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REGULAR ARTICLE

Soil characteristics determine the rhizobia in association with different species of *Mimosa* in central Brazil

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Abstract
Background and aims To evaluate the influence of soil type on the symbiosis between *Mimosa* spp. and rhizobia.
Methods A greenhouse experiment was carried out with trap plants using seeds of six species of *Mimosa* and soils from three different locations in central Brazil: Posse, Brasília and Cavalcante. Plant dry biomass and number of nodules were measured after four months. Symbiotic bacteria were isolated from nodules and their molecular identification was performed. Three housekeeping genes (16S rRNA, *recA* and *gyrB*) plus the *nodC* and *nifH* symbiotic genes were used to determine the identity of the symbionts and to reconstruct the phylogenetic relationships among the isolated nitrogen-fixing bacteria.
Results Rhizobia from the Betaproteobacterial genus *Paraburkholderia* (former *Burkholderia*) and the Alphaproteobacterial genus *Rhizobium* were isolated from different species of *Mimosa*. As in previous studies, the phylogenies of their symbiosis-essential genes, *nodC* and *nifH*, were broadly congruent with their core housekeeping genes (16S rRNA, *recA* and *gyrB*), which suggests limited or no horizontal gene transfer. Edaphic factors such as pH and fertility influenced the occurrence of these unrelated rhizobial types in the nodules on these *Mimosa* spp.

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Conclusions *Mimosa* species have the ability to associate with different types of rhizobia (α- and β-proteobacteria), suggesting low specificity between host and bacterium in experimental conditions. Soil factors such as pH, nitrogen and fertility seem to favour the predominance of certain types of rhizobia, thus influencing the establishment of symbiotic relationships.

Keywords Biological nitrogen fixation · Cerrado · Host-specificity · Nodulation · Rhizobia · β-rhizobia

Introduction

Leguminous plants are important for natural and agricultural ecosystems because of their ability to fix atmospheric N_2 in nodules formed on their roots via symbioses with diazotrophic bacteria (rhizobia). In this association, which is critical for plant nutrition and global N cycling, rhizobia provide nitrogen in exchange for carbon compounds supplied by the plant (Sprent 2009; Robledo et al. 2010).

All bacteria currently known to nodulate leguminous plants belong to the group of gram-negative proteobacteria. Until the beginning of the XXI century, bacteria of the order Rhizobiales, within the class α-proteobacteria, were considered the only ones that nodulated Leguminosae. However, since 2001, several reports have demonstrated that members of the β-proteobacteria class also nodulate and fix nitrogen in association with these plants (Chen et al. 2001; Vandamme et al. 2002; Chen et al. 2003; Elliott et al. 2007a, b, 2009; Bournaud et al. 2013; Liu et al. 2014; Lemaire et al. 2015). In particular, studies carried out with the pantropical *Mimosa* genus have been used to demonstrate that bacteria from the β-proteobacteria play a key role in nitrogen fixation in association with leguminous plants (Chen et al. 2005a, b; Elliott et al. 2009; Gyaneshwar et al. 2011; Bontemps et al. 2010; Reis Junior et al. 2010; Lammel et al. 2013; Platero et al. 2016). Indeed, *Mimosa* is now used as a model for studies involving these symbiotic relationships in natural ecosystems.

Previous studies have shown that bacteria from the genus *Paraburkholderia* (former *Burkholderia*) are the main symbionts of *Mimosa* species in the central region of Brazil (Chen et al. 2005a; Elliott et al. 2007a; Bontemps et al. 2010; Reis Junior et al. 2010), French Guiana, Costa Rica, and in areas where such plants were introduced such as Asia and Australia (Chen et al. 2005b; Barrett and Parker 2006; Parker et al. 2007; Liu et al. 2012; Mishra et al. 2012; Gehlot et al. 2013; Melkonian et al. 2014). A division of the genus *Burkholderia* was recently proposed, involving the creation of a novel genus *Paraburkholderia* containing the primarily environmental and plant-associated species which broadly corresponds to the "Plant Beneficial and Environmental (PBE)" clade of Suárez-Moreno et al. (2012), and which separates them from *Burkholderia* sensu stricto, which encompasses some environmental strains, but is particularly known for its human clinical and phytopathogenic species (Sawana et al. 2014; Beukes et al. 2017). All known nodulating *Burkholderia* species are currently placed in the genus *Paraburkholderia*, the exception being *B. symbiotica* which is most likely to be placed in a new genus along with some other members of the "Transition Group I" defined by Estrada-de los Santos et al. (2016), such as *B. caryophylli*, *B. soli* and *B. rhizoxinica*.

In Uruguay, the main symbionts associated with *Mimosa* belong to the genus *Cupriavidus* (Platero et al. 2016), which is related to *Paraburkholderia* in the β-proteobacteria class. In Mexico, an important center of *Mimosa* diversity, species of this genus are predominantly associated with α-proteobacteria from the genera *Rhizobium* and *Ensifer* (Bontemps et al. 2016). In India, the genus *Ensifer* is also predominant in two endemic species of *Mimosa* (Gehlot et al. 2013).

The preference of *Mimosa* for association with some distinct groups of rhizobia in different regions may be related to soil characteristics, such as pH and fertility, which influence symbiont selection (Elliott et al. 2009; Garau et al. 2009; Thrall et al. 2011; Liu et al. 2012, 2014; Mishra et al. 2012; Lammel et al. 2015; Stopnisek et al. 2014; Lemaire et al. 2015). For example, sites with high levels of heavy metals (zinc, copper and nickel) in soil tend to favour symbiosis with *Cupriavidus*, as verified in Uruguay (Platero et al. 2016) and New Caledonia (Klonowska et al. 2012). In central Brazil, where soils are generally acidic, *Paraburkholderia* spp. are the main *Mimosa* symbionts (Bontemps et al. 2010; Reis Junior et al. 2010). On the other hand, the predominance of α-proteobacteria in *Mimosa* species endemic to Mexico may be related to the presence of more fertile and pH neutral soils (Bontemps et al. 2016).

Symbiotic interactions generally depend on the expression of lipo-chitoligosaccharides (LCOs) or "Nod factors" produced by bacteria in response to plant secreted flavonoids, proteins and polysaccharides from the root surface. Any changes in the structures of these Nod factors can alter the specificity between plants and bacteria (Wang et al. 2012). In addition, this specificity varies among legumes, as some species are associated only with bacteria from one group (α- or β-proteobacteria), or with a few species of rhizobia, while others, more promiscuous, are associated with several types of rhizobia (Peix et al. 2015, Dall'Agnol et al. 2016). Factors that can influence the choice of symbionts by a plant species include the composition and diversity of the microbial community, soil characteristics, such as pH, salinity, nitrogen and nutrient levels, and altitude (Thrall et al. 2011; Lemaire et al. 2015, 2016).

In the case of *Mimosa*, it is also possible that the plants of each country or continent may have coevolved with their respective symbionts over millions of years resulting in increased specificity. For example, after the ancestors of the main lineages of *Mimosa* from Brazil and Mexico diverged, their descendants may have coevolved with the rhizobia of the local rhizosphere, *Paraburkholderia* in the case of the Cerrado and Caatinga biomes in Brazil, and *Rhizobium* and *Ensifer* in the central highlands of Mexico (Bontemps et al. 2010; Moulin et al. 2015; Bontemps et al. 2016; Sprent et al. 2017), which may have resulted in high rhizobia host-specificity. This might be particularly relevant for endemic plant species, which are restricted to very particular sites and narrow environmental conditions, and might therefore tend to restrict their symbiotic ability to a very limited range of rhizobia.

Despite the growing knowledge on the symbionts associated with leguminous plants in several ecosystems, the rhizobia host-specificity in natural environments and how different types of soil can affect this interaction are still poorly understood. In this study, we conducted an experiment to compare the nodulation of six native Brazilian species of the genus *Mimosa* in three soil types from central Brazil with distinct physicochemical characteristics. We hypothesized that endemic host legume species are expected to exhibit greater specificity in relation to their association with rhizobia, while widely distributed species may associate with a wide variety of symbionts. Regarding soil characteristics, according to recent literature (e.g. Elliott et al. 2009; Bontemps et al. 2016), it is expected that more fertile soils with high pH would favour association with α-proteobacteria, whereas more acidic and nutrient poor soils would result in nodulation with β-proteobacteria.

The aims of the present study were to verify the influence of soil type on nodulation and the composition of associated rhizobial species. Three housekeeping genes (16S rRNA, *recA* and *gyrB*) plus the *nodC* and *nifH* symbiosis-essential genes were used to determine the identity of the symbionts and to reconstruct the phylogenetic relationships among the isolated nitrogen-fixing bacteria.

Materials and methods

Collecting sites and plant species

In order to perform the nodulation tests, seeds of *Mimosa* species and soil samples containing their associated rhizosphere from three different sites in central Brazil with contrasting edaphic and vegetation characteristics, were used (Table 1). Soil samples from each locality were collected (0 to 20 cm depth) and analysed according to the protocols of Embrapa (1997).

A deciduous seasonal forest predominates in the collection site at Posse, with soil derived from limestone with high fertility and pH tending to neutral. The Brasília site is a typical woody savanna (cerrado) in a deep clay Oxisol, with low fertility and low pH. The Cavalcante site comprises an open savanna shrubby vegetation (cerrado rupestre) on sandy-rocky soil with low fertility and low pH (Table 1). Soil analysis shows high cation exchange capacity with elevated levels of Ca, Mg, K, P, nitrogen and organic matter in Posse, whereas the Brasília and Cavalcante sites have lower levels of cations, nitrogen and organic matter, but high concentrations of Al (Table 2).

Mature seeds of two *Mimosa* species per site were collected in the field. Plant species used in the study (Table S1) vary according to the extent of their geographic distribution: *M. xanthocentra* is a ruderal species of wide distribution in South America; *M. acutistipula* occurs in the Cerrado and Caatinga regions in Brazil often associated with fertile soils; *M. claussenii* and *M. radula* are widely distributed within the Cerrado region; *M. kalunga* and the undescribed *Mimosa* sp. are endemic to the region of Cavalcante in northern Goiás (Barneby 1991; Simon

Table 1 Description of the three sites in central Brazil (Posse, Brasília and Cavalcante) where seeds from *Mimosa* species and soil samples were collected

City/State	Site name	Coordinates	Soil classification*	Vegetation	Species collected
Posse/GO	Sabonete farm	14°04′00″S; 46°49′17″ W; 630 m	Oxisol (Nitisol) with limestone rock outcrop, high fertility and high pH	Seasonal Deciduous Forest (Mata Seca Decidual)	*Mimosa acutistipula* and *Mimosa xanthocentra*
Brasília/DF	Brasília National Park	15°43′39″S; 47°56′55″ W; 1038 m	Oxisol (Ferralsol), deep, low fertility and low pH	Woody Savanna (Cerrado)	*Mimosa claussenii* and *Mimosa radula*
Cavalcante/GO	RPPN Serra do Tombador	13°40′48″S; 47°49′21″ W; 794 m	Inceptsol (Cambisol) with rocky material derived from sandstone, low fertility and low pH	Shrubby Savannah (Cerrado Rupestre)	*Mimosa kalunga* and *Mimosa* sp.

*Soil classification: American System; between parenthesis World Reference Base

et al. 2010). The identity of the so far unidentified *Mimosa* sp. could not be determined as it might represent a new taxon. These species were chosen because of their abundance in each locality. They represent different lineages distributed throughout the phylogeny of the genus, with *M. claussenii*, *M. kalunga* and the *Mimosa* sp. belonging to the same clade (Simon et al. 2011).

Experiments with trap plants

Experiments with trap plants (Bontemps et al. 2016; Mishra et al. 2012) were performed to evaluate nodulation and growth of the *Mimosa* species cultivated in three different soil types, including the original soil where the seeds were collected. Seeds of six species of *Mimosa* were used (Table S1): two from Posse (*M. acutistipula* and *M. xanthocentra*), two from Brasília (*M. claussenii* and *M. radula*) and two from Cavalcante (*M. kalunga* and *Mimosa* sp.). Seeds were immersed in 70% alcohol for 30 s and sodium hypochlorite solution (2.5% active chlorine) for five minutes for surface sterilization. Subsequently, seeds were placed in a 23 mm mesh sieve to be washed five times with sterile distilled water. The dormancy was broken by scratching the testa of the seeds under sterile conditions. The seeds were then placed in Petri dishes with moistened filter paper, and after ten days, seedlings were transferred to 300 ml pots filled with soil (no fertilizer added), and cultivated for four months. Seedlings cultivated in sterilized sand were used as a negative control. Nodules from four to seven plants of each species were used for isolating rhizobia.

For comparison of plant growth (dry biomass) and nodulation among different types of soil an analysis of variance (ANOVA) was conducted and significant differences between means were assessed by Duncan's test at the 5% level of significance using the MSTAT-C software (Michigan State University). Means and standard errors were calculated from values of three replicates of each *Mimosa* spp. in each soil.

Rhizobia isolation

We randomly harvested one to six nodules per treatment (species/soil type), depending on their availability. Collected nodules were rehydrated in sterile distilled water for 2.5 h. Superficial sterilization of nodules was performed by soaking them in ethanol (95%) for 30 s, followed by immersion in 2.5% sodium hypochlorite solution for five minutes and then five washes in sterile water. After this procedure, 500 μl of sterile saline solution was added to 2 ml Eppendorf tubes and the nodules were crushed in this solution with sterile forceps. The resulting solution was serially diluted to 10^{-3} in sterile saline solution. Thereafter, 100 μl of the last two dilutions (10^{-2} and 10^{-3}) were plated on Medium 79 of Fred and Waskman (1928), otherwise known as YMA medium, with Congo red (Vincent 1970), using two replicates for each dilution. The plates were incubated for two to seven days at 30 °C and, after this, individual colonies were collected to obtain a pure culture of each bacterial isolate. Some nodules did not allow for the isolation of potentially symbiotic bacteria.

Table 2 Chemical and granulometric characteristics of the soil samples from the three collection sites (Posse, Brasília and Cavalcante), in the depth of 0 to 20 cm

Collection sites	Ca cmolc/dm3	Mg cmolc/dm3	Al cmolc/dm3	H+AL cmolc/dm3	K mg/dm3	CEC	P mg/dm3	OM %	$pHCaCl_2$	N %	Clay %	Silt %	Sand %
Posse	15.0	1.6	0.0	1.9	291	19.2	8.1	11.0	6.3	0.80	30	8	62
Brasília	0.6	0.2	0.6	7.5	48	8.4	0.5	4.5	4.2	0.14	48	12	40
Cavalcante	0.2	0.1	0.8	2.9	40	3.3	0.5	1.7	4.3	0.13	9	4	87

CEC, Cation exchange capacity; *OM*, Organic matter

DNA extraction, amplification and sequencing

Bacteria isolated from nodules formed during the experiment with trap plants were cultured in YMA solid medium with Congo red for 72 h at 28 °C. Thereafter, a purified colony was transferred into YMA liquid medium for 24 h at 28 °C. Subsequently, bacterial DNA was extracted using Pure Link Genomic DNA Kits (Invitrogen), following manufacturer's instructions.

The extracted DNA from each isolate was used as template for PCR reactions and the sequencing of five genes: 16S rRNA, *recA, gyrB*, *nodC* and *nifH*, which are widely used in phylogenetic studies with symbiotic bacteria (e.g. Peix et al. 2015). The PCR products were generated using Dr. Max DNA polymerase (MGMED, Co.) and sequenced in both directions (Macrogen, Korea). Primers used in gene amplification are listed in Table S2. The sequences obtained (GenBank accession numbers MG182874-MG183123) were compared to sequences deposited in the GenBank database (Table S3).

Phylogenetic analyses

To characterize the isolates at taxonomic level, representative sequences of several bacterial strains were obtained from GenBank and aligned with the sequences generated in this work using ClustalW, imported into BioEdit 4.8.4 (Hall 1999) and manually corrected. Phylogenetic analysis based on the 16S rRNA, *recA*, *gyrB*, *nodC* and *nifH* genes was performed following a maximum likelihood analysis (ML) implemented by the RAxML-HPC v.8 program using the GTR-CAT nucleotide substitution model (Stamatakis 2006) from the CIPRES portal (Miller et al. 2010). To obtain support values, data sets were retested a thousand times using the bootstrap method (Felsenstein 1985). Individual phylogenetic trees were constructed with the sequences aligned for each of the previously cited genomic DNA regions, inferring genetic distance and similarity among the studied bacteria. Sequences from reference strains closest to the genera *Paraburkholderia*, *Cupriavidus*, *Burkholderia*, *Rhizobium*, *Bradyrhizobium*, *Ensifer* and *Mesorhizobium* were included in the phylograms of the five genes. Whenever possible, sequences from the same reference species were used for all five genes. In addition, a phylogeny based on concatenated sequences of 16S rDNA, *recA* and *gyrB* was generated.

Evaluation of nodulation capacity

Confirmation of nodulation capacity was verified by means of an authentication experiment. The species *M. pudica* L. was chosen as a "model host" because it is a fast-growing species and has an ability to nodulate with a wide range of rhizobia, mainly β-proteobacteria (Chen et al. 2005a; Bontemps et al. 2010; Mishra et al. 2012), but also some *Rhizobium* species (Elliott et al. 2009; Baraúna et al. 2016). Seeds were sterilized and their dormancy was broken as cited before. Subsequently these seeds were pre-germinated on a cotton tray soaked with sterile dH_2O and incubated for 48 h at 27 °C.

Seedlings were tested in a perlite substrate according to Elliott et al. (2009). Glass tubes (50 ml volume) were half-filled with perlite and then autoclaved at 121 °C and 1 atm for 30 min. Sterilized Hoagland nutrient solution (Hoagland and Arnon 1938) without N was applied to the perlite until the saturation point. The seedlings were inoculated with one ml culture of the isolates grown in YMA liquid medium for 48 h. The possibility of cross-contamination was investigated using an uninoculated negative control randomly inserted among treatments. All plants remained in a growth chamber with controlled temperature (26 °C) and 16 h light / 8 h dark cycle. After one month the plants were collected and the presence / absence of nodules was recorded. Because the *Rhizobium* isolates did not nodulate effectively with *M. pudica*, we also used Siratro (*Macroptilium atropurpureum* Moc. & Sessé ex DC.) as an additional host, since this is a promiscuous papilionoid species with high capacity for nodulating with α-proteobacteria (Elliott et al. 2007b; Mishra et al. 2012). Comparisons between the vigour of inoculated and control plants, as well as observation of effective nodules, were used as qualitative evidence for the symbiotic capacity of rhizobial isolates.

Results

Plant biomass and number of nodules

All *Mimosa* species grew in their original and in the other tested soils (Fig. 1). *M. acutistipula* and *M. xanthocentra*, from Posse, produced higher biomass in their soil of origin compared to the biomass obtained in other soils (Table 3). *Mimosa kalunga* and *Mimosa* sp. from Cavalcante, and *M. claussenii* and *M. radula* from Brasília, also produced slightly more biomass in the soil from Posse, the most fertile of all soils, but these differences, with the exception of *M. kalunga*, were not statistically significant (Table 3).

Most *Mimosa* species were able to nodulate in the three soil types, the exceptions being *M. claussenii* and *M. xanthocentra*, which did not nodulate in soils from Posse and Cavalcante, respectively. The species from Posse, *M. acutistipula* and *M. xanthocentra*, showed higher number of nodules in their original soil than the other species growing with this same substrate. These two species also had higher nodulation in their original soil when compared to their nodulation in soils from Brasília and Cavalcante. The other species, with the exception of *M. claussenii*, did not have a notable nodulation difference when the three soils were compared (Table 3). There was no nodulation on plants grown in the control with sterilized sand.

Identification of rhizobia using sequences of the housekeeping genes 16S rRNA, *recA* and *gyrB*

The 54 isolates obtained in this study were identified at least to genus level by comparing their 16S rRNA and *recA* gene sequences with those in the GenBank database using the BLASTN algorithm (Altschul et al. 1990). The BLASTN results classified them in the α- and β-classes of Proteobacteria and showed that they shared a high similarity (97% - 100%) with already known species of bacteria (data not shown). Nine isolates were assigned to the genus *Rhizobium*, while the other 45 isolates belonged to the genus *Paraburkholderia*. All bacteria isolated from nodules of plants growing on soils from Brasília and Cavalcante were identified as *Paraburkholderia* spp. On the other hand, all isolates from Posse grouped within *Rhizobium*, except isolate POS_MSP2 that grouped with *Paraburkholderia* (Table 4).

Individual phylogenies based on housekeeping genes (16S rRNA, *recA* and *gyrB*) were highly congruent, although the 16S rRNA tree was less resolved (Figs. S1-S3). A phylogeny based on the concatenation of these three genes (Fig. 2) separated the isolates between the α- and β- proteobacteria, and confirmed that all tested *Mimosa* species associated with bacteria from both classes, except for *M. claussenii* which did not nodulate with *Rhizobium*.

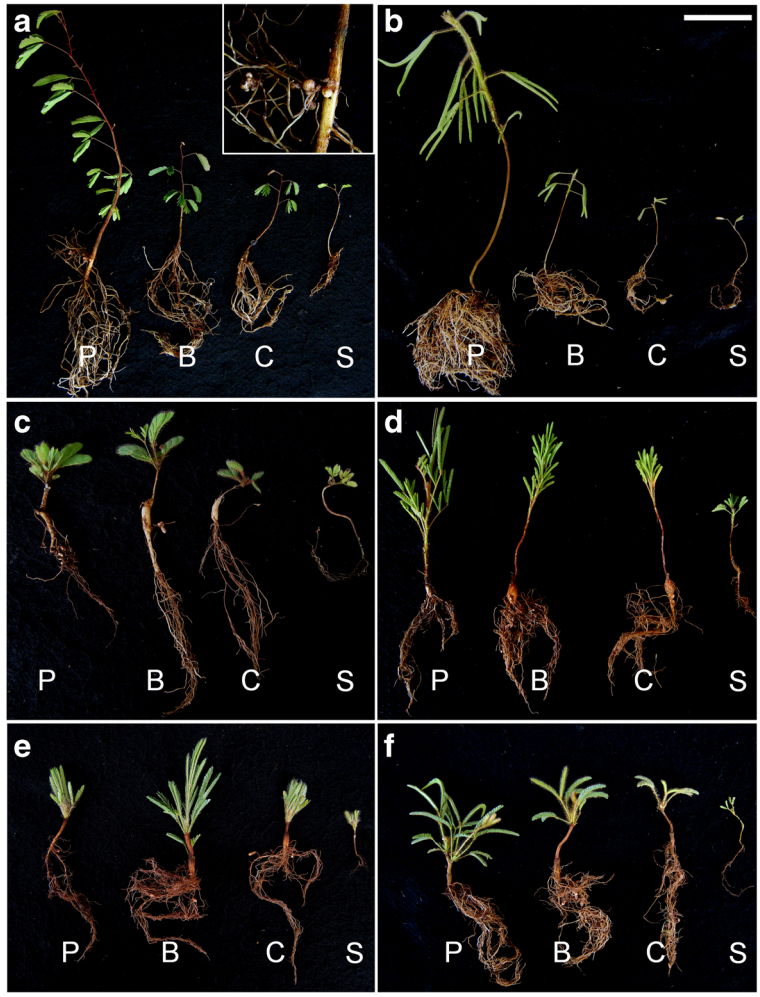


Fig. 1 Plants of six *Mimosa* species (**a** *M. acutistipula* and detail of nodules, **b** *M. xanthocentra*, **c** *M. radula*, **d** *Mimosa* sp., **e** *M. kalunga*, **f** *M. claussenii*) used in the trap experiment with soils from three different sites in central Brazil (P: Posse, B: Brasília, C: Cavalcante) (S: sand - negative control). Scale bar = 5 cm

The α-proteobacteria isolates grouped in a clade containing three *Rhizobium* strains from the study of Bontemps et al. (2016) and with *R. altiplani*, a newly-described species of *Rhizobium* associated with *M. pudica* in central Brazil (Baraúna et al. 2016) (Fig. 2). Isolates identified as β-proteobacteria

Table 3 Total biomass and number of nodules (mean ± standard deviation) of the six *Mimosa* species in the different soil types from central Brazil (Posse, Brasília and Cavalcante)

Species	Posse soil		Brasília soil		Cavalcante soil		Control (Sand)	
	Biomass* (g)	N° nodules	Biomass* (g)	N° nodules	Biomass* (g)	N° nodules	Biomass (g)	N° nodules
M. acutistipula	0.52a ±0.21	14.3a ±3.0	0.11b ±0.02	8.0b ±1.7	0.16b ±0.06	8.0b ±4.6	0.02 ± 0.01	0 ± 0
M. claussenii	0.70a ±0.39	0.0b ±0.0	0.53a ±0.13	7.3a ±2.5	0.24a ±0.03	7.0a ±2.3	0.05 ± 0.02	0 ± 0
M. kalunga	0.41a ±0.05	3.0a ±1.7	0.19b ±0.00	3.5a ±1.3	0.24b ±0.02	0.8a ±0.7	0.03 ± 0.01	0 ± 0
M. radula	0.20a ±0.20	1.8a ±2.4	0.11a ±0.03	2.0a ±1.8	0.06a ±0.02	0.6a ±0.5	0.02 ± 0.01	0 ± 0
Mimosa sp.	0.49a ±0.14	1.6a ±2.0	0.31a ±0.09	3.8a ±0.8	0.28a ±0.01	3.5a ±2.6	0.04 ± 0.02	0 ± 0
M. xanthocentra	2.31a ±0.72	120.6a ±16.7	0.14b ±0.00	8.2b ±7.1	0.04b ±0.01	0.0b ±0.0	0.02 ± 0.01	0 ± 0

*Values followed by the same letter, in the lines, are not different according to Duncan's test ($p < 0.05$)

Table 4 Bacterial (rhizobia) isolates obtained from a trap experiment using six species of *Mimosa* growing in soils from three different sites in central Brazil. Identification of the isolates at the genus level was based on the sequencing of five genes. The nodulation column indicates the result of an authentication experiment using *M. pudica* as plant host (+++ = effective nodulation; + = ineffective nodulation). For some *Rhizobium* isolates, an authentication experiment was also performed in the alternative host *Macroptilium atropurpureum* (nodulation effectiveness in parentheses)

Isolate	Identification (genus)	Host species	Site	Nodulation
POS_MAC1	*Rhizobium*	*M. acutistipula*	Posse	+ (+++)
POS_MAC2	*Rhizobium*	*M. acutistipula*	Posse	+
POS_MAC3	*Rhizobium*	*M. acutistipula*	Posse	+ (+++)
POS_MKA1	*Rhizobium*	*M. kalunga*	Posse	+
POS_MKA2	*Rhizobium*	*M. kalunga*	Posse	+ (+++)
POS_MRA1	*Rhizobium*	*M. radula*	Posse	+ (+++)
POS_MSP1	*Rhizobium*	*Mimosa* sp.	Posse	+ (+++)
POS_MSP2	*Paraburkholderia*	*Mimosa* sp.	Posse	+++
POS_MXA1	*Rhizobium*	*M. xanthocentra*	Posse	+
POS_MXA2	*Rhizobium*	*M. xanthocentra*	Posse	+
BSB_MAC1	*Paraburkholderia*	*M. acutistipula*	Brasília	+++
BSB_MAC2	*Paraburkholderia*	*M. acutistipula*	Brasília	+++
BSB_MAC3	*Paraburkholderia*	*M. acutistipula*	Brasília	+++
BSB_MCL1	*Paraburkholderia*	*M. claussenii*	Brasília	+++
BSB_MCL2	*Paraburkholderia*	*M. claussenii*	Brasília	+++
BSB_MCL3	*Paraburkholderia*	*M. claussenii*	Brasília	+++
BSB_MCL4	*Paraburkholderia*	*M. claussenii*	Brasília	+++
BSB_MKA1	*Paraburkholderia*	*M. kalunga*	Brasília	+++
BSB_MKA2	*Paraburkholderia*	*M. kalunga*	Brasília	+++
BSB_MKA3	*Paraburkholderia*	*M. kalunga*	Brasília	+++
BSB_MKA4	*Paraburkholderia*	*M. kalunga*	Brasília	+++
BSB_MKA5	*Paraburkholderia*	*M. kalunga*	Brasília	+++
BSB_MRA1	*Paraburkholderia*	*M. radula*	Brasília	+++
BSB_MRA2	*Paraburkholderia*	*M. radula*	Brasília	+++
BSB_MRA3	*Paraburkholderia*	*M. radula*	Brasília	+++
BSB_MRA4	*Paraburkholderia*	*M. radula*	Brasília	+++
BSB_MSP1	*Paraburkholderia*	*Mimosa* sp.	Brasília	+++
BSB_MSP2	*Paraburkholderia*	*Mimosa* sp.	Brasília	+++
BSB_MSP3	*Paraburkholderia*	*Mimosa* sp.	Brasília	+++
BSB_MSP4	*Paraburkholderia*	*Mimosa* sp.	Brasília	+++
BSB_MSP5	*Paraburkholderia*	*Mimosa* sp.	Brasília	+++
BSB_MSP6	*Paraburkholderia*	*Mimosa* sp.	Brasília	+++
BSB_MXA1	*Paraburkholderia*	*M. xanthocentra*	Brasília	+++
CAV_MAC1	*Paraburkholderia*	*M. acutistipula*	Cavalcante	+++
CAV_MAC2	*Paraburkholderia*	*M. acutistipula*	Cavalcante	+++
CAV_MAC3	*Paraburkholderia*	*M. acutistipula*	Cavalcante	+++
CAV_MAC4	*Paraburkholderia*	*M. acutistipula*	Cavalcante	+++
CAV_MCL1	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++
CAV_MCL2	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++
CAV_MCL3	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++
CAV_MCL4	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++

Table 4 (continued)

Isolate	Identification (genus)	Host species	Site	Nodulation
CAV_MCL5	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++
CAV_MCL6	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++
CAV_MCL7	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++
CAV_MCL8	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++
CAV_MCL9	*Paraburkholderia*	*M. claussenii*	Cavalcante	+++
CAV_MKA1	*Paraburkholderia*	*M. kalunga*	Cavalcante	+++
CAV_MKA2	*Paraburkholderia*	*M. kalunga*	Cavalcante	+++
CAV_MKA3	*Paraburkholderia*	*M. kalunga*	Cavalcante	+++
CAV_MSP1	*Paraburkholderia*	*Mimosa* sp.	Cavalcante	+++
CAV_MSP2	*Paraburkholderia*	*Mimosa* sp.	Cavalcante	+++
CAV_MSP3	*Paraburkholderia*	*Mimosa* sp.	Cavalcante	+++
CAV_MSP4	*Paraburkholderia*	*Mimosa* sp.	Cavalcante	+++

grouped in *Paraburkholderia*, forming two distinct clades: one of them containing *P. nodosa*, and the other with *P. tuberum* and *P. sprentiae*. The first clade contains mostly isolates from Brasília and the second from Cavalcante (Figs. 2, S1-S3).

Phylogenetic analysis of the symbiotic *nodC* and *nifH* genes

Sequences of the 54 isolates were obtained for *nodC*, but *nifH* sequences were obtained for only 46 of them due to problems with PCR amplification. The tree topologies based on these two genes involved in nodulation (*nodC*) and nitrogen fixation (*nifH*) were similar to that of the other housekeeping genes, with the isolates also distributed within their respective α- and β-rhizobial groups (Figs. 3 and 4).

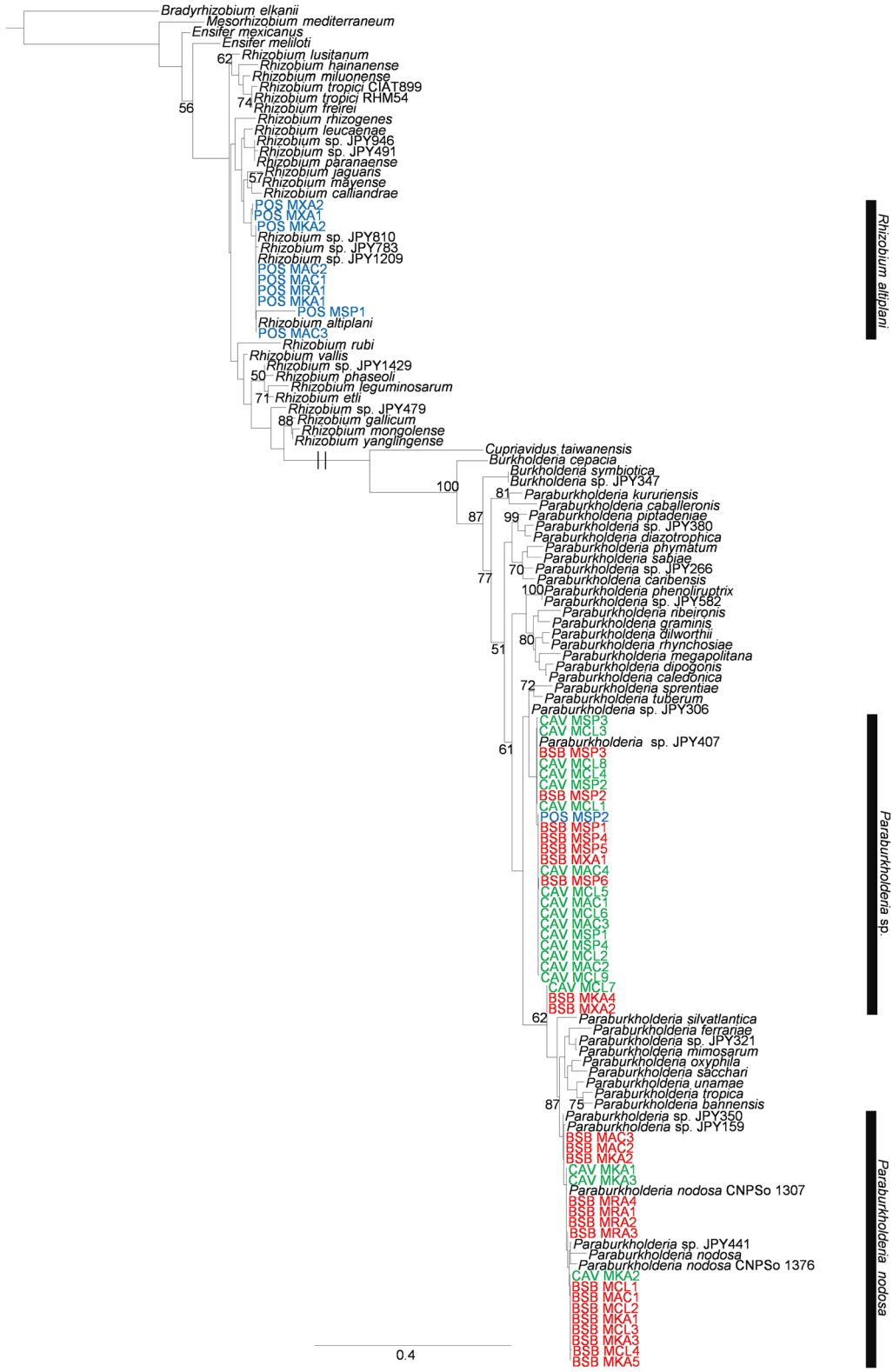
In the *nodC* and *nifH* phylogenies the isolates that grouped with α-proteobacteria were contained in a clade closest to *Rhizobium* sp. strain JPY479 which was isolated from *M. xanthocentra* by Bontemps et al. (2010). In general, the isolates identified as β-proteobacteria were grouped into two *nodC* clades, one closest to *P. tuberum* sv. mimosae and another to *P. nodosa*. For *nifH* two clades were also formed, one closest to *P. mimosarum* and *P. nodosa* and the other with strain JPY306 (Bontemps et al. 2010), which was subsequently placed in *P. tuberum* sv. mimosae by Mishra et al. (2012) along with several other strains from central Brazil.

Evaluation of nodulation capacity

The nodulation ability of all rhizobia isolated in the trap experiment was tested in *M. pudica*, a species known to be promiscuous. In this experiment all rhizobia confirmed their nodulation capacity, but the nine *Rhizobium* isolates (all obtained from Posse soil) formed small nodules with white coloration in their interior indicating ineffective nodulation. On the other hand, five of these *Rhizobium* isolates were tested on the alternative host Siratro and showed effective nodulation of it (Fig. S4). The 20 isolates from Cavalcante and the 24 isolates from Brasília soil, all belonging to the genus *Paraburkholderia*, resulted in effective nodulation in *M. pudica*, with nodules showing pinkish-red coloration in their interior, a sign of the presence of the symbiosis-essential protein leghemoglobin (Table 4). The strains of *Paraburkholderia* that nodulated *M. pudica* and those of *Rhizobium* that nodulated Siratro resulted in healthier (green leaves) and more vigorous plants compared to non-inoculated controls (which had no nodules and reduced growth; Fig. S4); taken together, these data provide compelling qualitative evidence for the symbiotic capacity of most of the isolates from this study.

Discussion

We observed a clear difference in the development of some species of *Mimosa* grown in different types of soil


Bradyrhizobium elkanii
Mesorhizobium mediterraneum
Ensifer mexicanus
Ensifer meliloti
62
Rhizobium lusitanum
Rhizobium hainanense
Rhizobium miluonense
Rhizobium tropici CIAT899
74
Rhizobium tropici RHM54
56
Rhizobium freirei
Rhizobium rhizogenes
Rhizobium leucaenae
Rhizobium sp. JPY946
Rhizobium sp. JPY491
Rhizobium paranaense
57
Rhizobium jaguaris
Rhizobium mayense
Rhizobium calliandrae
POS MXA2
POS MXA1
POS MKA2
Rhizobium sp. JPY810
Rhizobium sp. JPY783
Rhizobium sp. JPY1209
POS MAC2
POS MAC1
POS MRA1
POS MKA1
POS MSP1
Rhizobium altiplani
POS MAC3
Rhizobium rubi
Rhizobium vallis
Rhizobium sp. JPY1429
50
Rhizobium phaseoli
Rhizobium leguminosarum
71
Rhizobium etli
Rhizobium sp. JPY479
88
Rhizobium gallicum
Rhizobium mongolense
Rhizobium yanglingense
Cupriavidus taiwanensis
Burkholderia cepacia
Burkholderia symbiotica
Burkholderia sp. JPY347
100
81
Paraburkholderia kururiensis
Paraburkholderia caballeronis
87
99
Paraburkholderia piptadeniae
Paraburkholderia sp. JPY380
Paraburkholderia diazotrophica
Paraburkholderia phymatum
Paraburkholderia sabiae
70
Paraburkholderia sp. JPY266
77
Paraburkholderia caribensis
100
Paraburkholderia phenoliruptrix
Paraburkholderia sp. JPY582
Paraburkholderia ribeironis
Paraburkholderia graminis
80
Paraburkholderia dilworthii
51
Paraburkholderia rhynchosiae
Paraburkholderia megapolitana
Paraburkholderia dipogonis
Paraburkholderia caledonica
72
Paraburkholderia sprentiae
Paraburkholderia tuberum
Paraburkholderia sp. JPY306
CAV MSP3
CAV MCL3
61
Paraburkholderia sp. JPY407
BSB MSP3
CAV MCL8
CAV MCL4
CAV MSP2
BSB MSP2
CAV MCL1
POS MSP2
BSB MSP1
BSB MSP4
BSB MSP5
BSB MXA1
CAV MAC4
BSB MSP6
CAV MCL5
CAV MAC1
CAV MCL6
CAV MAC3
CAV MSP1
CAV MSP4
CAV MCL2
CAV MAC2
CAV MCL9
CAV MCL7
BSB MKA4
BSB MXA2
62
Paraburkholderia silvatlantica
Paraburkholderia ferrariae
Paraburkholderia sp. JPY321
Paraburkholderia mimosarum
Paraburkholderia oxyphila
Paraburkholderia sacchari
Paraburkholderia unamae
Paraburkholderia tropica
87
75
Paraburkholderia bannensis
Paraburkholderia sp. JPY350
Paraburkholderia sp. JPY159
BSB MAC3
BSB MAC2
BSB MKA2
CAV MKA1
CAV MKA3
Paraburkholderia nodosa CNPSo 1307
BSB MRA4
BSB MRA1
BSB MRA2
BSB MRA3
Paraburkholderia sp. JPY441
Paraburkholderia nodosa
Paraburkholderia nodosa CNPSo 1376
CAV MKA2
BSB MCL1
BSB MAC1
BSB MCL2
BSB MKA1
BSB MCL3
BSB MKA3
BSB MCL4
BSB MKA5
0.4
Rhizobium altiplani
Paraburkholderia sp.
Paraburkholderia nodosa

◀ **Fig. 2** Maximum-likelihood tree based on concatenated 16S rRNA, *recA* and *gyrB* gene sequences (2571 bp) showing the phylogenetic relationship between *Mimosa* nodulating rhizobia isolates (this work) and reference strains (GenBank). Support values (1000 bootstrap replicates >50%) are shown. Scale bar in number of substitutions per site. Isolates code: Sites/Soils: POS = Posse (blue); BSB = Brasília (red); CAV = Cavalcante (green). Host plant: MAC = *M. acutistipula*; MCL = *M. claussenii*; MKA = *M. kalunga*; MRA = *M. radula*; MSP = *Mimosa* sp.; MXA = *M. xanthocentra*

from central Brazil. Plants growing in the soil from Posse, which was the most fertile, tended to produce more biomass, which was statistically significant for the local species *M. acutistipula* and *M. xanthocentra*, as well as *M. kalunga* (Cavalcante endemic). Neither *M. acutistipula* nor *M. xanthocentra* performed well in the lower fertility Brasília and Cavalcante soils, even in the presence of potential symbiotic associations, suggesting that these plants are not well adapted to poor soils. On the other hand, most species native to Brasília and Cavalcante did not perform better when grown in richer soil (Posse), suggesting that they are better adapted to low fertility soils. Regarding the number of nodules, the mimosas did not show differences related to the soil types, except for the species from Posse, *M. acutistipula* and *M. xanthocentra*, which produced more nodules when growing in their original soil. Most likely, this large number of nodules found in an environment with high N and organic matter content may be related to the adaptation of both symbiotic partners (plant and rhizobia) to this environment. There are several studies supporting the concept that biological nitrogen fixation (BNF) is often (but not invariably) more active where the N supply is low (Vitousek et al. 2013). On the other hand, even in environments considered rich in N, BNF may be important to compensate for losses of this nutrient, thus avoiding its depletion in these soils (Pons et al. 2007).

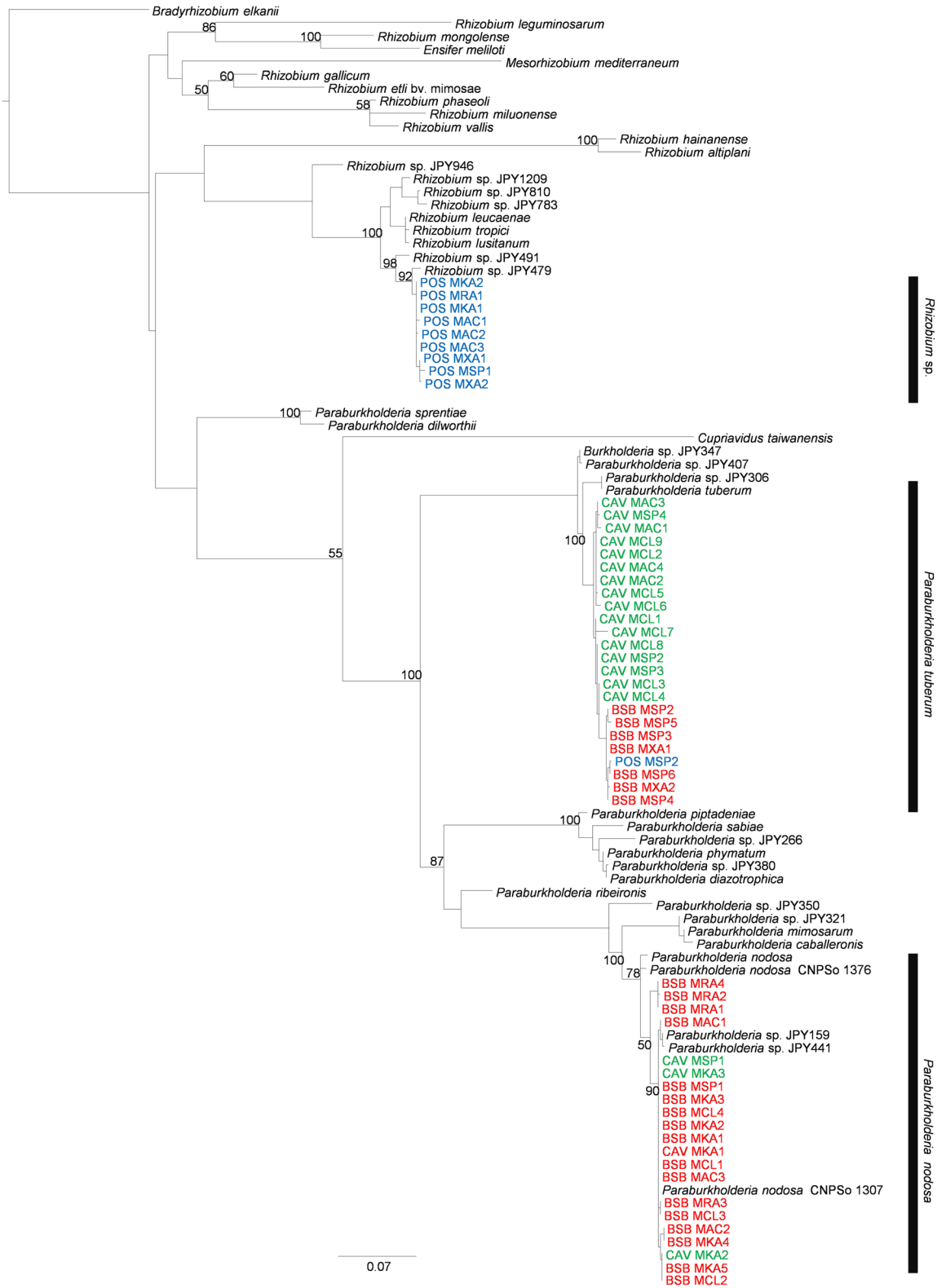
The analysis of the 16S rRNA, *recA* and *gyrB* genes demonstrated that α- and β-rhizobia were found in this study. Overall, the rhizobia found here are in accordance with the high incidence of both α- and β-proteobacteria in the soil microbiota as detected in metagenomics studies carried out in different sites across the Cerrado region (Quirino et al. 2009; Araújo et al. 2012; Castro et al. 2016).

Rhizobium strains that predominated in Posse were recovered in our phylogenetic analyses as closely related to *R. altiplani* and to other *Rhizobium* strains isolated from *Mimosa* species. *R. altiplani* was recorded associated with *M. pudica* growing in an anthropogenic neutral to alkaline soil (pH 7.7) in the Distrito Federal in central Brazil (Baraúna et al. 2016). Isolates obtained from nodules of *Mimosa* spp. growing in relatively fertile soils with neutral-alkaline pH in Mexico (Bontemps et al. 2016) also grouped within the clade that contains our *Rhizobium* isolates and *R. altiplani* (Fig. 2). These observations reinforce the preference of *Rhizobium* isolates for less acidic and more fertile soils.

The isolates of *Paraburkholderia* obtained in our experiment were closest to *P. nodosa*, *P. tuberum*, and *P. sprentiae*, and hence were similar to rhizobia isolated from other studies that sampled nodules from *Mimosa* in different countries, including many localities in Brazil (Chen et al. 2005a, 2006, 2007). For example, several strains of *P. nodosa* and *P. tuberum*-like bacteria, together with *P. mimosarum*, *P. diazotrophica*, and other unidentified *Paraburkholderia* isolates were obtained from different sites in the Cerrado and Caatinga biomes in central and north-eastern Brazil (Bontemps et al. 2010) where acidic soils with low amounts of available nutrients occur, such as those found in the Brasília and Cavalcante sites in the present study.

Paraburkholderia nodosa has *M. bimucronata* and *M. scabrella*, from south Brazil as its original hosts (Chen et al. 2005a, 2007; Lammel et al. 2013), but it is also capable of nodulating with many different *Mimosa* species (Bontemps et al. 2010), and is very widespread in South America. Indeed, *P. nodosa* was also the main species found in nodules of common bean (*Phaseolus vulgaris*) when it was used as trap plants, showing its ability to associate with other legumes in Cerrado soils (Dall'Agnol et al. 2016).

Along with *P. nodosa*, bacteria related to *P. tuberum* are among the most commonly found β-rhizobia associated with different *Mimosa* species in Brazil and elsewhere in South America and Mexico (Bontemps et al. 2010, 2016; Mishra et al. 2012; Lammel et al. 2013). Closely related bacteria belonging to *P. tuberum* sensu stricto were originally isolated from the papilionoid legume *Aspalathus carnosa* L. in South Africa (Vandamme et al. 2002), and the type strain of this species (STM678^T) is capable of nodulating many native South African papilionoid legumes (Elliott et al. 2007b; Beukes et al. 2013; Lemaire et al. 2015, 2016). Indeed, it has been proposed that *P. tuberum* has two symbiovars (sv. mimosae and sv. papilionoideae) in


Bradyrhizobium elkanii
Rhizobium leguminosarum
Rhizobium mongolense
Ensifer meliloti
Mesorhizobium mediterraneum
Rhizobium gallicum
Rhizobium etli bv. mimosae
Rhizobium phaseoli
Rhizobium miluonense
Rhizobium vallis
Rhizobium hainanense
Rhizobium altiplani
Rhizobium sp. JPY946
Rhizobium sp. JPY1209
Rhizobium sp. JPY810
Rhizobium sp. JPY783
Rhizobium leucaenae
Rhizobium tropici
Rhizobium lusitanum
Rhizobium sp. JPY491
Rhizobium sp. JPY479
POS MKA2
POS MRA1
POS MKA1
POS MAC1
POS MAC2
POS MAC3
POS MXA1
POS MSP1
POS MXA2
Paraburkholderia sprentiae
Paraburkholderia dilworthii
Cupriavidus taiwanensis
Burkholderia sp. JPY347
Paraburkholderia sp. JPY407
Paraburkholderia sp. JPY306
Paraburkholderia tuberum
CAV MAC3
CAV MSP4
CAV MAC1
CAV MCL9
CAV MCL2
CAV MAC4
CAV MAC2
CAV MCL5
CAV MCL6
CAV MCL1
CAV MCL7
CAV MCL8
CAV MSP2
CAV MSP3
CAV MCL3
CAV MCL4
BSB MSP2
BSB MSP5
BSB MSP3
BSB MXA1
POS MSP2
BSB MSP6
BSB MXA2
BSB MSP4
Paraburkholderia piptadeniae
Paraburkholderia sabiae
Paraburkholderia sp. JPY266
Paraburkholderia phymatum
Paraburkholderia sp. JPY380
Paraburkholderia diazotrophica
Paraburkholderia ribeironis
Paraburkholderia sp. JPY350
Paraburkholderia sp. JPY321
Paraburkholderia mimosarum
Paraburkholderia caballeronis
Paraburkholderia nodosa
Paraburkholderia nodosa CNPSo 1376
BSB MRA4
BSB MRA2
BSB MRA1
BSB MAC1
Paraburkholderia sp. JPY159
Paraburkholderia sp. JPY441
CAV MSP1
CAV MKA3
BSB MSP1
BSB MKA3
BSB MCL4
BSB MKA2
BSB MKA1
CAV MKA1
BSB MCL1
BSB MAC3
Paraburkholderia nodosa CNPSo 1307
BSB MRA3
BSB MCL3
BSB MAC2
BSB MKA4
CAV MKA2
BSB MKA5
BSB MCL2
0.07
Rhizobium sp.
Paraburkholderia tuberum
Paraburkholderia nodosa

◀ **Fig. 3** Maximum-likelihood tree based on the nodC gene sequences (610 bp) showing the phylogenetic relationship between *Mimosa* nodulating rhizobia isolates (this work) and reference strains (GenBank). Support values (1000 bootstrap replicates >50%) are shown. Scale bar in number of substitutions per site. Isolates code: Sites/Soils: POS = Posse (blue); BSB = Brasília (red); CAV = Cavalcante (green). Host plant: MAC = *M. acutistipula*; MCL = *M. claussenii*; MKA = *M. kalunga*; MRA = *M. radula*; MSP = *Mimosa* sp.; MXA = *M. xanthocentra*

terms of host, geographical distribution and nod gene phylogeny. The sv. papilionoideae strains from South Africa cannot nodulate with *Mimosa* (Elliott et al. 2007b; Mishra et al. 2012; Estrada-de los Santos et al. 2016; Lemaire et al. 2016; de Meyer et al. 2016).

Paraburkholderia sprentiae was originally isolated from root nodules of *Lebeckia ambigua* E. Mey. growing in the Western Cape of South Africa; it is most closely related to *P. tuberum* (De Meyer et al. 2013), and it has not previously been isolated from any legume outside South Africa. It should be noted, however, that the large species complex represented by *P. tuberum* and *P. sprentiae* is currently being revised (Venter, Steenkamp, James & de Meyer, unpublished), and it is likely that the Neotropical "*B. tuberum* - *B. sprentiae*" will be allocated to a new and separate species which preferentially nodulates *Mimosa* and related mimosoids.

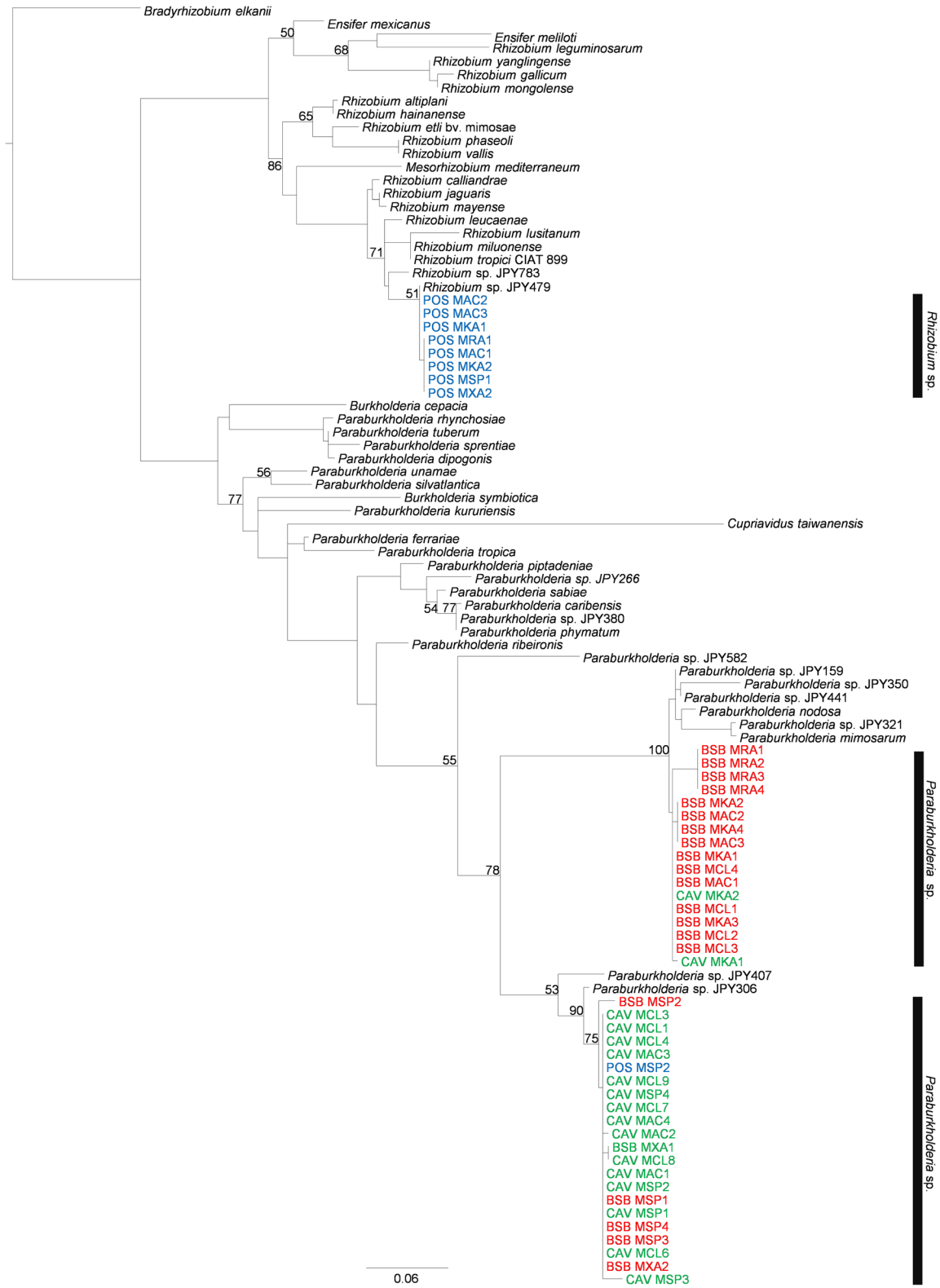
The *nodC* gene was sequenced for all isolates, indicating their capacity for nodulation. Probably owing to PCR amplification problems, we were unable to obtain amplicons of the *nifH* gene for some of the isolates. This does not imply that such isolates are ineffective symbionts that are not able to fix N_2, since unsuccessful PCR reactions may be attributed to different causes, such as primer mismatch or poor DNA quality (Howieson et al. 2013).

The strains that grouped with α-proteobacteria in the *nodC* and *nifH* phylogenies were closely related to *Rhizobium* sp. strain JPY479. This strain was originally isolated from nodules of *M. xanthocentra* in Mato Grosso – Brazil, and it is one of only two α-proteobacterial strains isolated from *Mimosa* spp. in the study of Bontemps et al. (2010). According to Bontemps et al. (2010) the *nifH* and *nodC* genes of these strains were related to previously described α-proteobacterial symbionts of *Mimosa* isolated elsewhere; they are close to *R. tropici* and group with *Rhizobium* strains commonly isolated from Mexican *Mimosa* species (Bontemps et al. 2016).

The authentication experiment confirmed that all isolates tested were able to nodulate with *M. pudica*. Although both *Paraburkholderia* and *Rhizobium* induced the formation of nodules in this host, the nodules formed by *Rhizobium* were small and did not appear to be effective. *Mimosa pudica* is known to be a trap plant suitable for β-rhizobia, but the same is not generally true for α-rhizobium, and this may have influenced the effectiveness of nodulation on *M. pudica* by the α-rhizobial strains (Chen et al. 2005a; Elliott et al. 2009; Bontemps et al. 2010, 2016; Mishra et al. 2012; Klonowska et al. 2012; Gehlot et al. 2013; Melkonian et al. 2014). On the other hand, five out of the nine *Rhizobium* isolates were tested and effectively nodulated the alternative host Siratro, which is a promiscuous papilionoid species, confirming that the *Rhizobium* strains obtained here are genuine legume symbionts.

Our study revealed that the predominance of certain rhizobia in *Mimosa* nodules depends on the soil properties. The most acidic and less fertile soils (Brasília and Cavalcante) favoured the association of *Mimosa* with *Paraburkholderia*, while the soil with pH close to neutral and with higher fertility (Posse) led to the association with *Rhizobium*. Only one *Paraburkholderia* strain (POS_MSP2) was isolated in Posse.

Previous studies have demonstrated that *Mimosa* species tend to associate with α-proteobacteria when growing in soils with neutral/alkaline pH, such as those from Posse. For example, mimosas in India, where soil pH ranged from 7.8 to 8.2 (Gehlot et al. 2013), and in Mexico from 6.5 to 7.8 (Bontemps et al. 2016), were found in association with α-proteobacteria (*Ensifer* and *Rhizobium*). Interestingly, reports of *Rhizobium* associated with *Mimosa* in Brazilian soils, and in the Cerrado in particular, are very rare (Bontemps et al. 2010; Reis Junior et al. 2010). In fact, most host species used in our experiment have already been reported as nodulating with *Paraburkolderia* (Bontemps et al. 2010; Reis Junior et al. 2010), but the only one that has been previously found nodulating with *Rhizobium* was *M. xanthocentra* (Bontemps et al. 2010). One possible reason for such high prevalence of *Paraburkholderia* is that previous studies have mainly sampled in sites of low fertility acidic soils, which are more typical in the Cerrado region, the main centre of *Mimosa* diversity (Simon and Proença 2000). For the very few previous records of *Rhizobium* isolation from *Mimosa* carried out in this biome, there is no specific information on soil


Bradyrhizobium elkanii
Ensifer mexicanus
Ensifer meliloti
Rhizobium leguminosarum
Rhizobium yanglingense
Rhizobium gallicum
Rhizobium mongolense
Rhizobium altiplani
Rhizobium hainanense
Rhizobium etli bv. mimosae
Rhizobium phaseoli
Rhizobium vallis
Mesorhizobium mediterraneum
Rhizobium calliandrae
Rhizobium jaguaris
Rhizobium mayense
Rhizobium leucaenae
Rhizobium lusitanum
Rhizobium miluonense
Rhizobium tropici CIAT 899
Rhizobium sp. JPY783
Rhizobium sp. JPY479
POS MAC2
POS MAC3
POS MKA1
POS MRA1
POS MAC1
POS MKA2
POS MSP1
POS MXA2
Rhizobium sp.
Burkholderia cepacia
Paraburkholderia rhynchosiae
Paraburkholderia tuberum
Paraburkholderia sprentiae
Paraburkholderia dipogonis
Paraburkholderia unamae
Paraburkholderia silvatlantica
Burkholderia symbiotica
Paraburkholderia kururiensis
Cupriavidus taiwanensis
Paraburkholderia ferrariae
Paraburkholderia tropica
Paraburkholderia piptadeniae
Paraburkholderia sp. JPY266
Paraburkholderia sabiae
Paraburkholderia caribensis
Paraburkholderia sp. JPY380
Paraburkholderia phymatum
Paraburkholderia ribeironis
Paraburkholderia sp. JPY582
Paraburkholderia sp. JPY159
Paraburkholderia sp. JPY350
Paraburkholderia sp. JPY441
Paraburkholderia nodosa
Paraburkholderia sp. JPY321
Paraburkholderia mimosarum
BSB MRA1
BSB MRA2
BSB MRA3
BSB MRA4
BSB MKA2
BSB MAC2
BSB MKA4
BSB MAC3
BSB MKA1
BSB MCL4
BSB MAC1
CAV MKA2
BSB MCL1
BSB MKA3
BSB MCL2
BSB MCL3
CAV MKA1
Paraburkholderia sp.
Paraburkholderia sp. JPY407
Paraburkholderia sp. JPY306
BSB MSP2
CAV MCL3
CAV MCL1
CAV MCL4
CAV MAC3
POS MSP2
CAV MCL9
CAV MSP4
CAV MCL7
CAV MAC4
CAV MAC2
BSB MXA1
CAV MCL8
CAV MAC1
CAV MSP2
BSB MSP1
CAV MSP1
BSB MSP4
BSB MSP3
CAV MCL6
BSB MXA2
CAV MSP3
Paraburkholderia sp.
0.06

◀ **Fig. 4** Maximum-likelihood tree based on the nifH gene sequences (300 bp) showing the phylogenetic relationship between *Mimosa* nodulating rhizobia isolates (this work) and reference strains (GenBank). Support values (1000 bootstrap replicates >50%) are shown. Scale bar in number of substitutions per site. Isolates code: Sites/Soils: POS = Posse (blue); BSB = Brasília (red); CAV = Cavalcante (green). Host plant: MAC = *M. acutistipula*; MCL = *M. claussenii*; MKA = *M. kalunga*; MRA = *M. radula*; MSP = *Mimosa* sp.; MXA = *M. xanthocentra*

characteristics, except for that of *R. altiplani*, which was isolated from *M. pudica* growing in an alkaline soil substrate in a disturbed area close to Brasília (Baraúna et al. 2016). It is important to consider that several patches of highly-fertile limestone soil associated with seasonally dry forests, such as those found in Posse, occur in central Brazil. Therefore, it is likely that in such localities a range of *Rhizobium* strains or even other genera from the α-proteobacteria, as well as less acid-tolerant β-proteobacteria like *Cupriavidus*, would be prevalent.

In addition to environmental factors, such as soil fertility and pH, nitrogen concentration also seems to play a role in rhizobial dominance. In a competition experiment between rhizobia strains, when *Mimosa* is growing in substrate with low concentration of N, the nodulation by *Paraburkholderia* stands out in relation to *Rhizobium*. This situation is only reversed when there is an increase of N concentration in the environment (Elliott et al. 2009; Suárez-Moreno et al. 2012). Our results support these findings since soils from Posse, where *Rhizobium* predominates, have six-fold higher concentrations of nitrogen than in Brasília or Cavalcante (Table 2).

Although *Paraburkholderia* is not restricted to acidic soils, species of this genus have acquired mechanisms to tolerate acidity that enabled them to grow on soils where many other rhizobia groups cannot survive well, which would result in competitive advantages (Stopnisek et al. 2014). A predominance of *Paraburkholderia* was also found in some legume genera in the Cape region of South Africa, where poor and acidic soils seem to favour the association of *Paraburkholderia* with endemic legumes (Garau et al. 2009; Lemaire et al. 2015, 2016).

Contrary to our expectations we did not find pronounced host specificity, since most *Mimosa* species tested were able to nodulate with bacteria from different classes. Most species nodulated with bacteria of both the genera *Paraburkholderia* and *Rhizobium*. The exception was *M. claussenii* which appears not to associate with *Rhizobium*, as evidenced by the fact that it was the only species that failed to nodulate in the soil of Posse wherein a predominance of *Rhizobium* was found. Interestingly, even among the two endemic species, *M. kalunga* and *Mimosa* sp., which are found only in the Cavalcante region, no specificity in terms of α and β-rhizobia was observed. These results partly contradict expectations about symbiotic relationships between rhizobia and endemic *Mimosa* spp. (Bontemps et al. 2010), supporting the view that these plants are more promiscuous than previously thought (Thrall et al. 2011).

This lack of specificity between plant species and bacteria may be related to the presence of different nodulation genes in the genome of several rhizobial strains, allowing a response to different types of flavonoids secreted by the plant, resulting in bacterial infection in different legume species (Peix et al. 2015; Sprent et al. 2017). From a host perspective, even habitat specialists and narrow endemic plant species seem to maintain their ability to communicate and associate with various types of rhizobia, even with those outside their restricted habitat of occurrence. In this case, the capacity of symbiotic association with different types of rhizobia remains dormant in the host genome, even after many generations of coevolution and strong habitat/symbiont specialization. This was observed, for example, with the symbionts of native and endemic *Mimosa* species in Mexico: although their dominant symbionts were α-rhizobia, species belonging to recently diverged clades (1–3 my old) with close relatives in South America had retained their ability to nodulate with *Paraburkholderia* whereas species belonging to clades which had evolved in Mexico for up to 20 my had lost that ability (Bontemps et al. 2016).

Lemaire et al. (2015, 2016) surveying symbiotic relationships in a range of South African native legumes found that the plant genera investigated showed large variation in symbiotic specificity. *Amphithalea* and *Podalyria* were exclusively nodulated by *Paraburkolderia*, whereas *Argyrolobium*, *Otholobium* and *Psoralea* were associated only with α-proteobacteria (*Mesorhizobium* and *Rhizobium*). In contrast, *Aspalathus* and *Indigofera* were associated with a wide diversity of rhizobia including both α- and β-proteobacteria. This large variation in symbiotic preference within these groups may be related to environmental conditions, such as pH, elevation, geology and biogeography (Lemaire et al. 2015, 2016), as previously

proposed in other studies (Mishra et al. 2012; Bournaud et al. 2013; Howieson et al. 2013; Gehlot et al. 2013).

Our results indicate that different *Mimosa* species have the ability to associate with different types of rhizobia (α- and β-proteobacteria), suggesting low specificity between host and bacterium. Even endemic plant species, when grown on soils from different localities, were able to nodulate with a range of bacteria present in non-native soil rhizospheres. Soil factors such as pH, nitrogen, organic matter and fertility seem to favour the predominance of certain types of rhizobia, irrespective of host identity, thus influencing the establishment of symbiotic relationships.

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