

## Supplementary Materials to

### ***Bradyrhizobium ivorense* sp. nov. as a potential local bioinoculant for *Cajanus cajan* cultures in Côte d'Ivoire**

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**Table S1.** Origin of the *C. cajan* nodule isolates proposed as *B. ivorensis* sp. nov. strains.

	Strain	Plant #	Field # (GPS coordinates)	Locality
1.	<b><u>CI-1B<sup>T</sup></u></b> *	1		Kossou-Bouafla
	<u>CI-4A2</u>	4	1 (N 7°17'45" - W 5°49'00")	
	<u>CI-4A3</u>			
	<u>CI-4C</u>			
5.	<u>CI-4D</u>			
	<b><u>CI-14A</u></b>	14		3 (N 6°51'19" - W 5°14'38")
	CI-14B	15		
	<u>CI-15A</u>			
	CI-15D			
10.	<u>CI-18C</u>	18	4 (N 6°51'02" - W 5°13'31")	
	CI-19A1	19		
	<b><u>CI-19D</u></b>			
	CI-19E			
	<u>CI-33F</u>	33	5 (N 7°55'28" - W 2°57'45")	Bondoukou
15.	CI-33K			
	<u>CI-35B</u>	35		
	CI-41B	41	6 (N 7°56'50" - W 2°56'26")	
	CI-41L			
19.	<b><u>CI-41S</u></b> *			

**Legend to Table S1.** Nodule isolates that were sequenced for multilocus sequence analyses (MLSA) are shown in bold and those for which genome data was collected are marked by an asterisk. Strains that are underlined were tested for nodulation and symbiotic nitrogen-fixation on *C. cajan* cvs. ILRI 16555 and "Light Brown". Strains in bold were also inoculated onto *Glycine max* cv. Davis, *Macroptilium atropurpureum* cv. Siratro, *Leucaena leucocephala*, *Tephrosia vogelii*, *Vigna radiata* cv. King and *Vigna unguiculata* cv. Red Caloona, and the phenotypes on these host plants can be found in Table S7.

**Table S2.** List of primers and sequences of *C. cajan* nodule isolates used in this study.

<b>Primer</b>	<b>5' to 3' sequence</b>	<b>Size (bases)</b>	<b>Amplicon (bp)</b>	<b>Reference</b>
dnaK-For	GGTGACCTTCGACATCGACG	20		this work
dnaK-Rev	CGGTGAACTCCGCGTCGAC	19	461	this work
glnII-For1	TGACCAAGTACAAGCTCGAGT	21		this work
glnII-Rev1	GAGAAGTTGGCGTGCATGCC	20	700	this work
gyrB-For1*	TTCGACCAGAACTCCTACAAGG	22		this work
gyrB-Rev1*	AGCTTGTCCTTGGTCTGCG	19	710	this work
recA-For	CAAGGGCTCGGTGATGAAGC	20		this work
recA-Rev	CGATGCGGCGGATGTCGAG	19	623	this work
rpoB-For	ACGGCACCGAGCGGTCAT	19		this work
rpoB-Rev	GTCGTCGATCTCGCCCTTGC	20	923	this work

<b>Strain</b>	<b>Gene</b>	<b>Size (bp)</b>	<b>Accession</b>	<b>Reference</b>
CI-1B	16S-ITS-23S	2540	KX396570	Fossou et al. 2016
	<i>dnaK</i>	422	MK376326	this work
	<i>glnII</i>	659	MH756157	this work
	<i>gyrB</i>	669	MH756161	this work
	<i>recA</i>	584	MK376330	this work
	<i>rpoB</i>	923	KX388393	Fossou et al. 2016
CI-14A	16S-ITS-23S	2540	KX396573	Fossou et al. 2016
	<i>dnaK</i>	422	MK376327	this work
	<i>glnII</i>	659	MH756158	this work
	<i>gyrB</i>	669	MH756162	this work
	<i>recA</i>	584	MK376331	this work
	<i>rpoB</i>	884	MK376334	this work
CI-19D	16S-ITS-23S	2539	KX396575	Fossou et al. 2016
	<i>dnaK</i>	422	MK376328	this work
	<i>glnII</i>	659	MH756159	this work
	<i>gyrB</i>	669	MH756163	this work
	<i>recA</i>	584	MK376332	this work
	<i>rpoB</i>	884	MK376335	this work
CI-41S	16S-ITS-23S	2540	KX396584	Fossou et al. 2016
	<i>dnaK</i>	422	MK376329	this work
	<i>glnII</i>	659	MH756160	this work
	<i>gyrB</i>	669	MH756164	this work
	<i>recA</i>	584	MK376333	this work
	<i>rpoB</i>	884	MK376336	this work

Legend to Table S2. \*GyrB-For1 and GyrB-Rev1 were derived from gyrB343F and gyrB1043R proposed by Martens et al. (2008).

**Table S3A.** List of the eight type strains and corresponding GenBank accessions used in this study as data for members of the *Bradyrhizobium japonicum* supergroup. The underlined accession includes one ambiguous (N) position.

Type strain	Type strain genome data	GenBank accession for corresponding gene							
		16S rRNA	<i>dnaK</i>	<i>glnII</i>	<i>gyrB</i>	<i>recA</i>	<i>rpoB</i>		
<i>B. arachidis</i> CBAU 051107 <sup>T</sup>	NZ_FPBG01000000	HM107167	JX437668	HM107251	JX437675	HM107233	JX437682		
<i>B. betae</i> LMG 21987 <sup>T</sup>	no genome available	<u>AY372184</u>	FM253303	AB353733	AB353735	AB353734	FM253260		
<i>B. canariense</i> BTA-1 <sup>T</sup>	no genome available	AJ558025	AY923047	AY386765	FM253220	AY591553	FM253263		
<i>B. diazoefficiens</i> USDA 110 <sup>T</sup>	NC_004463			gene sequences derived from archived genome data					
<i>B. iriomotense</i> EK05 <sup>T</sup>	no genome available	AB300992	JF308944	AB300995	AB300997	AB300996	HQ587646		
<i>B. japonicum</i> USDA 6 <sup>T</sup>	NC_017249			gene sequences derived from archived genome data					
<i>B. liaoningense</i> USDA 3622 <sup>T</sup>	no genome available	AF208513	FM253309	AY386775	FM253223	AY591564	EF190181		
<i>B. ottawense</i> OO99 <sup>T</sup>	CP029425	JN186270	JF308816	HQ587750	HQ873179	HQ587287	HQ587518		

**Table S3B.** Type strains of the *B. elkanii* supergroup and corresponding sequences selected for phylogenetic analyses. Accessions that differ from genome data are shown in bold and those with ambiguous nucleotides (e.g. N, R or Y) are underlined. Note that *recA* of *B. ferriligni* CCBAU 51502<sup>T</sup> (KJ818112) was identical to *recA* of *B. elkanii* CCBAU 05737 at both, genomic (NZ\_AJPV00000000) and single gene accessions (HM057521).

Type strain	Type strain genome data	GenBank accession for corresponding gene							
		16S rRNA	<i>dnaK</i>	<i>glnII</i>	<i>gyrB</i>	<i>recA</i>	<i>rhoB</i>		
<i>B. algeriense</i> RST89 <sup>T</sup>	NZ_PYCM01000000	FJ546419	n.a.	<b>FJ264924</b>	n.a.	<b>FJ264927</b>	n.a.		
<i>B. brasiliense</i> UFLA03-321 <sup>T</sup>	NZ_MPVQ01000000	<b>KF311068</b>	KF452791	n.a.	KF452827	KT793142	KF452879		
<i>B. centrolobii</i> BR 10245 <sup>T</sup>	NZ_LUUB01000000	<b>KF927049</b>	<b>KX527928</b>	KX527991	n.a.	<b>KX527954</b>	KF983827		
<i>B. elkanii</i> USDA 76 <sup>T</sup>	NZ_ARAG00000000	<b>HQ233240</b>	AM168363	AY599117	AB070584	AY591568	LC167350		
<i>B. embrapense</i> SEMIA 6208 <sup>T</sup>	NZ_LFIP00000000	<b>AY904773</b>	KP234519	GQ160500	<u>HQ634891</u>	<u>HQ634899</u>	<b>HQ634910</b>		
<i>B. erythrophlei</i> CCBAU 53325 <sup>T</sup>	no genome available	KF114645	MG811656	KF114693	KF114717	KF114669	MG811654		
<i>B. ferriligni</i> CCBAU 51502 <sup>T</sup>	no genome available	KX683400	MG811657	KJ818099	KJ818102	KJ818112	MG811655		
<i>B. icense</i> LMTR 13 <sup>T</sup>	CP016428	KF896156	KF896182	KF896175	KF896201	JX943615	n.a.		
<i>B. jicamae</i> PAC68 <sup>T</sup>	NZ_LLXZ01000000	<b>AY624134</b>	JN207408	FJ428204	HQ873309	HM590776	HQ587647		
<i>B. lablabi</i> CCBAU 23086 <sup>T</sup>	NZ_LLYB01000000	<b>GU433448</b>	KF962687	GU433498	JX437670	GU433522	JX437677		
<i>B. macuxiense</i> BR 10303 <sup>T</sup>	NZ_LNCU01000000	KX527919	KX527932	KX527995	KX528008	KX527958	KX527969		
<i>B. mercantei</i> SEMIA 6399 <sup>T</sup>	NZ_MKFI01000000	FJ025102	<b>KX690617</b>	KX690621	KX690623	<b>KX690615</b>	n.a.		
<i>B. namibiense</i> 5-10 <sup>T</sup>	no genome available	KX661401	KP402058	KM378440	KX661393	KM378377	KM378306		
<i>B. neotropicale</i> BR 10247 <sup>T</sup>	NZ_LSEF01000000	<b>KF927051</b>	KJ661693	KJ661700	KJ661707	<b>KJ661714</b>	KF983829		
<i>B. pachyrhizi</i> PAC48 <sup>T</sup>	NZ_LFIQ01000000	AY624135	JN207406	FJ428201	KF532651	HM047130	<b>LM994172</b>		
<i>B. paxllaeri</i> LMTR 21 <sup>T</sup>	NZ_MAXB01000000	AY923031	AY923038	KF896169	KF896195	JX943617	KP308154		
<i>B. retamae</i> Ro19 <sup>T</sup>	NZ_LLYA00000000	KC247085	LM994150	KC247108	KF962698	KC247094	LM994174		
<i>B. ripae</i> WR4 <sup>T</sup>	no genome available	MF593081	MF593102	MF593086	MF593094	MF593090	MF593098		
<i>B. tropiciagri</i> SEMIA 6148 <sup>T</sup>	NZ_LFLZ01000000	<b>AY904753</b>	<b>FJ391008</b>	FJ391048	<u>HQ634890</u>	<b>FJ391168</b>	<b>HQ634909</b>		
<i>B. valentinum</i> LmjM3 <sup>T</sup>	NZ_LLXX01000000	JX514883	n.a.	<b>JX518575</b>	n.a.	JX518589	n.a.		
<i>B. viridifuturi</i> SEMIA 690 <sup>T</sup>	NZ_LGTB01000000	FJ025107	<b>KR149128</b>	KR149131	KR149134	<b>KR149140</b>	KU724169		

*B. elkanii* super clade

**Table S4. Similarity levels between concatenated *dnaK-glnII-gyrB-recA-rpoB* (2,560 bp) sequences of bradyrhizobia.** Strains were ordered by highest similarity to *B. ivorensis* sp. nov. strains CI-1B<sup>T</sup> and CI-41S, except for *B. centrolobii* BR 10245<sup>T</sup> (#22) and *B. neotropicale* BR 10247<sup>T</sup> (#23) that better matched sequences of the *B. japonicum* super-clade representative strain USDA 6<sup>T</sup> (#24) shaded in light grey. DNA sequences were from type strains *B. algeriense* RST89<sup>T</sup> (#18), *B. brasiliense* UFLA03-321<sup>T</sup> (#7), *B. elkanii* USDA 76<sup>T</sup> (#5), *B. embrapense* SEMIA 6208<sup>T</sup> (#9), *B. erythrophlei* CCBAU 53325<sup>T</sup> (#13), *B. ferriligni* CCBAU 51502<sup>T</sup> (#12), *B. icense* LMTR 13<sup>T</sup> (#17), *B. jicamae* PAC68<sup>T</sup> (#16), *B. lablabi* CCBAU 23086<sup>T</sup> (#14), *B. macuxiense* BR 10303<sup>T</sup> (#11), *B. mercantei* SEMIA 6399<sup>T</sup> (#10), *B. namibiense* 5-10<sup>T</sup> (#19), *B. pachyrhizi* PAC48<sup>T</sup> (#6), *B. paxllaeri* LMTR 21<sup>T</sup> (#15), *B. retamae* Ro19<sup>T</sup> (#20), *B. ripae* WR4<sup>T</sup> (#4), *B. tropiciagri* SEMIA 6148<sup>T</sup> (#3), *B. valentinum* LmjM3<sup>T</sup> (#21) and *B. viridifuturi* SEMIA 690<sup>T</sup> (#8). Values above the 97% threshold proposed by Durán et al. (2014) are underlined. Levels of the highest similarity to CI-1B<sup>T</sup> and CI-41S are in bold.

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	CI-1B <sup>T</sup>	100																					
2	CI-41S	98.4	100																				
3	<i>B. tropiciagri</i>	<b>95.2</b>	<b>95.3</b>	100																			
4	<i>B. ripae</i>	95.1	95.1	96.6	100																		
5	<i>B. elkanii</i>	95.1	95.0	96.6	96.7	100																	
6	<i>B. pachyrhizi</i>	95.1	94.9	96.8	96.6	97.8	100																
7	<i>B. brasiliense</i>	95.0	95.1	96.8	96.5	97.9	99.1	100															
8	<i>B. viridifuturi</i>	94.9	94.8	97.2	96.2	96.5	96.5	96.5	100														
9	<i>B. embrapense</i>	94.5	94.5	96.4	95.6	96.2	96.1	96.2	100														
10	<i>B. mercantei</i>	94.5	94.3	96.7	96.1	96.3	96.3	96.7	95.5	100													
11	<i>B. macuxiense</i>	94.4	94.6	94.8	94.3	95.3	95.2	94.6	94.6	94.6	100												
12	<i>B. ferriligni</i>	93.8	93.9	96.2	95.7	96.9	97.1	95.9	95.6	95.8	94.0	100											
13	<i>B. erythrophlei</i>	93.7	93.9	93.9	93.6	94.1	93.7	93.9	93.8	93.6	94.1	93.0	100										
14	<i>B. lablabi</i>	91.0	91.3	91.1	90.7	91.5	91.3	91.4	91.3	91.1	91.3	90.4	90.4	100									
15	<i>B. paxllaeri</i>	91.0	91.3	91.1	91.1	91.3	91.1	91.3	91.2	91.1	91.2	90.7	90.4	97.1	100								
16	<i>B. jicamae</i>	90.7	91.1	91.0	90.6	91.3	91.0	90.9	90.9	91.0	91.0	90.5	90.4	96.6	97.4	100							
17	<i>B. icense</i>	90.8	91.0	90.9	91.0	91.5	91.2	91.4	91.2	91.1	90.7	90.8	90.5	94.5	94.3	94.2	100						
18	<i>B. algeriense</i>	90.4	90.2	90.4	90.0	90.2	90.1	90.4	90.0	89.8	90.5	89.6	90.2	92.4	92.2	92.2	92.2	100					
19	<i>B. namibiense</i>	90.3	90.8	90.6	90.0	91.0	90.4	90.5	90.6	90.3	90.7	90.1	90.4	92.2	92.5	92.8	92.3	90.0	100				
20	<i>B. retamae</i>	90.1	90.4	90.7	90.4	90.9	90.7	90.5	90.8	90.7	90.3	90.1	89.8	94.0	93.6	93.7	95.4	91.6	91.0	100			
21	<i>B. valentinum</i>	89.8	89.9	89.9	89.6	90.0	89.5	89.6	89.7	89.9	90.0	89.5	89.9	91.9	91.8	91.9	92.2	94.8	90.3	91.1	100		
22	<i>B. centrolobii</i>	90.7	90.8	90.5	90.7	91.0	90.7	90.8	90.6	90.5	91.1	89.8	91.1	90.1	89.9	89.9	89.5	88.9	90.5	88.8	88.8	100	
23	<i>B. neotropicale</i>	90.5	90.9	90.4	90.2	90.3	90.0	90.1	90.2	89.9	90.6	89.3	90.5	89.7	89.4	89.6	88.9	88.5	90.2	88.2	88.4	95.7	100
24	<i>B. japonicum</i>	90.5	90.7	91.0	90.9	91.0	90.7	90.8	91.0	90.7	90.9	90.0	90.1	89.8	89.5	89.6	89.4	89.0	89.5	88.9	89.3	93.7	93.6

**Table S5.** Overview and general characteristics of the CI-1B<sup>T</sup> and CI-41S draft genomes.

	<b>CI-1B<sup>T</sup></b>	<b>CI-41S</b>
Genome size (kb)	9,412	8,864
G+C content (mol%)	64.21	64.37
Number of contigs	44	59
Contig N <sub>50</sub>	466,280	253,924
Contig L <sub>50</sub>	4	11
Raw coverage	114.6 x	35.7 x
Number of predicted genes	8,546	8,123
Number of tRNAs	56	57

**Table S6.** Phenotypic characteristics of *B. ivorensis* sp. nov. strains and *B. elkanii* USDA 76<sup>T</sup>

Characteristic		CI-1B <sup>T</sup>	CI-14A	CI-19D	CI-41S	USDA 76 <sup>T</sup>
Growth in liquid RMS at (°C)	20	+	+	+	+	+
	25	+	+	+	+	+
	27	+	+	+	+	+
	30	+	+	+	+	+
	35	–	–	w	w	+
	40	–	–	–	–	–
Growth in liquid RMS at pH	4 to 5	–	–	–	–	–
	6 to 8	+	+	+	+	+
	9 to 12	–	–	–	–	–
Growth in RMM with 20 (*10) mM	fructose	w	n.t.	n.t.	w	w
	galactose	+	n.t.	n.t.	+	+
	glucose	+	n.t.	n.t.	+	w
	malate*	+	n.t.	n.t.	+	+
	maltose	–	n.t.	n.t.	–	–
	mannose	w	n.t.	n.t.	w	+
	pyruvate	+	n.t.	n.t.	+	+
	succinate*	+	n.t.	n.t.	+	+
	sucrose	–	n.t.	n.t.	–	–
Growth in YM at 27°C with NaCl at	0.04%	+	+	+	+	+
	0.25%	+	+	+	+	+
	0.5%	+	+	–	w	+
	0.75%	–	–	–	–	w
	1.0%	–	–	–	–	–
Growth on RMS plates with (µg per antibiotic disc)	ampicilin (100)	+	+	w	–	–
	chloramphenicol (500)	+	+	+	+	+
	erythromycin (50)	+	+	+	+	+
	gentamycin (50)	+	+	+	+	+
	kanamycin (100)	+	+	+	+	–
	penicillin (10)	+	+	+	+	+
	streptomycin (250)	+	+	+	+	+
	tetracycline (50)	+	+	+	+	+
Reaction of (API 20NE)	fermentation	–	–	–	n.t.	–
	esculin hydrolysis	w	–	–	n.t.	–
	gelatin hydrolysis	–	–	–	n.t.	–
	arginine di-hydrolase	+	+	+	n.t.	+
	indole production	–	–	–	n.t.	–
	NO <sub>3</sub> <sup>-</sup> reduced to NO <sub>2</sub> <sup>-</sup>	–	–	–	n.t.	+
	NO <sub>3</sub> <sup>-</sup> reduced to N <sub>2</sub>	–	–	–	n.t.	–
	urease	+	+	+	n.t.	+
	β-galactosidase (PNPG)	–	–	–	n.t.	–
Assimilation of (API 20NE)	adipic acid	+	+	+	n.t.	+
	capric acid	–	–	–	n.t.	–
	D-glucose	+	w	+	n.t.	w
	D-maltose	+	+	+	n.t.	w
	D-mannitol	+	+	+	n.t.	+
	D-mannose	+	+	+	n.t.	+
	L-arabinose	+	w	+	n.t.	w
	malic acid	w	w	w	n.t.	w
	N-acetylglucosamine	+	+	+	n.t.	w
	phenylacetic acid	w	w	w	n.t.	w
	potassium gluconate	+	+	+	n.t.	+
	trisodium citrate	w	w	w	n.t.	w

Legend to Table S6. +, positive for; –, negative for; w, weak for; n.t., not tested. For details on growth and resistance to antibiotics, see main text. All strains were negative for catalase and positive for oxidase reactions.



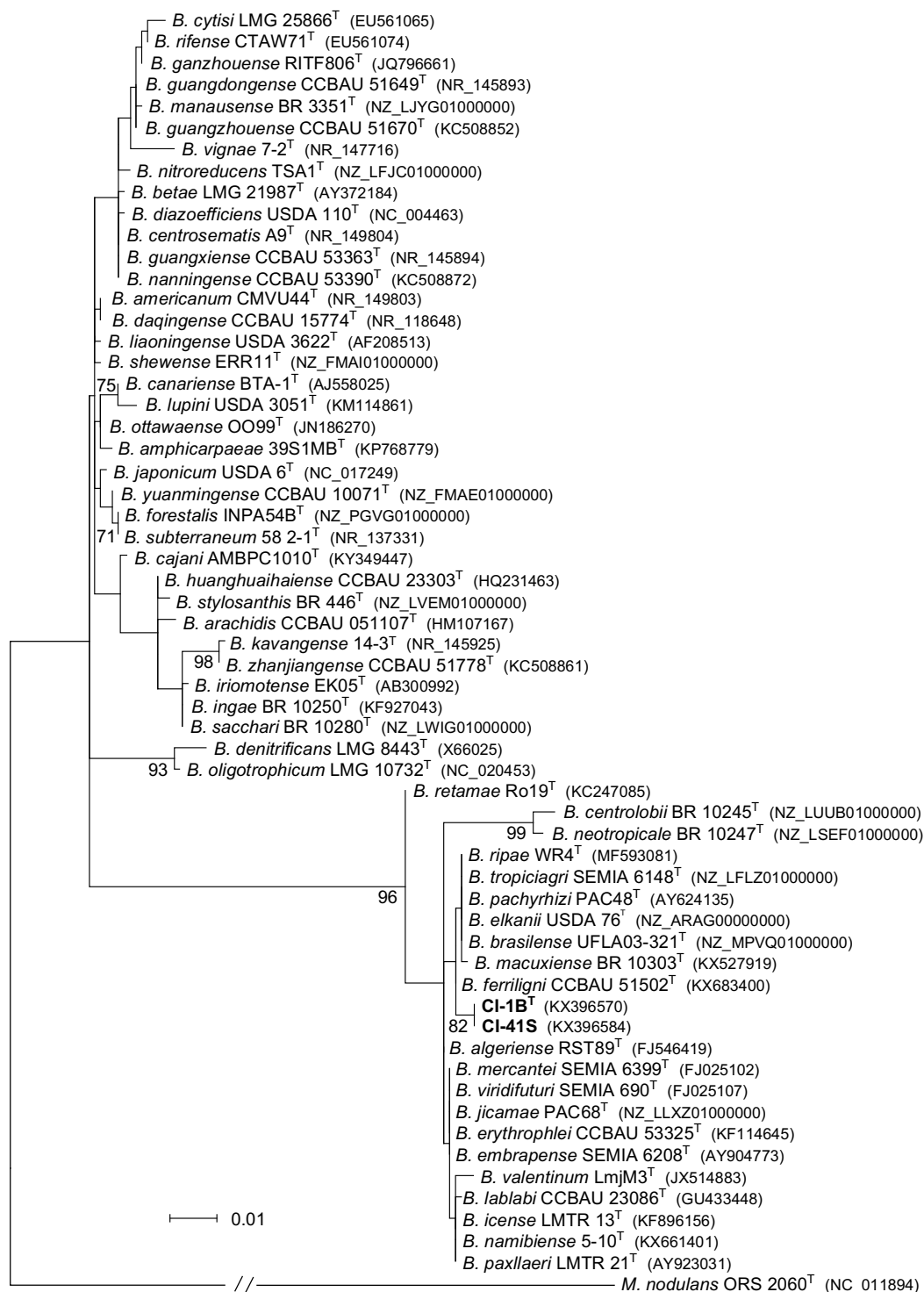
**Table S7.** Symbiotic phenotype of selected *B. ivorense* sp. nov. isolates on diverse legumes.

Inoculant	Host plant									
	Cc (42 dpi)	Gm (49 dpi)	Ma (42 dpi)	LI (98 dpi)	Tv (42 dpi)	Vr (35 dpi)	Vu (35 dpi)			
CI-1B <sup>T</sup>	Nod+ / Fix+	Nod+ / Fix-	Nod+ / Fix+	pNod	Nod+ / Fix+	Nod+ / Fix+	Nod+ / Fix+			
CI-14A	Nod+ / Fix+	Nod+ / Fix-	Nod+ / Fix+	n.t.	n.t.	Nod+ / Fix+	Nod+ / Fix+			
CI-19D	Nod+ / Fix+	Nod+ / Fix-	Nod+ / Fix+	n.t.	n.t.	Nod+ / Fix+	Nod+ / Fix+			
CI-41S	Nod+ / Fix+	Nod+ / Fix-	Nod+ / Fix <sup>red</sup>	pNod	Nod+ / Fix+	Nod+ / Fix <sup>red</sup>	Nod+ / Fix <sup>red</sup>			
USDA 76 <sup>T</sup>	Nod+ / Fix+	Nod+ / Fix+	Nod+ / Fix+	n.t.	n.t.	Nod± / Fix <sup>red</sup>	Nod+ / Fix+			
NGR234	Nod+ / Fix+	Nod-	Nod+ / Fix+	Nod+ / Fix+	Nod+ / Fix+	Nod+ / Fix+	Nod+ / Fix+			

**Legend to Table S7.** The following legumes were tested for nodulation: Cc, *Cajanus cajan* cv. ILRI 16555; Gm, *Glycine max* cv. Davis; Ma, *Macroptilium atropurpureum* cv. Siratro; LI, *Leucaena leucocephala*; Tv, *Tephrosia vogelii*; Vr, *Vigna radiata* cv. King; Vu, *Vigna unguiculata* cv. Red Caloona. Nodulation assays were conducted in Magenta jars with two plants per pot, and with a fixed inoculum of  $2 \times 10^8$  bacteria in 200  $\mu$ l of sterile water per seedling. When needed, plants were watered using B&D nitrogen-free solution. Depending on the host, plants were grown for 35 to 98 days post-inoculation (dpi) before being harvested to assess the symbiotic phenotype of inoculated strains. Capacity of isolates to nodulate was scored as Nod+ for those forming nodules on all inoculated roots, Nod± for sporadic nodules formation, and pNod for strains that made only pseudonodules. Symbiotic nitrogen fixation was scored as strains being capable of sustaining harmonious plant growth (Fix+), poor plant development (Fix<sup>red</sup>) or no growth beyond seed reserves (Fix-). As positive controls for nodulation and symbiotic nitrogen fixation, control plants were inoculated with strains *Bradyrhizobium elkanii* USDA 76<sup>T</sup> or *Sinorhizobium fredii* NGR234. Some plant/strain combinations were not tested (n.t.).

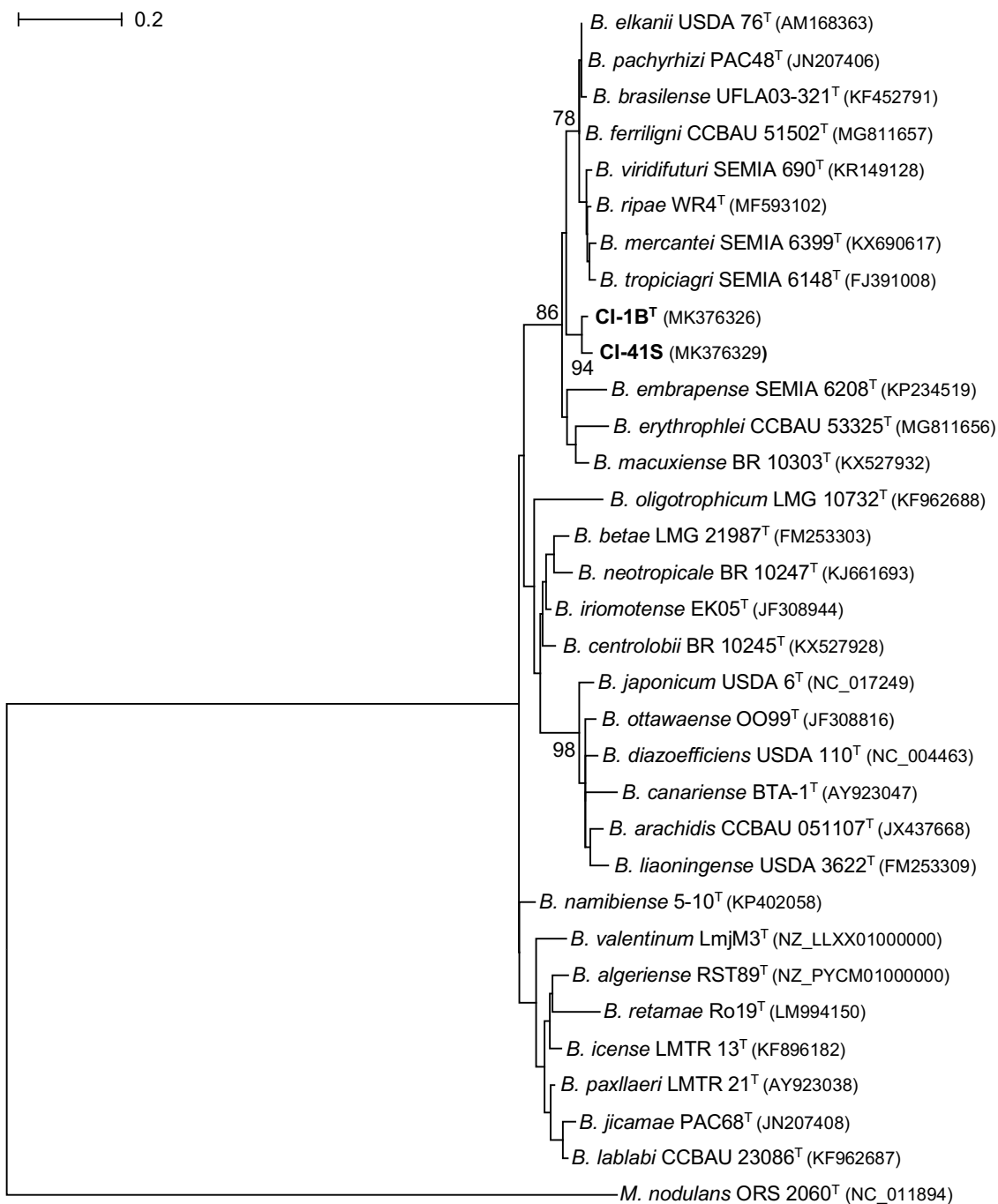
**Figure S1.** Maximum likelihood phylogram inferred from partial 16S rRNA gene sequences.

The unrooted maximum-likelihood phylogram was inferred from partial 16S rDNA sequences of 57 type strains of the *Bradyrhizobium* genus, of *B. ivorense* sp. nov. strains CI-1B<sup>T</sup> and CI-41S (shown in bold) and of *Methylobacterium nodulans* strain ORS 2060<sup>T</sup> that was used as the outgroup. Sequences were aligned with MAFFT version 7 using Q-INS-i. The T92+G+I model was used with 1,184 positions and 1,000 pseudoreplicates as parameters. Only bootstrap values >70% are shown at branch nodes. Scale bar indicates the number of substitutions per site.



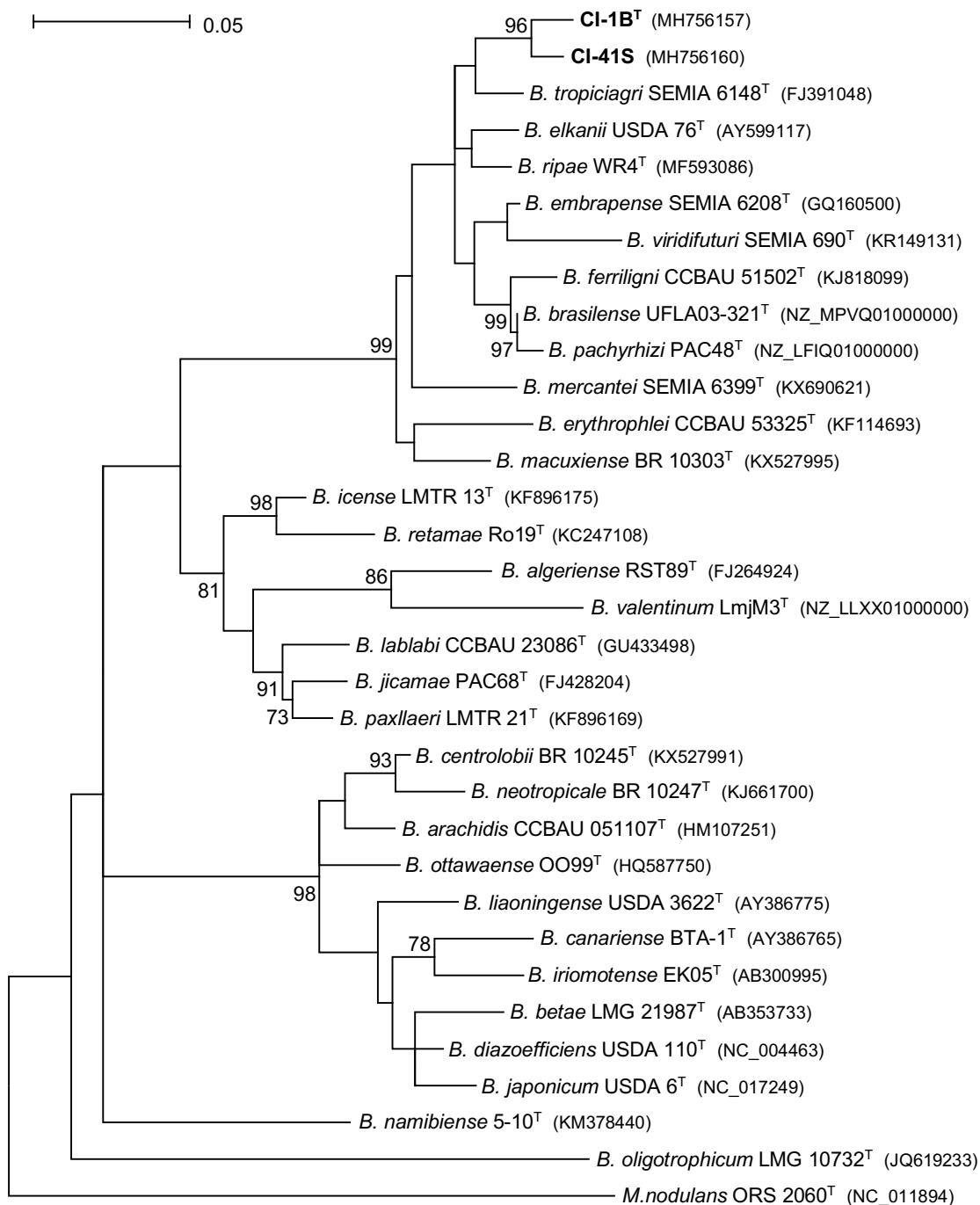
**Figure S2.** Maximum likelihood phylogram inferred from partial *dnaK* gene sequences.

The unrooted maximum-likelihood phylogram was inferred from 279 bp *dnaK* sequences of type strains of all species included in the *Bradyrhizobium elkanii* supergroup at the time of submission, of a selected number of additional *Bradyrhizobium* species, of *B. ivorense* sp. nov. strains CI-1B<sup>T</sup> and CI-41S (shown in bold) and of *Methylobacterium nodulans* strain ORS 2060<sup>T</sup> that was used as the outgroup. The TN93+G model was used with 279 positions and 1,000 pseudoreplicates as parameters. Only bootstrap values >70% are shown at branch nodes. Scale bar indicates the number of substitutions per site.



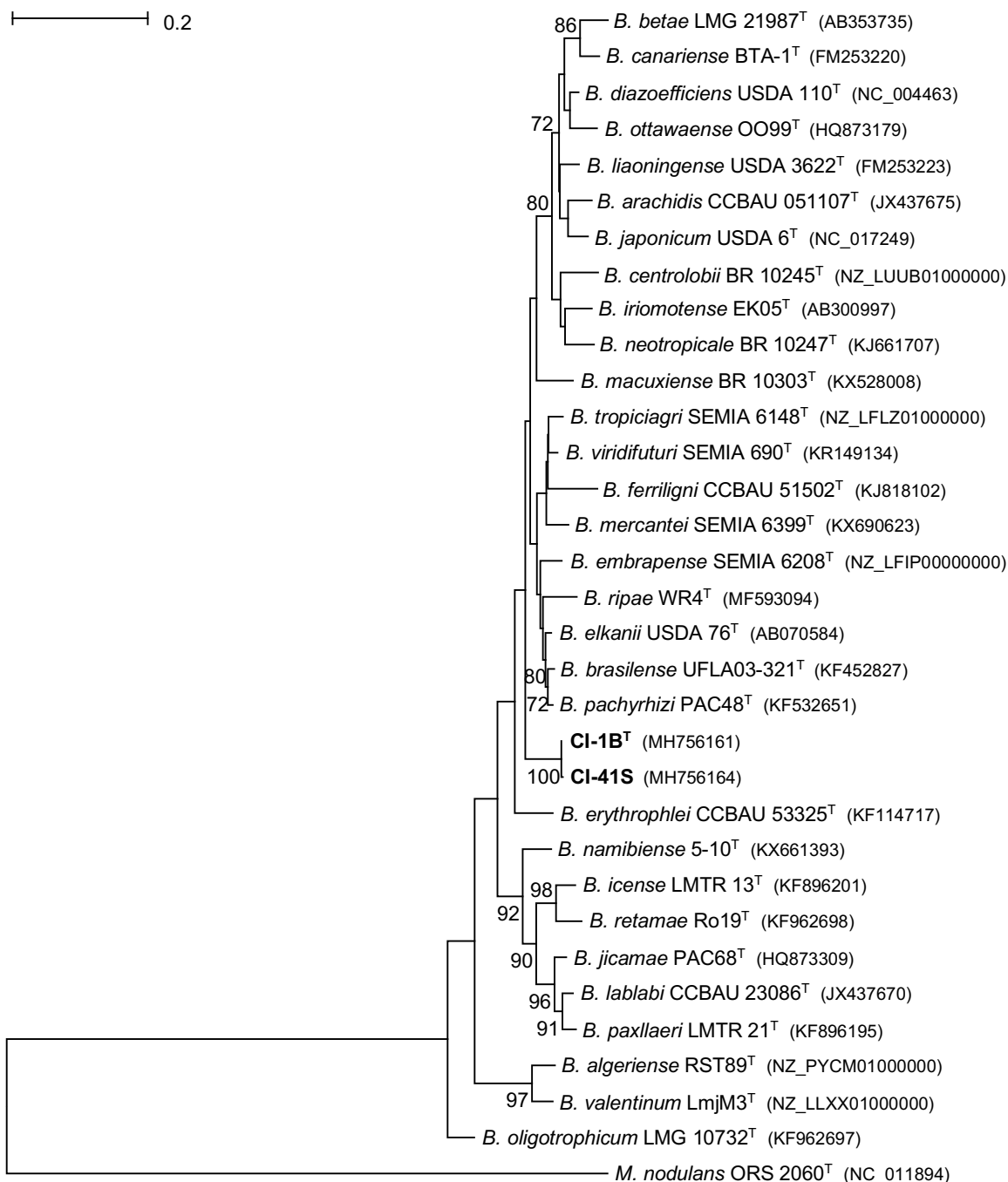
**Figure S3.** Maximum likelihood phylogram inferred from partial *glnII* gene sequences.

The unrooted maximum-likelihood phylogram was inferred from 540 bp *glnII* sequences of type strains of all species included in the *Bradyrhizobium elkanii* supergroup at the time of submission, of a selected number of additional *Bradyrhizobium* species, of *B. ivorense* sp. nov. strains CI-1B<sup>T</sup> and CI-41S (shown in bold) and of *Methylobacterium nodulans* strain ORS 2060<sup>T</sup> that was used as the outgroup. The GTR+G+I model was used with 540 positions and 1,000 pseudoreplicates as parameters. Only bootstrap values >70% are shown at branch nodes. Scale bar indicates the number of substitutions per site.



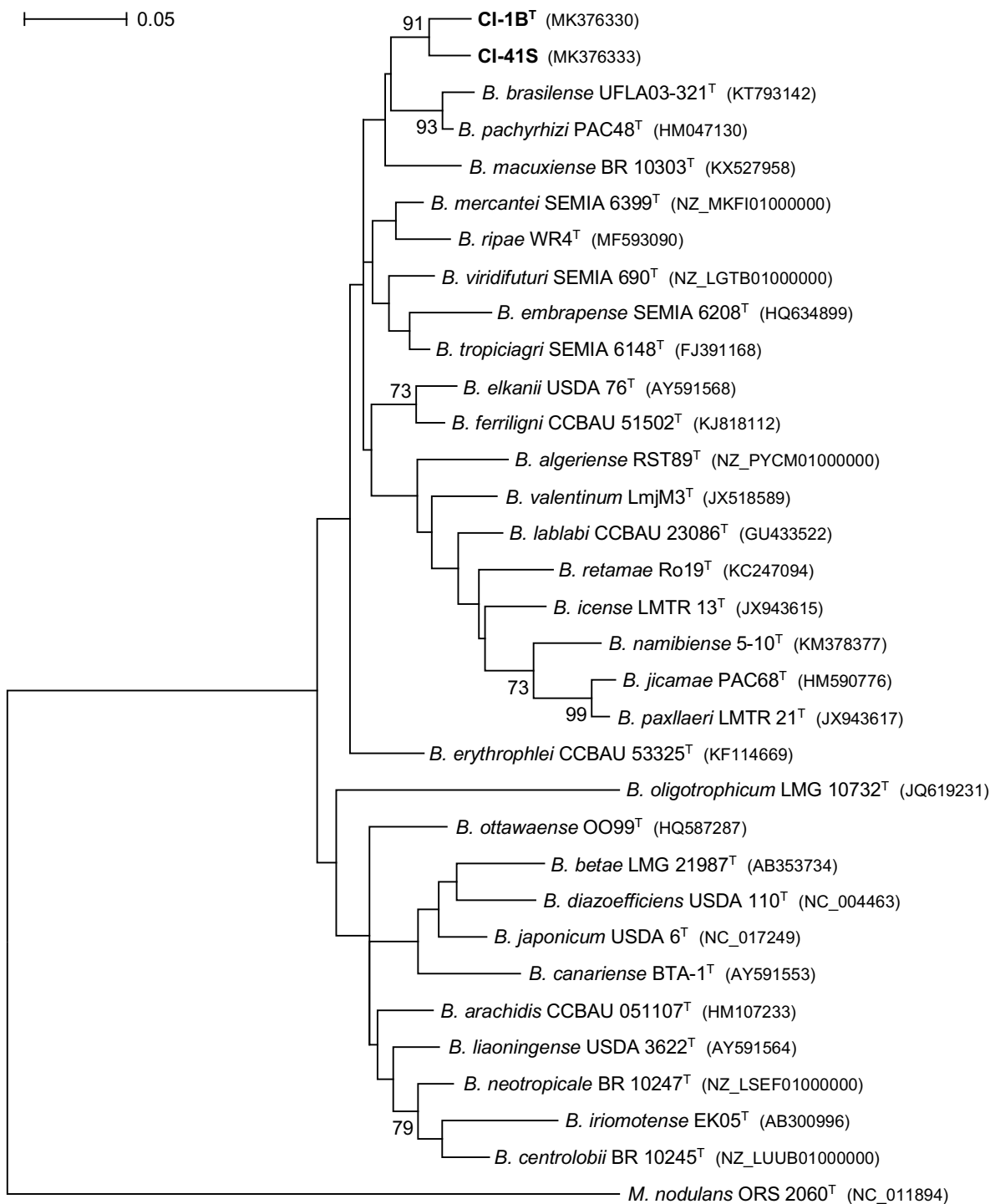
**Figure S4.** Maximum likelihood phylogram inferred from partial *gyrB* gene sequences.

The unrooted maximum-likelihood phylogram was inferred from 588 bp *gyrB* sequences of type strains of all species included in the *Bradyrhizobium elkanii* supergroup at the time of submission, of a selected number of additional *Bradyrhizobium* species, of *B. ivorense* sp. nov. strains CI-1B<sup>T</sup> and CI-41S (shown in bold) and of *Methylobacterium nodulans* strain ORS 2060<sup>T</sup> that was used as the outgroup. The T92+G model was used with 594 positions and 1,000 pseudoreplicates as parameters. Only bootstrap values >70% are shown at branch nodes. Scale bar indicates the number of substitutions per site.



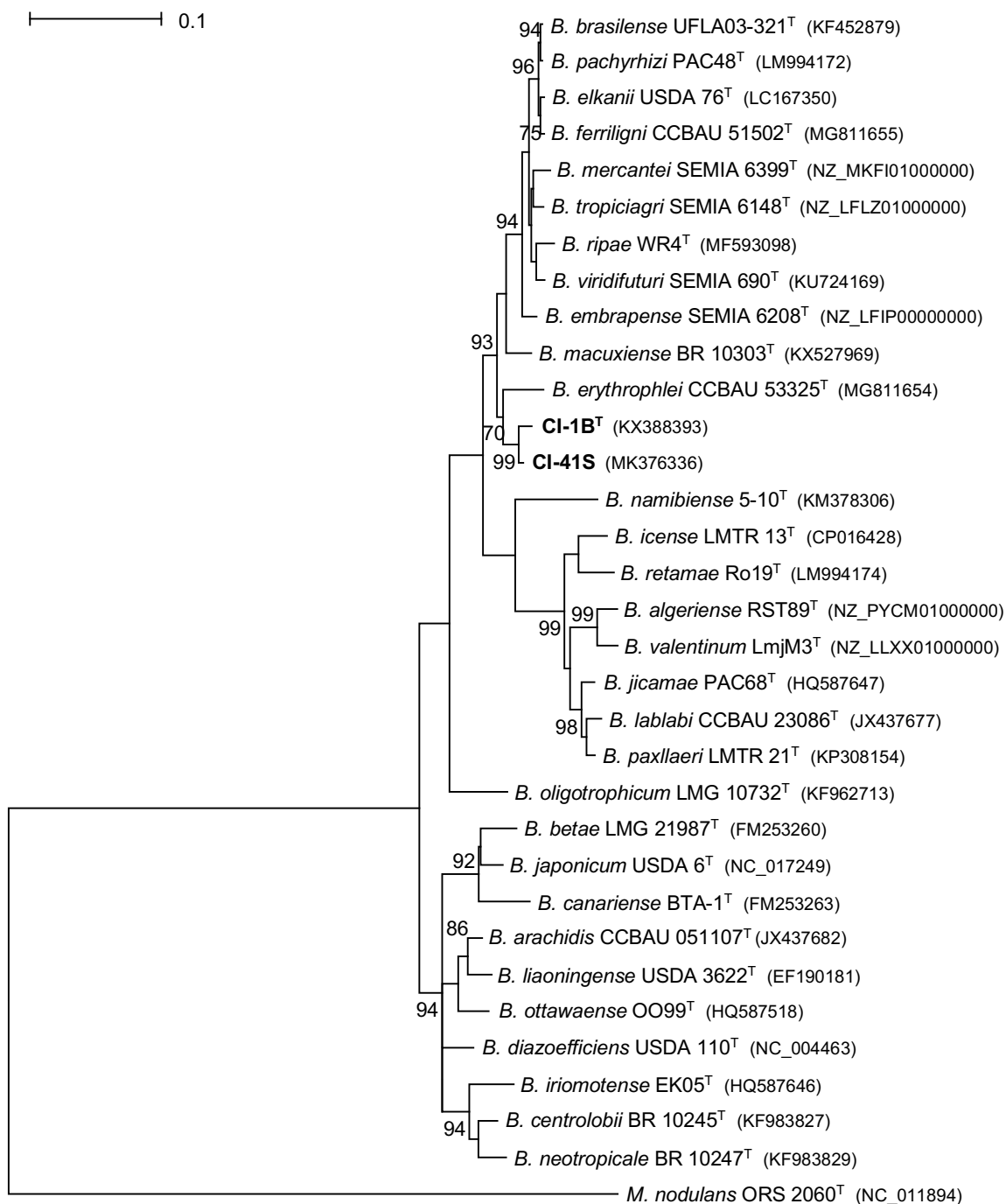
**Figure S5.** Maximum likelihood phylogram inferred from partial *recA* gene sequences.

The unrooted maximum-likelihood phylogram was inferred from 439 bp *recA* sequences of type strains of all species included in the *Bradyrhizobium elkanii* supergroup at the time of submission, of a selected number of additional *Bradyrhizobium* species, of *B. ivorense* sp. nov. strains CI-1B<sup>T</sup> and CI-41S (shown in bold) and of *Methylobacterium nodulans* strain ORS 2060<sup>T</sup> that was used as the outgroup. The T92+G model was used with 439 positions and 1,000 pseudoreplicates as parameters. Only bootstrap values >70% are shown at branch nodes. Scale bar indicates the number of substitutions per site.



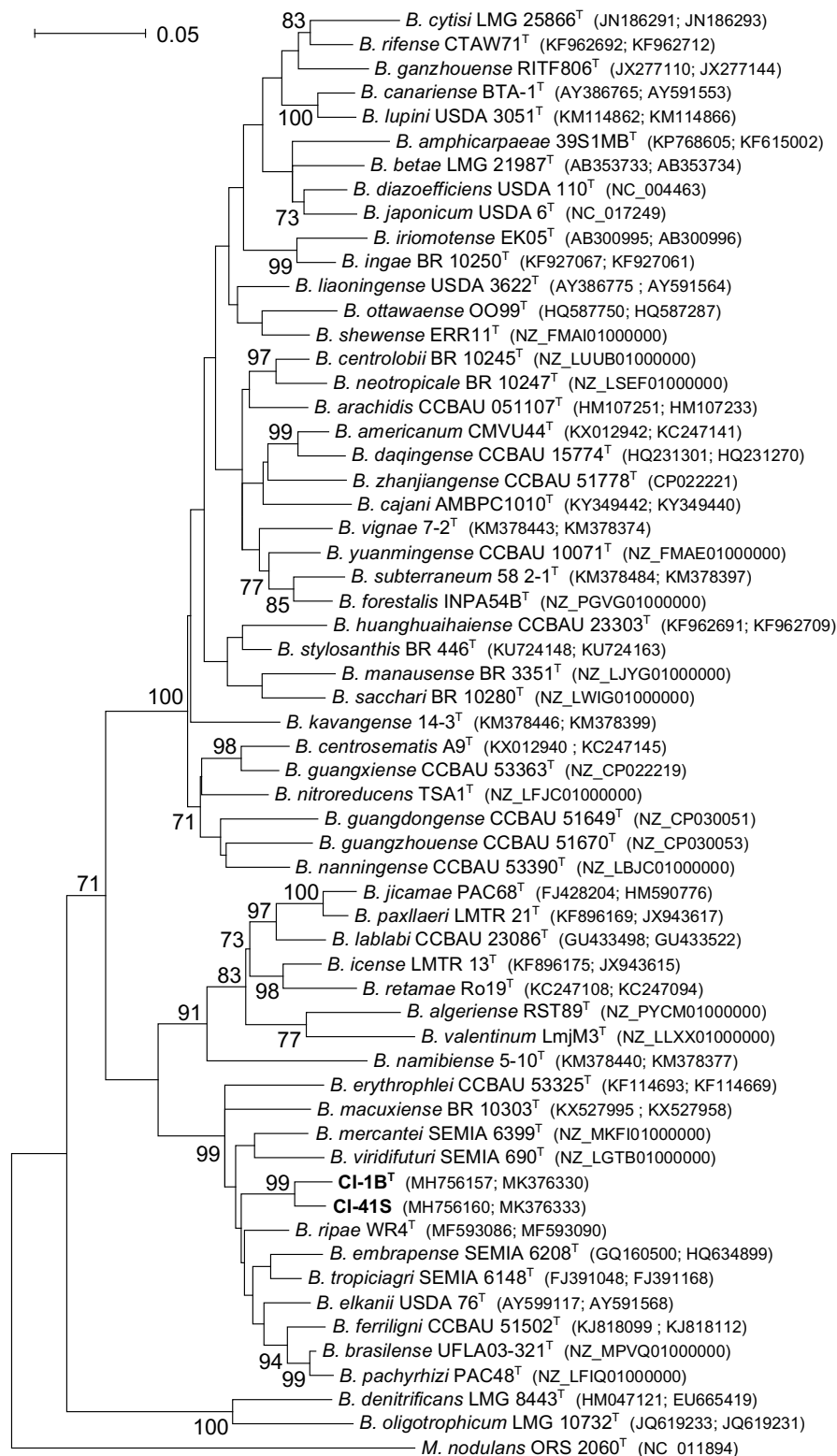
**Figure S6.** Maximum likelihood phylogram inferred from partial *rpoB* gene sequences.

The unrooted maximum-likelihood phylogram was inferred from 714 bp *rpoB* sequences of type strains of all species included in the *Bradyrhizobium elkanii* supergroup at the time of submission, of a selected number of additional *Bradyrhizobium* species, of *B. ivorense* sp. nov. strains CI-1B<sup>T</sup> and CI-41S (shown in bold) and of *Methylobacterium nodulans* strain ORS 2060<sup>T</sup> that was used as the outgroup. The GTR+G+I model was used with 714 positions and 1,000 pseudoreplicates as parameters. Only bootstrap values >70% are shown at branch nodes. Scale bar indicates the number of substitutions per site.



**Figure S7.** Maximum likelihood phylogram inferred from concatenated *glnII-recA* sequences.

The unrooted maximum-likelihood phylogram was inferred from concatenated partial *glnII* (522 bp) and *recA* (411 bp) sequences of 57 type strains of the *Bradyrhizobium* genus, of *B. ivorensis* sp. nov. strains CI-1B<sup>T</sup> and CI-41S (shown in bold) and of *Methylobacterium nodulans* strain ORS 2060<sup>T</sup> that was used as the outgroup. The GTR+G+I model was used with 933 positions and 1,000 pseudoreplicates as parameters. Only bootstrap values >70% are shown at branch nodes. Scale bar indicates the number of substitutions per site.





**Figure S8.** Maximum likelihood phylogram inferred from partial *nifH* gene sequences.

The rooted maximum-likelihood phylogram was inferred from 285 bp *nifH* sequences of type strains of all species included in the *Bradyrhizobium elkanii* supergroup at the time of submission, of a selected number of additional *Bradyrhizobium* species, of *B. ivorense* sp. nov. strains CI-1B<sup>T</sup> and CI-41S (shown in bold) and of *Methylobacterium nodulans* strain ORS 2060<sup>T</sup> that was used as the outgroup. The T92+G model was used with 285 positions and 1,000 pseudoreplicates as parameters. Only bootstrap values >70% are shown at branch nodes. Scale bar indicates the number of substitutions per site. Genomes of *B. centrolobii* BR 10245<sup>T</sup>, *B. neotropicale* BR 10247<sup>T</sup> and *B. oligotrophicum* LMG 10732<sup>T</sup> were found to code for two divergent copies of *nifH*, both of which were included in this phylogeny. No *nifH* record was found for *B. betae* LMG 21987<sup>T</sup>, *B. liaoningense* USDA 3622<sup>T</sup> and *B. ripae* WR4<sup>T</sup> strains that were included in other gene phylogenies, however.

