

# *Bradyrhizobium agreste* sp. nov., *Bradyrhizobium glycinis* sp. nov. and *Bradyrhizobium diversitatis* sp. nov., isolated from a biodiversity hotspot of the genus *Glycine* in Western Australia

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### Abstract

Strains of the genus Bradyrhizobium associated with agronomically important crops such as soybean (Glycine max) are increasingly studied; however, information about symbionts of wild *Glycine* species is scarce. Australia is a genetic centre of wild *Glycine* species and we performed a polyphasic analysis of three *Bradyrhizobium* strains—CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup>, and CNPSo 4019<sup>T</sup>—trapped from Western Australian soils with *Glycine clandestina*, *Glycine tabacina* and *Glycine max*, respectively. The phylogenetic tree of the 16S rRNA gene clustered all strains into the Bradyrhizobium japonicum superclade; strains CNPSo 4010<sup>T</sup> and CNPSo 4016<sup>T</sup> had *Bradyrhizobium yuanmingense* CCBAU 10071<sup>T</sup> as the closest species, whereas strain CNPSo 4019<sup>T</sup> was closer to Bradyrhizobium liaoningense LMG 18230<sup>T</sup>. The multilocus sequence analysis (MLSA) with five housekeeping genes—dnaK, glnII, gyrB, recA and rpoB—confirmed the same clusters as the 16S rRNA phylogeny, but indicated low similarity to described species, with nucleotide identities ranging from 93.6 to 97.6% of similarity. Considering the genomes of the three strains, the average nucleotide identity and digital DNA-DNA hybridization values were lower than 94.97 and 59.80%, respectively, with the closest species. In the nodC phylogeny, strains CNPSo 4010<sup>T</sup> and CNPSo 4019<sup>T</sup> grouped with Bradyrhizobium zhanjiangense and Bradyrhizobium ganzhouense, respectively, while strain CNPSo 4016<sup>T</sup> was positioned separately from the all symbiotic Bradyrhizobium species. Other genomic (BOX-PCR), phenotypic and symbiotic properties were evaluated and corroborated with the description of three new lineages of Bradyrhizobium. We propose the names of Bradyrhizobium agreste sp. nov. for CNPSo 4010<sup>T</sup> (=WSM 4802<sup>T</sup>=LMG 31645<sup>T</sup>) isolated from *Glycine clandestina*, *Bradyrhizobium glycinis* sp. nov. for CNPSo 4016<sup>T</sup> (=WSM 4801<sup>+</sup>=LMG 31649<sup>+</sup>) isolated from *Glycine tabacina* and *Bradyrhizobium diversitatis* sp. nov. for CNPSo 4019<sup>+</sup> (=WSM  $4799^{T}$  = LMG 31650<sup>T</sup>) isolated from *G. max*.

# **INTRODUCTION**

Balanced levels of N in soil are required to obtain high productivities and healthy plants. Biological nitrogen fixation (BNF) is carried out by a restricted group of prokaryotes able to reduce the atmospheric dinitrogen ( $N_2$ ) to ammonium (NH<sub>3</sub>), the assimilable form by plants [1, 2]. The highest level of evolution of BNF occurs with the symbiotic interaction between diazotrophic bacteria collectively known as rhizobia

and members of the family Fabaceae (=Leguminosae). The symbiosis involves a mutual exchange of molecular signals, culminating in the development of specialized structures on roots, and occasionally on stems, called nodules, in which the BNF process takes place [3, 4]. A variety of elite rhizobial strains have been introduced in agricultural systems for over a century as a sustainable way to improve soil fertility and crop yield, in addition to lowering costs, due to the replacement of

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Keywords: Bradyrhizobium; wild soybean; Glycine; nodulation; MLSA; ANI; dDDH.

Abbreviations: ANI, average nucleotide identity; BNF, biological nitrogen fixation; dDDH, digital DNA–DNA hybridization; DDH, DNA–DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; LB, Luria–Bertani; ML, maximum-likelihood; MLSA, multilocus sequence analysis; NI, nucleotide identity; UPGMA, unweighted pair group method with arithmetic mean algorithm; YMA, yeast–mannitol agar.

The following new sequences have been deposited at the GenBank/EMBL/DDBJ database: atpD of *B. agreste* CNPSo 4010<sup>T</sup> (MT683853); atpD of *B. glycinis* CNPSo 4016<sup>T</sup> (MT683854); atpD of *B. diversitatis* CNPSo 4019<sup>T</sup> (MT683855). Genome accession numbers of *B. agreste* CNPSo 4010<sup>T</sup> (JACCHP0000000000); *B. glycinis* CNPSo 4016<sup>T</sup> (JACCHQ000000000); *B. diversitatis* CNPSo 4019<sup>T</sup> (JACEGD000000000).

One supplementary table and nine supplementary figures are available with the online version of this article. 004742  ${}^{\odot}$  2021 The Authors

Table 1. Strains used in this st
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Species/strain name	Other nomenclatures	Original host species	Geographical origin	Reference
Bradyrhizobium agreste CNPSo 4010 <sup>T</sup>	WSM 4802 <sup>T</sup> =LMG 31645	Glycine clandestina	Kununurra, Australia	Helene <i>et al.</i> [16]
Bradyrhizobium glycinis CNPSo 4016 <sup>T</sup>	WSM 4801 <sup>T</sup> =LMG 31649 <sup>T</sup>	Glycine tabacina	Kununurra, Australia	Helene <i>et al.</i> [16]
Bradyrhizobium diversitatis CNPSo 4019 <sup>T</sup>	WSM 4799 <sup>T</sup> =LMG 31650 <sup>T</sup>	Glycine max	Nambung, Australia	Helene <i>et al</i> . [16]
Bradyrhizobium fredereickii CNPSo 3426 <sup>T</sup>	USDA 10052 <sup>T</sup> =U686 <sup>T</sup> =CL 20 <sup>T</sup>	Chamaecrista fasciculata	Missouri, USA	Urquiaga <i>et al</i> . [38]
Bradyrhizobium liaoningense LMG 18230 <sup>T</sup>	DSM 24092 <sup>T</sup> =CECT 4845 <sup>T</sup> =USDA 3622 <sup>T</sup>	Glycine max	China	Xu <i>et al.</i> [9]
Bradyrhizobium yuanmingense CCBAU 10071 <sup>T</sup>	CFNEB 101 <sup>T</sup> =CIP 108027 <sup>T</sup> =NBRC 100594 <sup>T</sup>	Lespedeza species	China	Yao et al. [59]

chemical N-fertilizers [5, 6]. One remarkable example relies on the symbiosis of *Bradyrhizobium* with the soybean (*Glycine max*) crop in Brazil, saving billions of dollars every year [7, 8].

Several *Bradyrhizobium* species have been isolated from nodules of soybean, mostly grown in China, the main genetic centre of this legume species [9–11]. However, *Bradyrhizobium* is a geographically widespread genus that nodulates several legume tribes, from herbaceous to trees, mainly in tropical regions [12–15]. Recently, Helene *et al.* [16] reported a high diversity of *Bradyrhizobium* strains isolated from Western Australian soils using species of *Glycine* species as trapping hosts. It is worth mentioning that the genus *Glycine* is split into two subgenera: *Soja*, with *Glycine max* and *Glycine* soja species, and *Glycine*, which includes 25 species of wild soybean, most indigenous to Australia [17–20]; for this reason, although not often mentioned, Australia is a great hotspot of diversity of the genus *Glycine* [21].

Populations of Australian indigenous *Glycine* species are distributed all over the country and are the wild relatives of the economically important soybean crop [17, 21, 22]. Due to the inhospitable conditions of most areas growing indigenous *Glycine* in Australia, bioprospecting their genomes may help to understand and improve the adaptation of soybean to climate change [21]. In addition, to investigate the diversity of rhizobia symbionts of indigenous *Glycine* species in soils of Western Australia should provide valuable information for biotechnological applications. Here, we report a polyphasic study with three lineages of *Bradyrhizobium* isolated from *Glycine clandestina*, *G. tabacina* and *G. max* that resulted in the description of three new species.

## **ISOLATION AND ECOLOGY**

Strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> were isolated from root nodules of three different species, *G. clandestina* JC Wendl [23], *G. tabacina* (Labill.) Benth [24] and *G. max*, respectively, used as trap plants in Western Australian soil, as previously reported by our group [16]. Information

about the strains used in this study as well as the sampling soil points are shown in Table 1.

The strains are deposited at the Diazotrophic and Plant Growth Promoting Bacteria Culture Collection of Embrapa Soja (WFCC Collection No. 1213, WDCM Collection No. 1054), in Londrina, State of Parana, Brazil; at the Western Australian Soil Microbiology Gene Bank (WSM Culture Collection); at the Belgian Coordinated Collections of Microorganisms (BCCM/LMG); and in other culture collections.

The strains were short-term maintained on modified yeast extract-mannitol agar (YMA) medium [25] at 4 °C in a cold room and periodically cultured, while for long-term preservation the cultures were stored in modified-YM with 30% glycerol (v/v) at -80 and -150 °C by cryopreservation, and lyophilized as previously described [26].

## PHYLOGENY

Sequences of the 16S rRNA and of four housekeeping genes (*dnaK*, *glnII*, *gyrB* and *recA*), as well as of the symbiotic gene nodC were obtained from a previous study [16], and their accession numbers are shown in Table S1. Amplicons for the housekeeping gene *atpD* were obtained using the pair of primers TSatpDf (5'-TCTGGTCCGYGGCCAGGAAG-3') and TSatpDr (5'-CGACACTTCCGARCCSGCCTG-3'), with the conditions described by Stepkowski et al. [27]. The Pure-Link kit (Invitrogen) was used following the manufacturer's instructions for the purification of the PCR products, which were sequenced in an ABI 3500XL (Applied Biosystems) capillary sequencer analyzer, as described by Delamuta et al. [28]. All sequences were deposited at the GenBank database (NCBI) and the accession numbers are listed in parentheses in the phylogenetic trees and in Table S1. The complete sequences of housekeeping genes atpD, dnaK, glnII, gyrB, recA and rpoB were also retrieved from the genome of the type strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> in order to build a robust multilocus sequence analysis (MLSA) phylogeny. Multiple sequence alignments were obtained

	Nucleotide identity (%)								
Strains	MLSA (five genes)	MLSA (six genes)	16S rRNA	atpD	dnaK	glnII	gyrB	recA	rpoB
			Bradyrhizob	ium agreste	CNPSo 401	<b>0</b> <sup>T</sup>			
<i>B. glycinis</i> CNPSo 4016 <sup>T</sup>	97.2	97.5	100	97.7	98.1	97.4	96.8	95.5	98.6
B. yuanmingense CCBAU $10071^{T}$	96.4	96.9	99.6	94.9	99	95.8	95.3	95.8	98
B. liaoningense LMG 18230 <sup>T</sup>	95.3	95.3	99.6	94.1	95.9	94.6	94.4	94.7	97.8
B. diversitatis CNPSo 4019 <sup>T</sup>	95.1	95.3	99.6	94.1	95.9	94.4	94.8	93.8	97.5
B. frederickii CNPSo 4026 <sup>T</sup>	93.6	94.7	99.5	94.6	95.4	93.8	91.3	93.8	95.6
			Bradyrhizobi	ium glycinis	CNPSo 401	<b>6</b> <sup>T</sup>			
<i>B. agreste</i> CNPSo $4010^{T}$	97.2	97.5	100	97.7	98.1	97.4	96.8	95.5	98.6
B. yuanmingense CCBAU $10071^{T}$	96.1	97.1	99.6	94.1	98.1	95.8	94.1	96.3	98.3
<i>B. diversitatis</i> CNPSo 4019 <sup>T</sup>	95.2	95.1	99.6	93.9	96.8	94.8	93.9	94.7	97.2
B. liaoningense LMG $18230^{T}$	94.9	95.1	99.6	94.1	96.8	94.2	93.9	94.4	97
B. frederickii CNPSo $4026^{T}$	93.6	94.5	99.5	95.1	95	94.2	90.7	93.8	95.9
			Bradyrhizobiu	m diversitat	is CNPSo 4	<b>)19</b> <sup>T</sup>			
B. liaoningense LMG $18230^{T}$	97.6	97.8	100	96.9	98.6	94.8	98.1	98.6	99.1
B. yuanmingense CCBAU $10071^{T}$	95.4	95.3	99.5	93.4	95.9	94.4	94.4	95.5	97.8
<i>B. glycinis</i> CNPSo 4016 <sup>T</sup>	95.2	95.1	99.6	94.1	95.9	94.4	94.8	93.8	97.5
<i>B. agreste</i> CNPSo $4010^{T}$	95.1	95.3	99.6	93.9	96.8	94.8	93.9	94.7	97.2
B. frederickii CNPSo $4026^{T}$	94.8	95.7	99.7	95.4	96.3	93.8	93.9	94.7	97

**Table 2.** Nucleotide identity among new lineages of *Bradyrhizobium* and closely related species, based on the sequences of single and concatenated housekeeping genes (*atpD*, *dnaK*, *glnII*, *gyrB*, *recA* and *rpoB*) and the 16S rRNA

with MUSCLE [29] and the best evolutionary distance model was inferred by the lowest Bayesian information criterion scores [30] for maximum-likelihood (ML) reconstructions in Molecular Evolutionary Genetics Analysis (MEGA) software version 7 [31]. The evolutionary models are described in the figure captions. The statistical support of the trees was estimated by bootstrap analysis [32] with 1000 re-samplings [33]. *Xanthobacter autotrophicus* Py2 was used as an outgroup for all phylogenies, except for the *nodC* tree. The sequences of housekeeping genes were concatenated with SeaView software version 4.7 [34] for the MLSA. Nucleotide identity was calculated with BioEdit software version 7.0.4.1 [35] for single and concatenated genes and the values are indicated in Table 2.

The 16S rRNA phylogeny traditionally splits the genus *Bradyrhizobium* into two well-supported superclades, *B. japonicum* and *B. elkanii* [36–38]. We included sequences of all described *Bradyrhizobium* species described at the time of writing and the three strains from our study fit into the *B. japonicum* superclade; strains CNPSo  $4010^{T}$  and CNPSo  $4016^{T}$  showed higher relatedness with *B. yuanmingense* CCBAU  $10071^{T}$  (99.6% of similarity), whereas the CNPSo  $4019^{T}$  showed 100% similarity to *B. liaoningense* LMG 18230<sup>T</sup> and 99.8% to *B. daqingense* CGMCC  $1.10947^{T}$  (Fig. 1). The

nucleotide identity values of the 16S rRNA genes among strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> ranged from 99.6 to 100% (Table 2). Considering the threshold of 98.65% for species boundary suggested by Kim *et al.* [39], these results corroborate that the 16S rRNA gene provides limited taxonomic information in the genus *Bradyrhizobium* [15, 40–42].

In the analysis of housekeeping genes, it is important to verify each single phylogeny to detect congruence with the 16S rRNA phylogeny and possible events of horizontal gene transfer and recombination [36, 43]. Therefore, single phylogenies of the genes atpD (398 bp), dnaK (221 bp), glnII (504 bp), gyrB (553 bp), recA (360 bp) and rpoB (371 bp) were built and are shown in Figs S1-S6 (available in the online version of this article). All phylogenies of single housekeeping genes clustered the strains from this study in clades separated from all described Bradyrhizobium species. However, some differences in the topology of the trees were verified on atpD (Fig. S1) and glnII (Fig. S3) phylogenies. The wellsupported B. japonicum and B. elkanii superclades were not detected in the *atpD* phylogeny, as previously reported by Menna et al. [36]. Therefore, in order to improve the phylogenetic information of strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup>, two alignments of concatenated

bium guangzhouense CCBAU 51670<sup>T</sup> (CP030053.1)

Bradyrhizobium manausense BR 3351<sup>T</sup> (HQ641226.2)

Bradyrhizobium ganzhouense RITF806<sup>T</sup> (JQ796661.2) Bradyrhizobium cytisi CTAW11<sup>T</sup> (EU561065.2)

Bradyrhizobium guangdongense CCBAU 51649<sup>T</sup> (CP030051.1)

Badyhtabian discatticien USBA 11 <sup>0</sup> (NC, 00448.3) Badyhtabian cartosama Al <sup>0</sup> (NC237115.1) Badyhtabian cartosama Al <sup>0</sup> (NC327115.1) Badyhtabian cartosama Al <sup>0</sup> (NC327115.1) Badyhtabian andipama CBAU 5336 <sup>0</sup> (NC 202211.1) Badyhtabian anginama CCAU 5336 <sup>0</sup> (NC3605.1) Badyhtabian align magnitum CCAU 5330 <sup>0</sup> (NC33447.1) Badyhtabian magna MBPC1010 <sup>1</sup> (NC33447.1) Badyhtabian magna KM 10250 <sup>1</sup> (NC33447.1) Badyhtabian magna MBP 10250 <sup>1</sup> (NC37043.1) Badyhtabian machar BR 10250 <sup>1</sup> (NC47049.1) Badyhtabian machar BR 10250 <sup>1</sup> (NC47049.1) Badyhtabian machar BR 10250 <sup>1</sup> (NC47049.1) Badyhtabian machar BR 1026 <sup>1</sup> (NC47049.1) Badyhtabian machar BR 44 <sup>0</sup> (NC47031.1) Badyhtabian machar BR 1043 <sup>0</sup> (NC41000000.1) Badyhtabian machar BR 44 <sup>0</sup> (NC41000000.1) Badyhtabian machar BR 44 <sup>0</sup>		Bradyrhizobium rifense CTAW71 (EU561074.2)
Badyhitobian militi (CPS-344 <sup>6</sup> (RC70000000) Badyhitobian guargianse CGBAU 5338 <sup>7</sup> (NZ, CP022211.) Badyhitobian guargianse CGBAU 5338 <sup>7</sup> (NZ, CP022211.) Badyhitobian diamangraine CCBAU 5338 <sup>7</sup> (NZ, CP022211.) Badyhitobian diamangraine CGBAU 5338 <sup>7</sup> (NZ, CP022211.) Badyhitobian militiane LGM 443 <sup>7</sup> (CP03120.1) Badyhitobian militiane CGBAU 5339 <sup>7</sup> (NZ, CP022221.1) Badyhitobian machine RUS <sup>6</sup> (NK57646) Badyhitobian machine RUS <sup>6</sup> (NK57646) Badyhitobian machine RUS <sup>6</sup> (NK57646) Badyhitobian machine RUS <sup>6</sup> (NK57646) Badyhitobian machine RUS <sup>6</sup> (NK57647) Badyhitobian machine RUS <sup>6</sup> (NK57670) Badyhitobian machine RUS <sup>6</sup> (NK57670) Badyhitobian machine R		93 Bradyrhizobium diazoefficiens USDA 110 <sup>T</sup> (NC_004463.1)
Badyhizabian cambama Ad <sup>6</sup> (C227115.1) Badyhizabian mamiganas CCBAU S330 <sup>6</sup> (UZ_EBICO100082.1) Eadyhizabian mitoradacem TSA <sup>11</sup> (AB4228.1) Badyhizabian mitoradacem TSA <sup>11</sup> (AB4228.1) Badyhizabian mitoradacem TSA <sup>11</sup> (AB4228.1) Badyhizabian mitoradacem TSA <sup>11</sup> (AB4228.1) Badyhizabian makamamas 14.3 (OS40447.1) Badyhizabian makamamas 14.3 (OS407447.1) Badyhizabian makamamas 14.3 (OS407447.1) Badyhizabian makamamas 14.3 (OS707710) Badyhizabian makamamas 14.3 (OS707710) Badyhizabian makamamas 05.10 (OS707701) Badyhizabian makamamas 05.00 (MK87040) Badyhizabian makamamas 05.00 (MK87040) Badyhizabian makamamas 05.00 (MK87040) Badyhizabian makamama 05.01 (NZ_EDOT000000.1) Badyhizabian makamama 05.01 (NZ_ENOT000000.1) Badyhizabian makamama 05.00 (NZ_ENOT000000.1) Badyhizabian makamama 05.00 (NZ_ENOT000000.1) Badyhizabia		Bradyrhizobium niftali CNPSo3448 <sup>T</sup> (SPQT00000000)
Badyhitabian guargimma CCBAU S305 (%C_PC02211.)     Badyhitabian minumac CCBAU S305 (%C_PC020002.)     Badyhitabian minumaccGAU S305 (%C_PC02021.)     Badyhitabian minumaccam TSA1 <sup>1</sup> (AB54236.1)     Badyhitabian makufanama CGBU 3303 (%C_PC0222.1.)     Badyhitabian makufanama CGBU 3303 (%CP00000.)     Badyhitabian makufanama CGBU 3303 (%CP00000.)     Badyhitabian makufanama CGBU 3303 (%CP00000.)     Badyhitabian makufanama CGBU 3203 (%CP0022.1.)     Badyhitabian makufanama CGBU 3203 (%CP00000.)     Badyhitabian makufanama CGBU 3203 (%CP000000.)     Badyhitabian makufanama B2 44 <sup>6</sup> (%CP0000000.)     Badyhitabian makufana B1 44 <sup>6</sup> (%CP000000.)     Badyhitabian makufana B1 <sup>6</sup> (%CUN0000000.)     Badyhitabian maku		Bradyrhizobium centrosemae A9 <sup>T</sup> (KC247115.1)
DODO     Padyhizobim mamingma CCBAU 3330 <sup>2</sup> (%Z_EBC0100002.1)     Badyhizobim mikoraduam IKaH <sup>1</sup> (AB45228.1)     Badyhizobim mikoraduam UKB1072 <sup>10</sup> (02(F96000000.1)     Badyhizobim makamgana 143 <sup>2</sup> (WB492.1)     Badyhizobim makamgana 143 <sup>2</sup> (WB492.1)     Badyhizobim makamgana 143 <sup>2</sup> (WB492.1)     Badyhizobim makamgana 143 <sup>2</sup> (WB998.2)     Badyhizobim makamgana 143 <sup>2</sup> (WB2.2F092.221.1)     Badyhizobim makamgana 143 <sup>2</sup> (WB30642)     Badyhizobim makamgana 295 <sup>1</sup> (WZ 79214)     Badyhizobim makamgana 295 <sup>1</sup> (WZ 79214)     Badyhizobim makamgana 292 <sup>1</sup> (WZ 79		Bradyrhizobium guangxiense CCBAU 53363 <sup>T</sup> (NZ_CP022219.1)
0020       Badyhizobim molosicomi TSA <sup>1</sup> (AB54238.1)         0020       Badyhizobim molosicophicum LMG B443 (C68025.1)         0020       Badyhizobim molosicophicum LMG 1073 <sup>2</sup> (C619220.1)         Badyhizobim molosicophicum LMG 1073 <sup>2</sup> (C619222.1)       Badyhizobim manghushamaras CCBAU 23303 (H0231483.1)         un Badyhizobim machania CCBAU 1370 <sup>2</sup> (C702222.1)       Badyhizobim machania CCBAU 1370 <sup>2</sup> (K702022.1)         Badyhizobim machania CCBAU 12303 (H0221483.1)       Badyhizobim machania CCBAU 12303 (H0221483.1)         Badyhizobim machania CNP3 (MKF060)       Badyhizobim machania CNP3 (MKF060)         Badyhizobim maniferame LMG 120 <sup>2</sup> (KF11301.3)       Badyhizobim maniferame CMU44 (C009183.1)         Badyhizobim maniferame CMU44 (C009183.1)       Badyhizobim maniferame CMU44 (C009183.1)         Badyhizobim maniferame CMU44 (C009183.1)       Badyhizobim maniferame CMU44 (C009183.1)         Badyhizobim maniferame CMU44 (C0091083.1)       Badyhizobim maniferame CMU44 (C009183.1)         Badyhizobim maniferame CMU3 210 <sup>2</sup> (VL01000000.1)       Badyhizobim maniferame CMU3 (C000000.1)         Badyhizobim maniferame CMU3 (C0011827.1)       Badyhizobim maniferame CMU3 (C0011827.1)         Badyhizobim maniferame CMU3 (C00100000.1)       Badyhizobim maniferame CMU3 (C0000000.1)         Badyhizobim machani B14 4.04 (C109100000.1)       Badyhizobim machani B2 -1 (C10000000.1)         Badyhizobim machani B12 (C100000000.1)       Badyhizobim machani B12 (C10000000.1		Bradyrhizobium nanningense CCBAU 53390 <sup>T</sup> (NZ_LBJC01000082.1)
0.020 <ul> <li>Badyhizobim damifican LMG 143<sup>2</sup> (0601230.1)</li> <li>Badyhizobim diwagani MBCP1010<sup>2</sup> (0424447.1)</li> <li>Badyhizobim hanginganes CGAU 05110<sup>2</sup> (02, CP02000000.1)</li> <li>Badyhizobim hanginganes CGAU 05170<sup>2</sup> (02, CP02221.1)</li> <li>Badyhizobim hanginganes CGAU 05170<sup>2</sup> (02, CP02221.1)</li> <li>Badyhizobim dwaringens 14-3<sup>3</sup> (0989562.1)</li> <li>Badyhizobim hanginganes CGAU 05170<sup>2</sup> (02, CP02221.1)</li> <li>Badyhizobim dwaringens CGAU 05170<sup>2</sup> (02, CP02221.1)</li> <li>Badyhizobim dwaringens CGAU 05170<sup>3</sup> (02, CP02221.1)</li> <li>Badyhizobim microhyses CGAU 0500<sup>3</sup> (MK87044)</li> <li>Badyhizobim microhyses CGAU 050<sup>3</sup> (MK87044)</li> <li>Badyhizobim microhyses CGAU 05170<sup>3</sup> (02, CP022010.1)</li> <li>Badyhizobim microhyses CGAU (00000.1)</li> <li>Badyhizobim microhyses CGAU (007<sup>4</sup> (02, CP02701))</li> <li>Badyhizobim microhyses CGAU (007<sup>4</sup> (MK87044)</li> <li>Badyhizobim microhyses CGAU (007<sup>4</sup> (MK87041))</li> <li>Badyhizobim microhyses R140<sup>4</sup> (02, CP0000000.1)</li> <li>Badyhizobim microhyses R14<sup>4</sup> (02, CP0000000.1)</li> <li>Badyhizobim microhyses R10<sup>4</sup> (02, CP0000000.1)</li> <li>Badyhizobim microh</li></ul>		Bradyrhizobium nitroreducens TSA1 <sup>T</sup> (AB542368.1)
0020         00200         00200         00200         00200         00200		Bradyrhizobium denitrificans LMG 8443 <sup>T</sup> (X66025.1)
D020 Bradyhizobium Gaini MMPC1010 <sup>1</sup> (VT 349447.1) Bradyhizobium machida CGBU U0510 <sup>7</sup> (VZ_FPE00000000.1) Bradyhizobium Haumagness 14.3 <sup>1</sup> (VF990582.1) Bradyhizobium Haumagness CGBU B1720 <sup>1</sup> (VZ_CPC02221.1) Bradyhizobium Haumagness CGBU B1720 <sup>1</sup> (VZ_CPC02221.1) Bradyhizobium Anayingeness CGBU B1720 <sup>1</sup> (VZ_CPC02221.1) Bradyhizobium devariated CKBS 040 <sup>2</sup> (MK97064) Bradyhizobium devariated CKBS 040 <sup>2</sup> (MK97064) Bradyhizobium anghicaguess 205 (MS00080) Bradyhizobium anghicaguess 205 (MS00201) Bradyhizobium anghicaguess 205 (MS00201) Bradyhizobium anghicaguess 205 (MS00201) Bradyhizobium anghicaguess 205 (MS002000) Bradyhizobium anghicaguess 205 (MS002000) Bradyhizobium gaybiantha B14 M6 (V2) VEAU 000000) Bradyhizobium gaybiantha B14 M6 (V2) VEAU 0000000) Bradyhizobium gaybiantha B14 M6 (V2) VEAU 0000000) Bradyhizobium gaybiantha B14 M6 (V2) VEAU 0000000.1) Bradyhizobium maching B2 -1 <sup>1</sup> (V2) VA0000000.1) Bradyhizobium maching B2 -1 <sup>1</sup> (V2) VA00000000.1) Bradyhizobium maching B2 VI (V2) VE00000000.1) Bradyhizobium maching B2 VI (V2) VE00000000.1) Bradyhizobium maching VEAU 010 <sup>2</sup> (V2) VE00000000.1) Bradyhizobium maching R2 VEAU 010 <sup>2</sup> (V2) VE00000000.1) Bradyhizobium maching R2 VEAU 010 <sup>2</sup> (V2) V		98 Bradyrhizobium cligotrophicum LMG 10732 <sup>T</sup> (JQ619230.1)
D020     Bradyrhacbium machida CCBU 05107 <sup>1</sup> (NZ_FPB00000000.1)     Bradyrhacbium manghuaimiense CCBAU 23303 <sup>2</sup> (HC22148.1)     arg. Bradyrhacbium Managhua H-14 (PG90562.1)     Bradyrhacbium machina Regno (F42 PC90508.2)     Bradyrhacbium federicki (F42 PC9110.2)     Bradyrhacbium federicki (F42 PC9110.2)     Bradyrhacbium synainer UKG 2280 <sup>2</sup> (V42 PC913.1)     Bradyrhacbium federicki (F42 PC914.2)     Bradyrhacbium federicki (F42 PC914.2)     Bradyrhacbium synainer UKG 2280 <sup>2</sup> (V42 PC913.1)     Bradyrhacbium federicki (F42 PC914.2)     Bradyrhacbium federicki (F42 PC914.2)     Bradyrhacbium synainer UKG 2280 <sup>2</sup> (V42 PC914.2)     Bradyrhacbium synainer UKG 248 <sup>2</sup> (V42 PC914.2)     Bradyrhacbium genetic UKS 26 <sup>3</sup> (V42 PC914.2)     Bradyrhacbium genetic UKS 26 <sup>3</sup> (V42 PC914.2)     Bradyrhacbium machina Res 10 <sup>4</sup> (V42 PC9150.2)     Bradyrhacbium machina Res 10 <sup>4</sup> (V42 PC9150.1)     Bradyrhacbium		- Bradyrhizobium cajani AMBPC1010 <sup>T</sup> (KY349447.1)
0.020 Badyritzobium hanafbuahainese CCBAU 23303 (HC22148.1) Badyritzobium hanafbuahainese CCBAU 51771 (M2_CP022221.1) Badyritzobium ingen BR 10205 (KF92704.1) Badyritzobium ingen BR 10205 (KF92704.1) Badyritzobium athewanse ERR11 (N2_FMA01000001.1) Bradyritzobium athewanse ERR11 (N2_FMA01000000.1) Bradyritzobium athewanse ERR11 (N2_FMA0100000.1) Bradyritzobium athewanse ERR11 (N2_FMA0100000.1) Bradyritzobium athewanse ERR11 (N2_FMA0100000.1) Bradyritzobium athewanse ERR11 (N2_FMA0100000.1) Bradyritzobium igenose CMB0 (N118927.0.1) Bradyritzobium igenose CMB0 (N118927.0.1) Bradyritzobium igenose CMB0 (N118927.0.1) Bradyritzobium igene LMG 2286 (N4562637) Bradyritzobium igene LMG 2286 (N4562637) Bradyritzobium igene LMG 2287 (N2_FOR000000.1) Bradyritzobium igene LMG 2371 (N2_FOR000000.1) Bradyritzobium igene LMG 2371 (N2_FOR000000.1) Bradyritzobium meatingenese CCBAU 10071 (AF192818) Bradyritzobium meatingenese CH80 0321 (N2_FOR0000000.1) Bradyritzobium meatingenese CH80 03112 (N400000000.1) Bradyritzobium meatingenese CH80 03112 (N400000000.1) Bradyritzobium meatingenese CH80 03112 (N22_FOR0000000.1) Bradyritzobium meatingenese CH80 0311 (N22_FOR0000000.1) Bradyritzobium meatingenese CH80 0311 (N22_FOR0000000.1) Bradyritzobium meatingenese CH80 03112 (N22_FOR0000000.1) Bradyritzobium meatingenese CH80 03112 (N22_FOR0000000.1) Bradyritzobium meatingenese CH80 03112 (N22_FOR0000000.1) Bradyritzobium meatingenese CH80 0311 (N22_FOR0	H	Bradyrhizobium arachidis CCBALL051107 <sup>T</sup> (NZ EPBO000000001)
<pre>cm provide the set of the se</pre>	0.020	Bradymizobium arachius COBAC COTTOT (N2_11 BCCCCCCCC.1)
<pre>a f adoptinzbolum Analysingense CGRU D19726 (WZ, CP022221.1) Badyrinzbolum images BR 10250 (GF02703.1) Badyrinzbolum images BR 10250 (GF02703.1) Bradyrinzbolum diversitätis CNPSo 4015 (MK67604) Bradyrinzbolum diversitätis CNPSo 4015 (MK67607) Bradyrinzbolum diversitätis CNPSo 4015 (MK672937) Bradyrinzbolum fredericki CNPSo 3420 (MK672937) Bradyrinzbolum fredericki CNPSo 3420 (MK672937) Bradyrinzbolum misse LMG 2276 (MK67047) Bradyrinzbolum methodia BR 10047 (MZ LBEF00000000.1) Bradyrinzbolum methodia BR 10032 (MK67047).1) Bradyrinzbolum methodia BR 10032 (MK67047).1) Bradyrinzbolum methodia BR 10032 (MK704075.1) Bradyrinzbolum methodia BR 10032 (MZ LMC00000000.1) Bradyrinzbolum methodia BR 40 40 42 (MZ MK770200.1) Bradyrinzbolum methodia BR 40 41 44 (MZ MK770502) Bradyrinzbolum methodia BR 40</pre>		Diadymizobium noanginaanse CCBA0 23303 (HC231403.1)
Headyrizabian Zukawa Kito (KPS20143)     Badyrizabian izometane Kito <sup>1</sup> (AB300992)     Badyrizabian izometane Kito <sup>1</sup> (AB30092)     Badyrizabian izometane Kito <sup>2</sup> (AB3211)     Badyrizabian izometane Kito <sup>2</sup> (AB3211)     Badyrizabian izometane Kito <sup>2</sup> (AB3211)     Badyrizabian izometane Kito <sup>2</sup> (AB32137)     Badyrizabian izometane Kito <sup>2</sup> (AB40000001)     Badyrizabian izometane Kito <sup>2</sup> (AB2000000001)     Badyrizabian izometane Kito <sup>2</sup> (AB2000000001)     Badyrizabian izometane Kito <sup>2</sup> (AB2000000001)     Badyrizabian izometane Kito <sup>3</sup> (AB2000000001)     Badyrizabian izometane Kito <sup>3</sup> (AB2000000001)     Badyrizabian metane Kito <sup>3</sup> (AB200000001)     Badyrizabian metane Kito <sup>3</sup> (AB2000000001)     Badyrizabian metane Kito <sup>3</sup> (AB200000001)     Badyrizabian metane Kito <sup>3</sup> (AB200000001)     Badyrizabian metane Kito <sup>3</sup> (AB200000001		98 Bradymizobium kavangense 14-3 (KP899562.1)
Comparison of the second seco		Bradyrhizobium zhanjiangense CCBAU 51778 (NZ_CP022221.1)
Badyrhizobium sichers EKG (AB30092)     Bradyrhizobium ackarlar BL Neg2 (KF109013)     Bradyrhizobium ackarlar BL Neg2 (KF20913)     Bradyrhizobium americanum CMVU4* (KU991833.1)     Bradyrhizobium amplicangena SUB16 <sup>1</sup> (KF20878)     Bradyrhizobium amplicangena SUB16 <sup>1</sup> (KF20870)     Bradyrhizobium amplicangena SUB16 <sup>1</sup> (KF20870)     Bradyrhizobium amplicangena SUB16 <sup>1</sup> (KF20870)     Bradyrhizobium amplicangena SUB10 <sup>1</sup> (KF1081818)     Bradyrhizobium amplicangena SUB10 <sup>1</sup> (KF208000000.1)     Bradyrhizobium amplicangena SUB10 <sup>1</sup> (KF208000000.1)     Bradyrhizobium amethysine RN 10744 <sup>1</sup> (MK570000000.1)     Bradyrhizobium metangena CH80 <sup>2</sup> (US12100000000.1)     Bradyrhizobium metangena CH80 <sup>2</sup> (VS10000000.1)     Bradyrhizobium metangena SUB <sup>1</sup> (CS10000000.1)     Bradyrhizobium metangena SUB <sup>1</sup> (CS10000000.1)     Bradyrhizobium metangena SUB <sup>1</sup> (CS40000000.1)     Bradyrhizobium ametangena SUB <sup>1</sup> (CS40000000.1)     Bradyrhizobium metametame SUB <sup>1</sup> (CS40000000.1		L Bradyrhizobium ingae BR 10250 (KF927043.1)
<sup>1</sup> Bradyrhizobium sevena ERR1 (VZ, FNAIO 100001.1) Bradyrhizobium diversitatis CRV50.4015 (MK67040) Bradyrhizobium diversitatis CRV50.4015 (MK67040) Bradyrhizobium amphicapaea 393.118 (CVP58779) Bradyrhizobium amphicapaea 393.118 (CVP58779) Bradyrhizobium setuciarum CMV144 (CU991833.1) Bradyrhizobium setuciarum CMV144 (CU991833.1) Bradyrhizobium setuciarum CMV144 (CU978779) Bradyrhizobium setuciarum CMV144 (CU978779.1) Bradyrhizobium synbiodeficiene 8551MB (CVP58779.1) Bradyrhizobium synbiodeficiene 8551MB (CVP58779.1) Bradyrhizobium isplasmit BR 14262 (VX12184) Bradyrhizobium isplasmit BR 14262 (VX114861.1) Bradyrhizobium isplasmit BR 1426 (K0220.1) Bradyrhizobium atyleamthis BR 446 (K0224) Bradyrhizobium setters and Br 1024 (VX2, EVK0000000.1) Bradyrhizobium setters and BR 1024 (VX2, EVK0000000.1) Bradyrhizobium achesian URP 426 (VX2, EVK0000000.1) Bradyrhizobium metaries BR 10247 (VX2, EVK00000000.1) Bradyrhizobium metaries BR 10247 (VX2, EVK00000000.1) Bradyrhizobium metaries BR 10247 (VX2, EVK00000000.1) Bradyrhizobium metaries BR 10247 (VX2, EVK000000000.1) Bradyrhizobium metaries BR 10247 (VX2, EVK00000000.1) Bradyrhizobium metaries BR 10247 (VX2, EVK0000000.1) Bradyrhizobium metaries BR 10247 (VX2, EVK0000000.1) B		Bradyrhizobium iriomotense EK05 (AB300992)
Bradyrhizobium shawanes ERS11 <sup>1</sup> (KZ_FMA0100001.1) Bradyrhizobium ilaciniganis LMS (19230 <sup>7</sup> (AF205613) Bradyrhizobium ilaciniganis CMS (19230 <sup>7</sup> (AZ_VLKL01000000) Bradyrhizobium amplicapaese 3951MB <sup>7</sup> (KY272184) Bradyrhizobium aphlicapaese 3951MB <sup>7</sup> (KY262787) Bradyrhizobium isponicul USDA 3051 <sup>7</sup> (KM118881.1) Bradyrhizobium isponicul USDA 3051 <sup>7</sup> (KM118881.1) Bradyrhizobium isponicul USDA 3051 <sup>7</sup> (KM118881.1) Bradyrhizobium isponicul USDA 3051 <sup>7</sup> (KM12807) Bradyrhizobium isponicul USDA 3051 <sup>7</sup> (KM2120000000.1) Bradyrhizobium isponicul USDA 3051 <sup>7</sup> (KM210000000.1) Bradyrhizobium more LMS 21971 <sup>7</sup> (XZ_DEPG0000000.1) Bradyrhizobium more times BR 146 <sup>7</sup> (XZ_EVEM01000003.1) Bradyrhizobium more the S12 <sup>-11</sup> (KP306152.1) - Bradyrhizobium more KD150 <sup>7</sup> (KZ_EDEF00000000.1) Bradyrhizobium more R015 <sup>7</sup> (VZ_LUKP00000000.1) Bradyrhizobium more R015 <sup>7</sup> (VZ_LUKP00000000.1) Bradyrhizobium more R015 <sup>7</sup> (VZ_LUKP00000000.1) Bradyrhizobium more R015 <sup>7</sup> (VZ_LUKP00000000.1) Bradyrhizobium maieme BR 1032 <sup>7</sup> (VZ_LUKP00000000.1) Bradyrhizobium maieme BR 1024 <sup>7</sup> (VZ_LUKP00000000.1) Bradyrhizobium maieme BR 10303 <sup>7</sup> (VZ_LUKP0000000.1) Bradyrhizobium maiemes BR 103030 <sup>7</sup> (VZ_LUKP0000000.1) Bradyrhizobium maiemes BR		Bradyrhizobium sacchari BR 10280' (KF113091.3)
Bradyrhizobium Waraitatic CNPSo 4019 <sup>4</sup> (MK870049) Bradyrhizobium ampicanama CMUV4 <sup>4</sup> (KU991833.1) Bradyrhizobium ampicanamae 3951MB <sup>6</sup> (KP98770) Bradyrhizobium ampicanamae 3951MB <sup>6</sup> (KP98770) Bradyrhizobium symbioteficiena 551MB <sup>7</sup> (KY27140) Bradyrhizobium symbioteficiena 551MB <sup>7</sup> (KY27140) Bradyrhizobium symbioteficiena 551MB <sup>7</sup> (KY27140) Bradyrhizobium fawaenaso COPa <sup>6</sup> (MK872937) Bradyrhizobium fawaenaso COPa <sup>6</sup> (MK872937) Bradyrhizobium fawaenaso COPa <sup>6</sup> (MK872937) Bradyrhizobium inganoicum USDA s <sup>6</sup> (K86024) Bradyrhizobium symbioteficiena 592.1 <sup>1</sup> (KZ_2VEM1000003.1) Bradyrhizobium symaetung 2005 <sup>1</sup> (KM114861.1) Bradyrhizobium symaetung 2005 <sup>1</sup> (KK114861.1) Bradyrhizobium symaetung 2005 <sup>1</sup> (KZ_2VEM1000003.1) Bradyrhizobium symaetung 2007 <sup>1</sup> (KZ_2VEM0000000.1) Bradyrhizobium symaetung 2017 <sup>1</sup> (KZ_2VEM0000000.1) Bradyrhizobium symaetung 2017 <sup>1</sup> (KZ_2VEM0000000.1) Bradyrhizobium achestalin ENPA-641 <sup>1</sup> (KS76047) 71a Bradyrhizobium achestalin ENPA-641 <sup>1</sup> (KS76047) 71b Bradyrhizobium achestalin ENPA-641 <sup>1</sup> (KS76047) 71b Bradyrhizobium achestalin ENPA-045 <sup>1</sup> (KS60801.1) Bradyrhizobium achestalines ENFL0.032 <sup>1</sup> (KZ_1NFU00000000.1) Bradyrhizobium achestalines ENFL0.032 <sup>1</sup> (KZ_1NFU00000000.1) Bradyrhizobium achestalines ENFL0.032 <sup>2</sup> (KH140801.1) Bradyrhizobium achestalines ENFL0.032 <sup>3</sup> (KZ_1NFU00000000.1) Bradyrhizobium achestalines ENFL0.032 <sup>3</sup> (KZ_1NFU00000000.1) Bradyrhizobium achestalines ENFL0.032 <sup>3</sup> (KZ_1NFU00000000.1) Bradyrhizobium achestalines ENFL0.032 <sup>3</sup> (KH14041.1) Bradyrhizobium achestalines ENFL0.032 <sup>3</sup> (KH14041.1) Bradyrhizobium achestalines ENFL0.032 <sup>3</sup> (KH14041.1) Bradyrhizobium achestalines ENFL0.032 <sup>3</sup>		Bradyrhizobium shewense ERR11 (NZ_FMAI01000001.1)
Bradyrhizobium anaicanum CMVU44 <sup>1</sup> (KU991833.1) Bradyrhizobium anaicanum CMVU44 <sup>1</sup> (KU991833.1) Bradyrhizobium anaphicarpasea 3851Ma <sup>2</sup> (KP7688779) Bradyrhizobium anaphicarpasea 3851Ma <sup>2</sup> (KP768873) Bradyrhizobium symbiodeficiane BSISMa <sup>2</sup> (KP76873.1) Bradyrhizobium iaponicaum USDA 3005 <sup>1</sup> (KM114881.1) Bradyrhizobium iaponicaum USDA 3005 <sup>1</sup> (KM114881.1) Bradyrhizobium iaponicaum USDA 3005 <sup>1</sup> (KM114881.1) Bradyrhizobium iaponicaum USDA 6 <sup>1</sup> (KK270397) Bradyrhizobium iaponicaum USDA 6 <sup>1</sup> (KK270397) Bradyrhizobium iaponicaum USDA 6 <sup>1</sup> (KK270397) Bradyrhizobium ingeneticau 23879 <sup>1</sup> (KZ, FROF00000000.1) Bradyrhizobium ingeneticau 23879 <sup>1</sup> (KZ, FROF00000000.1) Bradyrhizobium gerset CMPSo 4010 <sup>2</sup> (MK676049) Bradyrhizobium gareste CMPSo 4010 <sup>2</sup> (MK676049) Bradyrhizobium gareste CMPSo 4010 <sup>2</sup> (MK676049) Bradyrhizobium anathema FRO <sup>1</sup> (KZ, EUCM0000000.1) Bradyrhizobium achetaypum WSM 1744 <sup>1</sup> (MK676049) Bradyrhizobium achetaypum WSM 1744 <sup>1</sup> (MK676049) Bradyrhizobium methorgae BR 10342 <sup>1</sup> (KP92749.1) Bradyrhizobium methorgae BR 10342 <sup>1</sup> (KP92749.1) Bradyrhizobium methorgae BR 10342 <sup>1</sup> (KP90753.1) Bradyrhizobium methorgae BR 10342 <sup>1</sup> (KZ, USEF00000000.1) Bradyrhizobium methorgae BR 10303 <sup>2</sup> (KZ, USEF00000000.1) Bradyrhizobium methorgae SR 100 <sup>2</sup> (KY841465.1) Bradyrhizobium methorgae S		Bradyrhizobium diversitatis CNPSo 4019 <sup>T</sup> (MK676046)
<pre>1 Bradyrhizobium danigense CGMCG 1.1047<sup>1</sup> (KZ_VLKU01000000) Bradyrhizobium ampicagenese 3951MB<sup>1</sup> (KP28778) Bradyrhizobium sphibodeficiens 8951MB<sup>1</sup> (KP28783.1) Bradyrhizobium sphibodeficiens 8951MB<sup>1</sup> (KP28783.1) Bradyrhizobium sphibodeficiens 8951MB<sup>1</sup> (KP28783.1) Bradyrhizobium inpointium USDA 612256<sup>5</sup> (M580225.1) Bradyrhizobium inpointium USDA 612256<sup>5</sup> (M580225.1) Bradyrhizobium inpointium USDA 612256<sup>5</sup> (M580225.1) Bradyrhizobium inpointium USDA 61 (XE2UCM1000003.1) Bradyrhizobium inpointium USDA 61 (XE2UCM1000003.1) Bradyrhizobium inpointium USDA 61 (XE2UCM10000000.1) Bradyrhizobium inpointium USDA 61 (XE2UCM10000000.1) Bradyrhizobium inpointium USDA 61 (XE2UCM10000000.1) Bradyrhizobium inpointium USDA 61 (KR576049) Bradyrhizobium gytcinis CNP56 4010<sup>5</sup> (MK676049) Bradyrhizobium relamase CCBAU 10071<sup>1</sup> (AF193818) Bradyrhizobium inpointium USDA 61 (VK876047) Bradyrhizobium inpointium USDA 61 (VK876047) Bradyrhizobium inpae WRA1 144<sup>4</sup> (MK676045) Bradyrhizobium relamase Ro19<sup>1</sup> (VZ_LUX00000000.1) Bradyrhizobium relamase Ro19<sup>1</sup> (VZ_LUX00000000.1) Bradyrhizobium inpae WRA1 (MF580681.1) Bradyrhizobium inpae WRA1 (MF580681.1) Bradyrhizobium meaturese BR 10347<sup>4</sup> (VZ_UMPV00000000.1) Bradyrhizobium meaturese BR 10347<sup>4</sup> (VZ_UMPV00000000.1) Bradyrhizobium inpae WRA1 (MF58067.1) Bradyrhizobium apertyrhizi PAC46<sup>1</sup> (XY244135) Bradyrhizobium meaturese RS180<sup>2</sup> (VC11445.1) Bradyrhizobium meaturese RS180<sup>2</sup> (VC11445.1) Bradyrhizobium meanse RS180<sup>2</sup> (VC11445.1) Bradyrhizobium meanses RS180<sup>2</sup></pre>		Bradyrhizobium liaoningense LMG 18230 <sup>T</sup> (AF208513)
Bradyrhizobium apairose CGMCC 1.1047 <sup>1</sup> (NZ_VLKL0100000) Bradyrhizobium aphiciarpases 3951M <sup>2</sup> (KP327184) Bradyrhizobium betae LMG 2189 <sup>7</sup> (AV327184) Bradyrhizobium outpiciolises 8551M <sup>2</sup> (KP372837) Bradyrhizobium canairose LMG 2226 <sup>7</sup> (AU5807281) Bradyrhizobium iupicii USDA 3051 <sup>7</sup> (XM1148611) Bradyrhizobium vigne LMG 2879 <sup>31</sup> (NZ_FOCR0000000.1) Bradyrhizobium ingenicum USDA 6 <sup>7</sup> (X68024) Bradyrhizobium vigne LMG 2879 <sup>31</sup> (NZ_FOCR0000000.1) Bradyrhizobium macratella INPA45 <sup>1</sup> (NZ_FOCR0000000.1) Bradyrhizobium gareste CMP50 4016 <sup>7</sup> (MK676049) Bradyrhizobium gareste CMP50 4016 <sup>7</sup> (MK676049) Bradyrhizobium macratemae R0 9 <sup>1</sup> (NZ_LIVEM0100000.1) Bradyrhizobium macratemae R0 9 <sup>1</sup> (NZ_LIVEM0100000.1) Bradyrhizobium neutonolobii BR 10246 <sup>7</sup> (NZ_2SEF00000000.1) Bradyrhizobium neutonolobii BR 10246 <sup>17</sup> (NZ_LIVEM0100000.1) Bradyrhizobium neutonolobii BR 10246 <sup>17</sup> (NZ_LIVEM0100000.1) Bradyrhizobium neutonolobii BR 10247 <sup>1</sup> (NZ_SEF00000000.1) Bradyrhizobium neutonolobii BR 10247 <sup>1</sup> (NZ_LIVEM0100000.1) Bradyrhizobium macrateres BR 10303 <sup>1</sup> (NZ_LIVEM0100000.1) Bradyrhizobium macrateres BR 10303 <sup>1</sup> (NZ_LIVEM0100000.1) Bradyrhizobium algerieses BR 10303 <sup>1</sup> (NZ_LIVEM0100000.1) Bradyrhizobium algerieses BR 10303 <sup>1</sup> (NY_LIVEN0100000.1) Bradyrhizobium algerieses BR 10303 <sup>1</sup> (NY_LIVEN0100000.1) Bradyrhizobium algerieses CNR 1030 <sup>1</sup> (NY_LIVEN0100000.1) Bradyrhizobium algerieses CNR 1030 <sup>1</sup> (NY CR062) Bradyrhizobium algerieses ENR 10 <sup>3</sup> (NY 64773.1) Bradyrhizobium algerieses ENR 10 <sup>3</sup> (NY 64773.1) Bradyrhizobium algerieses ENR 10 <sup>3</sup> (NY 647602) Bradyrhizobium algerieses ENR 10 <sup>3</sup> (NY 647602) Br		Bradyrhizobium americanum CMVU44 <sup>T</sup> (KU991833.1)
Bradyhizobium aphicapiaae 3951MB <sup>1</sup> (VP76779) Bradyhizobium symbiodhiciana BS51MB <sup>1</sup> (VP768779) Bradyhizobium symbiodhiciana BS51MB <sup>1</sup> (VP768793)) Bradyhizobium canariense LMG 22265 <sup>1</sup> (MS58025.1) Bradyhizobium tederickii CNP50-3426 <sup>1</sup> (MK11481.1) Bradyhizobium tederickii CNP50-3426 <sup>1</sup> (MK172397) Bradyhizobium styloanthi BR 446 <sup>1</sup> (MZ _UVEM01000003.1) Bradyhizobium syloanthi BR 446 <sup>1</sup> (MZ _UVEM01000003.1) Bradyhizobium syloanthi BR 446 <sup>1</sup> (MZ _UVEM01000000.1) Bradyhizobium syloanthi BR 446 <sup>1</sup> (MZ _UVEM0100000.1) Bradyhizobium syloanthi BR 446 <sup>1</sup> (MZ _UVEM0100000.1) Bradyhizobium setrease RD 50 <sup>1</sup> (MK576047) 7a Bradyhizobium achetypum WSM 1744 <sup>1</sup> (MK678065) Bradyhizobium neteranee RD 10 <sup>1</sup> (MZ _UXEM0000000.1) Bradyhizobium metamae RD 10 <sup>1</sup> (MZ _UXEM0000000.1) Bradyhizobium metamae RD 10 <sup>1</sup> (MZ _UXEM00000000.1) Bradyhizobium metamisese BT 10327 <sup>1</sup> (MZ _USEF00000000.1) Bradyhizobium macuniense BT 10333 <sup>1</sup> (MZ _UXEM00000000.1) Bradyhizobium macuniense BT 1033 <sup>1</sup> (MZ _UXEM01000091.1) Bradyhizobium macuniense BT 1033 <sup>1</sup> (MZ _UXEM01000091.1) Bradyhizobium macuniense BT 1033 <sup>1</sup> (MX _UXEM01000091.1) Bradyhizobium macuniense BT 103 <sup>1</sup> (MX 67602) Bradyhizobium macuniense BT 10 <sup>2</sup> (VF14445.1) Bradyhizobium macuniense BT 10 <sup>2</sup> (VF14451.1) Bradyhizobium macuniense BT 10 <sup>2</sup> (VF1466150) Bradyhizobium macuniense BT 10 <sup>2</sup> (VF1466150) Bradyhizobium macuniense BT 10 <sup>2</sup> (VX 541432) Bradyhizobium macuniense BT 10 <sup>2</sup> (VX 561510.2) Bradyhizobi		<sup>8</sup> Bradyrhizobium daqingense CGMCC 1.10947 <sup>T</sup> (NZ_VLKL01000000)
The set of the		Bradyrhizobium amphicarpaeae 39S1MB <sup>T</sup> (KP768779)
Partyhizobium symbiodhiciems BSSIMB (R7568783.1) Bradyhizobium clawaense OO96 <sup>7</sup> (N1186270.1) Bradyhizobium idevici (NPSo 3426 <sup>7</sup> (MK11481.1) Bradyhizobium idevici (NPSo 3426 <sup>7</sup> (MK12397) Bradyhizobium idevici (NPSo 3426 <sup>7</sup> (MK12397) Bradyhizobium igaonicum USDA 6 <sup>7</sup> (K86024) Bradyhizobium wignae LMG 228791 <sup>7</sup> (NZ_PCO0000000.1) Bradyhizobium wignae LMG 28791 <sup>7</sup> (NZ_PCO0000000.1) Bradyhizobium gestatis INPA6H <sup>6</sup> (NZ_PCO40000000.1) Bradyhizobium gestatis UNPA6H <sup>6</sup> (NZ_PCO40000000.1) Bradyhizobium gestatis UNPA6H <sup>6</sup> (NZ_PCO40000000.1) Bradyhizobium achestypum WSM 1744 <sup>7</sup> (MK676047) Ta Bradyhizobium achestypum WSM 1744 <sup>7</sup> (MK676047) Bradyhizobium nechospicale RR 10247 <sup>7</sup> (NZ_LSEF00000000.1) Bradyhizobium nechospicale RR 10247 <sup>7</sup> (NZ_LSEF00000000.1) Bradyhizobium mechospicale RR 10247 <sup>7</sup> (NZ_LNCU00000000.1) Bradyhizobium mechospicale RR 1030 <sup>7</sup> (NZ_LNCU00000000.1) Bradyhizobium mechose RST89 <sup>6</sup> (PY00100000.1) Bradyhizobium mechose RST89 <sup>6</sup> (PY00100000.1) Bradyhizobium mechospicale SRM 6300 <sup>7</sup> (PI025102.1) Bradyhizobium mechose RST89 <sup>6</sup> (PY00100000.1) Bradyhizobium mechospicale SRM 6300 <sup>7</sup> (FI14441.1) Bradyhizobium mechospicale SRM 6300 <sup>7</sup> (FI14441.1) Bradyhizobium mechospicale SRM 6300 <sup>7</sup> (FI025102.1) Bradyhizobium mechospicale SRM 6300 <sup>7</sup> (FI025107.1) Bradyhizobium mechospicale SRM 630 <sup>7</sup> (FI025107.1) Bradyhizobium mechospicale SRM 630 <sup>7</sup> (FI025107.1) Bradyhizobium mechospicale SRM 630 <sup>7</sup> (FI04682.2) Bradyhizobium mechospicale SRM 630 <sup>7</sup> (FI04682.2) Bradyhizobium mechospicale SRM 630 <sup>7</sup> (FI04682.2) Bradyhizobium mechos		Bradyrhizobium betae I MG 21987 (AY372184)
Bradyrhizobium ottawaenes OO99 <sup>7</sup> (N188 (CT-0047) Bradyrhizobium inpini USDA 305 <sup>1</sup> (VM114801.1) Bradyrhizobium inpini USDA 305 <sup>1</sup> (VM114801.1) Bradyrhizobium inponicum USDA 6 <sup>1</sup> (M6024) Bradyrhizobium subteranum USDA 6 <sup>1</sup> (M6024) Bradyrhizobium one LMG 28791 <sup>1</sup> (WZ_FOUG000000.1) Bradyrhizobium one LMG 28791 <sup>1</sup> (WZ_FOUG000000.1) Bradyrhizobium subteranum 58 2-1 <sup>1</sup> (KP080152.1) Bradyrhizobium greate LMS 28791 <sup>1</sup> (WZ_FOUG000000.1) Bradyrhizobium greate LMSA 40 <sup>1</sup> (WZ_FOUG000000.1) Bradyrhizobium greate CKPS-0 4016 <sup>1</sup> (MK67047) To Bradyrhizobium greate CKPS-0 4016 <sup>1</sup> (MK67047) To Bradyrhizobium netropicale BR 10247 <sup>1</sup> (WK57048) Bradyrhizobium netropicale BR 10247 <sup>1</sup> (WZ_ESE0000000.1) Bradyrhizobium metropicale BR 10303 <sup>1</sup> (NZ_LISE00000000.1) Bradyrhizobium metropicale CLAU 51502 <sup>1</sup> (VB18809.1) Bradyrhizobium metropicale CLAU 51502 <sup>1</sup> (VB1809.1) Bradyrhizobium metropicale CLAU 53325 <sup>1</sup> (VF11445.1) Bradyrhizobium metropicale CLAU 53325 <sup>1</sup> (VF11		98 Bradychizobium symbiodeficiens 85S1MB <sup>T</sup> (KP768783.1)
The second seco		Bradyrhizobium offauroppe OOPP <sup>T</sup> (IN186270.1)
<pre>l l andyritzobium dipie USA 2005 (VGN14801.1)     Bradyrhizobium inderickii CNPSo 3428<sup>1</sup> (MK672337)     Bradyrhizobium signe LGS 2971<sup>1</sup> (NZ_VEN01000003.1)     Bradyrhizobium wigne LGS 2971<sup>1</sup> (NZ_VEN01000003.1)     Bradyrhizobium wigne LGS 2971<sup>1</sup> (NZ_VEN01000000.1)     Bradyrhizobium morestalis INPA54B<sup>1</sup> (NZ_POF00000000.1)     Bradyrhizobium gestes CNPSo 4016<sup>1</sup> (MK676048)     Bradyrhizobium dentersates R10<sup>1</sup> (NZ_ULVA00000000.1)     Bradyrhizobium dentersates R110<sup>1</sup> (NZ_ULV00000000.1)     Bradyrhizobium dentersates R110<sup>1</sup> (NZ_ULV00000000.1)     Bradyrhizobium dentersates R110<sup>1</sup> (NZ_ULV00000000.1)     Bradyrhizobium entersates R110<sup>1</sup> (NZ_ULV00000000.1)     Bradyrhizobium metarise R110<sup>1</sup> (NZ_ULV00000000.1)     Bradyrhizobium metarises R110<sup>1</sup> (NZ_ULV00000000.1)     Bradyrhizobium achropicale R1 1024<sup>7</sup> (NZ_USEF00000000.1)     Bradyrhizobium acuranes R110<sup>9</sup> (PC00000000.1)     Bradyrhizobium acuranes R110<sup>9</sup> (PC0000000.1)     Bradyrhizobium acuranes R110<sup>9</sup> (PC0000000.1)     Bradyrhizobium acuranes R110<sup>9</sup> (PC0000000.1)     Bradyrhizobium mercanes R110<sup>9</sup> (PC0000000.1)     Bradyrhizobium mercanes R110<sup>9</sup> (PC0000000.1)     Bradyrhizobium mercanes R110<sup>9</sup> (PC0000000.1)     Bradyrhizobium mercanes SNH509<sup>1</sup> (PC0000000.1)     Bradyrhizobium mercanes SNH509<sup>1</sup> (PC0000000.1)     Bradyrhizobium mercanes SNH509<sup>1</sup> (PC0000000.1)     Bradyrhizobium mercanes SNH509<sup>1</sup> (PO0000000.1)     Bradyrhizobium mercanes SNH509<sup>1</sup> (PO0000000.1)     Bradyrhizobium mercanes SNH509<sup>1</sup> (PO1<sup>1</sup> (MK676067)     Br</pre>		
Bradyrhizobium investini (SMI 14861.)) Bradyrhizobium inport USDA 4001 (KMI 14861.)) Bradyrhizobium igaonicum USDA 6 <sup>1</sup> (X68024) Bradyrhizobium igana LMG 28791 <sup>1</sup> (NZ_RDCF00000000.1) Bradyrhizobium inport SMI 87 446 <sup>1</sup> (NZ_PCG00000000.1) Bradyrhizobium inport SMI 87 446 <sup>1</sup> (NZ_PCG00000000.1) Bradyrhizobium isubternanum 58 2-1 <sup>1</sup> (KP306152.1) Bradyrhizobium gueste LMS 28791 <sup>1</sup> (NZ FDG0000000.1) Bradyrhizobium gueste CDPS 04010 <sup>1</sup> (MK676047) To Bradyrhizobium archetypum WSM 1744 <sup>1</sup> (MK676047) To Bradyrhizobium archetypum WSM 1744 <sup>1</sup> (MK676047) Bradyrhizobium netrame Ro10 <sup>1</sup> (NZ LLYA00000000.1) Bradyrhizobium netrapeare Ro10 <sup>1</sup> (NZ LLYA00000000.1) Bradyrhizobium netrapeare Ro10 <sup>2</sup> (NZ LLYA00000000.1) Bradyrhizobium netrapeare Ro10 <sup>2</sup> (NZ LSEF00000000.1) Bradyrhizobium metamae Ro10 <sup>1</sup> (NZ LYA00000000.1) Bradyrhizobium metames Ro10 <sup>3</sup> (NZ LNC0000000.1) Bradyrhizobium metames B Ro1030 <sup>3</sup> (NZ LNC00000000.1) Bradyrhizobium macuriense BR 10303 <sup>1</sup> (NZ LNC00000000.1) Bradyrhizobium macuriense BR 10303 <sup>1</sup> (NZ LNC00000000.1) Bradyrhizobium in alexiense UFLA 03-321 <sup>1</sup> (NZ MPV00000000.1) Bradyrhizobium in alexiense BR 10303 <sup>1</sup> (NZ LNC00000000.1) Bradyrhizobium inalexiense UFLA 03-3164 <sup>1</sup> (NZ VR4P01000093.1) Bradyrhizobium inalexiense UFLA 03-164 <sup>1</sup> (NZ VR4P01000993.1) Bradyrhizobium metamies SEMB 630 <sup>1</sup> (F1025101.1) Bradyrhizobium metamiese UFLA 13 <sup>1</sup> (MK676067) Bradyrhizobium metamiese UFLA 13 <sup>1</sup> (MK676067) Bradyr		Disadymizobium canariense EMG 22266 (AJ568026.1)
Bradyrhizobium ipaonicum USDA 6 <sup>1</sup> (X8024)         Bradyrhizobium aponicum USDA 6 <sup>1</sup> (X8024)         Bradyrhizobium sylosanthis BR 446 <sup>1</sup> (N2_LVEM0100000.1)         Bradyrhizobium sylosanthis BR 446 <sup>1</sup> (N2_LVEM0100000.1)         Bradyrhizobium sylosanthis BR 446 <sup>1</sup> (N2_LVEM0100000.1)         Bradyrhizobium subtraneum B8 2-1 <sup>1</sup> (N2_PGV600000000.1)         Bradyrhizobium genesi CCBA1 1007 <sup>1</sup> (AF193818)         Bradyrhizobium genesi CCBA5 04010 <sup>1</sup> (MK676048)         Bradyrhizobium acretargem Rola <sup>1</sup> (N2_LLYA0000000.1)         Bradyrhizobium netropicale BR 10247 <sup>1</sup> (N2_ESF00000000.1)         Bradyrhizobium netropicale BR 10247 <sup>1</sup> (NZ_EFF00000000.1)         Bradyrhizobium netropicale BR 10247 <sup>1</sup> (NZ_MV20000000.1)         Bradyrhizobium metropicale BR 10247 <sup>1</sup> (NZ_MV20000000.1)         Bradyrhizobium misselmese UFA 3321 <sup>1</sup> (NZ_MV20000000.1)         Bradyrhizobium misselmese UFA 3321 <sup>1</sup> (NZ_MV0000000.1)         Bradyrhizobium misselmese UFA 3321 <sup>1</sup> (NZ_MV0000000.1)         Bradyrhizobium misselmese UFA 331 <sup>1</sup> (S2_MV0000000.1)         Bradyrhizobium misselmese UFA 331 <sup>1</sup> (NZ_MV0000000.1)         Bradyrhizobium macrisese BR 1030 <sup>2</sup> (NZ_LLVE00000000.1)         Bradyrhizobium materisese RST89 <sup>2</sup> (PYC001000001.1)		Bradyrhizobium lupini USDA 3051 (KM114861.1)
Bradyrhizobium stylosanthis BR 446 <sup>1</sup> (NZ_LVEM01000003.1)         Bradyrhizobium stylosanthis BR 446 <sup>1</sup> (NZ_PGVG0000000.1)         Bradyrhizobium signae LMG 28791 <sup>1</sup> (NZ_PGVG0000000.1)         Bradyrhizobium agresste CNPSo 4010 <sup>1</sup> (MK676048)         se Bradyrhizobium dycinis CNPSo 4016 <sup>1</sup> (MK676047)         7a       Bradyrhizobium retmage N16 <sup>1</sup> (NZ_U1X00000000.1)         9       Bradyrhizobium centrobibi BR 10245 <sup>1</sup> (KF2704.1)         9       Bradyrhizobium centrobibi BR 10245 <sup>1</sup> (KYE92704.1)         9       Bradyrhizobium metamage N16 <sup>1</sup> (NZ_UX00000000.1)         9       Bradyrhizobium intext compoicale BR 10247 <sup>1</sup> (NZ_LSEF000000000.1)         9       Bradyrhizobium machines BR 10303 <sup>1</sup> (NZ_UNCU00000000.1)         9       Bradyrhizobium macurianes BR 10303 <sup>1</sup> (NZ_UNCU0000000.1)         9       Bradyrhizobium macur		Bradyrhizobium frederickii CNPSo 3426 (MK672937)
<ul> <li>Bindyrhizobium sylamathis BR 446<sup>1</sup> (N2_WEM01000001:)</li> <li>Bradyrhizobium ingrastalis INPA64<sup>1</sup> (N2_FOG0000000.1)</li> <li>Bradyrhizobium forstalis INPA64<sup>1</sup> (N2_FOG0000000.1)</li> <li>Bradyrhizobium subternanum 58 2-1<sup>1</sup> (K9308152.1)</li> <li>Bradyrhizobium givesta CNPSo 4010<sup>1</sup> (MK676047)</li> <li>Bradyrhizobium agrests CNPSo 4010<sup>1</sup> (MK676047)</li> <li>Bradyrhizobium agrests CNPSo 4016<sup>1</sup> (N2_LUX0000000.1)</li> <li>Bradyrhizobium agrest CNPSo 4016<sup>1</sup> (N2_LUX0000000.1)</li> <li>Bradyrhizobium achetypum WSM 1744<sup>1</sup> (MK676047)</li> <li>Bradyrhizobium achetypum WSM 1744<sup>1</sup> (MK676047)</li> <li>Bradyrhizobium achetypum WSM 1744<sup>1</sup> (MK2_USEF00000000.1)</li> <li>Bradyrhizobium neotropicale RR 10245<sup>1</sup> (N2_LUX00000000.1)</li> <li>Bradyrhizobium neotropicale RR 10245<sup>1</sup> (N2_LUSEF00000000.1)</li> <li>Bradyrhizobium basilense UFLA 03-321<sup>1</sup> (N2_USEF00000000.1)</li> <li>Bradyrhizobium macuriense BR 10303<sup>1</sup> (N2_LUC00000000.1)</li> <li>Bradyrhizobium macuriense BR 10303<sup>1</sup> (N2_LUC00000000.1)</li> <li>Bradyrhizobium in achetypuin (CRAU 5135)</li> <li>Bradyrhizobium internieni CCRAU 51302<sup>1</sup> (V3 B18096.1)</li> <li>Bradyrhizobium meses RST89<sup>1</sup> (PO201000001.1)</li> <li>Bradyrhizobium meses BR 5189<sup>1</sup> (PO201000001.1)</li> <li>Bradyrhizobium meses RST89<sup>1</sup> (PO201000001.1)</li> <li>Bradyrhizobium meses CNF05 2833<sup>1</sup> (XP114445.1)</li> <li>Bradyrhizobium methapense CNF05 2833<sup>1</sup> (XP114445.1)</li> <li>Bradyrhizobium methatises MSM 1791<sup>1</sup> (MK676067)</li> <li>Bradyrhizobium methatises MKM 6390<sup>1</sup> (FJ025107.1)</li> <li>Bradyrhizobium methatises LMR 6390<sup>1</sup> (FJ025107.1)</li> <li>Bradyrhizobium methapense CNF05 2833<sup>1</sup> (XP04773.1)</li> <li>Bradyrhizobium methapense CNF05 2833<sup>1</sup> (XP04773.1)</li> <li>Bradyrhizobium methatises LMR 6390<sup>1</sup> (FJ056067)</li> <li>Bradyrhizobium methatises LMR 6390<sup>1</sup> (FJ057007)</li> <li>Bradyrhizobium methapense LMR 13<sup>1</sup> (KF38062)</li> <li>Bradyrhizobium methapense CNF</li></ul>		Bradyrhizobium japonicum USDA 6 (X66024)
Imadyhizobium vigane LMG 28791 (NZ, EPCF00000000.1)         Bradyrhizobium forestalis INPA54B <sup>T</sup> (NZ, EPCF000152.1)         Bradyrhizobium subtarnawn BS 2-1 <sup>T</sup> (KP608152.1)         Bradyrhizobium gycanningense CCBAU 10071 <sup>T</sup> (AF193818)         Imadyrhizobium returnae Ro15 <sup>T</sup> (NZ_LLYA0000000.1)         Imadyrhizobium neetropicale BR 10247 <sup>T</sup> (NZ_LSEF00000000.1)         Imadyrhizobium neetropicale DR 10247 <sup>T</sup> (NZ_LSEF00000000.1)         Imadyrhizobium neetropicale DR 10247 <sup>T</sup> (NZ_LSEF00000000.1)         Imadyrhizobium torpiciagri CNPSo1112 <sup>T</sup> (AY904753.1)         Bradyrhizobium neetropicale BR 10303 <sup>T</sup> (NZ_LNCU0000000.1)         Imadyrhizobium macuinese BR 10303 <sup>T</sup> (NZ_LNCU0000000.1)         Bradyrhizobium autense UFLA 03-184 <sup>T</sup> (NZ_VKHP01000993.1)         Bradyrhizobium autenses UFLA 30-184 <sup>T</sup> (NZ_VKHP01000993.1)         Bradyrhizobium maturatese SBR 630 <sup>T</sup> (F1		Bradyrhizobium stylosanthis BR 446 (NZ_LVEM01000003.1)
Bradyrhizobium forestalis INPAS4B <sup>1</sup> (NZ_PGVG0000000.1) Bradyrhizobium submaningense CCBAU 10071 <sup>1</sup> (AF193818) Bradyrhizobium agreste CNPSo 4010 <sup>5</sup> (MK676048) Bradyrhizobium agreste CNPSo 4016 <sup>5</sup> (MK676048) Bradyrhizobium retamae Ro 15 <sup>1</sup> (NZ_LLYA0000000.1) Bradyrhizobium nectropicale BR 10247 <sup>5</sup> (NZ_SEF0000000.1) Bradyrhizobium nectropicale BR 10247 <sup>5</sup> (NZ_SEF00000000.1) Bradyrhizobium mectropicale BR 10247 <sup>5</sup> (NZ_SEF00000000.1) Bradyrhizobium mectropicale BR 10247 <sup>5</sup> (NZ_SEF00000000.1) Bradyrhizobium mectropicale BR 10303 <sup>7</sup> (NZ_LNCU00000000.1) Bradyrhizobium mectropicale BR 10303 <sup>7</sup> (NZ_LNCU00000000.1) Bradyrhizobium achines UFLA 03-521 <sup>7</sup> (NZ_NUPV00000000.1) Bradyrhizobium ageriense BR 10303 <sup>7</sup> (NZ_LNCU00000000.1) Bradyrhizobium mectropheir CCBAU 53025 <sup>7</sup> (KF114645.1) Bradyrhizobium mectropheir CCBAU 53025 <sup>7</sup> (KF114645.1) Bradyrhizobium methylapheir SCBA 630 <sup>6</sup> (F1025102.1) Bradyrhizobium methylapheir SCBAU 23008 <sup>7</sup> (KF10677) Bradyrhizobium methylapheir SCBAU 2303		Bradyrhizobium vignae LMG 28791 <sup>T</sup> (NZ_RDQF00000000.1)
Bradyrhizobium subterraneum 58 2-1 <sup>1</sup> (KP308152.1) Bradyrhizobium agreste CNPSO 4010 <sup>7</sup> (MF39818) Bradyrhizobium agreste CNPSO 4010 <sup>7</sup> (MK576048) Bradyrhizobium agreste CNPSO 4015 <sup>7</sup> (MK576047) Tal Bradyrhizobium achetypum WSM 1744 <sup>1</sup> (MK676065) Bradyrhizobium centrolobii BR 10245 <sup>7</sup> (KZ_LSF00000000.1) Bradyrhizobium elkanii USDA 76 <sup>7</sup> (U35000) Bradyrhizobium metropicale BR 10247 <sup>7</sup> (KZ_LSF00000000.1) Bradyrhizobium metropicale BR 10247 <sup>7</sup> (KZ_LSF00000000.1) Bradyrhizobium metropicale CNPSO 1112 <sup>7</sup> (MY204753.1) Bradyrhizobium metropicale CNPSO 112 <sup>7</sup> (MZ_LSF00000000.1) Bradyrhizobium metura Bradi (MF593081.1) Bradyrhizobium metura Bradi (MF593081.1) Bradyrhizobium metura Bradi (MZ S00000000.1) Bradyrhizobium metura Bradi (MZ S000000000.1) Bradyrhizobium metura Bradi (MZ S00000000.1) Bradyrhizobium metura Bradi (MZ S0000000.1) Bradyrhizobium metura SBM 6390 <sup>7</sup> (F10464.1) Bradyrhizobium metura SMM 6390 <sup>7</sup> (F1045102.1) Bradyrhizobium metura SMM 6390 <sup>7</sup> (F1045002.1) Bradyrhizobium metura SMM 6390 <sup>7</sup> (F1045007) Bradyrhizobium metura SMM 7191 <sup>7</sup> (MK676067) Bradyrhizobium metura SMM 7191 <sup>7</sup> (MK676067) Bradyrhizobium mathina set IMT 13 <sup>7</sup> (K749832) Bradyrhizobium mathina set		Bradyrhizobium forestalis INPA54B <sup>T</sup> (NZ_PGVG00000000.1)
Bradyrhizobium greate CVBS0 4010 <sup>11</sup> (AF193818) Bradyrhizobium agreste CVBS0 4016 <sup>7</sup> (MK676048) Bradyrhizobium archetypum WSM 1744 <sup>7</sup> (MK676047) Bradyrhizobium retamae Ro16 <sup>7</sup> (NZ_LLYA00000000.1) Bradyrhizobium retamae Ro16 <sup>7</sup> (NZ_LLYA00000000.1) Bradyrhizobium neatorpicale BR 10245 <sup>7</sup> (KZ_SEF000000000.1) Bradyrhizobium ineatorpicale BR 10247 <sup>7</sup> (NZ_SEF000000000.1) Bradyrhizobium ineatorpicale BR 10303 <sup>7</sup> (NZ_LNCU00000000.1) Bradyrhizobium ineatorpicale BR 10303 <sup>7</sup> (NZ_LNCU0000000.1) Bradyrhizobium metarpanes CNP50 2833 <sup>7</sup> (XP114445.1) Bradyrhizobium metarpanes CNP50 2833 <sup>7</sup> (XP01000093.1) Bradyrhizobium ineatorpicale SEMA 6390 <sup>7</sup> (F1025107.1) Bradyrhizobium ineatorpicale SEMA 6390 <sup>7</sup> (F1025107.1) Bradyrhizobium autainese VSM 1791 <sup>7</sup> (MK676067) Bradyrhizobium autainese VSM 1791 <sup>7</sup> (MK676067) Bradyrhizobium metarpanes LMR 21 <sup>7</sup> (VF20301) Bradyrhizobium metarpanes LMR 13 <sup>7</sup> (KF980150) Bradyrhizobium materiace MIR 13 <sup>7</sup> (KF980150) Bradyrhizobium materiace MIR 13 <sup>7</sup> (KF980150) Bradyrhizobium materiace MIR 12 <sup>7</sup> (Y220301) Antrobacter autoropricus P/2 (NC_009720.1)		Bradyrhizobium subterraneum 58 2-1 <sup>T</sup> (KP308152.1)
<pre>Bradyrhizobium agreste CNPSo 4010<sup>*</sup> (MK676049) Bradyrhizobium archatypum VSM 1744<sup>*</sup> (MK676047) Bradyrhizobium archatypum VSM 1744<sup>*</sup> (MK676065) Bradyrhizobium retamae Ro10<sup>‡</sup> (NZ_LLYA00000000.1) Bradyrhizobium retamae Ro10<sup>‡</sup> (NZ_LLYA00000000.1) Bradyrhizobium nectropicale BR 10247<sup>‡</sup> (NZ_LSEF00000000.1) Bradyrhizobium topicalegie R 10247<sup>‡</sup> (NZ_LSEF00000000.1) Bradyrhizobium topicalegie CNPSo1112<sup>‡</sup> (AY904753.1) Bradyrhizobium topicalegie CNPSo1112<sup>‡</sup> (AY904753.1) Bradyrhizobium topicalegie R 10303<sup>‡</sup> (NZ_LNCU00000000.1) Bradyrhizobium activates BR 10303<sup>‡</sup> (NZ_LNCU00000000.1) Bradyrhizobium algeriense RS189<sup>‡</sup> (PYCM01000001.1) Bradyrhizobium augrese CI-18<sup>‡</sup> (NX396570.1) Bradyrhizobium mercantei SEMA 6399<sup>‡</sup> (PYCM01000091.1) Bradyrhizobium mercantei SEMA 6399<sup>‡</sup> (PYCM01000091.1) Bradyrhizobium mercantei SEMA 6399<sup>‡</sup> (PYCM01000091.1) Bradyrhizobium mercantei SEMA 6399<sup>‡</sup> (PYCM01000001.1) Bradyrhizobium mercantei SEMA 6399<sup>‡</sup> (PYCM01000000.1) Bradyrhizobium mercantei SEM</pre>		Bradyrhizobium yuanmingense CCBAU 10071 <sup>T</sup> (AF193818)
as Bradyrhizobium glycinis CNPSo 4016 <sup>°</sup> (MK676047) Ta Bradyrhizobium retamae Ro 15 <sup>°</sup> (NZ_LLYA0000000.1) as Construction of the second secon		Bradyrhizobium agreste CNPSo 4010 <sup>T</sup> (MK676048)
Bradyrhizobium achetypum WSM 1744 <sup>1</sup> (MK678065) Bradyrhizobium centrolobii BR 10245 <sup>1</sup> (KPL1VA00000000.1) Bradyrhizobium entrolopicale BR 10245 <sup>1</sup> (KPL2V60000000.1) Bradyrhizobium elexini USDA 76 <sup>1</sup> (U35000) Bradyrhizobium moreiogial CNPS01112 <sup>1</sup> (AY904753.1) Bradyrhizobium maeixerse URLA 03-321 <sup>1</sup> (KPL000000000.1) Bradyrhizobium maeixerse URLA 03-321 <sup>1</sup> (KPL000000000.1) Bradyrhizobium macuriense BR 10303 <sup>1</sup> (NZ_LNCU00000000.1) Bradyrhizobium macuriense BR 10303 <sup>1</sup> (NZ_LNCU00000000.1) Bradyrhizobium icense BR 10303 <sup>1</sup> (NZ_LNCU00000000.1) Bradyrhizobium icense BR 10303 <sup>1</sup> (NZ_LNCU00000000.1) Bradyrhizobium icense BR 1030 <sup>1</sup> (NZ_LNCU00000000.1) Bradyrhizobium icense BR 1030 <sup>1</sup> (NZ_LNCU00000000.1) Bradyrhizobium icense BR 1030 <sup>1</sup> (NZ_UKHP01000093.1) Bradyrhizobium mercanets SEMB 6309 <sup>1</sup> (FPU50100001.1) Bradyrhizobium mercanets SEMB 6309 <sup>1</sup> (FJ025102.1) Bradyrhizobium icense CNE92 2833 <sup>1</sup> (AY9404773.1) Bradyrhizobium icense CNE92 2833 <sup>1</sup> (AY9404773.1) Bradyrhizobium icense SMI 1791 <sup>1</sup> (MK676067) Bradyrhizobium michanese CNE92 2833 <sup>1</sup> (AY940773.1) Bradyrhizobium michanese CNE92 2833 <sup>1</sup> (AY940773.1) Bradyrhizobium michanese CNE92 2833 <sup>1</sup> (AY940773.1) Bradyrhizobium icense LMR 13 <sup>1</sup> (KF14483.2) Bradyrhizobium michanese LMR 13 <sup>1</sup> (KF14483.2) Bradyrhizobium machanese LMR 13 <sup>1</sup> (KF890616) Bradyrhizobium machanese LMR 13 <sup>1</sup> (KF90616) Bradyrhizobium manufanese LMR 13 <sup>1</sup> (KF902031) Cantrobacter autoropricus P/2 (NC_009720.1)		88 Bradyrhizobium glycinis CNPSo 4016 (MK676047)
Bradyrhizobium retamae Ro16 <sup>T</sup> (NZ_LLYA00000000.1)     Bradyrhizobium neotropicale BR 10245 <sup>T</sup> (KF927049.1)     Bradyrhizobium neotropicale BR 10247 <sup>T</sup> (NZ_LSEF00000000.1)     Bradyrhizobium tekaniu USDA 76 <sup>T</sup> (U35000)     Bradyrhizobium tekaniu USDA 76 <sup>T</sup> (U350000000.1)     Bradyrhizobium maturianse BR 10303 <sup>T</sup> (NZ_LNCU0000000.1)     Bradyrhizobium maturianse BR 10303 <sup>T</sup> (NZ_LNCU0000000.1)     Bradyrhizobium maturianse UFLA 03-321 <sup>T</sup> (NZ_UNCU000000.1)     Bradyrhizobium maturianse UFLA 03-184 <sup>T</sup> (NZ_VKHP01000093.1)     Bradyrhizobium autense UFLA 03-184 <sup>T</sup> (NZ_VKHP01000093.1)     Bradyrhizobium maturianse CNP30 2833 <sup>T</sup> (XP114445.1)     Bradyrhizobium micrapense CNP30 2833 <sup>T</sup> (XP04773.1)     Bradyrhizobium micrapense CNP30 2833 <sup>T</sup> (XP040773.1)     Bradyrhizobium incatanies USMA 6300 <sup>T</sup> (F1025107.1)     Bradyrhizobium incatanies USMA 6301 <sup>T</sup> (F104682.2)     Bradyrhizobium micrapense UNP3 1791 <sup>T</sup> (MK676067)     Bradyrhizobium micrapense LMFR 13 <sup>T</sup> (KP590160)     Bradyrhizobium micrapense LMFR 13 <sup>T</sup> (KP590160)     Bradyrhizobium maturianese LMFR 13 <sup>T</sup> (KP590160)     Bradyrhizobium maturianese LMFR 13 <sup>T</sup> (KP590160)     Bradyrhizobium maturianese LMFR 13 <sup>T</sup> (KP590160)     Bradyrhizobium mataliense LMFR 13 <sup>T</sup> (KP590160)     Bradyrhizobium maturianese LMFR 13 <sup>T</sup> (KP590160)     Bradyrhizobium maturianum LMFR 13 <sup>T</sup> (KF590160)     Bradyrhizo		78 Bradyrhizobium archetypum WSM 1744 <sup>T</sup> (MK676065)
Bradyrhizobium centrolobii BR 10245 <sup>7</sup> (KF927049.1)     Bradyrhizobium neotropicale BR 10247 <sup>7</sup> (NZ_LSEF00000000.1)     Bradyrhizobium teariu USDA 76 <sup>7</sup> (U35000)     Bradyrhizobium tipae WF4 <sup>7</sup> (MF930801.1)     Bradyrhizobium tipae WF4 <sup>7</sup> (MF930801.1)     Bradyrhizobium taxilense UFLA 03-321 <sup>7</sup> (NZ_LNCU00000000.1)     Bradyrhizobium taxilense UFLA 03-321 <sup>7</sup> (NZ_LNCU00000000.1)     Bradyrhizobium taxilense UFLA 03-321 <sup>7</sup> (NZ_LNCU00000000.1)     Bradyrhizobium taxilense BR 10303 <sup>7</sup> (NZ_LNCU00000000.1)     Bradyrhizobium auxiense BR 10303 <sup>7</sup> (NZ_LNCU00000000.1)     Bradyrhizobium algariense RST89 <sup>7</sup> (PYCM01000001.1)     Bradyrhizobium angleriense RST89 <sup>7</sup> (PYCM01000093.1)     Bradyrhizobium mercantei SEMA 6399 <sup>6</sup> (FI025102.1)     Bradyrhizobium mercantei SEMA 6399 <sup>6</sup> (FI025102.1)     Bradyrhizobium icenare CNPS0 2833 <sup>7</sup> (AY904773.1)     Bradyrhizobium icenare NSM 1791 <sup>7</sup> (MK676067)     I Bradyrhizobium australiense WSM 1791 <sup>7</sup> (MK676067)     I Bradyrhizobium australiense USM 1791 <sup>7</sup> (MK676067)     I Bradyrhizobium australiense LMR 123 <sup>1</sup> (KF98065)     Bradyrhizobium australiense ELMR 123 <sup>1</sup> (KF98065)     Bradyrhizobium australiense ELMR 131 <sup>7</sup> (MK676062)     Bradyrhizobium australiense ELMR 131 <sup>7</sup> (MK676067)     I Bradyrhizobium australiense ELMR 131 <sup>7</sup> (KF98065)     Bradyrhizobium mercanteise ELMR 131 <sup>7</sup> (KF98065)     Bradyrhizobium australiense ELMR 131 <sup>7</sup> (KF98065)     Bradyrhizobium australiense ELMR 131 <sup>7</sup> (KF98065)     Bradyrhizobium market REM 131 <sup>7</sup> (KF980510)     Bradyrhizobium market REM		Bradyrhizobium retamae Ro19 <sup>T</sup> (NZ_LLYA00000000.1)
Bradythizobium neotropicale BR 10247 <sup>7</sup> (NZ_LSEF00000000.1) Bradythizobium elkanii USDA 76 <sup>7</sup> (U35000) Bradythizobium topiciagri CNP6o1112 <sup>7</sup> (NY904753.1) Bradythizobium tipae WRA <sup>7</sup> (MF693081.1) Bradythizobium macuiense BR 10303 <sup>7</sup> (NZ_UNCU0000000.1) Bradythizobium macuiense BR 10303 <sup>7</sup> (NZ_UNCU0000000.1) Bradythizobium macuiense BR 10303 <sup>7</sup> (NZ_UNCU0000000.1) Bradythizobium algeriense RST89 <sup>7</sup> (PCM01000001.1) Bradythizobium algeriense RST89 <sup>7</sup> (PCM01000001.1) Bradythizobium mercanse CI-TB <sup>7</sup> (KX36457.0.1) Bradythizobium mercanse CI-TB <sup>7</sup> (KY44541.1) Bradythizobium mercanse SCM8 6390 <sup>7</sup> (PCM01000093.1) Bradythizobium mercanses CNF90 2833 <sup>7</sup> (NF114645.1) Bradythizobium mercanses CNF90 2833 <sup>7</sup> (NF114645.1) Bradythizobium mercanses CNF90 2833 <sup>7</sup> (NF114645.1) Bradythizobium methagenese CNF90 2833 <sup>7</sup> (NF14645.1) Bradythizobium icanse CNF90 2833 <sup>7</sup> (NF14645.1) Bradythizobium methagenese CNF90 2833 <sup>7</sup> (NF14645.1) Bradythizobium materiatinese CNF90 2833 <sup>7</sup> (NF067067) Bradythizobium materiatinese CNF91 <sup>7</sup> (MK676067) Bradythizobium materiatinese BITR 13 <sup>7</sup> (KF164615.2) Bradythizobium materiatinese F-10 <sup>7</sup> (CK681401.2) Bradythizobium materiatinese F-10 <sup>7</sup> (C		Bradyrhizobium centrolobii BR 10245 <sup>T</sup> (KF927049.1)
Bulky/Hazabium elkanii USDA 76 <sup>7</sup> (U3S00)         Fradyrhizabium ispainee WR4 <sup>7</sup> (MF93000)         Bradyrhizabium trasienes UFLA 03-321 <sup>7</sup> (NZ_MPVQ0000000.1)         Bradyrhizabium brasienes UFLA 03-321 <sup>7</sup> (NZ_LNCU0000000.1)         Bradyrhizabium ispainees UFLA 03-321 <sup>7</sup> (NZ_LNCU0000000.1)         Bradyrhizabium machynia PAC48 <sup>7</sup> (AV524135)         Bradyrhizabium ispainees UFLA 03-321 <sup>7</sup> (NZ_LNCU0000000.1)         Bradyrhizabium ispainees UFLA 03-321 <sup>7</sup> (NZ_LNCU0000000.1)         Bradyrhizabium ispainees UFLA 03-3164 <sup>7</sup> (NY536570.1)         Bradyrhizabium ispainees UFLA 03-184 <sup>7</sup> (NZ VKHP01000093.1)         Bradyrhizabium metricates UFLA 03-184 <sup>7</sup> (NZ VKHP01000000.1)         Bradyrhizabium metricates USM 1791 <sup>7</sup> (INK670670         Bradyrhizabium autainees UFLA 13 <sup>7</sup> (NY 200000000.1)         Bradyrhizabium machates USM 1791 <sup>7</sup> (MK670682)         Bradyrhizabium metricates USM 1791 <sup>7</sup> (MK670682)         Bradyrhizabium metricates USM 1791 <sup>7</sup> (MK6780		Bradyrhizobium neotropicale BR 10247 <sup>T</sup> (NZ LSEE00000000 1)
<ul> <li>Bildynizzobium topicagri CNPSo112<sup>2</sup> (AY904753.1)</li> <li>Bradyrhizobium topicagri CNPSo112<sup>2</sup> (AY904753.1)</li> <li>Bradyrhizobium topicagri CNPSo112<sup>2</sup> (AY904753.1)</li> <li>Bradyrhizobium torasilense WR4<sup>1</sup> (MF593081.1)</li> <li>Bradyrhizobium torasilense WR4<sup>1</sup> (MF593081.1)</li> <li>Bradyrhizobium torasilense WR4 (VE24155)</li> <li>Bradyrhizobium macuiense B10303<sup>7</sup> (NZ_LNCU00000000.1)</li> <li>Bradyrhizobium inverse CI-15<sup>1</sup> (XX396570.1)</li> <li>Bradyrhizobium autense UFLA 03-81<sup>4</sup> (NZ_VKHP0100093.1)</li> <li>Bradyrhizobium autense UFLA 03-164<sup>7</sup> (NZ_VKHP0100093.1)</li> <li>Bradyrhizobium mercantei SEMA 6993<sup>7</sup> (F025102.1)</li> <li>Bradyrhizobium mercantei SEMA 6993<sup>2</sup> (F025102.1)</li> <li>Bradyrhizobium autense VNA 690<sup>3</sup> (F1025107.1)</li> <li>Bradyrhizobium autense VNM 1791<sup>4</sup> (MK676067)</li> <li>Bradyrhizobium autenses VNM 1791<sup>4</sup> (MK676067)</li> <li>Bradyrhizobium autenses LIMR 13<sup>3</sup> (KS14883.2)</li> <li>Bradyrhizobium invanetatiense VNM 1741<sup>4</sup> (MK676062)</li> <li>Bradyrhizobium invalentimus LIMR 13<sup>4</sup> (KS76067)</li> <li>Bradyrhizobium invalentimus EMR 163<sup>4</sup> (KS76067)</li> <li>Bradyrhizobium invalentimus EMR 163<sup>4</sup> (KS76067)</li> <li>Bradyrhizobium invalentimus EMR 13<sup>4</sup> (KS76067)</li></ul>		- Bradyhizobium alkanii LISDA 76 <sup>T</sup> (125000)
<ul> <li>Badyritizzbium ingeukgin-VRF4 (WF93081.1)</li> <li>Bradyritizzbium ingeukgin-VRF4 (WF93081.1)</li> <li>Bradyritizzbium ingeukgin-VRF4 (WF93081.1)</li> <li>Bradyritizzbium macuyinse UFLA 03-321<sup>T</sup> (NZ_MPV00000000.1)</li> <li>Bradyritizzbium macuyinse BR 10303<sup>T</sup> (NZ_LNCU00000000.1)</li> <li>Bradyritizzbium macuyinse BR 10303<sup>T</sup> (NZ_LNCU00000000.1)</li> <li>Bradyritizzbium inverses CI-15<sup>T</sup> (K3308570.1)</li> <li>Bradyritizzbium algeriense RST83<sup>T</sup> (PYCM01000001.1)</li> <li>Bradyritizzbium algeriense RST83<sup>T</sup> (PYCM01000093.1)</li> <li>Bradyritizzbium merchenic SEMA 6393<sup>T</sup> (IP205102.1)</li> <li>Bradyritizzbium merchenic SEMA 6393<sup>T</sup> (IP205102.1)</li> <li>Bradyritizzbium indirane PAC88<sup>T</sup> (AY904773.1)</li> <li>Bradyritizzbium uitarianes UFLA 03-164<sup>T</sup> (K14685.1)</li> <li>Bradyritizzbium witarianes UFLA 03-164<sup>T</sup> (K15483.2)</li> <li>Bradyritizzbium uitarialense UFMA 6901<sup>T</sup> (IP305107.1)</li> <li>Bradyritizzbium uitarialense UFMA 1791<sup>T</sup> (MK676067)</li> <li>Bradyritizzbium incense LIMR 163<sup>T</sup> (K146876062)</li> <li>Bradyritizzbium incense LIMR 163<sup>T</sup> (K146876062)</li> <li>Bradyritizzbium incense LIMR 163<sup>T</sup> (K7496156)</li> <li>Bradyritizzbium incense E-IMR 163<sup>T</sup> (K7496156)</li> <li>Bradyritizzbium incense E-IMR 163<sup>T</sup> (K7496156)</li> <li>Bradyritizzbium incense E-IMR 163<sup>T</sup> (K7496150)</li> </ul>		Productionality and a series of CNIPS at 110 <sup>T</sup> (AV004752.1)
Figury/fizzbium brasilerse UFLA 03-321 <sup>*</sup> (NZ_MPVQ00000000.1) Brady/fizzbium brasilerse UFLA 03-321 <sup>*</sup> (NZ_MPVQ00000000.1) Brady/fizzbium macuziense BR 10303 <sup>*</sup> (NZ_LNCU00 <sup>*</sup> (VB18096.1) Brady/fizzbium incense BR 10303 <sup>*</sup> (NZ_LNCU00 <sup>*</sup> (VB18096.1) Brady/fizzbium incense CI-16 <sup>*</sup> (XS396570.1) Brady/fizzbium ingeriense RST89 <sup>*</sup> (PCVM0100000.1) Brady/fizzbium usiense UFLA 03-184 <sup>*</sup> (NZ_VKHP01000983.1) Brady/fizzbium mercantei SEMA 6399 <sup>*</sup> (F014645.1) Brady/fizzbium mercantei SEMA 6399 <sup>*</sup> (F025102.1) Brady/fizzbium mercantei SEMA 6399 <sup>*</sup> (F025102.1) Brady/fizzbium inatienses CNP50-2833 <sup>*</sup> (X904773.1) Brady/fizzbium inatienses CNP50-2833 <sup>*</sup> (X904773.1) Brady/fizzbium usianiense VSMI 1791 <sup>*</sup> (MK676067) Brady/fizzbium usianiense VSMI 1791 <sup>*</sup> (MK676067) Brady/fizzbium usianiense USMI 1791 <sup>*</sup> (MK676067) Brady/fizzbium inatieniense LMR 121 <sup>*</sup> (KF14883.2) Brady/fizzbium incense LMRT 13 <sup>*</sup> (KF896156) Brady/fizzbium inatienium cmNI <sup>3</sup> (KF1489.2) Brady/fizzbium inatienium cmNI <sup>3</sup> (KF3661401.2) Brady/fizzbium inatienium inatienii SEMA 6301 <sup>*</sup> (KF1469.1) Brady/fizzbium manifisnes E LITR 13 <sup>*</sup> (KF896156) Brady/fizzbium manifisnes E LITR 13 <sup>*</sup> (KF3061401.2) Brady/fizzbium manifisnes E LITR 13 <sup>*</sup> (KF3061401.2) Brady/fizzbium manifisnes E LITR 13 <sup>*</sup> (KF3061401.2) Brady/fizzbium manifisnes E LI <sup>*</sup> (XF20301) Xanthobacter autotrophicus Py2 (NC_009720.1)		Bradyhizobium uopiciagii GNP301112 (A1904753.1)
B The Bradynizobium basienee UFLA 3-321 (VZ_MPK0000000.1) Bradynizobium pachynizi PAC48 <sup>1</sup> (AY624135) + Bradynizobium macuianes BR 10303 <sup>1</sup> (VZ_LNCU00000000.1) Bradynizobium inorense CI-1B <sup>1</sup> (VX396570.1) Bradynizobium algenizense RST89 <sup>1</sup> (PYCM01000001.1) Bradynizobium algenizense RST89 <sup>1</sup> (PYCM01000001.1) Bradynizobium algenizense RST89 <sup>2</sup> (PYCM01000001.1) Bradynizobium algenizense RST89 <sup>2</sup> (FYCM01000001.1) Bradynizobium algenizense RST89 <sup>2</sup> (FYCM01000093.1) Bradynizobium algenizense RST89 <sup>2</sup> (FYCM0100093.1) Bradynizobium algenizense RST89 <sup>2</sup> (FYCM0100093.1) Bradynizobium algenizense RST89 <sup>2</sup> (FYCM0173.1) Bradynizobium micraaperse CNPS0 2833 <sup>2</sup> (AY604773.1) Bradynizobium igamae PAC88 <sup>3</sup> (AY604773.1) Bradynizobium autaliense WSM 1791 <sup>-1</sup> (MK676067) Bradynizobium autaliense WSM 1791 <sup>-1</sup> (MK676067) Bradynizobium autaliense WSM 1791 <sup>-1</sup> (MK676062) Bradynizobium algelabi CCBAU 2308 <sup>2</sup> (XE144) Bradynizobium algelabi CCBAU 2308 <sup>2</sup> (XE1490000000.1) Bradynizobium namidaci WSM 1741 <sup>-1</sup> (MK676062) Bradynizobium namidaci WSM 1741 <sup>-1</sup> (MK676062) Bradynizobium namidaci WSM 1741 <sup>-1</sup> (MK676012) Bradynizobium namidaci WSM 1741 <sup>-1</sup> (WS90116) Bradynizobium namidaci WSM 1741 <sup>-1</sup> (WS90110) Bradynizobium namida		Bradyrhizobium ripae WR4 (MF593081.1)
Bradyrhizobium pachyrhiz PAC48 <sup>°</sup> (Xr624135)         Bradyrhizobium macuiense BR 10303 <sup>°</sup> (NZ_LNCU00000000.1)         Bradyrhizobium ivorense CI-1B <sup>°</sup> (KX396570.1)         Bradyrhizobium algeriense RST89 <sup>°</sup> (PVCM01000001.1)         Bradyrhizobium algeriense RST89 <sup>°</sup> (PVCM01000093.1)         Bradyrhizobium ervtrophelic CCBAU 51302 <sup>°</sup> (KV14P6100093.1)         Bradyrhizobium mercantei SEMM 6390 <sup>°</sup> (Fl025102.1)         Bradyrhizobium mercantei SEMM 6393 <sup>°</sup> (Fl025102.1)         Bradyrhizobium mercantei SEMM 6393 <sup>°</sup> (Fl025107.1)         Bradyrhizobium understeinse VSM 1791 <sup>°</sup> (MK676067)         Bradyrhizobium australienses VSM 1791 <sup>°</sup> (MK676067)         Bradyrhizobium mercanteise SMM 1741 <sup>°</sup> (MK676062)         Bradyrhizobium mercanteise SIMM 630 <sup>°</sup> (K21488.2)         Bradyrhizobium mercanteises VSM 1791 <sup>°</sup> (MK676062)         Bradyrhizobium mercanteises VSM 1741 <sup>°</sup> (MK676062)         Bradyrhizobium mercanteises F-10 <sup>°</sup> (KK681401.2)         Bradyrhizobium mercanteises F-10 <sup>°</sup> (KK68101.2)         Bradyrhizobium mathem se -10 <sup>°</sup> (KK68101.2)         Bra		Bradyrhizobium brasilense UFLA 03-321 (NZ_MPVQ00000000.1)
Bradyrhizobium macuziense BR 10303 <sup>(</sup> NZ_LNCU000000.1)         Bradyrhizobium ierniligni CCBAU 51502 <sup>(</sup> (XJB18096.1)         Bradyrhizobium ierniligni CCBAU 51502 <sup>(</sup> (XJB18096.1))         Bradyrhizobium algeriense RSTB <sup>5</sup> <sup>(</sup> (PYCM01000001.1)         Bradyrhizobium algeriense RSTB <sup>5</sup> <sup>(</sup> (PYCM01000093.1)         Bradyrhizobium mercanse UFLB <sup>(</sup> (XJB676.1))         Bradyrhizobium mercanses VERA 6393 <sup>(</sup> (PI205102.1))         Bradyrhizobium mercanses CNP50 2833 <sup>(</sup> (XY904773.1))         Bradyrhizobium icamae PAC88 <sup>(</sup> (XY624134))         Bradyrhizobium australiense WSM 1791 <sup>(</sup> (MK676067))         Bradyrhizobium australiense WSM 1791 <sup>(</sup> (MK676067))         Bradyrhizobium murdachi WSM 1741 <sup>(</sup> (MK676062))         Bradyrhizobium icanse LMR 13 <sup>(</sup> (KF969156))         Bradyrhizobium icanse LMR 13 <sup>(</sup> (KF96156))         Bradyrhizobium icanse LMR 13 <sup>(</sup> (KF96165))         Bradyrhizobium icanse LMR 13 <sup>(</sup> (KF96161))         Bradyrhizobium icanse LMR 12 <sup>(</sup> (XP92301))		Bradyrhizobium pachyrhizi PAC48' (AY624135)
Bradyrhizobium familiani CCBAU 51502 <sup>1</sup> (KJB18096.1)     Bradyrhizobium ivoranse CI-1B <sup>7</sup> (KX396570.1)     Bradyrhizobium auiense UFLA 03-1B4 <sup>7</sup> (NZ_VKHP01000993.1)     Bradyrhizobium auiense UFLA 03-1B4 <sup>7</sup> (NZ_VKHP01000993.1)     Bradyrhizobium embrapense CNP50-2833 <sup>7</sup> (XF114445.1)     Bradyrhizobium embrapense CNP50-2833 <sup>7</sup> (XF114445.1)     Bradyrhizobium auiense CNP50-2833 <sup>7</sup> (XF04713.1)     Bradyrhizobium icamae PAC88 <sup>7</sup> (AF04713.1)     Bradyrhizobium icamae PAC88 <sup>7</sup> (AF04713.1)     Bradyrhizobium auiense VSM 1791 <sup>7</sup> (MK676067)     Bradyrhizobium auieniane WSM 1791 <sup>7</sup> (MK676067)     Bradyrhizobium auieniane UMIS1 (XF14832.2)     Bradyrhizobium auieniane UMIS1 (XF14832.2)     Bradyrhizobium auienianes LMIR 13 <sup>7</sup> (KF896156)     Bradyrhizobium ane LMTR 13 <sup>7</sup> (KF896156)     Bradyrhizobium ane LMTR 13 <sup>7</sup> (KF20301)     Zardyrhizobium analyticanes LMTR 21 <sup>7</sup> (AY23031)		Bradyrhizobium macuxiense BR 10303 <sup>1</sup> (NZ_LNCU00000000.1)
L Bradyrhizobium ivorense Cl-18 <sup>1</sup> (XX396570.1) Bradyrhizobium uaiense UFL 03-184 <sup>1</sup> (NZ_VK4P01000993.1) Bradyrhizobium eythrophici CCBAU 5325 <sup>2</sup> (KY14645.1) Bradyrhizobium mercantei SEMIA 6393 <sup>1</sup> (FJ025102.1) Bradyrhizobium mercantei SEMIA 6393 <sup>1</sup> (FJ025102.1) Bradyrhizobium windifuturi SEMIA 6393 <sup>1</sup> (FJ025107.1) Bradyrhizobium windifuturi SEMIA 690 <sup>7</sup> (FJ025107.1) Bradyrhizobium australiense VVSM 1791 <sup>4</sup> (MK676067) Bradyrhizobium murchochi WSM 1741 <sup>7</sup> (MK676067) Bradyrhizobium murchochi WSM 1741 <sup>7</sup> (MK676062) Bradyrhizobium materaliense 5-10 <sup>7</sup> (KK681401.2) Bradyrhizobium materaliense 5-10 <sup>7</sup> (KK681401.2) Bradyrhizobium materaliense 5-10 <sup>7</sup> (KK92031) — Xarthobacter autotrophicus Py2 (NC_009720.1)		Bradyrhizobium ferriligni CCBAU 51502 <sup>T</sup> (KJ818096.1)
Bradyrhizobium algeriense RST89 <sup>1</sup> (PYCM01000001.1) Bradyrhizobium algeriense RST89 <sup>1</sup> (PYCM01000093.1) Bradyrhizobium erythrophiai CCBAU 53325 <sup>1</sup> (KF114645.1) Bradyrhizobium mercanies ISEMA 6399 <sup>2</sup> (FI025102.1) Bradyrhizobium iembrapense CNPSo 2833 <sup>3</sup> (AY904773.1) Bradyrhizobium australiense WSM 1791 <sup>1</sup> (MK576067) Bradyrhizobium murdochi WSM 1741 <sup>1</sup> (MK676062) Bradyrhizobium icense LIMR 13 <sup>3</sup> (KF896156) Bradyrhizobium namibiense 5-10 <sup>5</sup> (KK581401.2) Bradyrhizobium padlaeri LIMR 21 <sup>1</sup> (MK923031) — Xarthobacter autotrophicus Py2 (NC_009720.1)		Bradyrhizobium ivorense CI-1B <sup>T</sup> (KX396570.1)
Bradyrhizobium ualense UFLA 03-184 <sup>T</sup> (NZ_VK4P01000993.1) Bradyrhizobium erythrophiei CCBAU 5332 <sup>E</sup> (KF114645.1) Bradyrhizobium mencharpense CNP50-2833 <sup>T</sup> (K9904773.1) Bradyrhizobium windfulturi SEMM 690 <sup>T</sup> (FJ025107.1) Bradyrhizobium windfulturi SEMM 690 <sup>T</sup> (FJ025107.1) Bradyrhizobium australiense WSM 1791 <sup>T</sup> (MK676067) Bradyrhizobium australiense WSM 1791 <sup>T</sup> (MK676067) Bradyrhizobium murdochi WSM 1741 <sup>T</sup> (MK676062) Bradyrhizobium michabi/ CCBAU 2308 <sup>E</sup> (KZ_LLYB00000000.1) Bradyrhizobium naise LMTR 13 <sup>T</sup> (KF96156) Bradyrhizobium naiser LMTR 13 <sup>T</sup> (KF96156) Bradyrhizobium maliaer LMTR 21 <sup>T</sup> (AY23031) - Xanthobacter autotrophicus Py2 (NC_009720.1)		Bradyrhizobium algeriense RST89 <sup>T</sup> (PYCM01000001.1)
Bradyrhizobium erythrophlei CCBAU 53325 <sup>T</sup> (KF114645.1) Bradyrhizobium mercantei SEMIA 6399 <sup>T</sup> (FJ025102.1) Bradyrhizobium picamae PAC89 <sup>T</sup> (K904773.1) - Bradyrhizobium yicifuturi SEMIA 680 <sup>T</sup> (FJ025107.1) Bradyrhizobium vairdifuturi SEMIA 680 <sup>T</sup> (FJ025107.1) Bradyrhizobium valentinum LnjM3 <sup>T</sup> (X514883.2) Bradyrhizobium murdochi VSMI 1741 <sup>T</sup> (MK676062) Bradyrhizobium murdochi VSMI 1741 <sup>T</sup> (MK676062) Bradyrhizobium icense LMTR 13 <sup>T</sup> (KF896156) Bradyrhizobium icense E-I0 <sup>T</sup> (KK676010.2) Bradyrhizobium pasiliaeri LMTR 21 <sup>T</sup> (KY923031) - Xarthobacter autotrophicus Py2 (NC_009720.1)		Bradyrhizobium uaiense UFLA 03-164 <sup>T</sup> (NZ VKHP01000993.1)
Bradyrhizobium mercantei SEMIA 6393 <sup>T</sup> (F025102.1) Bradyrhizobium embrapense CNPSo 2833 <sup>T</sup> (AY904773.1) Bradyrhizobium iotifuturi SEMIA 6393 <sup>T</sup> (F12025102.1) Bradyrhizobium witifuturi SEMIA 600 (FJ025107.1) Bradyrhizobium australiense VNSM 1791 <sup>T</sup> (MK676067) Bradyrhizobium murdochi WSM 1741 <sup>T</sup> (MK676062) Bradyrhizobium murdochi WSM 1741 <sup>T</sup> (MK676062) Bradyrhizobium michaes E-10 <sup>T</sup> (KK96166) Bradyrhizobium aattenese E-10 <sup>T</sup> (KK961610.2) Bradyrhizobium pasilaeri LMTR 21 <sup>T</sup> (KY923031) — Xarthobacter autotrophicus Py2 (NC_009720.1)		Bradyrbizobium erythrophlei CCBAU 53325 <sup>T</sup> (KE114645.1)
Bradyrhizobium embragenese CNPS0 2833 (V320173.1) Bradyrhizobium gicamae PAC68 <sup>7</sup> (AY624134) Bradyrhizobium uiethiluuri SEMA 690 <sup>7</sup> (FJ025107.1) Bradyrhizobium australiense WSM 1791 <sup>7</sup> (MK676067) Bradyrhizobium murduchi WSM 1741 <sup>7</sup> (MK676062) Bradyrhizobium murduchi WSM 1741 <sup>7</sup> (MK676062) Bradyrhizobium icense LMTR 13 <sup>7</sup> (KF896156) Bradyrhizobium pauliaeri LMTR 21 <sup>7</sup> (AY923031) - Xanthobacter autotrophicus Py2 (NC_009720.1)		Bradychizabium marcantai SEMIA 6299 <sup>T</sup> (El025102.1)
Bradyrhizobium jicamae PAC86 <sup>†</sup> (AY624134)     Bradyrhizobium jicamae PAC86 <sup>†</sup> (AY624134)     Bradyrhizobium widfilutri SEMM 690 <sup>-1</sup> (FJ025107.1)     Bradyrhizobium australiense WSM 1791 <sup>-1</sup> (MK676067)     Bradyrhizobium valentinum LmjM3 <sup>†</sup> (XX514883.2)     Bradyrhizobium lablabi CCBAU 23086 <sup>†</sup> (NZ_LLYB00000000.1)     Bradyrhizobium icense LMTR 13 <sup>†</sup> (KF960f56)     Bradyrhizobium namibierse 5-10 <sup>†</sup> (CX661401.2)     Bradyrhizobium paullaeri LMTR 21 <sup>†</sup> (AY923031)     — Xarthobacter autotrophicus Py2 (NC_009720.1)		Diadymizobian metaaner CENIX 0000 (1020102.1)
Bradyrhizobium jeamae PAC88 (Xr824134)     Bradyrhizobium viridifuturi SEMA 690 <sup>7</sup> (FJ025107.1)     J Eradyrhizobium australiense VSM 1791 <sup>7</sup> (MK676067)     Eradyrhizobium valentinum LnjM3 <sup>7</sup> (XS14883.2)     Eradyrhizobium murdochi VSM 1741 <sup>7</sup> (MK676062)     Eradyrhizobium murdochi VSM 1741 <sup>7</sup> (MK676062)     Bradyrhizobium icense LMTR 13 <sup>4</sup> (KF896156)     Bradyrhizobium namibiense 5-10 <sup>7</sup> (XK681401.2)     Bradyrhizobium mater LMTR 13 <sup>7</sup> (KF920301)     — xarthobacter autotrophicus Py2 (NC_009720.1)		Bradymizobium embrapense CNPS0 2833 (A1904773.1)
Bradyrhizobium vielfulturi SEMM 690 (FJ025107.1)         Bradyrhizobium austaniense WSM 1791 <sup>T</sup> (MK676067)         Bradyrhizobium valentum mulmis <sup>1</sup> (LK14883.2)         Bradyrhizobium murdochi WSM 1741 <sup>T</sup> (MK676062)         Bradyrhizobium murdochi WSM 1741 <sup>T</sup> (MK676062)         Bradyrhizobium babai CCBAU 23086 <sup>T</sup> (XZ_LLYB0000000.1)         Bradyrhizobium familianes et IMTR 13 <sup>T</sup> (KF896156)         Bradyrhizobium pasilaeri LMTR 21 <sup>T</sup> (AY923031)         — Xanthobacter autotrophicus Py2 (NC_009720.1)		Bradyrhizobium jicamae PAC68' (AY624134)
In Bradynhizobium australiense WVSM 1791 <sup>1</sup> (MK676067)         Eradynhizobium valentinum LmJNG <sup>1</sup> (X514883.2)         Bradynhizobium murdochi WSM 1741 <sup>1</sup> (MK676062)         Eradynhizobium lablabi CCBAU 23086 <sup>5</sup> (NZ_LLYB00000000.1)         Bradynhizobium icense LMTR 13 <sup>1</sup> (KF986156)         Bradynhizobium namibiense 5-10 <sup>7</sup> (KX661401.2)         Bradynhizobium parllaeri LMTR 21 <sup>1</sup> (AY923031)		Bradyrhizobium viridifuturi SEMIA 690' (FJ025107.1)
Bradyrhizobium valentinum LnjM3 <sup>T</sup> (XK514883.2)         Bradyrhizobium murdochi VISM 174.1 (MK676062)         Bradyrhizobium lablabi CCBAU 23086 <sup>T</sup> (NZ_LLYB00000000.1)         Bradyrhizobium icense LMTR 13 <sup>T</sup> (KF696156)         Bradyrhizobium namibiense 5-10 <sup>T</sup> (KC61401.2)         Bradyrhizobium pasllaeri LMTR 21 <sup>T</sup> (AY923031)		Bradyrhizobium australiense WSM 1791 (MK676067)
Bradyrhizobium murdochi WSM 1741 <sup>T</sup> (MK676062)         Bradyrhizobium lablaki CCBAU 2308 <sup>6</sup> (NZ_LLYB0000000.1)         Bradyrhizobium icense LMTR 13 <sup>T</sup> (KF696156)         Bradyrhizobium namibianes F-10 <sup>T</sup> (KS61401.2)         Bradyrhizobium pasllaeri LMTR 21 <sup>T</sup> (AY923031)		Bradyrhizobium valentinum LmjM3 <sup>T</sup> (JX514883.2)
Bradyrhizobium lablabi CCBAU 23086 <sup>7</sup> (NZ_LLYB0000000.1) Bradyrhizobium icense LMTR 13 <sup>7</sup> (KF696156) Bradyrhizobium namibiense 5-10 <sup>7</sup> (KX661401.2) Bradyrhizobium paxllaeri LMTR 21 <sup>7</sup> (AY923031) — Xanthobacter autotrophicus Py2 (NC_009720.1)		Bradyrhizobium murdochi WSM 1741 <sup>T</sup> (MK676062)
4 Bradyrhizobium icense LMTR 13 <sup>T</sup> (KF898156) Bradyrhizobium namibiense 5-10 <sup>T</sup> (KX661401.2) Bradyrhizobium paxllæri LMTR 21 <sup>T</sup> (AY923031) — Xanthobacter autotrophicus Py2 (NC_009720.1)		Bradyrhizobium lablabi CCBAU 23086 <sup>T</sup> (NZ_LLYB00000000.1)
Bradyrhizobium namibiense 5-10 <sup>7</sup> (KX681401.2) Bradyrhizobium pasllaeri LMTR 21 <sup>7</sup> (AY923031) — Xanthobacter autotrophicus Py2 (NC_009720.1)		Bradyrhizobium icense LMTR 13 <sup>T</sup> (KF896156)
Bradyrhizobium pasilaeri LMTR 21 <sup>T</sup> (AY923031) ————————————————————————————————————		Bradyrhizobium namibiense 5-10 <sup>T</sup> (KX861401.2)
• eraeyrnizooium paxilaen Lmirt z1 (419/23031) — Xanthobacter autotrophicus Py2 (NC_009720.1)		Production for an INTE OF (AV002021)
Xanthobacter autotrophicus Py2 (NC_009720.1)		· Dradyrnizobium paxilaeri LMTR 21 (AY 923031)
	L	Xanthobacter autotrophicus Py2 (NC_009720.1)

**Fig. 1.** Maximum-likelihood phylogeny based on 16S rRNA alignment (1312 bp), using the T92 (Tamura three-parameter+G+I) model by MEGA version 7. Accession numbers are indicated in parentheses and in Table S1. The novel species are shown in bold. Bootstrap values >70% are indicated at the nodes. *Xanthobacter autotrophicus* Py2 was used as an outgroup. Bar indicates two substitutions per 100 nucleotide positions.

housekeeping genes were performed for the MLSA; the first including *dnaK+glnII+gyrB+recA+rpoB* genes (2009 bp), and the second with complete sequences of *atpD+dnaK+ glnII+gyrB+recA+rpoB* genes (11704 bp), and they are shown in Figs 2 and S7, respectively. The resulting concatenated phylograms maintained the main groups of strains observed in the 16S rRNA phylogeny, but improved the species delineation. Also, the similar topology of both MLSA trees demonstrated that this approach represents a reliable buffer against possible events of recombination at a single locus [44].

In the MLSA with five housekeeping genes (Fig. 2), strains CNPSo 4010<sup>T</sup> and CNPSo 4016<sup>T</sup> remained in a consistent cluster with 99% bootstrap support, with *B. yuanmingense* CCBAU 10071<sup>T</sup> as the closest species with 96.4 and 96.1% nucleotide identity, respectively (Fig. 2, Table 2). Concerning strain CNPSo 4019<sup>T</sup>, *B. liaoningense* LMG 18230<sup>T</sup> was the closest species with 99% bootstrap support and 97.6% similarity; another close species was *B. frederickii* CNPSo 3426<sup>T</sup>, sharing 94.8% nucleotide identity. In addition, the nucleotide identity values among strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> ranged from 95.1 to 97.2%.

Based on an MLSA with five housekeeping genes, Durán et al. [45] proposed the threshold of 97% similarity for Bradyrhizobium species delineation. Even though the strains of this study showed nucleotide identity values slightly above this threshold in the MLSA, other species also shared nucleotide identity values higher than 97%; for example, B. amphicarpaeae 39S1MB<sup>T</sup> and B. symbiodeficiens 85S1MB<sup>T</sup> (97.1%), B. elkanii USDA 76<sup>T</sup> and B. pachyrhizi PAC  $48^{T}$  (97.5%), *B. elkanii* USDA  $76^{T}$  and *B. brasilense* UFLA03-321<sup>T</sup> (97.6%) (data not shown). Recently, also in studies with Bradyrhizobium, Klepa et al. [15], Helene et al. [46] and Fossou et al. [47] reported values higher than 97% when considering five concatenated housekeeping genes. Therefore, as the nucleotide identity is a mathematic parameter and does not take into account specific mutations on gene sequences, we suggest that the threshold of Bradyrhizobium species delineation should be revised.

The symbiotic process between rhizobia and legumes relies on a complex molecular signal exchange that activates several genes responsible for nodule development [48-50]. Phylogeny of nodulation genes does not elucidate the taxonomic status; however, it is important to reveal the evolutionary history of symbiotic relationships, host-range specificity and symbiovar definitions [3, 28, 46, 51, 52]. In this study, the *nodC* gene analysis revealed that CNPSo 4010<sup>T</sup> from G. clandestina clustered in a well-supported clade with B. zhanjiangense CCBAU 51778<sup>T</sup> isolated from nodules of Arachis hypogaea in southeast China [53], CNPSo 4019<sup>T</sup> from *G. max* clustered with 100% bootstrap support with *B. ganzhouense* RITF806<sup>T</sup> isolated from nodules of Acacia melanoxylon grown in China [54]; whereas CNPSo 4016<sup>T</sup>, isolated from G. tabacina, occupied an isolated position (Fig. 3). Note that the phylogenetic position of each of the three strains in the *nodC* tree was different. Comparing the core 16S rRNA and housekeeping genes with the *nodC* gene phylogenies, a different evolutionary pattern

4



**Fig. 2.** Maximum-likelihood phylogeny based on alignment of *dnaK+glnll+gyrB+recA+rpoB* concatenated genes (2009 bp), using the GTR (general time reversible)+G+I model by MEGA version 7. Accession numbers are indicated in Table S1. The novel species are shown in bold. Bootstrap values >70% are indicated at the nodes. *Xanthobacter autotrophicus* Py2 was used as an outgroup. Bar indicates five substitution per 100 nucleotide positions.

was detected in symbiotic phylogeny, suggesting different evolutionary histories, confirming previous reports of other species within the genus *Bradyrhizobium* [16, 28, 51].

# **GENOME FEATURES**

Total DNA of strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> was extracted and used to reconstruct the sequence libraries according to the manufacturer's protocol of Nextera XT kit. The genome sequencing was performed using the MiSeq platform (Illumina) at Embrapa Soja. De novo sequence assemblies were carried out by A5-MiSeq pipeline version 20140604. The genomes were annotated with RAST version 2.0 [55], using default parameters. The draft genome of CNPSo 4010<sup>T</sup> (JACCHP00000000) presented about 7877331 bp with 75 contigs and an N50 value of 251874 bp. The genome size of CNPSo 4016<sup>T</sup> (JACCHQ00000000) was estimated at 7794411 bp, containing 83 contigs and an N50 value of 202190 bp. The genome of CNPSo 4019<sup>T</sup> (JACEGD00000000) was estimated at 8450368 bp, with 133 contigs and an N50 value of 198871 bp. The sequence coverages of genomes CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> were estimated at 95-, 91-, and 94-fold, respectively. Coding DNA sequences were identified as 7883 for CNPSo  $4010^{T}$ , as 7987 for CNPSo  $4016^{T}$  and 8603 for CNPSo  $4019^{T}$ .

Genomic-based measurements of similarity simplify and ensure robust data for microbial taxonomy as a replacement for the traditional DNA-DNA hybridization (DDH) technique [56, 57]. We used average nucleotide identity (ANI) and the digital DNA-DNA hybridization (dDDH) analyses for species delineation considering the suggested cut-off values of 95-96 and 70%, respectively [56, 58, 59]. The genomes of strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> were compared with the closest related strains according to the phylogenetic analyses: B. frederickii CNPSo 3426<sup>T</sup> (SPQS0000000), B. liaoningense LMG 18230<sup>T</sup> (project ID: 1052895 - JGI) and B. yuanmingense CCBAU 10071<sup>T</sup> (FMAE01000000). ANI values were calculated using the ANI calculator [60] and the dDDH values were estimated with the Genome-to-Genome Distance Calculator (GGDC) version 2.1 [61], using the recommended 'formula 2' (identities/ HSP length). Strains CNPSo 4010<sup>T</sup> and CNPSo 4016<sup>T</sup> shared 94.18% of ANI and 55.50% of dDDH to each other and both strains showed values lower than 93.32% for ANI and 51.80% for dDDH when compared with *B. yuanmingense* CCBAU 10071<sup>T</sup> isolated from nodules of *Lespedeza* species plants in China [62] (Table 3). The ANI and dDDH values between CNPSo 4019<sup>T</sup> and *B. liaoningense* LMG 18230<sup>T</sup> isolated from nodules of G. max grown in China [9] were of 94.97 and 59.80 %, respectively. The values below the threshold for species delineation recorded for the three Australian strains isolated from Glycine species confirm that they belong to new species.

The genome G+C contents were calculated using the SEED platform [55] and estimated to be 63.8, 63.7 and 63.8 mol% for CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup>, respectively.



**Fig. 3.** Maximum-likelihood phylogeny based on *nodC* alignment (426 bp), using the T92 (Tamura three-parameter+G+I) model by MEGA version 7. Accession numbers are indicated in parentheses. The novel species are shown in bold. Bootstrap values >70% are indicated at the nodes. Bar indicates five substitutions per 100 nucleotide positions.

	Bradyrhizobi CNPSo	ium agreste 4010 <sup>T</sup>	Bradyrhizol CNPSo	oium glycinis o 4016 <sup>T</sup>	Bradyrhizobiı CNPSo	um diversitatis 9 4019 <sup>T</sup>
Strains	ANI %	dDDH %	ANI %	dDDH %	ANI %	dDDH %
B. fredereickii CNPSo 3426 <sup>T</sup> (SPQS00000000)	88.20	35.80	88.13	35.60	88.66	36.90
B. liaoningense LMG 18230 <sup>™</sup> (JGI Project Id: 1052895)	89.20	38.40	89.19	38.60	94.97	59.80
B. yuanmingense CCBAU $10071^{T}$ (FMAE01000000)	92.62	48.60	93.32	51.80	89.21	38.40
B. agreste CNPSo $4010^{\mathrm{T}}$ (JACCHP000000000)	-	-	94.18	55.50	89.25	38.30
B. glycinis CNPSo 4016 <sup>T</sup> (JACCHQ000000000)	94.18	55.50	-	-	89.28	38.50
B. diversitatis CNPSo 4019 <sup>T</sup> (JACEGD000000000)	89.26	38.40	89.28	38.40	-	-

Table 3. ANI and dDDH values among new lineages of Bradyrhizobium and closely related Bradyrhizobium species

Genomic diversity at strain level was determined by BOX-PCR, using BOX-A1R primer (5'-CTACGGCAAGGCGACG CTGACG-3') [63], with the conditions described by Chibeba *et al.* [64]. The BOX-PCR profiles of strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> and the most related species based on the MLSA, *B. liaoningense* LMG 18230<sup>T</sup> and *B. yuan-mingense* CCBAU 10071<sup>T</sup>, were analysed and compared with the Bionumerics software version 7.6 (Applied Mathematics) using the UPGMA (unweighted pair-group method with arithmetic mean) [65] and the Jaccard coefficient [66], with 2% tolerance. The profiles shared less than 50% similarity among the strains of this study, confirming high diversity, and also varied in relation to the closest *Bradyrhizobium* species (Fig. S8).

Therefore, this is the first study describing novel species isolated from wild *Glycine* (*G. clandestina* and *G. tabacina*) indigenous to Australia, which are usually neglected compared to the economically important soybean crop. Even though these wild species are not cultivated, they are considered as a secondary genetic source for desirable agronomic traits such as drought tolerance and disease resistance, due the closeness to soybean [17, 20, 22]. Considering the symbiosis with compatible and well-adapted *Bradyrhizobium*, the nodulated wild *Glycine* may represent an interesting option to restore degraded habitats in Australia.

The phylogenetic relationships of the genus *Glycine* have not yet been fully explained. Whereas *G. max* was domesticated in China around 5000 years ago, through the crossing of wild soybean species [67–69], Australia harbours most of the wild species of the genus *Glycine* [17–21]. Some events during the Earth's evolution have been hypothesized to explain the diversification of these legumes. After the Pangea breakup, the Gondwana supercontinent was formed, connecting Australia and Antarctica; these landmasses separated and Australia moved northwards close to Asia. This moment possibly allowed the successfully entry of Asian legumes in Australia and, consequently, their symbionts [14, 70–72]. Taking into account that China is the original centre of soybean, the region might also be a diversification centre for compatible rhizobia [73–75]. The high similarity of CNPSo  $4010^{T}$  and CNPSo  $4016^{T}$  to *B. yuanmingense* CCBAU  $10071^{T}$  and CNPSo  $4019^{T}$  to *B. liaoningense* LMG  $18230^{T}$  may be related to dispersal events. Although *B. yuamingense* CCBAU  $10071^{T}$  was the original symbiont of *Lespezeda* species in China [62], many strains of this species have been isolated from soybean in Asian soils [74–76]. Also, *B. liaoningense* is a widespread soybean symbiont in India and China [9, 77, 78]. Therefore, our findings support the hypothesis of legume and symbionts exchange between Asian and Australian continents.

## PHENOTYPIC CHARACTERIZATION

Several morphophysiological evaluations were performed and compared with strains CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup> and closest species *B. liaoningense* LMG 18230<sup>T</sup> and *B. yuanmingense* CCBAU 10071<sup>T</sup>. Except where indicated, the tests were assessed on modified-YMA medium at 28 °C [25]. Colony morphology was analysed using Congo red and acid and alkaline reaction using bromothymol blue after 7–10 days of growth. In order to evaluate growth under different conditions, the strains were cultured with 1% NaCl, at 37 °C, on Luria-Bertani (LB) medium, at pH 4.0 and 8.0, adapted from Hungria et al. [79]. The urease activity was tested using 2% urea and phenol red as indicator. Carbohydrate metabolism was detected by the API 50CH kit platform (bioMérieux), according to the manufacturer's instructions and using modified-YM-minus-mannitol with bromothymol blue. Tolerance of antibiotics was determined by the discdiffusion technique proposed by Bauer et al. [80] using ampicillin (10 µg), bacitracin (10 U), cefuroxime (30 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), neomycin (30 µg), penicillin (10 U), streptomycin (10 µg), tetracycline  $(30 \,\mu g)$  and erythromycin  $(15 \,\mu g)$ . All tests were conducted in duplicate.

In general, the phenotypic results are in agreement with those commonly found in the genus Bradyrhizobium. However, it is worth mentioning some unusual features detected in this study: CNPSo 4010<sup>T</sup> showed a neutral reaction on modified-YMA with bromothymol blue as indicator and the three strains from this study presented optimal growth on modified-YMA at 37 °C in 3-4 days, indicating a possible mechanism of high temperature tolerance. CNPSo  $4019^{T}$  was able to grow in a large pH range, pH 4.0-8.0, while strain CNPSo 4016<sup>T</sup> grew well at pH 8.0. Carbohydrate metabolism by API 50CH was heterogeneous among the strains and revealed slightly incongruences with the universal culture medium used to grow rhizobia (YMA), since the CNPSo strains weakly used D-mannitol and also glycerol, another C source commonly used for this culture media. Interestingly, CNPSo 4016<sup>T</sup> was positive or weak for metabolism of all carbohydrate sources available on the kit. Although our findings revealed different metabolic features, the phenotypic characteristics are generally encoded by the accessory genome, being unstable over time. Also, they may present different results according to the laboratory conditions [81, 82]. The differential phenotypical features among the strains from this study and closest species are reported in Table 4.

Nodulation and nitrogen fixation assays were carried out in Leonard jars with soybean commercial cultivar BRASMAX Potência RR and with the promiscuous papilionoid siratro (*Macroptilium atropurpureum*). Each jar was sterilized with sand, vermiculite (2:1, v:v) and the N-free nutrient solution described by Broughton and Dilworth [83]. The strains were cultured in modified-YM medium [25] and inoculated on the seeds (1 ml) after planting. The plants grew under controlled glasshouse conditions for 30 days. All strains were able to form effective red colour nodules with siratro but only strain CNPSo 4019<sup>T</sup> was able to effectively nodulate soybean, its original host (Fig. S9).

Therefore, the phylogenetic, genomic and phenotypic data accomplished in this study support the proposed descriptions of the novel species *Bradyrhizobium agreste* sp. nov., *Bradyrhizobium glycinis* sp. nov. and *Bradyrhizobium diversitatis* sp. nov., isolated from three different *Glycine* species, for which the type strains are CNPSo 4010<sup>T</sup>, CNPSo 4016<sup>T</sup> and CNPSo 4019<sup>T</sup>, respectively.

# DESCRIPTION OF *BRADYRHIZOBIUM AGRESTE* SP. NOV.

*Bradyrhizobium agreste* (a.gres'te. L. neut. adj. *agreste*, wild, referring to the importance of isolation from wild species, such as the *Glycine clandestina* from this study).

Cells are Gram-stain-negative, aerobic and non-sporeforming. Colonies on modified-YMA medium at pH 6.8–7.0 and Congo red are slightly pink, less than 1 mm in diameter, circular, opaque, with low mucus production and a gummy consistency after 7 days of growth at 28 °C. The strain shows a neutral reaction on modified-YMA with bromothymol blue as indicator and weak urease activity. CNPSo 4010<sup>T</sup> shows weak growth at pH 4.0 and 8.0. The strain is able to grow at 37 °C for 4 days, but unable to grow on solid LB medium and modified-YMA containing 1% NaCl. With respect to carbon sources in the API test, the strain is able to use starch, glycogen, D-se, L-fucose and potassium 2-ketogluconate; weakly uses glycerol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, methyl  $\beta$ -D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, D-mannitol, N-acetylglucosamine, aesculin ferric citrate, D-fucose, D-arabitol and potassium 5-ketogluconate; does not use erythritol, D-adonitol, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl a-D-mannopyranoside, methyl a-Dglucopyranoside, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, xylitol, gentiobiose, turanose, D-tagatose, L-arabitol and potassium gluconate. The strain is tolerant to the antibiotics ampicillin (10 µg), bacitracin (10 U), chloramphenicol  $(30 \mu g)$ , nalidixic acid  $(30 \mu g)$ and penicillin G (10 U), moderately sensitive to neomycin  $(30 \,\mu g)$  and tetracycline  $(30 \,\mu g)$  and sensitive to cefuroxime  $(30 \,\mu g)$ , erythromycin  $(15 \,\mu g)$  and streptomycin  $(10 \,\mu g)$ . The strain is able to form effective nitrogen-fixing nodules with Macroptilium atropurpureum, but does not nodulate Glycine max.

The type strain, CNPSo  $4010^{T}$  (=WSM  $4802^{T}$ =LMG  $31645^{T}$ ), was isolated from a nodule of *Glycine clandestina* in Kununurra, Australia. The DNA G+C content of strain CNPSo  $4010^{T}$  is 63.8 mol%.

# DESCRIPTION OF *BRADYRHIZOBIUM GLYCINIS* SP. NOV.

*Bradyrhizobium glycinis* (gly.ci'nis. N.L. gen. n. *glycinis*, of the genus *Glycine*, a genus that encompasses host plants of several *Bradyrhizobium* species, including this new species, isolated from *G. tabacina*).

Cells are Gram-stain-negative, aerobic and non-sporeforming. Colonies on modified-YMA medium at pH 6.8-7.0 and Congo red are slightly pink, less than 1 mm in diameter, circular, opaque and exhibit low mucus production with a gummy consistency after 7 days of growth at 28 °C. The strain shows an alkaline reaction on modified-YMA with bromothymol blue and weak urease activity. CNPSo 4016<sup>T</sup> shows weak growth at pH 4.0 but grows well at pH 8.0 and at 37 °C after 4 days. The strain is unable to grow on solid LB medium and modified-YMA containing 1% NaCl. With respect to carbon sources in the API test, strain CNPSo 4016<sup>T</sup> is able to use D-arabinose, amygdalin, aesculin ferric citrate, starch, turanose, D-ose, D-fucose, L-fucose, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate; weakly uses glycerol, erythritol, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl

#### Table 4. Distinctive phenotypical properties of new lineages of Bradyrhizobium and closely related strains

Strains: 1, Bradyrhizobium agreste CNPSo 4010<sup>T</sup>; 2, Bradyrhizobium glycinis CNPSo 4016<sup>T</sup>; 3, Bradyrhizobium diversitatis CNPSo 4019<sup>T</sup>; 4, Bradyrhizobium liaoningense LMG 18230<sup>T</sup>; 5, Bradyrhizobium yuanmingense CCBAU 10071<sup>T</sup>. Data are evaluated as: +, growth; w, weakly positive; –, no growth. NA, No data available.

Characteristic	1	2	3	4*	5†
Carbon source utilization:					
Erythritol	-	W	-	-	-
D-Arabinose	W	+	W	+	w
L-Arabinose	W	W	W	+	+
D-Ribose	W	W	W	+	+
D-Xylose	W	+	W	+	+
l-Xylose	W	+	+	+	+
D-Adonitol	-	W	-	-	-
Methyl β-D-xylopyranoside	W	W	W	-	w
D-Galactose	W	W	-	w	w
D-Fructose	W	W	-	-	w
L-Sorbose	W	W	w	-	-
L-Rhamnose	-	W	W	w	w
Dulcitol	-	-	W	-	-
Inositol	-	W	-	-	-
D-Mannitol	W	W	W	-	w
D-Sorbitol	-	W	W	-	-
Methyl α-D-mannopyranoside	-	-	W	-	-
Methyl a-d-glusopyranoside	-	W	W	-	-
N-Acetylglucosamine	W	-	W	-	-
Amygdalin	-	+	w	-	-
Arbutin	-	W	w	-	-
Aesculin ferric citrate	W	+	+	+	+
Salicin	-	W	w	-	-
Cellobiose	-	W	w	-	-
Maltose	-	W	w	-	w
Lactose	-	W	w	-	-
Melibiose	-	W	w	-	-
Sucrose	-	W	W	-	-
Trehalose	-	W	W	W	-
Inulin	-	W	W	-	-
Melezitose	-	W	W	-	-
Raffinose	-	W	W	-	-
Glycogen	+	W	+	+	-
Xylitol	-	W	-	_	-

Continued

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max.	
The type strain CNPSo 4016 <sup>T</sup> (=WSM 4801 <sup>T</sup> =LMG 31649 <sup>T</sup> ) was isolated from a nodule of <i>Glycine tabacina</i> in Kununurra,	

Characteristic	1	2	3	4*	5†
Gentiobiose	_	w	w	-	-
Turanose	-	+	w	-	-
D-Lyxose	+	+	w	+	+
D-Tagatose	-	W	w	-	-
D-Fucose	w	+	w	+	+
d-Arabitol	w	W	w	w	-
L-Arabitol	_	+	_	-	-
Potassium gluconate	-	+	+	-	+
Potassium 2-ketogluconate	+	+	+	-	+
Potassium 5-ketogluconate	w	+	+	-	+
Enzymatic activity:					
Urease	w	w	+	+	NA
Growth at/in:					
pH 4	w	w	+	+	-
рН 8	w	+	+	+	+
Alkaline reaction	w	+	+	+	+
37°C	+ (4 days)	+ (4 days)	+ (3 days)	-	+
Tolerance to antibiotics:					
Ampicillin (10µg)	+	-	+	-	W
Bacitracin (10 U)	+	+	+	+	NA
Erythromycin (15 µg)	-	-	+	+	+
Neomycin (30 µg)	w	w	+	-	-
Streptomycin (10 µg)	-	-	-	-	+
Tetracycline (30 µg)	W	-	+	+	+

\*Data obtained from Urquiaga *et al.* [42].

†Data obtained from Delamuta et al. [37]; Yao et al. [62] and Grönemeyer et al. [84].

α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, glycogen, xylitol, gentiobiose, D-tagatose and D-arabitol. The strain is tolerant to bacitracin (10 U), chloramphenicol (30 μg), nalidixic acid (30 μg) and penicillin G (10 U), moderately sensitive to neomycin (30 μg) and sensitive to ampicillin (10 μg), cefuroxime (30 μg), erythromycin (15 μg), streptomycin (10 μg) and tetracycline (30 μg). The strain is able to form effective nitrogen-fixing nodules with *Macroptilium atropurpureum* but does not nodulate *Glycine max*. Australia. The DNA G+C content of strain CNPSo  $4016^{T}$  is 63.7 mol%.

# DESCRIPTION OF BRADYRHIZOBIUM DIVERSITATIS SP. NOV.

*Bradyrhizobium diversitatis* (di.ver.si.ta'tis. L. fem. n. *diversitas* diversity, N.L. gen. n. *diversitatis*, of diversity, referring to the importance of studies on microbial diversity revealing, as in the case of this study, genetic richness of *Bradyrhizobium* isolated from *Glycine max*).

Cells are Gram-stain-negative, aerobic and non-sporeforming. Colonies on modified-YMA medium at pH 6.8–7.0 and Congo red are slightly pink, less than 1 mm in diameter,

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circular, opaque and exhibit low mucus production with a gummy consistency after 7 days of growth at 28 °C. The strain shows alkaline reaction on modified-YMA with bromothymol blue, is urease-positive and able to grow at pH 4.0 and 8.0. CNPSo 4019<sup>T</sup> is able to grow at 37 °C after 3 days. The strain is unable to grow on solid LB medium and modified-YMA containing 1% NaCl. With respect to carbon sources in the API test, strain CNPSo 4019<sup>T</sup> is able to use L-xylose, aesculin ferric citrate, starch, glycogen, L-fucose, potassium gluconate, potassium 2-ketogluconate, and potassium 5-ketogluconate; weakly uses glycerol, D-arabinose, L-arabinose, D-ribose, D-xylose, D-adonitol, methyl β-D-xylopyranoside, D-glucose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl a-D-mannopyranoside, methyl a-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose and D-arabitol; does not use erythritol, D-galactose, D-fructose, xylitol and L-arabitol. The strain is tolerant to the antibiotics ampicillin (10 µg), bacitracin (10 U), chloramphenicol (30 µg), erythromycin (15 µg), nalidixic acid (30 µg), neomycin (30 µg), penicillin G (10 U) and tetracycline (30 µg); and is sensitive to cefuroxime  $(30 \mu g)$  and streptomycin  $(10 \mu g)$ . The strain is able to form nitrogen-fixing nodules with Macroptilium *atropurpureum* and *Glycine max*.

The type strain, CNPSo  $4019^{T}$  (=WSM  $4799^{T}$ =LMG  $31650^{T}$ ), was isolated from a nodule of *Glycine max* in Nambung, Australia. The DNA G+C content of strain CNPSo  $4019^{T}$  is 63.8 mol%.

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#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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