Supplementary Material

Native Rhizobia Improve Plant Growth, Fix N2, and Reduce Greenhouse Emissions of Sunnhemp More than Commercial Rhizobia Inoculants in Florida Citrus Orchards

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Table S1. Soil physicochemical properties of the studied sites.

	Citrus orchard A	Citrus orchard B
Soil texture	Sandy	Sandy
Sand (%)	93.5	95.2
Silt (%)	5.5	4.0
Clay (%)	1	0.8
Soil organic matter (%)	2.2	1.1
Ammonium (mg/kg)	6.7	2.4
Nitrate (mg/kg)	8.4	2.6
Soil pH	7.4	7.1

Table S2. Identification of strains from nodules of sunnhemp (*Crotalaria juncea* L.). Closest relative type strains of the genera *Rhizobium* and *Bradyrhizobium* based on different targeted genes. Identity values are shown in brackets.

Rhizobacterial strain	Closest relative type strains (Identity values are shown in brackets)					
	Targeted gene					
	16S rRNA	*recA*	*glnII*	*atpD*	*nifH*	*nodC*
COA3	*R. tropici* CIAT 899^T (99.06%), *R. freirei* PRF 81^T (99.25%), and *R. hainanense* CCBAU 57015^T (99.7%)	*R. tropici* CIAT 899^T (95.7%)	*R. tropici* CIAT 899^T (96.7%)	*R. tropici* CIAT 899^T (95.9%)	*R. tropici* CIAT 899^T (99.2%), *R. multihospitium* CCBAU 83401^T (99.2%), and *R. lusitanum* P1-7^T (99.2%)	*R. tropici* CIAT 899^T (99.1%)
COA6	*R. tropici* CIAT 899^T (99.43%), *R. freirei* PRF 81^T (99.33%), and *R. hainanense* CCBAU 57015^T (99.43%)	*R. tropici* CIAT 899^T (99.6%)	*R. tropici* CIAT 899^T (99.7%)	*R. tropici* CIAT 899^T (99.7%)	*R. tropici* CIAT 899^T (99.2%), *R. multihospitium* CCBAU 83401^T (99.2%), and *R. lusitanum* P1-7^T (99.2%)	*R. tropici* CIAT 899^T (99.2%)
COB5	*R. tropici* CIAT 899^T (99.42%), *R. freirei* PRF 81^T (99.32%), and *R. hainanense* CCBAU 57015^T (99.41%)	*R. tropici* CIAT 899^T (99.6%)	*R. tropici* CIAT 899^T (99.8%)	*R. tropici* CIAT 899^T (99.6%)	*R. tropici* CIAT 899^T (99.2%), *R. multihospitium* CCBAU 83401^T (99.2%), and *R. lusitanum* P1-7^T (99.2%)	*R. tropici* CIAT 899^T (99.2%)
COB6	*R. tropici* CIAT 899^T (99.52%), *R. freirei* PRF 81^T (99.38%), and *R. hainanense* CCBAU 57015^T (99.38%)	*R. tropici* CIAT 899^T (99.6%)	*R. tropici* CIAT 899^T (99.7%)	*R. tropici* CIAT 899^T (99.6%)	*R. tropici* CIAT 899^T (99.2%), *R. multihospitium* CCBAU 83401^T (99.2%), and *R. lusitanum* P1-7^T (99.2%)	*R. tropici* CIAT 899^T (99.2%)
MI13	*B. japonicum* USDA6^T (99.85%), and *B. liaoningense* LMG18230^T (99.93%)	*B. japonicum* USDA6^T (99.5%)	*B. japonicum* USDA6^T (99.6%)	-	*B. daqingense* CCBAU 15774^T (99.9%), *B. huanghuaihainense* CCABU 23303^T (99.9%), *B. ottawaense* OO99^T, *B. liaoningense* LMG 18230^T (99.9%), and *B. japonicum* USDA6^T (99.9%)	*B. yuanmingense* CCBAU10071^T (99.9%)

Table S3. N_2-fixation, nitrous oxide (N_2O), and dinitrogen (N_2) rates of rhizobial strains from nodules of sunnhemp (*Crotalaria juncea* L.) inoculated with soil from commercial orchards A and B (COA and COB, respectively) and the microbial inoculant (MI). Values represent the mean ± standard error. For each gas analysis, numbers in a column followed by the same letter are not significantly different according to one-way ANOVA test (Tukey's HSD, $p < 0.05$).

Treatment	Nodules		
	N_2-fixation (nmol C_2H_4 h^{-1} mL^{-1})	N_2O production (nmol N_2O h^{-1} mL^{-1})	N_2 production (nmol N_2O h^{-1} mL^{-1})
COA	102.7 ± 15.8a	18.7 ± 5.6b	1.5 ± 0.8a
COB	140.4 ± 28.1a	22.8 ± 6.2b	1.0 ± 0.6a
MI	48.2 ± 16.6b	39.7 ± 8.5a	1.1 ± 0.9a
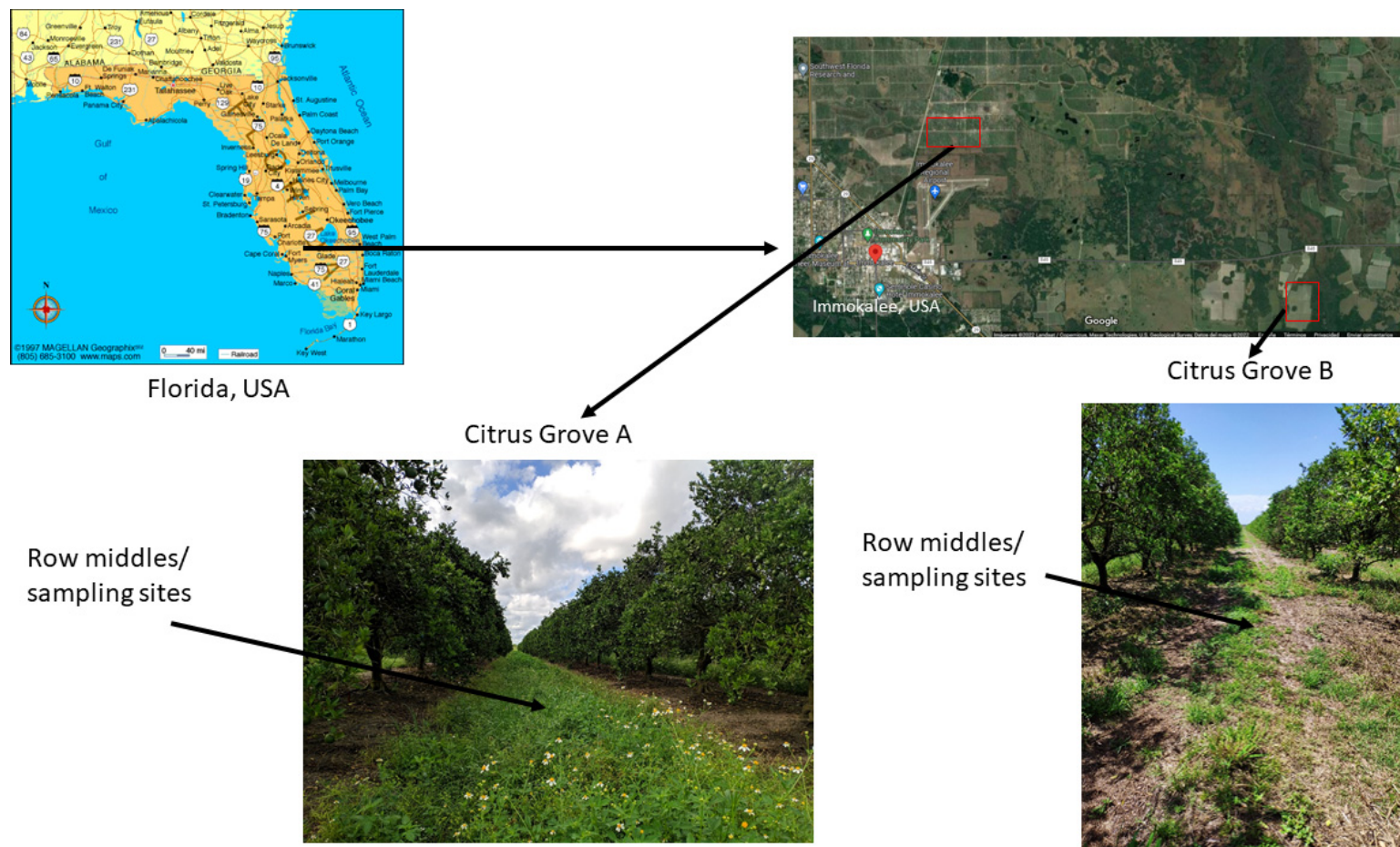
Figure S1. Map showing the location of the sampling sites within Citrus orchard A and Citrus orchard B.

Rhizobium indicum JKLM 12A2^T (MK949132)
Rhizobium acidisoli FH13^T (KJ921033)
Rhizobium hidalgonense FH14^T (KJ921034)
Rhizobium ruizarguesonis UMP1133^T (MG904297)
Rhizobium leguminosarum bv. viciae USDA 2370^T (NR118339)
Rhizobium laguerreae FB206^T (JN558651)
Rhizobium anhuiense CCBAU 23252^T (KF11868)
Rhizobium sophorae CCBAU 03386^T (KJ831229)
Rhizobium esperanzae CNPSo 668^T (KC293513)
Rhizobium pisi DSM 30132^T (AY509899)
Rhizobium fabae CCBAU 33202^T (DQ835306)
Rhizobium phaseoli ATCC 14482^T (EF141340)
Rhizobium ecuadorense CNPSo 671^T (JN129381)
Rhizobium pisi C5^T (KJ438829)
Rhizobium aegyptiacum 1010^T (NR137399)
Rhizobium binae BLR195^T (NR137242)
Rhizobium bangladeshense BLR175^T (JN648931)
Rhizobium sophoriradicis CCBAU 03470^T (KJ831225)
Rhizobium lentis BLR27^T (JN648905)
Rhizobium etli CFN 42^T (U28916)
Rhizobium endophyticum CCGE2052^T (EU867317)
Rhizobium dioscoreae S-93^T (LC498520)
Rhizobium paranaense PRF 35^T (EU488753)
Rhizobium jaguaris CCGE525^T (CP032694)
Rhizobium vallis CCBAU 65647 (FJ839677)
Rhizobium calliandrae CCGE524^T (JX855162)
Rhizobium leucaenae CFN299^T (EU488741)
Rhizobium lusitanum P1-7^T (AY738130)
Rhizobium rhizogenes NBRC 13257^T (D14501)
Rhizobium mayense CCGE526^T (JX855172)
Rhizobium miluonense HAMBI 2971^T (EF061096)
Rhizobium multihospitium HAMBI 2975^T (EF035074)
Rhizobium freirei PRF 81^T (EU488742)
COA3 (OL907126)
Rhizobium hainanense CCBAU 57015^T (U71078)
Rhizobium tropici CIAT 899^T (CP004015)
COB5 (OL907125)
COA6 (OL907128)
COB6 (OL907127)
Rhizobium sullae IS 123^T (Y10170)
Rhizobium indigoferae CCBAU 71042^T (AF364068)
Rhizobium mongolense USDA 1844^T (NR026066)
Rhizobium gallicum R602sp^T (U86343)
Rhizobium sphaerophysae CCNWGS0238^T (FJ154088)
Rhizobium giardinii H152^T (EU488750)
Rhizobium vignae CCBAU 05176^T (GU128881)
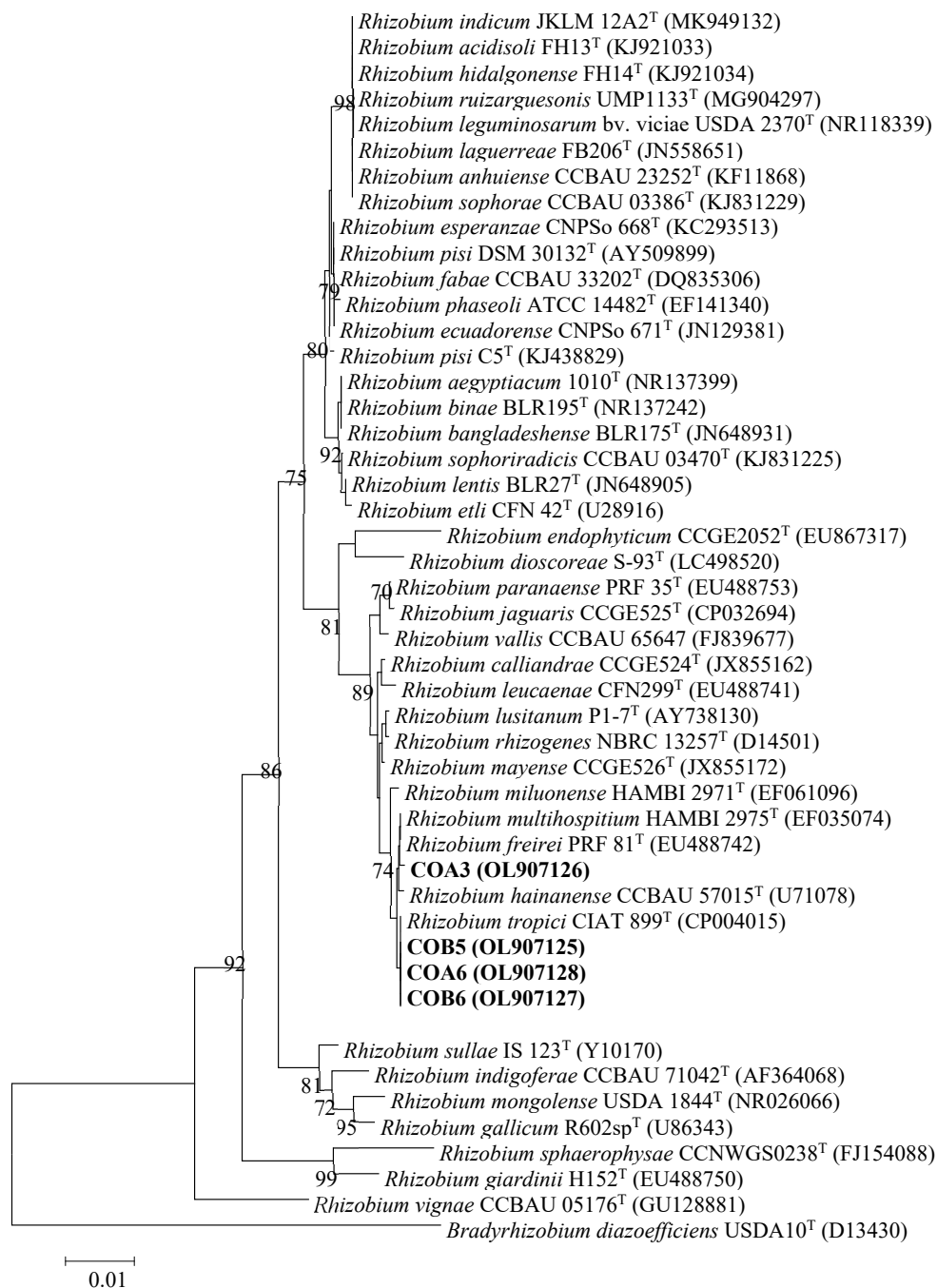*Bradyrhizobium diazoefficiens* USDA10^T (D13430)

0.01

Figure S2. ML phylogenetic tree based on partial 16S rRNA sequences of strains from nodules of sunnhemp (*Crotalaria juncea* L.) and phylogenetically related species within the genus *Rhizobium*. The analysis was based on 1420 nucleotides. Isolates are denoted in bold. Bootstrap values are indicated as percentages derived from 1000 replications. Values lower than 70 are not shown. Bar, 1 nucleotides substitution per 100 nucleotides. The tree is rooted with *Bradyrhizobium* diazoefficiens USDA10^T.

Bradyrhizobium cytisi CTAW11^T (EU561065)

Bradyrhizobium rifense CTAW71^T (EU561074)

Bradyrhizobium ganzhouense RITF806^T (JQ796661)

75 — Bradyrhizobium manausense BR3351^T (HQ641226)

Bradyrhizobium vignae 7-2^T (KP899563)

Bradyrhizobium guangxiense CCBAU 53363^T (KC508877)

84 — Bradyrhizobium guangdongense CCBAU 51649^T (KC508877)

Bradyrhizobium centrosematis A9^T (KC247115)

71 — Bradyrhizobium betae LMG 21987^T (AY372184)

Bradyrhizobium diazoefficiens USDA10^T (D13430)

70 — Bradyrhizobium stylosanthis BR446^T (KU724142)

Bradyrhizobium huanghuaihaiense CCBAU23303^T (HQ231463)

Bradyrhizobium arachidis CCBAU051107^T (HM107167)

Bradyrhizobium kavangense 14-3^T (KP899562)

74 — Bradyrhizobium iriomotense EK05^T (AB300992)

98 — Bradyrhizobium ingae BR10250^T (KF927043)

76

83 — Bradyrhizobium lupini USDA3051^T (KM114861)

Bradyrhizobium canariense BTA1^T (AJ558025)

Bradyrhizobium ottawaense OO99^T (JN186270)

75 — Bradyrhizobium subterraneum 58 2-1^T (KP308152)

Bradyrhizobium yuanmingense CCBAU10071^T (AF193818)

Bradyrhizobium japonicum USDA6^T (AB231927)

100

75 — **MI13 (OL907124)**

Bradyrhizobium liaoningense LMG18230^T (AF208513)

Bradyrhizobium daqingense CCBAU15774^T (HQ231274)

71 — Bradyrhizobium americanum CMVU44^T (KU991833)

Bradyrhizobium oligotrophica LMG 10732^T (JQ619230)

99 — Bradyrhizobium denitrificans LMG 8443^T (X66025)

Bradyrhizobium retamae Ro19^T (KC247085)

Bradyrhizobium neotropicale BR10247^T (KF927051)

Bradyrhizobium algeriense RST89^T (FJ546419)

97

Bradyrhizobium jicamae PAC68^T (AY624134)

Bradyrhizobium erythrophlei CCBAU 53325^T (KF114645)

80 — Bradyrhizobium viridifuturi SEMIA 690^T (FJ025107)

Bradyrhizobium embrapense SEMIA 6208^T (AY904773)

82 — Bradyrhizobium valentinum LmjM3^T (JX514883)

Bradyrhizobium icense LMTR13^T (KF896156)

Bradyrhizobium paxllaeri LMTR 21^T (AY923031)

Bradyrhizobium ferriligni CCBAU51502^T (KJ818096)

88 — Bradyrhizobium pachyrhizi PAC48^T (AY624135)

74 — Bradyrhizobium tropiciagri SEMIA 6148^T (AY904753)

Bradyrhizobium elkanii USDA76^T (U35000)

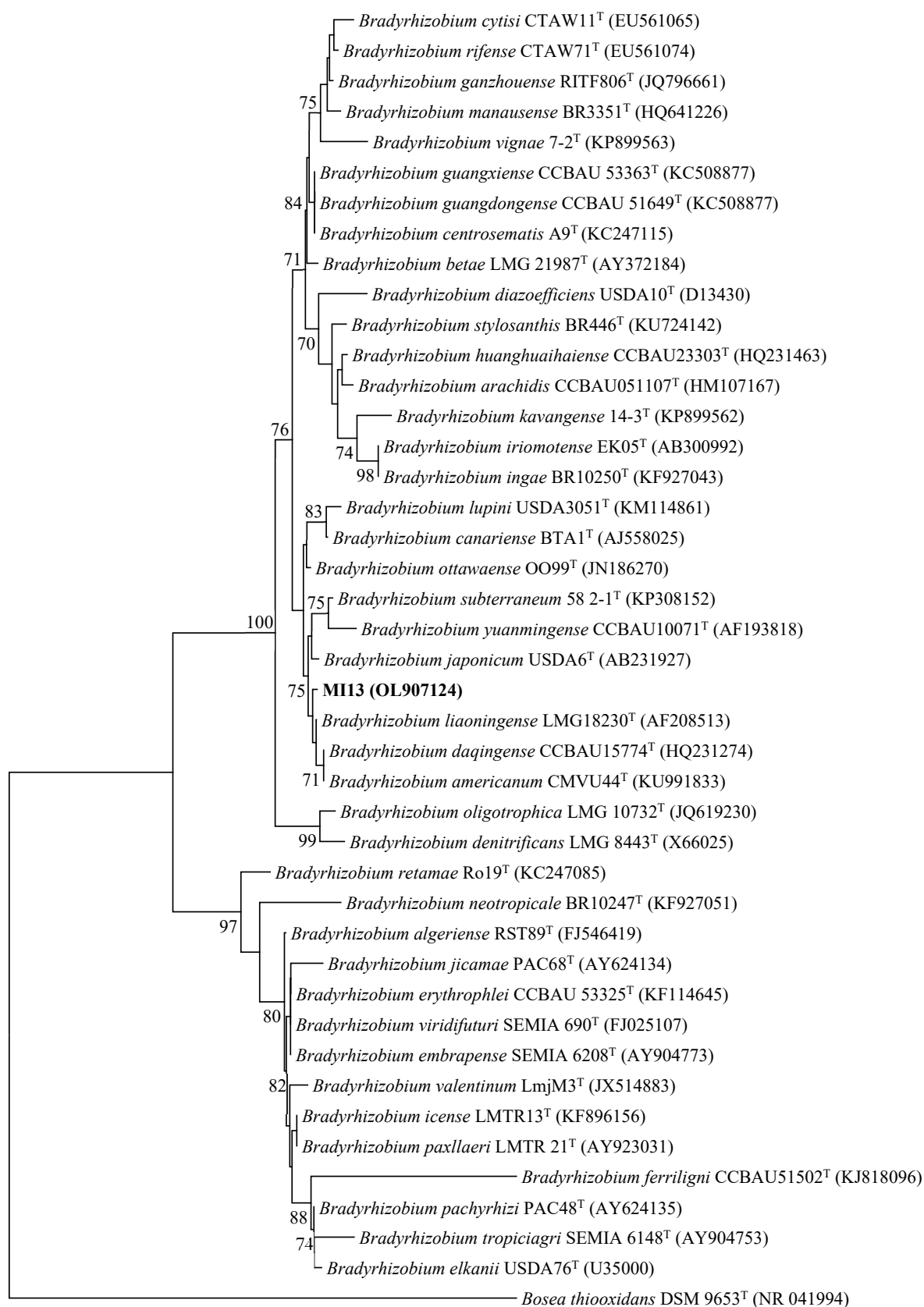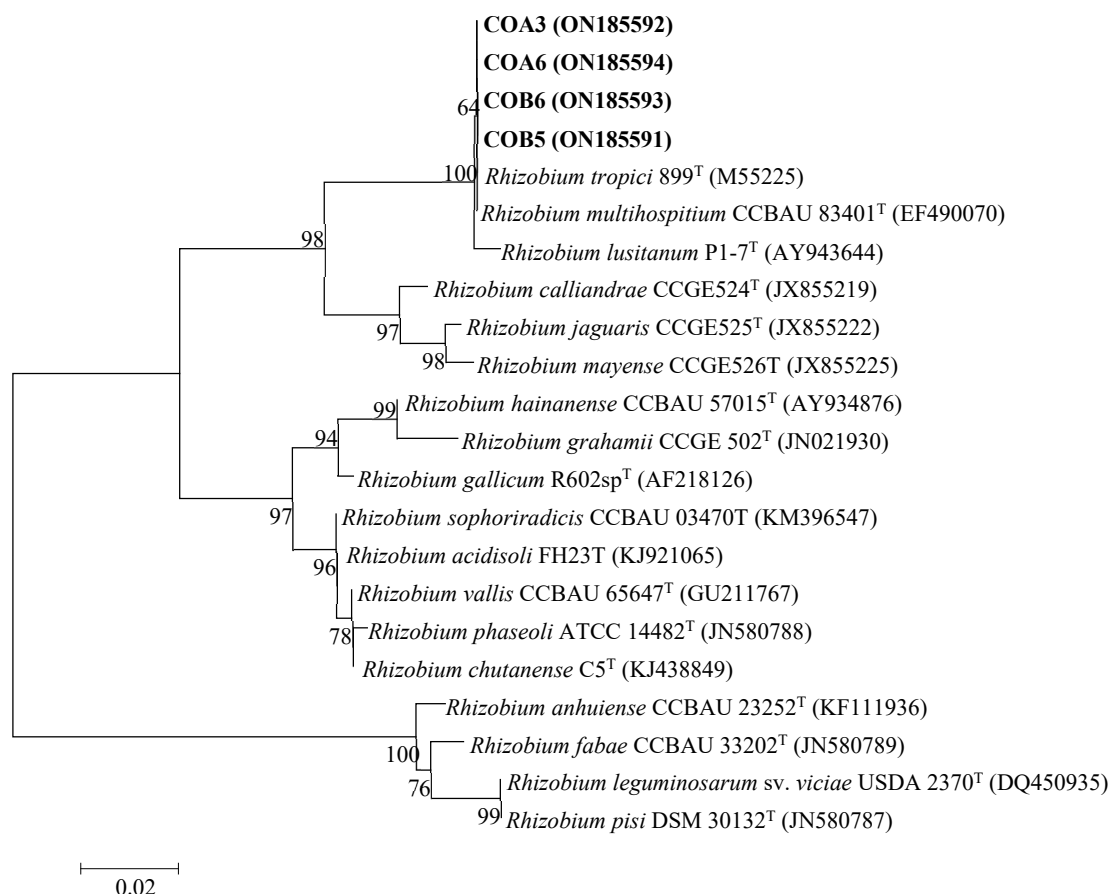Bosea thiooxidans DSM 9653^T (NR 041994)

0.01

Figure S3. ML phylogenetic tree based on partial 16S rRNA sequences of strains from nodules of sunnhemp (*Crotalaria juncea* L.) and phylogenetically related species within the genus *Bradyrhizobium*. The analysis was based on 1415 nucleotides. Isolates are denoted in bold. Bootstrap values are indicated as percentages derived from 1000 replications. Values lower than 70 are not shown. Bar, 1 nucleotides substitution per 100 nucleotides. The tree is rooted with *Bosea thiooxidans* DSM 9653[T].
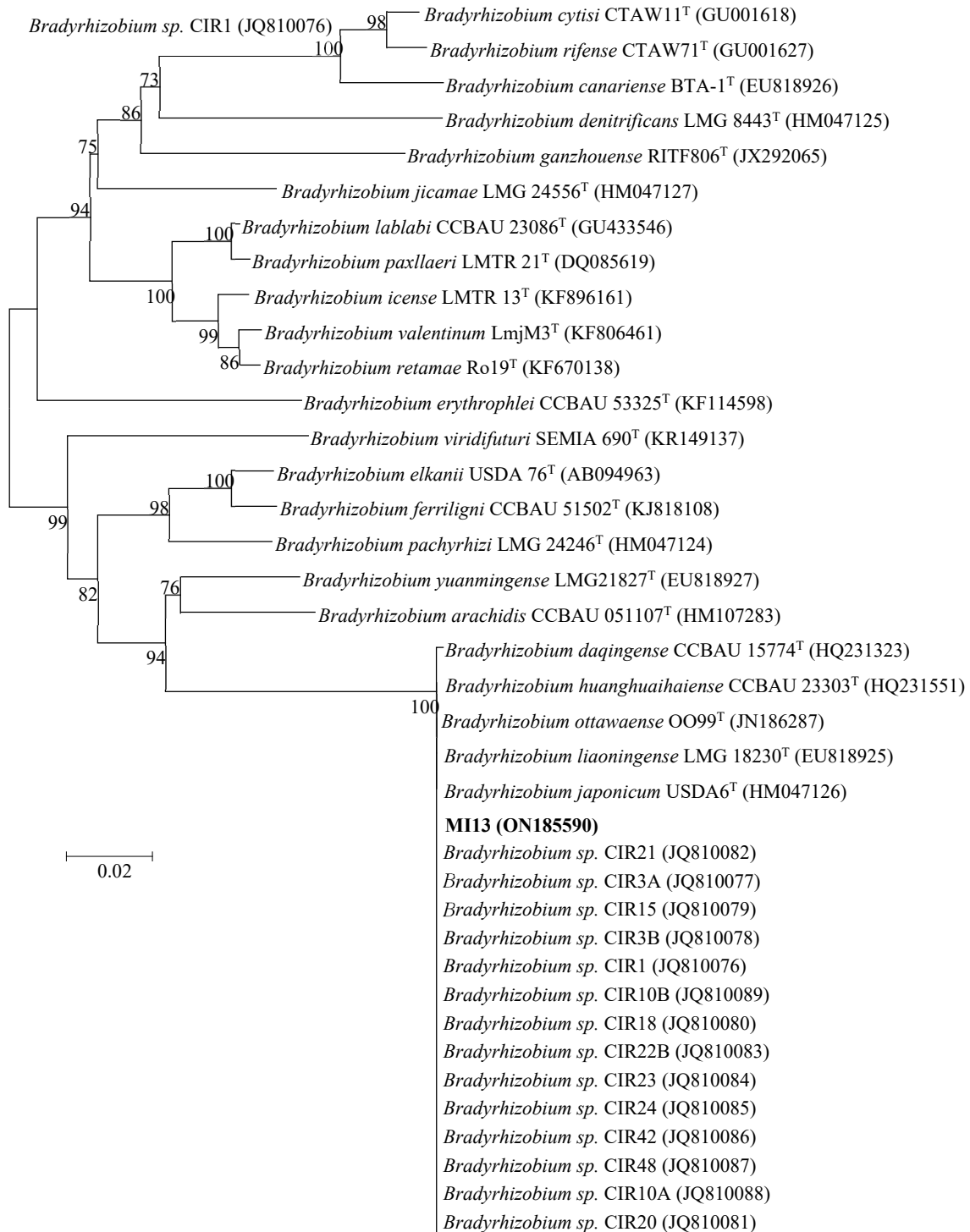

Figure S4. ML phylogenetic tree based on *nifH* sequences of strains from nodules of strains from nodules of sunnhemp (*Crotalaria juncea* L.) and phylogenetically related species within the genus *Rhizobium*. The analysis was based on 340 nucleotides. Isolates are denoted in bold. Bootstrap values are indicated as percentages derived from 1000 replications. Values lower than 70 are not shown. Bar, 2 nucleotide substitution per 100 nucleotides.

Figure S5. ML phylogenetic tree based on *nifH* sequences of strains from nodules of strains from nodules of sunnhemp (*Crotalaria juncea* L.) and phylogenetically related species within the genus *Bradyhizobium*. The analysis was based on 420 nucleotides. Isolates are denoted in bold. Bootstrap values are indicated as percentages derived from 1000 replications. Values lower than 70 are not shown. Bar, 2 nucleotide substitution per 100 nucleotides.