Nodules of *Medicago* spp. Host a Diverse Community of Rhizobial Species in Natural Ecosystems

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Figure S1 Cluster analysis of phenotypic features by UPGMA. The sampled plant populations and elevation (in meters) are written in parentheses, next to the rhizobial isolate names. The isolates followed by an asterisk (*) were not included in the genetic analyses. The reference strains are written in dark blue.

Figure S2 Basic descriptive statistics of inoculation experiments. Left: mean total dry weight per plant (g). Right: Mean number of pink nodules per plant. Symbol shapes and colors are the same as in Figure 1 (triangle = *Medicago lupulina*, square = *M. falcata*, circle = *M. sativa*; blue = *Sinorhizobium meliloti*, yellow = *S. medicae*, green = *Rhizobium leguminosarum*, magenta = *Mesorhizobium* sp.). The horizontal line inside the boxplot represents the median, the red circle represents the mean and the red bars represent the standard error of the mean. Outliers are represented as black dots. Each symbol (circle, square or triangle) represents an individual value. Please note the difference in y-axis scales between graphs.

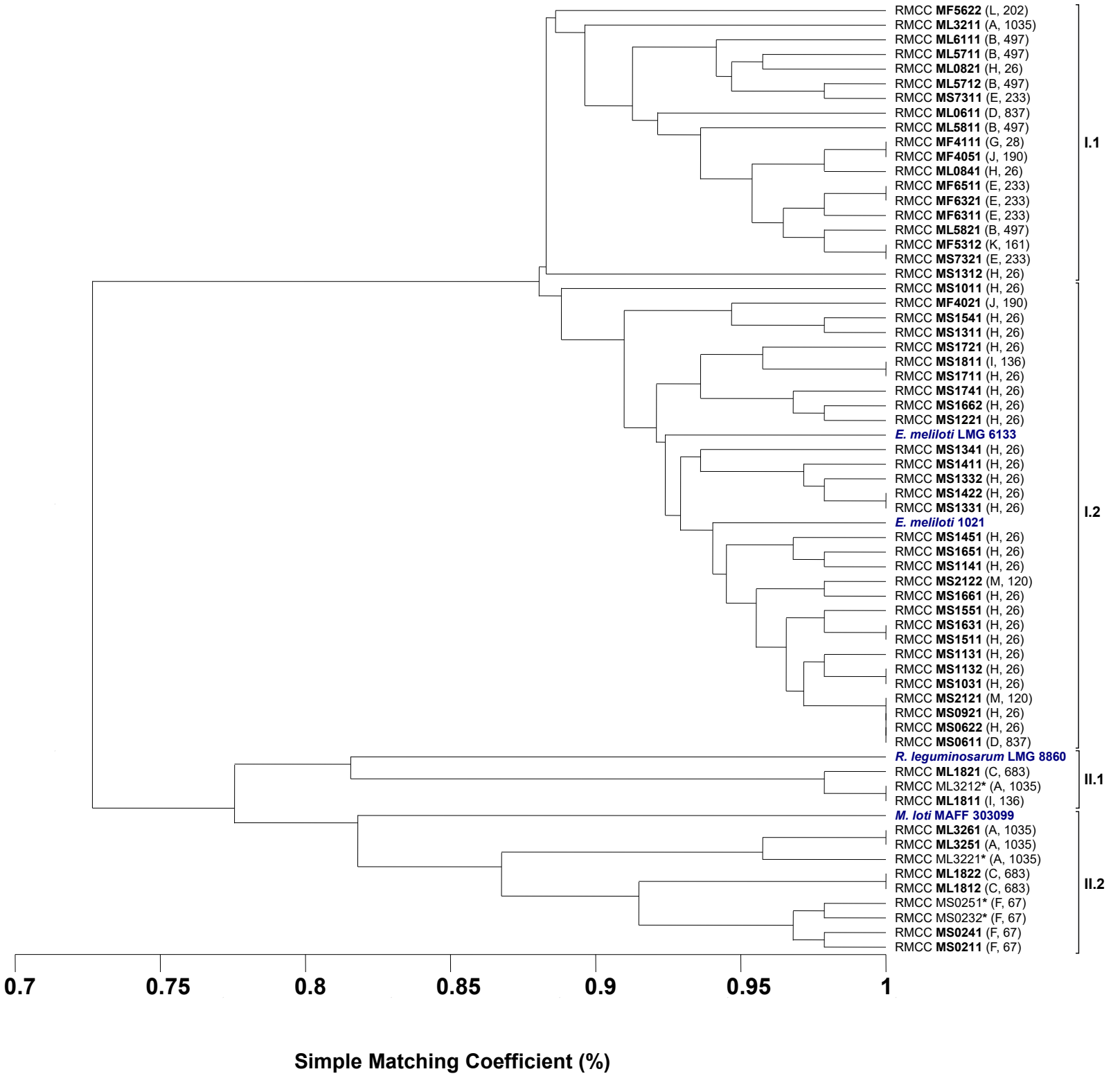
Figure S3 Maximum Likelihood tree of the *atpD* sequences (435 nt); the best fit model was found to be Tamura 3-parameter, and a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories). Bootstrap values greater than 70% are shown on branch nodes. The isolates from this study are in bold type. The three main rhizobial groups are shown in Roman numerals (Ia, Ib, II, III). The 'MF', 'ML', and 'MS' in the rhizobial isolates names stand for the three *Medicago* species.

Figure S4 Maximum Likelihood tree of the *glnII* sequences (588 nt); the best fit model was found to be Tamura 3-parameter, and a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories). Bootstrap values greater than 70% are shown on branch nodes. The isolates from this study are in bold type.

Figure S5 Maximum Likelihood tree of the *recA* sequences (330 nt); the best fit model was found to be Tamura 3-parameter, and a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories). Bootstrap values greater than 70% are shown on branch nodes. The isolates from this study are in bold type.

Figure S6 Maximum Likelihood tree of the *nifH* sequences (168 nt); the best fit model was found to be Kimura 2-parameter. Bootstrap values greater than 70% are shown on branch nodes. The isolates from this study are in bold type.

Figure S1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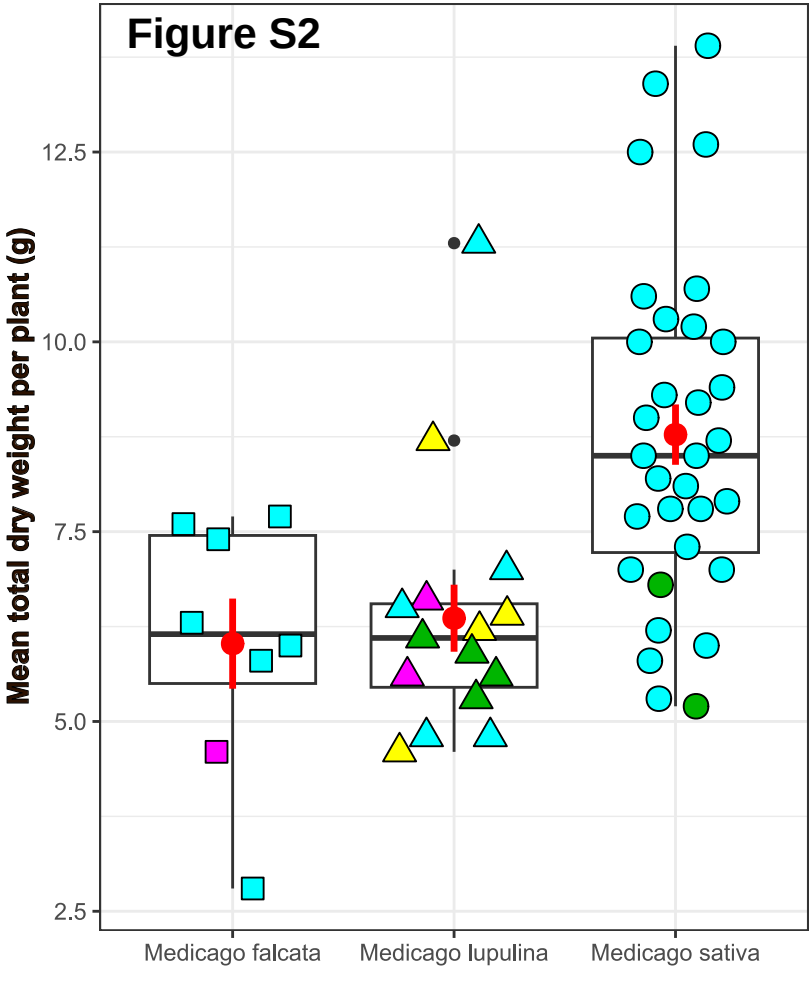
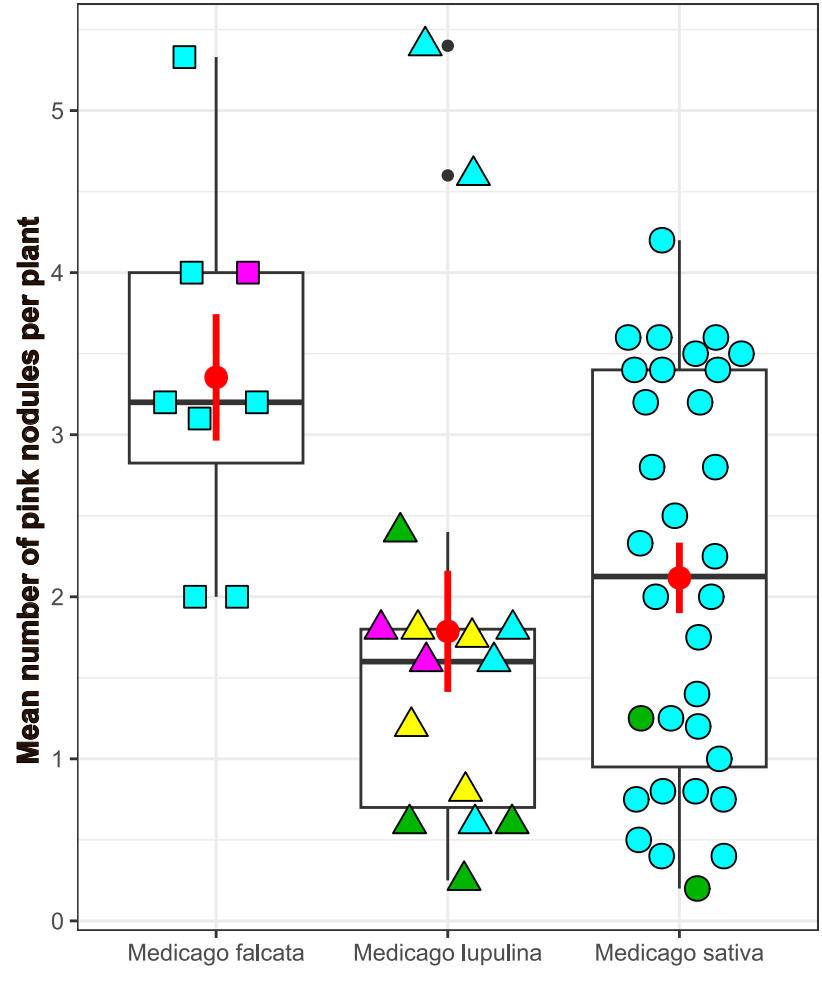
RMCC ML3211 (A, 1035)
RMCC ML6111 (B, 497)
RMCC ML5711 (B, 497)
RMCC ML0821 (H, 26)
RMCC ML5712 (B, 497)
RMCC MS7311 (E, 233)
RMCC ML0611 (D, 837)
RMCC ML5811 (B, 497)
RMCC MF4111 (G, 28)
RMCC MF4051 (J, 190)
RMCC ML0841 (H, 26)
RMCC MF6511 (E, 233)
RMCC MF6321 (E, 233)
RMCC MF6311 (E, 233)
RMCC ML5821 (B, 497)
RMCC MF5312 (K, 161)
RMCC MS7321 (E, 233)
RMCC MS1312 (H, 26)
RMCC MS1011 (H, 26)
RMCC MF4021 (J, 190)
RMCC MS1541 (H, 26)
RMCC MS1311 (H, 26)
RMCC MS1721 (H, 26)
RMCC MS1811 (I, 136)
RMCC MS1711 (H, 26)
RMCC MS1741 (H, 26)
RMCC MS1662 (H, 26)
RMCC MS1221 (H, 26)
E. meliloti LMG 6133
RMCC MS1341 (H, 26)
RMCC MS1411 (H, 26)
RMCC MS1332 (H, 26)
RMCC MS1422 (H, 26)
RMCC MS1331 (H, 26)
E. meliloti 1021
RMCC MS1451 (H, 26)
RMCC MS1651 (H, 26)
RMCC MS1141 (H, 26)
RMCC MS2122 (M, 120)
RMCC MS1661 (H, 26)
RMCC MS1551 (H, 26)
RMCC MS1631 (H, 26)
RMCC MS1511 (H, 26)
RMCC MS1131 (H, 26)
RMCC MS1132 (H, 26)
RMCC MS1031 (H, 26)
RMCC MS2121 (M, 120)
RMCC MS0921 (H, 26)
RMCC MS0622 (H, 26)
RMCC MS0611 (D, 837)
R. leguminosarum LMG 8860
RMCC ML1821 (C, 683)
RMCC ML3212* (A, 1035)
RMCC ML1811 (I, 136)
M. loti MAFF 303099
RMCC ML3261 (A, 1035)
RMCC ML3251 (A, 1035)
RMCC ML3221* (A, 1035)
RMCC ML1822 (C, 683)
RMCC ML1812 (C, 683)
RMCC MS0251* (F, 67)
RMCC MS0232* (F, 67)
RMCC MS0241 (F, 67)
RMCC MS0211 (F, 67)
I.1
I.2
II.1
II.2
0.7
0.75
0.8
0.85
0.9
0.95
1
Simple Matching Coefficient (%)

Figure S2





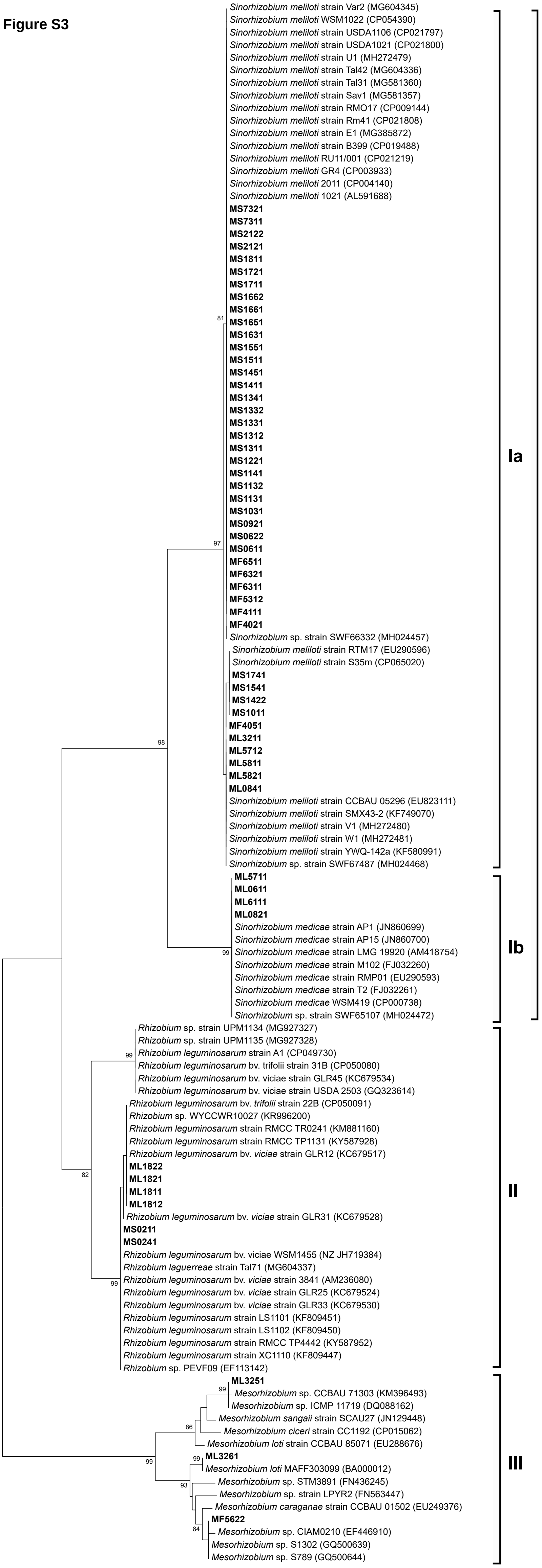
Plant species

- □ *Medicago falcata*
- △ *Medicago lupulina*
- ○ *Medicago sativa*

Treatment

- *Sinorhizobium meliloti*
- *Sinorhizobium medicae*
- *Rhizobium leguminosarum*
- *Mesorhizobium* sp.

Figure S3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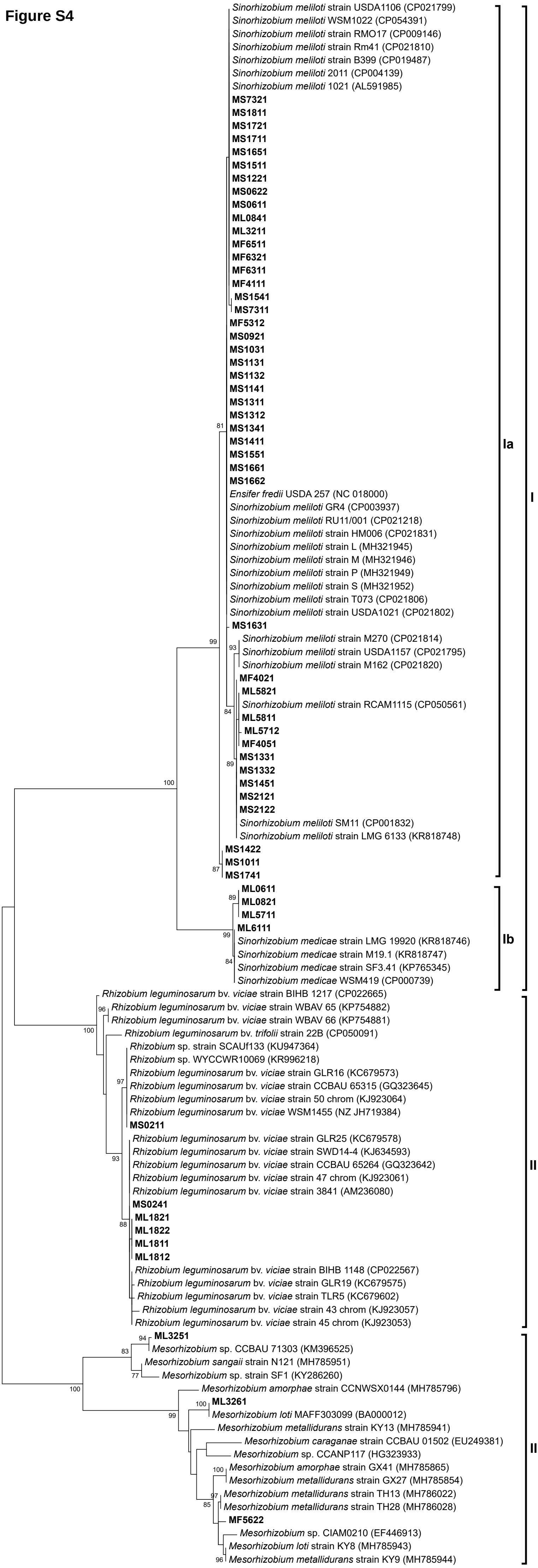
Sinorhizobium meliloti strain USDA1021 (CP021800)
Sinorhizobium meliloti strain U1 (MH272479)
Sinorhizobium meliloti strain Tal42 (MG604336)
Sinorhizobium meliloti strain Tal31 (MG581360)
Sinorhizobium meliloti strain Sav1 (MG581357)
Sinorhizobium meliloti strain RMO17 (CP009144)
Sinorhizobium meliloti strain Rm41 (CP021808)
Sinorhizobium meliloti strain E1 (MG385872)
Sinorhizobium meliloti strain B399 (CP019488)
Sinorhizobium meliloti RU11/001 (CP021219)
Sinorhizobium meliloti GR4 (CP003933)
Sinorhizobium meliloti 2011 (CP004140)
Sinorhizobium meliloti 1021 (AL591688)
MS7321
MS7311
MS2122
MS2121
MS1811
MS1721
MS1711
MS1662
MS1661
81
MS1651
MS1631
MS1551
MS1511
MS1451
MS1411
MS1341
MS1332
MS1331
MS1312
MS1311
MS1221
MS1141
MS1132
MS1131
MS1031
MS0921
MS0622
97
MS0611
MF6511
MF6321
MF6311
MF5312
MF4111
MF4021
Sinorhizobium sp. strain SWF66332 (MH024457)
Sinorhizobium meliloti strain RTM17 (EU290596)
Sinorhizobium meliloti strain S35m (CP065020)
MS1741
MS1541
MS1422
MS1011
MF4051
98
ML3211
ML5712
ML5811
ML5821
ML0841
Sinorhizobium meliloti strain CCBAU 05296 (EU823111)
Sinorhizobium meliloti strain SMX43-2 (KF749070)
Sinorhizobium meliloti strain V1 (MH272480)
Sinorhizobium meliloti strain W1 (MH272481)
Sinorhizobium meliloti strain YWQ-142a (KF580991)
Sinorhizobium sp. strain SWF67487 (MH024468)
ML5711
ML0611
ML6111
ML0821
Sinorhizobium medicae strain AP1 (JN860699)
Sinorhizobium medicae strain AP15 (JN860700)
99
Sinorhizobium medicae strain LMG 19920 (AM418754)
Sinorhizobium medicae strain M102 (FJ032260)
Sinorhizobium medicae strain RMP01 (EU290593)
Sinorhizobium medicae strain T2 (FJ032261)
Sinorhizobium medicae WSM419 (CP000738)
Sinorhizobium sp. strain SWF65107 (MH024472)
Rhizobium sp. strain UPM1134 (MG927327)
Rhizobium sp. strain UPM1135 (MG927328)
99
Rhizobium leguminosarum strain A1 (CP049730)
Rhizobium leguminosarum bv. trifolii strain 31B (CP050080)
Rhizobium leguminosarum bv. viciae strain GLR45 (KC679534)
Rhizobium leguminosarum bv. viciae strain USDA 2503 (GQ323614)
Rhizobium leguminosarum bv. *trifolii* strain 22B (CP050091)
Rhizobium sp. WYCCWR10027 (KR996200)
Rhizobium leguminosarum strain RMCC TR0241 (KM881160)
Rhizobium leguminosarum strain RMCC TP1131 (KY587928)
Rhizobium leguminosarum bv. *viciae* strain GLR12 (KC679517)
ML1822
82
ML1821
ML1811
ML1812
Rhizobium leguminosarum bv. *viciae* strain GLR31 (KC679528)
MS0211
MS0241
Rhizobium leguminosarum bv. viciae WSM1455 (NZ JH719384)
Rhizobium laguerreae strain Tal71 (MG604337)
99
Rhizobium leguminosarum bv. *viciae* strain 3841 (AM236080)
Rhizobium leguminosarum bv. *viciae* strain GLR25 (KC679524)
Rhizobium leguminosarum bv. *viciae* strain GLR33 (KC679530)
Rhizobium leguminosarum strain LS1101 (KF809451)
Rhizobium leguminosarum strain LS1102 (KF809450)
Rhizobium leguminosarum strain RMCC TP4442 (KY587952)
Rhizobium leguminosarum strain XC1110 (KF809447)
Rhizobium sp. PEVF09 (EF113142)
ML3251
99
Mesorhizobium sp. CCBAU 71303 (KM396493)
Mesorhizobium sp. ICMP 11719 (DQ088162)
Mesorhizobium sangaii strain SCAU27 (JN129448)
86
Mesorhizobium ciceri strain CC1192 (CP015062)
Mesorhizobium loti strain CCBAU 85071 (EU288676)
99
ML3261
99
Mesorhizobium loti MAFF303099 (BA000012)
Mesorhizobium sp. STM3891 (FN436245)
93
Mesorhizobium sp. strain LPYR2 (FN563447)
Mesorhizobium caraganae strain CCBAU 01502 (EU249376)
MF5622
84
Mesorhizobium sp. CIAM0210 (EF446910)
Mesorhizobium sp. S1302 (GQ500639)
Mesorhizobium sp. S789 (GQ500644)
Ia
Ib
II
III
I
0,05

Figure S4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Sinorhizobium meliloti strain Rm41 (CP021810)
Sinorhizobium meliloti strain B399 (CP019487)
Sinorhizobium meliloti 2011 (CP004139)
Sinorhizobium meliloti 1021 (AL591985)
MS7321
MS1811
MS1721
MS1711
MS1651
MS1511
MS1221
MS0622
MS0611
ML0841
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MF6311
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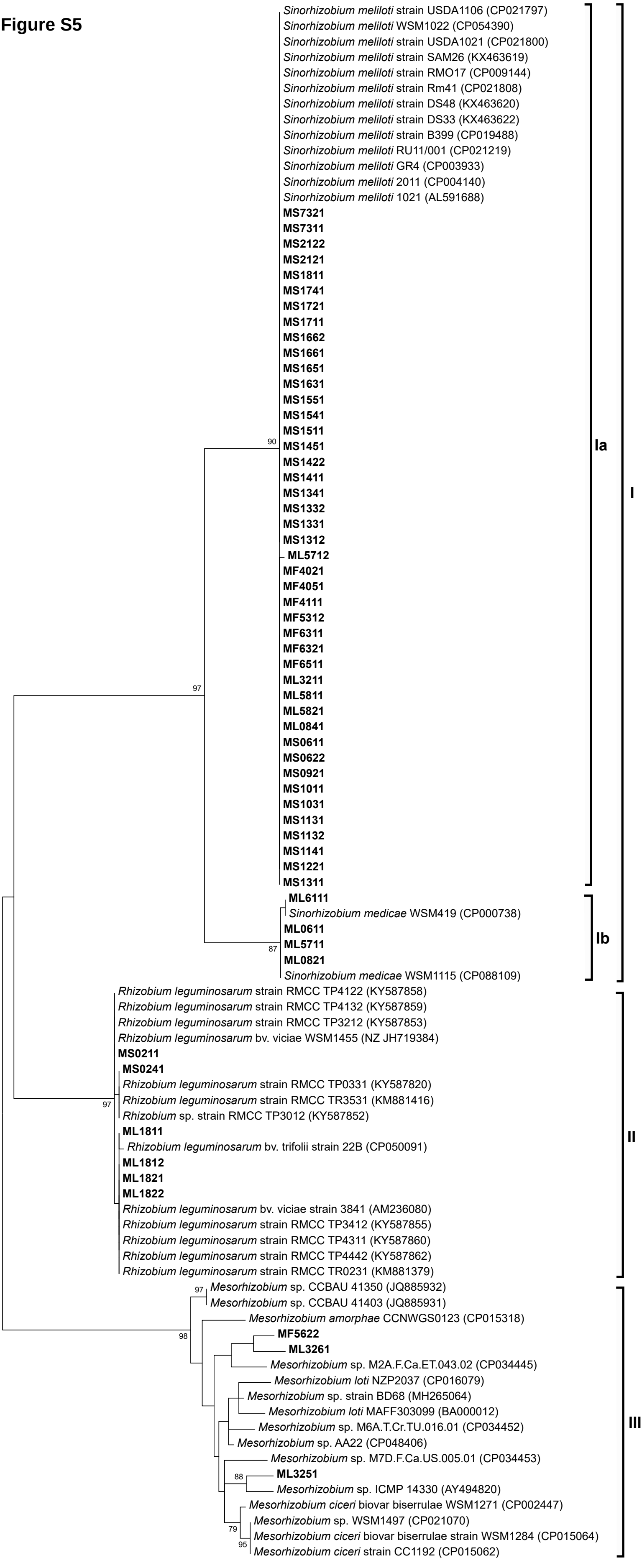
MF5312
MS0921
MS1031
MS1131
MS1132
MS1141
MS1311
MS1312
81
MS1341
MS1411
MS1551
MS1661
MS1662
Ensifer fredii USDA 257 (NC 018000)
Sinorhizobium meliloti GR4 (CP003937)
Sinorhizobium meliloti RU11/001 (CP021218)
Sinorhizobium meliloti strain HM006 (CP021831)
Sinorhizobium meliloti strain L (MH321945)
Sinorhizobium meliloti strain M (MH321946)
Sinorhizobium meliloti strain P (MH321949)
Sinorhizobium meliloti strain S (MH321952)
Sinorhizobium meliloti strain T073 (CP021806)
Sinorhizobium meliloti strain USDA1021 (CP021802)
MS1631
99
Sinorhizobium meliloti strain M270 (CP021814)
93
Sinorhizobium meliloti strain USDA1157 (CP021795)
Sinorhizobium meliloti strain M162 (CP021820)
MF4021
ML5821
Sinorhizobium meliloti strain RCAM1115 (CP050561)
84
ML5811
ML5712
MF4051
MS1331
89
MS1332
100
MS1451
MS2121
MS2122
Sinorhizobium meliloti SM11 (CP001832)
Sinorhizobium meliloti strain LMG 6133 (KR818748)
MS1422
MS1011
87
MS1741
ML0611
89
ML0821
ML5711
ML6111
99
Sinorhizobium medicae strain LMG 19920 (KR818746)
Sinorhizobium medicae strain M19.1 (KR818747)
84
Sinorhizobium medicae strain SF3.41 (KP765345)
Sinorhizobium medicae WSM419 (CP000739)
Rhizobium leguminosarum bv. *viciae* strain BIHB 1217 (CP022665)
96
Rhizobium leguminosarum bv. *viciae* strain WBAV 65 (KP754882)
Rhizobium leguminosarum bv. *viciae* strain WBAV 66 (KP754881)
100
Rhizobium leguminosarum bv. *trifolii* strain 22B (CP050091)
Rhizobium sp. strain SCAUf133 (KU947364)
Rhizobium sp. WYCCWR10069 (KR996218)
Rhizobium leguminosarum bv. *viciae* strain GLR16 (KC679573)
97
Rhizobium leguminosarum bv. *viciae* strain CCBAU 65315 (GQ323645)
Rhizobium leguminosarum bv. *viciae* strain 50 chrom (KJ923064)
Rhizobium leguminosarum bv. *viciae* WSM1455 (NZ JH719384)
MS0211
Rhizobium leguminosarum bv. *viciae* strain GLR25 (KC679578)
Rhizobium leguminosarum bv. *viciae* strain SWD14-4 (KJ634593)
93
Rhizobium leguminosarum bv. *viciae* strain CCBAU 65264 (GQ323642)
Rhizobium leguminosarum bv. *viciae* strain 47 chrom (KJ923061)
Rhizobium leguminosarum bv. *viciae* strain 3841 (AM236080)
MS0241
ML1821
88
ML1822
ML1811
ML1812
Rhizobium leguminosarum bv. *viciae* strain BIHB 1148 (CP022567)
Rhizobium leguminosarum bv. *viciae* strain GLR19 (KC679575)
Rhizobium leguminosarum bv. *viciae* strain TLR5 (KC679602)
Rhizobium leguminosarum bv. *viciae* strain 43 chrom (KJ923057)
Rhizobium leguminosarum bv. *viciae* strain 45 chrom (KJ923053)
94
ML3251
83
Mesorhizobium sp. CCBAU 71303 (KM396525)
Mesorhizobium sangaii strain N121 (MH785951)
77
Mesorhizobium sp. strain SF1 (KY286260)
100
Mesorhizobium amorphae strain CCNWSX0144 (MH785796)
100
ML3261
99
Mesorhizobium loti MAFF303099 (BA000012)
Mesorhizobium metallidurans strain KY13 (MH785941)
Mesorhizobium caraganae strain CCBAU 01502 (EU249381)
Mesorhizobium sp. CCANP117 (HG323933)
100
Mesorhizobium amorphae strain GX41 (MH785865)
Mesorhizobium metallidurans strain GX27 (MH785854)
97
Mesorhizobium metallidurans strain TH13 (MH786022)
85
Mesorhizobium metallidurans strain TH28 (MH786028)
MF5622
Mesorhizobium sp. CIAM0210 (EF446913)
Mesorhizobium loti strain KY8 (MH785943)
96
Mesorhizobium metallidurans strain KY9 (MH785944)
Ia
Ib
I
II
III
0,05

Figure S5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Sinorhizobium meliloti strain Rm41 (CP021808)
Sinorhizobium meliloti strain DS48 (KX463620)
Sinorhizobium meliloti strain DS33 (KX463622)
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Sinorhizobium meliloti RU11/001 (CP021219)
Sinorhizobium meliloti GR4 (CP003933)
Sinorhizobium meliloti 2011 (CP004140)
Sinorhizobium meliloti 1021 (AL591688)
MS7321
MS7311
MS2122
MS2121
MS1811
MS1741
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MS0622
MS0921
MS1011
MS1031
MS1131
MS1132
MS1141
MS1221
MS1311
Ia
I
ML6111
Sinorhizobium medicae WSM419 (CP000738)
ML0611
ML5711
87
ML0821
Sinorhizobium medicae WSM1115 (CP088109)
Ib
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MS0211
MS0241
Rhizobium leguminosarum strain RMCC TP0331 (KY587820)
Rhizobium leguminosarum strain RMCC TR3531 (KM881416)
97
Rhizobium sp. strain RMCC TP3012 (KY587852)
ML1811
Rhizobium leguminosarum bv. trifolii strain 22B (CP050091)
ML1812
ML1821
ML1822
Rhizobium leguminosarum bv. viciae strain 3841 (AM236080)
Rhizobium leguminosarum strain RMCC TP3412 (KY587855)
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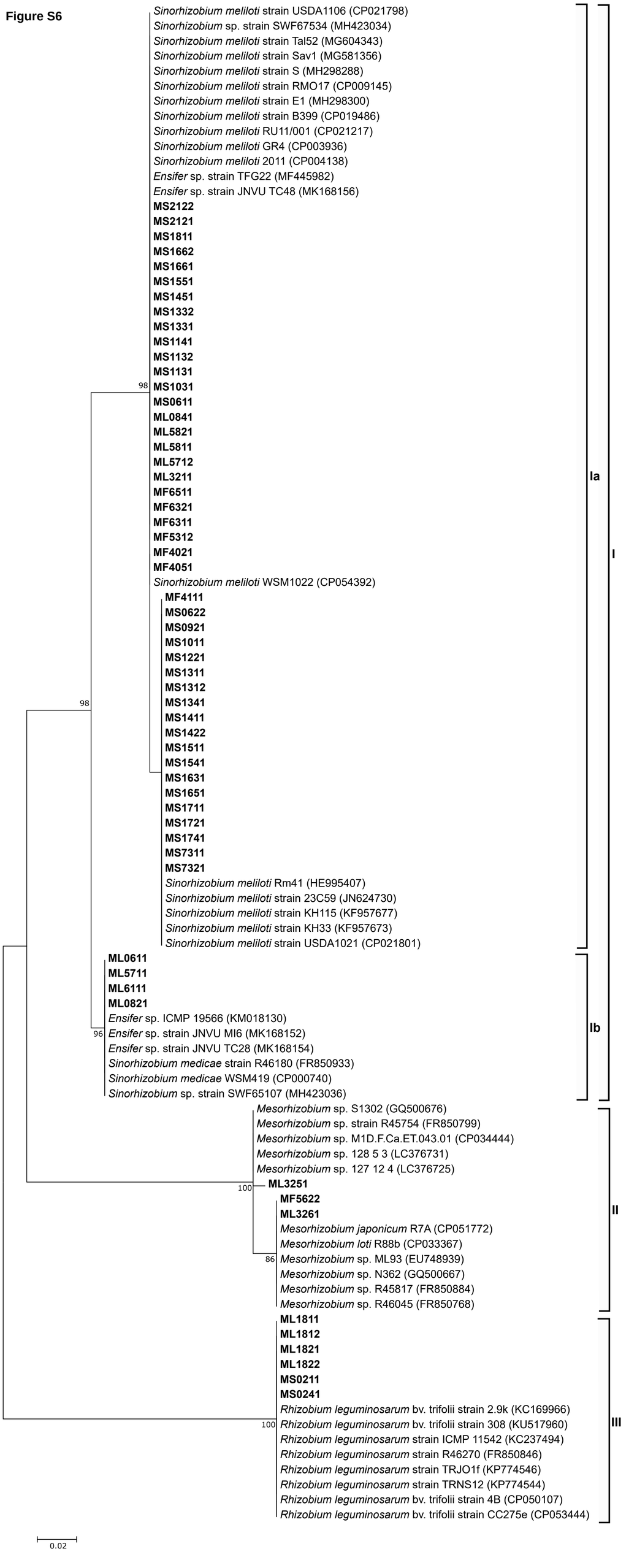
II
97
Mesorhizobium sp. CCBAU 41350 (JQ885932)
Mesorhizobium sp. CCBAU 41403 (JQ885931)
Mesorhizobium amorphae CCNWGS0123 (CP015318)
98
MF5622
ML3261
Mesorhizobium sp. M2A.F.Ca.ET.043.02 (CP034445)
Mesorhizobium loti NZP2037 (CP016079)
Mesorhizobium sp. strain BD68 (MH265064)
Mesorhizobium loti MAFF303099 (BA000012)
Mesorhizobium sp. M6A.T.Cr.TU.016.01 (CP034452)
Mesorhizobium sp. AA22 (CP048406)
III
Mesorhizobium sp. M7D.F.Ca.US.005.01 (CP034453)
88
ML3251
Mesorhizobium sp. ICMP 14330 (AY494820)
Mesorhizobium ciceri biovar biserrulae WSM1271 (CP002447)
Mesorhizobium sp. WSM1497 (CP021070)
79
Mesorhizobium ciceri biovar biserrulae strain WSM1284 (CP015064)
95
Mesorhizobium ciceri strain CC1192 (CP015062)
0.05

Figure S6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Sinorhizobium meliloti strain S (MH298288)
Sinorhizobium meliloti strain RMO17 (CP009145)
Sinorhizobium meliloti strain E1 (MH298300)
Sinorhizobium meliloti strain B399 (CP019486)
Sinorhizobium meliloti RU11/001 (CP021217)
Sinorhizobium meliloti GR4 (CP003936)
Sinorhizobium meliloti 2011 (CP004138)
Ensifer sp. strain TFG22 (MF445982)
Ensifer sp. strain JNVU TC48 (MK168156)
MS2122
MS2121
MS1811
MS1662
MS1661
MS1551
MS1451
MS1332
MS1331
MS1141
MS1132
MS1131
98
MS1031
MS0611
ML0841
ML5821
ML5811
ML5712
ML3211
MF6511
MF6321
MF6311
MF5312
MF4021
MF4051
Sinorhizobium meliloti WSM1022 (CP054392)
MF4111
MS0622
MS0921
MS1011
MS1221
MS1311
MS1312
98
MS1341
MS1411
MS1422
MS1511
MS1541
MS1631
MS1651
MS1711
MS1721
MS1741
MS7311
MS7321
Sinorhizobium meliloti Rm41 (HE995407)
Sinorhizobium meliloti strain 23C59 (JN624730)
Sinorhizobium meliloti strain KH115 (KF957677)
Sinorhizobium meliloti strain KH33 (KF957673)
Sinorhizobium meliloti strain USDA1021 (CP021801)
Ia
ML0611
ML5711
ML6111
ML0821
Ensifer sp. ICMP 19566 (KM018130)
96
Ensifer sp. strain JNVU MI6 (MK168152)
Ensifer sp. strain JNVU TC28 (MK168154)
Sinorhizobium medicae strain R46180 (FR850933)
Sinorhizobium medicae WSM419 (CP000740)
Sinorhizobium sp. strain SWF65107 (MH423036)
Ib
I
Mesorhizobium sp. S1302 (GQ500676)
Mesorhizobium sp. strain R45754 (FR850799)
Mesorhizobium sp. M1D.F.Ca.ET.043.01 (CP034444)
Mesorhizobium sp. 128 5 3 (LC376731)
Mesorhizobium sp. 127 12 4 (LC376725)
100
ML3251
MF5622
ML3261
Mesorhizobium japonicum R7A (CP051772)
Mesorhizobium loti R88b (CP033367)
86
Mesorhizobium sp. ML93 (EU748939)
Mesorhizobium sp. N362 (GQ500667)
Mesorhizobium sp. R45817 (FR850884)
Mesorhizobium sp. R46045 (FR850768)
II
ML1811
ML1812
ML1821
ML1822
MS0211
MS0241
Rhizobium leguminosarum bv. trifolii strain 2.9k (KC169966)
100
Rhizobium leguminosarum bv. trifolii strain 308 (KU517960)
Rhizobium leguminosarum strain ICMP 11542 (KC237494)
Rhizobium leguminosarum strain R46270 (FR850846)
Rhizobium leguminosarum strain TRJO1f (KP774546)
Rhizobium leguminosarum strain TRNS12 (KP774544)
Rhizobium leguminosarum bv. trifolii strain 4B (CP050107)
Rhizobium leguminosarum bv. trifolii strain CC275e (CP053444)
III
0.02