gram-negative, aerobic, non-motile, non-spore-forming-rods (0.86 × 0.45 μm). Colonies on PY are circular, convex, white–yellow, semi-translucent, shiny, smooth-edged, opalescent and usually 2.5–3 mm in diameter after 3 days incubation at 28 °C. The optimum temperature for growth is 28–30 °C; cannot grow at temperatures above 37 °C. Can grow at pH 4.5 and 5 but not at pH 4. No growth in Luria–Bertani (LB) medium. Produces polysaccharides on YM and acid is produced. Utilizes sucrose and sodium citrate as sole carbon sources, but not D-sodium gluconate, L-proline or L-aspartic acid. Can use L-arginine, but not L-aspartic acid as a sole nitrogen source. Sensitive to 0.25, 0.5 and 1 M NaCl, to cefalotin (30 μg ml^{-1}), cefotaxime (30 μg ml^{-1}), chloramphenicol (30 μg ml^{-1}), cefotetan (30 μg ml^{-1}), cefaclor (30 μg ml^{-1}), cefazolin (30 μg ml^{-1}), nitrofurantoin (300 μg ml^{-1}), piperocillin–tazobactam (100/10 μg ml^{-1}), cefoperazone (75 μg ml^{-1}) and ampicillin–sulbactam (20 μg ml^{-1}); weakly resistant to ceftriaxone (30 μg ml^{-1}), cefepime (30 μg ml^{-1}), pefloxacin (5 μg ml^{-1}) and trimethoprim–sulfamethoxazole (25 μg ml^{-1}).

The type strain is CCGE525^T (=ATCC BAA-2445^T=CIP 110453^T=SJP1-2^T) isolated from an effective nodule of *Calliandra grandiflora*.

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