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1 # Diverse novel mesorhizobia nodulate New Zealand native *Sophora*
2 species
3
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12
13 A B S T R A C T
14
15 Forty eight rhizobial isolates from New Zealand (NZ) native *Sophora* spp. growing in natural
16 ecosystems were characterised. Thirty eight isolates across five groups showed greatest similarity to
17 *Mesorhizobium ciceri* LMG 14989[T] with respect to their 16S rRNA and concatenated *recA*, *glnII* and
18 *rpoB* sequences. Seven isolates had a 16S rRNA sequence identical to *M. amorphae* ATCC 19665[T]
19 but showed greatest similarity to *M. septentrionale* LMG 23930[T] on their concatenated *recA*, *glnII* and
20 *rpoB* sequences. All isolates grouped closely together for their *nifH, nodA* and *nodC* sequences,
21 clearly separate from all other rhizobia in the Genbank database. None of the type strains closest to
22 the *Sophora* isolates based on 16S rRNA sequence similarity nodulated *Sophora microphylla* but they
23 all nodulated their original host. Twenty one *Sophora* isolates selected from the different 16S rRNA
24 groupings produced $N_2$-fixing nodules on three *Sophora* spp. but none nodulated any host of the type
25 strains for the related species. DNA hybridisations indicated that these isolates belong to novel
26 *Mesorhizobium* spp. that nodulate NZ native *Sophora* species.
27
28 *Keywords*:
29 *Mesorhizobium*
30 *Sophora*
31 Phylogeny
32 Symbiosis genes
33
34 **Introduction**
35
36 Most legumes (plant family Fabaceae) have the capacity to fix atmospheric nitrogen ($N_2$) via
37 symbiotic bacteria (generally termed 'rhizobia') in root nodules which gives them an advantage under
38 low soil nitrogen (N) conditions if other factors are favourable for growth [1,2,21]. There are four
39 genera of native legume of the sub-family Papilionoideae on the main New Zealand (NZ) islands.
40 These genera are the closely related *Carmichaelia* (23 endemic species), *Clianthus* (2 endemic
41 species) and *Montigena* (1 endemic species) in the 'Carmichaelinae' clade, tribe Galegeae, and
42 *Sophora* (8 endemic species) in the tribe Sophoreae [11,12,13,27]. All four genera are capable of
43 forming nodules [21], but genotypic data on the rhizobia which induce nodules on the *Sophora* spp.
44 are limited.

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45 Previously, sequences were obtained for the small subunit ribosomal RNA (16S rRNA) gene of five
46 isolates of *Sophora* spp. growing in natural ecosystems [34]. Four isolates were most closely
47 grouped to the *Mesorhizobium ciceri* and *M. loti* type strains while the fifth isolate, aligned closely with
48 *Rhizobium leguminosarum* strains. This study did not assess the ability of the isolated strains to
49 nodulate NZ native legumes or sequence any of their $N_2$-fixation (*nif*) or nodulation (*nod*) genes but
50 subsequent work showed that although the *R. leguminosarum* strain (ICMP 14642) produced nodules
51 on *Sophora microphylla*, these nodules did not fix $N_2$ [33, http://scd.landcareresearch.co.nz].
52 Tan et al. [25] examined ten bacterial strains isolated from NZ *Carmichaelinae* growing in natural
53 ecosystems which grouped close to the *M. huakuii* type strain in relation to their 16S rRNA and
54 nitrogenase iron protein gene (*nifH*) sequences. These strains showed novel DNA recombinase A
55 gene (*recA*), glutamine synthetase II gene (*glnII*), N-acyltransferase nodulation protein A gene (*nodA*)
56 and N-acetylglucosaminyltransferase nodulation protein C gene (*nodC*) sequences. Seven strains
57 selected produced functional nodules on *Carmichaelia* spp. and *Clianthus puniceus* but did not
58 nodulate two *Sophora* species indicating that within NZ native legumes, at least some bacterial strains
59 are specific to Carmichaelinae species. Tan et al. [24] tested the ability of eleven isolates, two from
60 *Montigena* and three each from *Carmichaelia* spp., *Clianthus puniceus* and *Sophora* spp. to nodulate
61 *Montigena*. Two of the *Sophora* strains were taken from a previous study [34] and showed 16S rRNA
62 similar to *M. loti/ M. ciceri* but the third had a 16S rRNA sequence similar to *M. huakuii*. All three
63 isolates from *Sophora* spp. were shown to nodulate their original host. Only isolates from
64 *Carmichaelia* spp. and *Clianthus puniceus* that produced functional nodules on their original host and
65 strains from *Montigena* produced functional nodules on *Montigena*. Strains that nodulated *Montigena,*
66 *Carmichaelia* spp. and *Clianthus puniceus* had variable 16S rRNA, *recA* and *glnII* gene sequences,
67 but specific *nifH, nodA* and *nodC* gene sequences different from those of the *Sophora* isolates.
68 These results indicate that *Montigena*, *Carmichaelia* spp. and *Clianthus* spp. share at least some
69 rhizobia with each other but not with *Sophora* spp. and that the ability of different rhizobial strains to
70 produce functional nodules on the different NZ native legume genera is associated with specific
71 symbiosis genes. Here we focus on bacterial isolates from *Sophora* spp. growing in natural
72 ecosystems and characterise them with respect to their 16S rRNA, *recA, glnII, rpoB, nifH, nodA* and
73 *nodC* gene sequences, ability to nodulate a range of legume species and DNA hybridisation tests with
74 their most closely related rhizobial type strains on the basis of gene sequence similarity.
75
76 **Materials and methods**
77
78 *Bacterial isolates*
79
80 Forty five bacterial isolates from *Sophora* spp. sampled in April 2011 or April 2012 under natural
81 conditions in the current study plus the three *Sophora* isolates previously shown to nodulate their host
82 [24] were studied. Here, bacteria were isolated from plants sampled at six sites in the South Island:
83 alluvial limestone river terrace, Waima/Ure River, Marlborough (41° 52´S 174° 0´E, 147m; Field site
84 1); alluvial outwash river fan, Pororari River, Westland (42° 6S´ 171° 20´E, 1m; Field site 2); margin of
85 estuary, Saltwater Creek, Greymouth, Westland (42° 30´S 171° 9´E, 2m; Field site 3); margin of
86 Greywacke rock outcrop, Kowai River, Springfield, Canterbury (43° 19´S 171° 46´E, 612m; Field site

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87 4); alluvial Greywacke river terrace, upper Rakaia River, Canterbury (43° 26´S 171° 34´E, 357m; Field
88 Site 5) and among Haast Schist rock outcrop, Waitaki River, Otago (44° 53´S 170° 48´E, 126m; Field
89 site 6). Field sites were selected to represent low rainfall in the eastern South Island (< 1000 mm per
90 year; sites 1, 4-6) and high rainfall in the western South Island (> 2500 mm per year, sites 2, 3) [16].
91 All isolates are deposited in the International Collection of Microorganisms from Plants (ICMP),
92 Landcare Research, Auckland, NZ. The ICMP number, *Sophora* species host and field site sampled
93 are given for the isolates on the phylogenetic trees. The type strains of *M. amorphae* (ICMP 15022 =
94 ACCC 19665$^T$), *M. ciceri* (ICMP 13641 = LMG 14989$^T$), *M. huakuii* (ICMP 11069 = IAM 14158$^T$) and
95 *M. loti* (ICMP 4682 = LMG 6125$^T$) were obtained from the ICMP collection directly.
96 For bacteria isolated in this study, root nodules were surface sterilised, crushed in sterile water and
97 the bacterial suspension was streaked onto yeast mannitol agar (YMA) [26] and incubated at 25°C in
98 the dark for 5 days as described previously [25]. A purified culture was obtained by sub-culture from
99 each plate. Each culture was inoculated into a suspension of yeast mannitol broth (YMB) [26] and
100 used for preparation of subcultures for DNA extraction or inoculum.
101
102 *DNA sequencing*
103
104 DNA was extracted from the bacterial cultures grown in YMB using the standard Qiagen-Gentra
105 PUREGENE DNA Purification Kit for gram-negative bacteria. The 16S rRNA, *recA*, *glnII*, *nifH*, *nodA*
106 and *nodC* genes were amplified with appropriate primer sets and PCR conditions as described
107 previously [25] except that for most strains, the *nodC* primers NodCfor540 (TGA-TYG-AYA-TGG-
108 ART-AYT-GGC-T) and NodCrev1160 (CGY-GAC-ARC-CAR-TCG-CTR-TTG) [20] were used. The
109 *rpo*B83F (CCT- SAT-CGA-GGT-TCA-CAG-AAG-GC) and *rpo*B1061R (AGC-GTG-TTG-CGG-ATA-
110 TAG-GCG) primers [17] were used for *rpoB* amplification. The PCR products were separated by
111 electrophoresis in 1% (w/v) agarose gels, stained with ethidium bromide (0.5 $\mu$g mL$^{-1}$) and viewed
112 under UV light. PCR products of the expected size were sequenced by the Bio-Protection Research
113 Centre Sequencing Facility, Lincoln University. DNA sequence data were viewed via Sequence
114 Scanner v 1.0 software (©Applied Biosystems) and edited and assembled using DNAMAN Version 6
115 (©Lynnon Biosoft Corporation).
116
117 *Phylogenetic analyses*
118
119 DNA sequences of individual genes and concatenated *recA, glnII* and *rpoB* for the *Sophora* isolates
120 were aligned and Maximum Likelihood (ML) trees constructed using the partial deletion method with
121 an 80% cut off in MEGA5 software [23]. Bootstrap support for each node was evaluated with 500
122 replicates. The most closely related *Mesorhizobium* type strains on the Genbank sequence database
123 [www.ncbi.nlm.nih.gov/genbank] were used for all trees. In addition, sequences of closely related
124 non-type strains were included in the *nifH* and *nodC* trees and those of strains shown to produce $N_2$
125 fixing nodules on NZ *Carmichaelinae* species in the *nodA and nodC* trees. Only bootstrap values
126 ≥50% are shown for each tree. MEGA5 model test was performed to select a model of nucleotide
127 substitution and the 'best' model (lowest Bayesian Information Criterion (BIC) score) used for each
128 gene. GenBank accession numbers for all genes are shown in the figures.



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129
130 *Nodulation and $N_2$ fixation studies*
131
132 All bacterial strains isolated in the current study were inoculated onto their original host legume
133 species. In addition, twenty one of the isolates of variable 16S rRNA, *recA, glnII* and *rpoB* gene
134 sequence groups, but which all nodulated their host *Sophora* sp. were inoculated onto *Sophora*
135 *microphylla, Sophora prostrata, Sophora longicarinata*, *Carmichaelia australis* and *Clianthus*
136 *puniceus*. *Mesorhizobium amorphae* ACCC 19665$^T$, *M. ciceri* LMG 14989$^T$, *M. huakuii* IAM 14158$^T$
137 and *M. loti* LMG 6125$^T$ were inoculated onto the three *Sophora* species and the *Sophora* isolates
138 were also inoculated onto *Amorpha fruticosa, Cicer arietinum, Astragalus sinicus* and *Lotus*
139 *corniculatus* the legume species from which respectively, the *M. amorphae* [29], *M. ciceri* [18], *M.*
140 *huakuii* [5] and *M. loti* [14] type strains were originally isolated. The type strains were inoculated onto
141 their original host as a positive control. Seeds were obtained from different sources: *Carmichaelia*
142 *australis* from Proseed, North Canterbury, NZ; *Clianthus puniceus* and all *Sophora* spp. from New
143 Zealand Tree Seeds, Rangiora, NZ; *Amorpha fruticosa* from the Agroforestry Research Trust, Devon,
144 UK; *Astragalus sinicus* and *Lotus corniculatus* from the Margot Forde Germplasm Centre, Palmerston
145 North, NZ and *Cicer arietinum* from the Binn Inn, Christchurch, NZ.
146 All plant procedures were carried out under gnotobiotic conditions. All seeds, except those of
147 *Cicer arietinum* were, in sequence, soaked in concentrated sulphuric acid for 30-60 minutes, rinsed
148 with sterile water, soaked in hot (~ 60°C) sterile water which was left at room temperature overnight
149 then transferred to 1.5% water agar plates and kept in the dark to germinate. After germination, each
150 seedling was transferred to a polyethylene terephthalate jar containing vermiculite and supplied with a
151 complete nutrient solution [25]. Plants were grown in a chamber with a 16 hour photoperiod (400
152 $\mu$mol photons $m^{-2}$ $s^{-1}$) at a constant 22°C. Seeds of *Cicer arietinum* were soaked in 20% commercial
153 bleach (0.5 g $L^{-1}$ sodium hypochlorite) for 20 min, rinsed with 96% ethanol, air dried then soaked in
154 hot sterile water and left to cool overnight. *Cicer arietinum* was germinated and grown in 0.75 L pots
155 (four seedlings per pot) containing autoclaved N-free potting mix under natural daylight in a
156 glasshouse. The potting mix base was 80% composted bark and 20% pumice (1-4 mm) to which was
157 added 1 g $L^{-1}$ agricultural lime (primarily calcium carbonate), 0.3 g $L^{-1}$ superphosphate (9P-11S-20Ca;
158 Ravensdown, NZ), and 0.3 g $L^{-1}$ Osmocote (6 months, ON-OP-37K), 0.3 g $L^{-1}$ Micromax trace
159 elements and 1 g $L^{-1}$ Hydraflo, all three obtained from Everris International, Geldermalsen, The
160 Netherlands. The pH of the medium was 5.8.
161 At 5-10 days after sowing, seedlings were inoculated with 10 ml of the appropriate bacterial strain
162 grown to log phase. Uninoculated plants supplied with YMB only were used as controls. There were
163 3 replicates per treatment. All plants were supplied with sterile water as required. Plants were
164 inspected at three weekly intervals for nodulation and at 70-90 days after inoculation were tested for
165 nitrogenase activity using the acetylene reduction assay (ARA) [6]. All values taken as negative were
166 similar to controls and in the range 2.1 to 4.5 $\mu$L ethylene $L^{-1}$. All values taken as positive were one to
167 two orders of magnitude greater than this (ranging from 198.9 to 2107.3 $\mu$L ethylene $L^{-1}$). After the
168 ARA, rhizobial strains were isolated from three to six nodules per treatment and their 16S rRNA and

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169 *nodC* genes sequenced. In all cases, the 16S rRNA and *nodC* sequences for the strain recovered
170 from nodules from the inoculation assays were identical to those of the strain used as inoculant.
171
172 *DNA-DNA hybridisation*
173
174 For DNA-DNA hybridisation, high-molecular weight DNA was prepared [19]. DNA-DNA hybridisations
175 were performed using a microplate method and biotinylated probe DNA [7]. The hybridisation
176 temperature was 48 °C ± 1 °C. Reciprocal reactions (A × B and B × A) were performed in
177 quadruplicate for each DNA pair and their variation was within the limits of this method [10].
178
179 **Results and discussion**
180
181 In the current study, forty eight bacterial isolates from *Sophora* spp. were shown to produce $N_2$-fixing
182 nodules on their host species. These isolates separated into three major groupings on the basis of
183 their 16S rRNA sequences (Fig. 1). Group one, comprising thirty eight isolates from four different
184 *Sophora* species across eight different field sites separated into five sub-groups with high similarity
185 (99.60-100% similarity, 1260 bp) to *M. ciceri* LMG 14989$^T$ isolated from *Cicer arietinum* in Spain [18].
186 The second group, comprising seven isolates of *Sophora longicarinata* from Field site 1, showed 16S
187 rRNA sequences (1260 bp) identical (100% similarity) to that of the *M. amorphae* type strain which
188 was isolated from *Amorpha fruticosa* growing in Beijing, China, although there is evidence that its
189 origin was the native range of *Amorpha fruticosa* which is South Eastern and Mid-Western USA
190 [28,29]. The third group contains three isolates (ICMP 19550, ICMP 19559 and ICMP 19567) aligning
191 closely, based on 16S rRNA gene analysis, with *M. huakuii* IAM 14158$^T$ which was isolated from
192 *Astragalus sinicus* nodules sampled in Nanjing province, China [5]. These data indicate that *Sophora*
193 spp. and Carmichaelinae species in NZ [24,25] can be nodulated by *Mesorhizobium* spp. with 16S
194 rRNA gene sequences similar to *M. ciceri, M. huakuii* and *M. amorphae*.
195 The forty eight mesorhizobial isolates from *Sophora* spp. separated into eight groups and three
196 individual isolates (ICMP 11719, ICMP 19513 and ICMP 19550) on the basis of their concatenated
197 *recA, glnII* and *rpoB* sequences, separate from all *Mesorhizobium* type strains. Generally, isolates
198 from the same field site grouped together. This apparent link between *recA, glnII* and *rpoB*
199 sequences and field site may be at least in part, due to adaptation of the bacteria to local conditions
200 outside the host plant and this warrants further study. Seven of the groups and the three individual
201 isolates (40 isolates) clustered around *M. ciceri* LMG 14989$^T$ (84.5-86.5 % similarity, 1800 bp), while
202 one group of eight isolates, all from *Sophora longicarinata* sampled at field site 1, was closest to
203 (87.9% similarity, 1800 bp) *M. septentrionale* LMG 23930$^T$ isolated from *Astragalus adsurgens*
204 growing in Northern China [8]. This second group contained all seven isolates with a 16S rRNA
205 sequence identical to *M. amorphae* ACCC 19665$^T$ and ICMP 19567 which had a 16S rRNA sequence
206 closest to *M. huakuii* IAM 14158.
207 The *nifH* gene sequences for all forty eight isolates clustered together (97.4-100% similarity, 280
208 bp) closest to but separate from (92.5-95.6% similarity, 280 bp) *M. alhagi* CCNWXJ12-2$^T$ isolated
209 from *Alhagi sparsifolia* in Xinjiang province, China [3]; *Mesorhizobium* strain CCNWSX672 isolated
210 from *Coronilla varia* in Shaanxi province, China [35]; and *M. camelthorni* CCNWX340-4$^T$ isolated from

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211 *Alhagi sparsifolia* in Xinjiang Province, China [4] (Fig. 3). The *nifH* sequences of the forty eight
212 *Sophora* isolates differed (92.5-94.7% similarity, 280 bp) from the *nifH* sequences of the seven strains
213 previously shown to induce $N_2$ fixing nodules on *Carmichaelinae* but not *Sophora* species which were
214 identical (six strains) or very similar to that of the *M. huakuii* type strain [24,25].
215 The *nodA* sequences for the *Sophora* isolates showed 97.4-100% similarity to each other (420 bp)
216 and clearly separated from those of all *Mesorhizobium* type strains and rhizobial symbionts of NZ
217 Carmichaelinae species (Fig. 4A). The strains aligned closest to (86.5-88.4% similarity, 420 bp) the
218 *M. albiziae* type strain isolated from *Albizia kalkora* in Suchian province, Southern China [30,31]. The
219 *nodC* sequences for the *Sophora* isolates showed 98.9-100% similarity to each other (650 bp) and as
220 for *nodA* sequences were clearly separated from all *Mesorhizobium* type strains and rhizobial
221 symbionts of NZ Carmichaelinae species (Fig. 4B). Here, the isolates aligned closest to *M. albizeae*
222 CCBAU 61158$^T$ (91.5-91.7% similarity, 650 bp) and *Mesorhizobium* strain CBAU03074 (93.5-94.2%
223 similarity, 650 bp) isolated from *Astragalus scaberrimus* in temperate China which had a 16S rRNA
224 sequence identical to *M. septentrionale* [37]. Overall, the data obtained in the current and previous
225 studies provide strong evidence that mesorhizobia are the major, if not exclusive, rhizobial partners of
226 *Sophora* spp. in NZ. These mesorhizobia had variable 16S rRNA, *recA*, *glnII* and *rpoB* but specific
227 *nifH, nodA* and *nodC* gene sequences. A similar generalisation was made for rhizobia that nodulate
228 NZ Carmichaelinae species [25] but the symbiosis genes of the two groups are very different from
229 each other. Considering work on *Sophora* outside NZ, 16S rRNA and *recA* gene sequences of 75
230 rhizobial isolates indicated that *Mesorhizobium* sp., *Agrobacterium* sp., *Ensifer* sp., *Phyllobacterium*
231 sp. and *Rhizobium* sp. could effectively nodulate *Sophora alopecuroides* grown in different regions of
232 the Loess Plateau in China [36]. These isolates had diverse *nifH* and *nodA* genes similar to those of
233 rhizobial isolates from a range of legume genera in the same region indicating that these legumes
234 may be able to share these rhizobia. However, as for rhizobia from NZ *Sophora*, there was a
235 correlation between genotype and geographical origin for rhizobia isolated from *Sophora*
236 *alopecuroides*.
237 Previously, rhizobia shown to produce $N_2$ fixing nodules on Carmichaelinae species did not
238 nodulate NZ *Sophora* spp. [24,25]. Here, twenty one *Sophora* isolates from the different 16S rRNA/
239 housekeeping gene groupings were inoculated onto *Sophora microphylla, Sophora prostrata,*
240 *Sophora longicarinata, Clianthus puniceus* and *Carmichaelia australis* (Table 1). All *Sophora* rhizobial
241 isolates tested, regardless of their 16S rRNA or concatenated *recA, glnII* and *rpoB* gene grouping,
242 produced $N_2$ fixing nodules on the three *Sophora* species but none induced $N_2$ fixing nodules on
243 *Carmichaelia australis*. However, eighteen of the twenty one isolates produced $N_2$ fixing nodules on
244 *Clianthus puniceus*. *Clianthus puniceus*, therefore, can share some rhizobia with *Sophora* spp. and
245 others with *Carmichaelia* spp. (Table 1) and *Montigena* [24]. Host range in rhizobia is at least in part
246 determined by the structure of the lipo-chitin oligosaccharide 'Nod factors' synthesised by the
247 products of the nodulation genes such as *nodA* and *nodC* [6,15]. Therefore, the substantial
248 differences in the *nodA* and *nodC* gene sequences between isolates that produce $N_2$ fixing nodules
249 on NZ *Sophora* spp. or *Carmichaelia* spp. and *Montigena* are likely to be important in determining the
250 specificity of the different groups of strains. It is thus unexpected that rhizobial symbionts of *Sophora*

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251 spp., and *Carmichaelia* spp. and *Montigena* can induce functional nodules on *Clianthus puniceus*.
252 This indicates that *Clianthus puniceus* is a more promiscuous rhizobial host than *Carmichaelia* spp.,
253 *Montigena* or *Sophora* spp. Previously, *M huakuii* IAM 14158$^T$ was shown to nodulate *Carmichaelia*
254 spp. and *Clianthus puniceus* [25]. Here, none of the type strains closest to the *Sophora* isolates on
255 16S rRNA sequences (*M. ciceri, M. amorphae* or *M. huakuii* depending on strain) nor the *M. loti* type
256 strain, isolated from nodules of *Lotus corniculatus* in Wanganui, NZ [14], produced nodules on
257 *Sophora microphylla, Sophora prostrata* or *Sophora longicarinata* (Table 1) but they all induced $N_2$
258 fixing nodules on their original host (data not shown). Also, none of the 21 *Sophora* isolates
259 nodulated any of the type strain hosts (data not shown) indicating a specific symbiosis with *Sophora*
260 spp.

261 It is generally accepted that strains within a species should exhibit more than 70% DNA
262 hybridisation [9,22,32]. In the current study, DNA-DNA hybridisation values ranged from 30.1-52.1%
263 between the five *Sophora* isolates selected from the different 16S rRNA/ housekeeping gene groups
264 indicating that these groups are quite different from each other (Table 2). Additionally, these strains
265 showed only 26.1-36.3% similarity to *M. ciceri* LMG 14989$^T$. Also, isolate ICMP 19515 which was
266 identical to *M. ciceri* LMG 14989$^T$ on 16S rRNA sequence and closest to *M. ciceri* on concatenated
267 housekeeping gene sequences showed only 28.9% similarity to *M. ciceri* LMG 14989$^T$. Similarly,
268 strain ICMP 19557 which had a 16S rRNA sequence identical to *M. amorphae* ACCC 19665$^T$ and
269 showed greatest similarity to *M. septentrionale* LMG 23930 on concatenated *recA, glnII* and *rpoB*
270 gene sequences showed 28.8% similarity to *M. septentrionale* LMG 23930$^T$ and 32.3% similarity to
271 ICMP 19515. Thus, DNA-DNA hybridisations indicate that the isolates belong to novel
272 *Mesorhizobium* spp.

273 In this study, DNA sequencing, DNA hybridisations and inoculation experiments have revealed that
274 a significant diversity of novel *Mesorhizobium* spp. nodulate NZ native *Sophora* spp. The 'drivers' for
275 this diversity are unknown and require further study. Abiotic characteristics of the sampling sites may
276 be important drivers as the field sites represent a variety of South Island habitats, including parent
277 rock type (e.g. schist, greywacke and limestone), substrate (alluvium and rock outcrop), and rainfall (>
278 2500 mm in western South Island, < 1000 mm in eastern South Island).
279

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281

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284

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286

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396 **Legends to Figures**

397

398 **Fig. 1**. 16S rRNA gene maximum likelihood (ML) tree (ca. 1260 bp) of bacterial strains isolated from
399 New Zealand native *Sophora* spp. and selected *Mesorhizobium* type strains. Sl = *Sophora*
400 *longicarinata*; Sm = *Sophora microphylla*; Sp = *Sophora prostrata*; St = *Sophora tetraptera*. The
401 Kimura 2-parameter + Gamma distribution (K2 + G) model was used to construct the tree. Numbers
402 on branches are bootstrap % from 500 replicates (shown only when ≥ 50%). The tree was rooted
403 with *Bradyrhizobium elkani* USDA 76$^T$. Scale bar = 2% sequence divergence (2 substitutions per 100
404 nucleotides). *Isolated in previous study [34]. FS = field site.

405

406 **Fig. 2**. Concatenated *recA*, *glnII* and *rpoB* gene maximum likelihood (ML) tree (ca. 1800 bp) of
407 bacterial strains isolated from New Zealand native *Sophora* spp. and selected *Mesorhizobium* type
408 strains. Sl = *Sophora longicarinata*; Sm = *Sophora microphylla*; Sp = *Sophora prostrata*; St = *Sophora*
409 *tetraptera*. The Tamura-Nei with gamma distribution (TN93 + G) model was used to construct the
410 tree. Numbers on branches are bootstrap % from 500 replicates (shown only when ≥ 50%). The
411 trees were rooted with *Bradyrhizobium elkani* USDA 76$^T$. Scale bar = 5% sequence divergence (5
412 substitutions per 100 nucleotides). *Isolated in previous study [34]. FS = field site.

413

414 **Fig. 3**. *nifH* gene maximum likelihood (ML) tree (ca. 280 bp) of bacterial strains isolated from New
415 Zealand native *Sophora* spp. and selected *Mesorhizobium* type and non-type strains. Sl = *Sophora*
416 *longicarinata*; Sm = *Sophora microphylla*; Sp = *Sophora prostrata*; St = *Sophora tetraptera*. The
417 Tamura 3-parameter with gamma distribution (T92 + G) model was used to construct the tree.
418 Numbers on branches are bootstrap % from 500 replicates (shown only when ≥ 50%). The trees
419 were rooted with *Azorhizobium caulinodans* ORS571$^T$. Scale bar = 5% sequence divergence (5
420 substitutions per 100 nucleotides). *Isolated in previous study [34]. FS = field site.

421

422 **Fig. 4**. *nodA* (A) and *nodC* (B) gene maximum likelihood (ML) trees (ca. 420 bp and 650 bp
423 respectively) of bacterial strains isolated from New Zealand native *Sophora* spp., selected
424 *Mesorhizobium* type and non-type strains and strains shown to produce functional nodules on NZ
425 Carmichaelinae species (superscript C). Sl = *Sophora longicarinata*; Sm = *Sophora microphylla*; Sp =
426 *Sophora prostrata*; St = *Sophora tetraptera*. The Tamura 3-parameter with gamma distribution and
427 invariant sites (T92 + G + I) model was used to construct the trees. Numbers on branches are
428 bootstrap % from 500 replicates (shown only when ≥ 50%). The trees were rooted with
429 *Azorhizobium caulinodans* ORS571$^T$. Scale bars = 1% sequence divergence (1 substitution per 100
430 nucleotides). *Isolated in previous study [34]. FS = field site.

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433 **Table 1**. Host specificity of rhizobial strains used in this study.
434
435

| 16S rRNA grouping | Strain | Host | microphylla | Sophora prostrata | longicarinata | Clianthus puniceus | Carmichaelia australis |
|---|---|---|---|---|---|---|---|
| *M. ciceri* | ICMP 11719 | *S. tetraptera* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix- |
| *M. ciceri* | ICMP 14330 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix- |
| *M. ciceri* | ICMP 19513 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix- |
| *M. ciceri* | ICMP 19514 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19515 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19519 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19520 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix- |
| *M. ciceri* | ICMP 19560 | *S. longicarinata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19561 | *S. longicarinata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19545 | *S. prostrata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19546 | *S. prostrata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19523 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19528 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. ciceri* | ICMP 19535 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. amorphae* | ICMP 19557 | *S. longicarinata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. amorphae* | ICMP 19558 | *S. longicarinata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. amorphae* | ICMP 19568 | *S. longicarinata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. amorphae* | ICMP 19569 | *S. longicarinata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod- |
| *M. huakuii* | ICMP 19559 | *S. longicarinata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix- | Nod+Fix- |
| *M. huakuii* | ICMP 19567 | *S. longicarinata* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix- | Nod+Fix- |
| *M. huakuii* | ICMP 19550 | *S. microphylla* | Nod+Fix+ | Nod+Fix+ | Nod+Fix+ | Nod+Fix- | Nod+Fix- |
| *M. ciceri*$^{T}$ | ICMP 13641 | *Cicer arietinum* | Nod- | Nod- | Nod- | N.D. | N.D. |
| *M. amorphae*$^{T}$ | ICMP 15022 | *Amorpha fruticosa* | Nod- | Nod- | Nod- | N.D. | N.D. |
| *M. huakuii*$^{T}$ | ICMP 11069 | *Astragalus sinicus* | Nod- | Nod- | Nod- | N.D. | N.D. |
| *M. loti*$^{T}$ | ICMP 4682 | *Lotus corniculatus* | Nod- | Nod- | Nod- | N.D. | N.D. |

436

437 Nod+ = all plants nodulated; Nod- = no plants nodulated; Fix+ = $N_2$ fixing nodules; Fix- = nodules not fixing $N_2$

438 N.D. Not determined

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439 **Table 2. % DNA-DNA hybridisation between pairs of *Sophora* isolates and *Sophora* isolates and their closest *Mesorhizobium* type strain.**

440

| Strains | ICMP 19512 | ICMP 19535 | ICMP 19523 | ICMP 19545 | ICMP 19560 | LMG 14989 | ICMP 19515 | ICMP 19557 | LMG 23930 |
|---|---|---|---|---|---|---|---|---|---|
| ICMP 19512 | 100 | | | | | | | | |
| ICMP 19535 | 43.6 | 100 | | | | | | | |
| ICMP 19523 | 52.1 | 38.2 | 100 | | | | | | |
| ICMP 19545 | 41.1 | 32.4 | 32.8 | 100 | | | | | |
| ICMP 19560 | 36.1 | 30.1 | 36.6 | 38.4 | 100 | | | | |
| *M. ciceri* LMG14989$^T$ | 29.2 | 31.1 | 27.0 | 26.1 | 36.3 | 100 | | | |
| ICMP 19515 | N.D. | N.D. | N.D. | N.D. | N.D. | 28.9 | 100 | | |
| ICMP 19557 | N.D. | N.D. | N.D. | N.D. | N.D. | 29.2 | 31.8 | 100 | |
| *M. septentrionale* LMG 23930$^T$ | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 32.3 | 28.8 | 100 |

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442 N.D. Not determined

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Fig. 1
16S rRNA

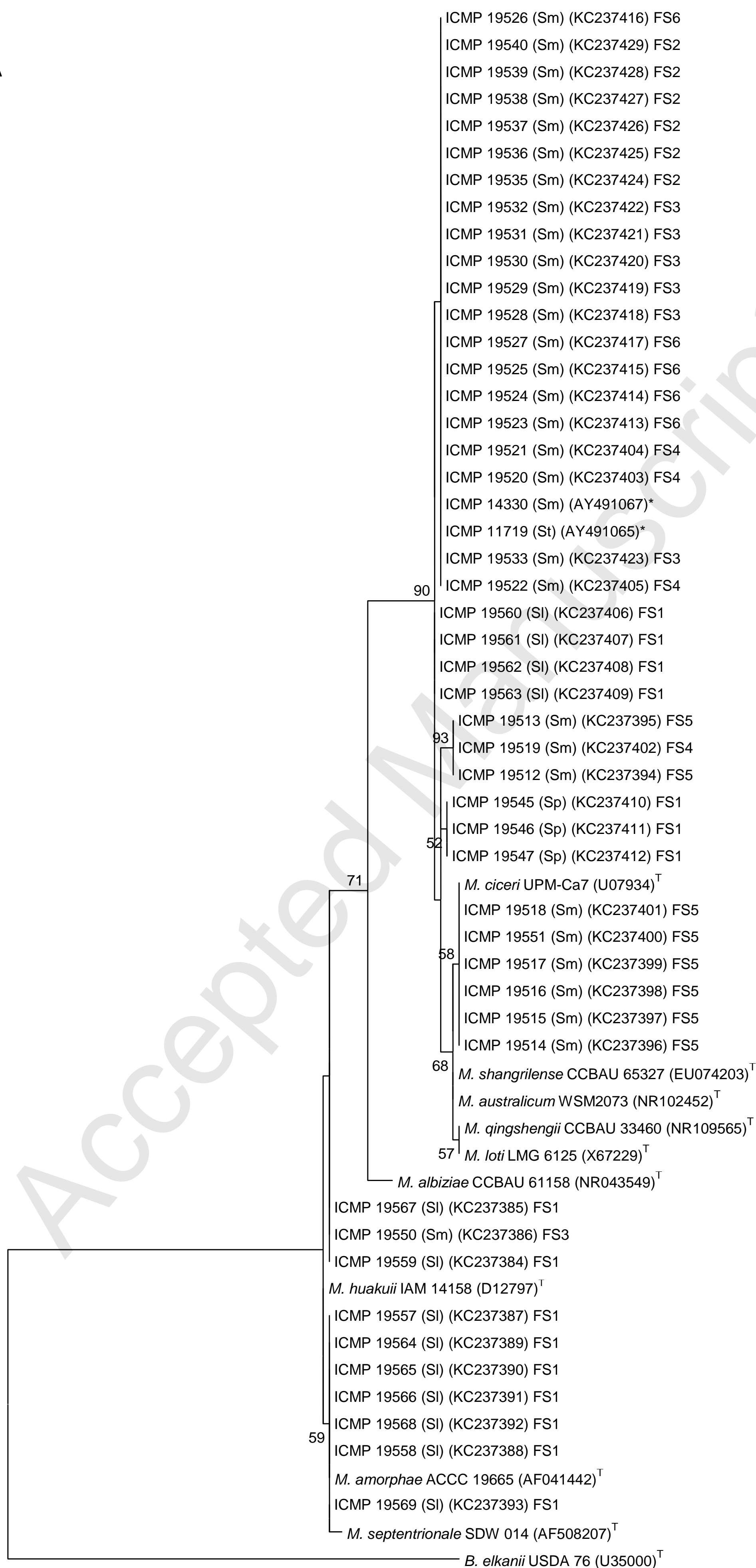


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Fig. 2
*recA + glnII + rpoB*

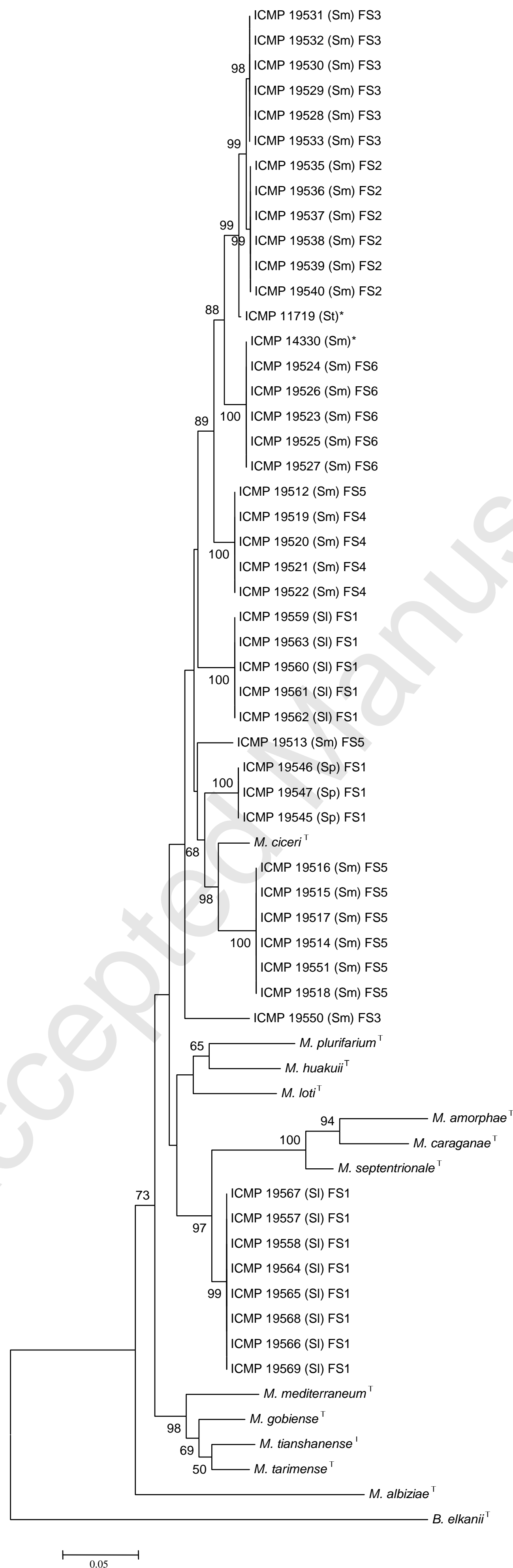


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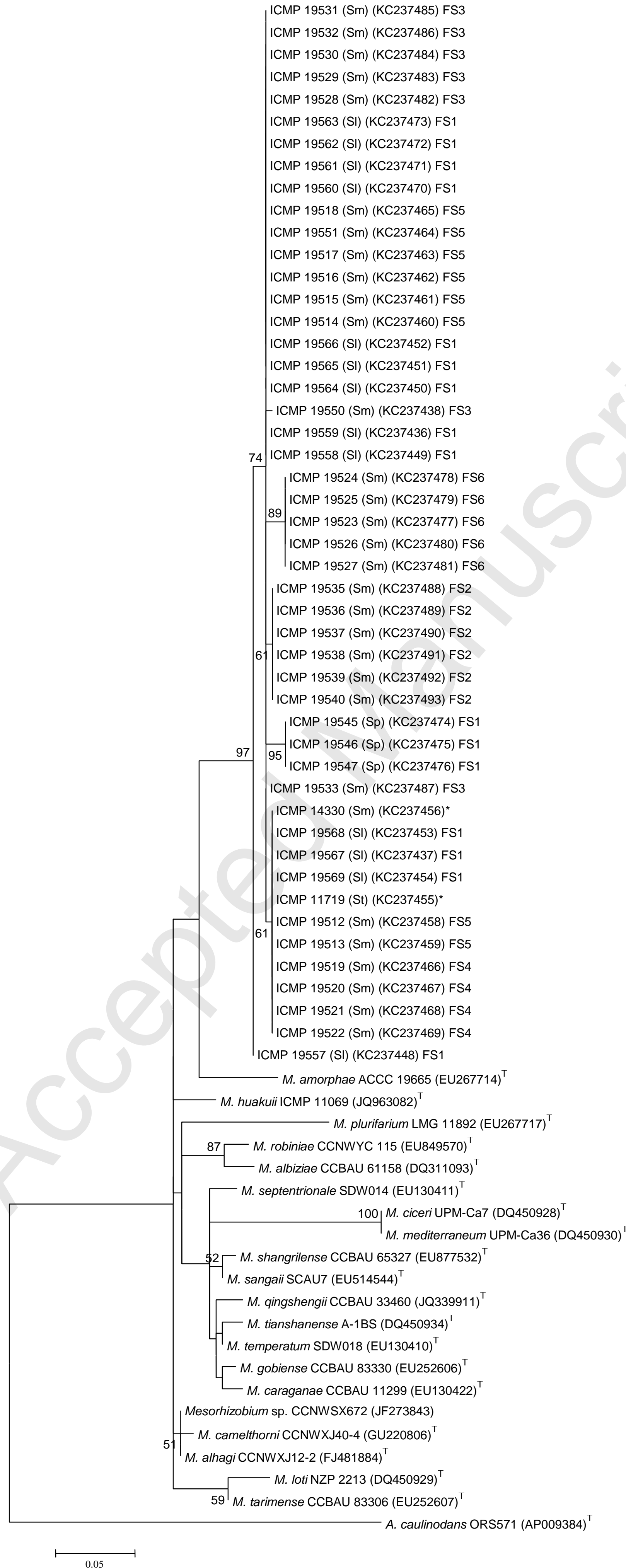
Fig. 3
*nifH*

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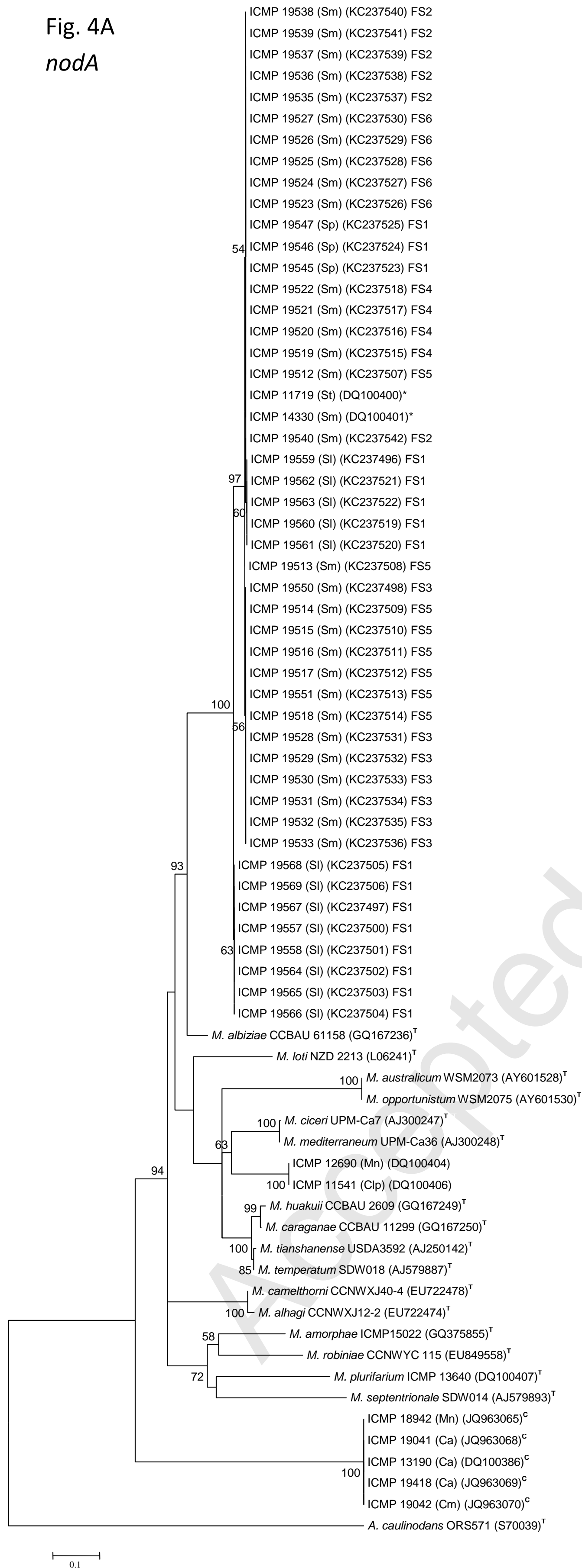

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