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1 **‘*Candidatus Moeniiplasma glomeromycotorum*’, an endobacterium of**
2 **arbuscular mycorrhizal fungi**

3
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16
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SUMMARY

Arbuscular mycorrhizal fungi (AMF, phylum *Glomeromycota*) are symbionts of most terrestrial plants. They commonly harbour endobacteria of a largely unknown biology, referred to as MRE (*Mollicutes*/mycoplasma-related endobacteria). Here, we propose to accommodate MRE in the novel genus ‘*Candidatus Moeniiplasma*.’ Phylogeny reconstructions based on the 16S rRNA gene sequences cluster ‘*Ca. Moeniiplasma*’ with representatives of the class *Mollicutes*, whereas phylogenies derived from amino acid sequences of 19 genes indicate that it is a discrete lineage sharing ancestry with the members of the family *Mycoplasmataceae*. Cells of ‘*Ca. Moeniiplasma*’ reside directly in the host cytoplasm and have not yet been cultivated. They are coccoid, ~500 nm in diameter, with an electron-dense layer outside the plasma membrane. However, the draft genomes of ‘*Ca. Moeniiplasma*’ suggest that this structure is not a Gram-positive cell wall. The evolution ‘*Ca. Moeniiplasma*’ appears to be driven by an ultrarapid rate of mutation accumulation related to the loss of DNA repair mechanisms. Moreover, molecular evolution patterns suggest that, in addition to vertical transmission, ‘*Ca. Moeniiplasma*’ is able to transmit horizontally among distinct *Glomeromycota* host lineages and exchange genes. On the basis of these unique lifestyle features, the new species ‘*Candidatus Moeniiplasma glomeromycotorum*’ is proposed.

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF, phylum *Glomeromycota*) are obligate biotrophs forming symbiotic associations with the roots of most terrestrial plants (Smith & Read, 2008; Gutjahr & Parniske, 2013). They improve plant mineral nutrient uptake in exchange for photosynthates and are important members of terrestrial ecosystems. Based on electron microscopy studies, it has been known for decades that AMF harbour endobacteria in the cytoplasm of their hyphae and spores, referred to as bacterium-like organelles, or BLOs (Mosse, 1970; MacDonald & Chandler, 1981; MacDonald *et al.*, 1982; Scannerini & Bonfante, 1991). These bacteria display diverse morphologies, including coccoid cells that remain unclassified and are referred to as *Mollicutes*/mycoplasma-related endobacteria or MRE, based on the 16S rRNA gene phylogenies that cluster them with members of the class *Mollicutes* (Naumann *et al.*, 2010). MRE have been found in AMF from nearly all major lineages of *Glomeromycota* surveyed to date (Naumann *et al.*, 2010; Desirò *et al.*, 2013; Desirò *et al.*, 2014; Toomer *et al.*, 2015). The MRE genomes are characterized by a highly reduced gene content that is indicative of metabolic dependence on the fungal host (Naito *et al.*, 2015; Torres-Cortés *et al.*, 2015). For example, MRE are incapable of amino acid and nucleic acid biosynthesis, and so these metabolites must be obtained from the AMF host cytoplasm. Similarly, the MRE genomes do not encode enzymes catalyzing the TCA cycle and oxidative phosphorylation. Remarkably, the MRE genomes harbour multiple genes horizontally acquired from AMF (Naito *et al.*, 2015; Torres-Cortés *et al.*, 2015). While the role of MRE in the biology of AMF is unknown, their broad distribution across the host taxa suggests that MRE may modulate the impact of AMF on terrestrial ecology. To recognize this unique

73 lineage of endosymbionts, we propose the new genus ‘*Candidatus Moeniiplasma*’ and the new
74 species ‘*Candidatus Moeniiplasma glomeromycotorum*.’

75

76 **METHODS**

77 **16S rRNA gene and multilocus phylogenies.** To elucidate the relationship between MRE and
78 other lineages within the *Mollicutes* class, we conducted phylogenetic reconstructions based on
79 the sequences of 16S rRNA gene and proteins encoded by 19 conserved genes (*dnaG*, *infC*,
80 *nusA*, *rplA*, *rplB*, *rplC*, *rplE*, *rplF*, *rplM*, *rplN*, *rplP*, *rplT*, *rpmA*, *rpsB*, *rpsC*, *rpsE*, *rpsJ*, *rpsS*,
81 *smpB*), selected based on the Genomic Encyclopaedia of Bacteria and Archaea, GEBA (Wu *et*
82 *al.*, 2009). Sequences of these genes were extracted from the *de novo* sequenced metagenomes
83 of MRE associated with *Dentiscutata heterogama* (Torres-Cortés *et al.*, 2015), *Racocetra*
84 *verrucosa*, and *Rhizophagus clarus* (Naito *et al.*, 2015). Sequences from non-MRE species were
85 obtained from IMG (Markowitz *et al.*, 2012). The 16S rRNA and amino acid sequences were
86 aligned using MUSCLE (Edgar, 2004). Sequence alignments were adjusted manually. Amino
87 acid sequence alignments were concatenated in Geneious 9.1.2 (Biomatters Ltd). Bayesian
88 analyses were performed in MrBayes 3.2 (Ronquist *et al.*, 2012). 16S rRNA gene sequences
89 were analyzed under the nucleotide substitution model GTR+I+ Γ (Tavaré, 1986) in a run of
90 1,000,000 generations with 25% burn-in. Amino acid sequences were examined under the model
91 mixed+I+ Γ in a run of 100,000 generations with 25% burn-in. The average standard deviation of
92 split frequencies was used as a convergence diagnostic. Maximum Likelihood analyses were
93 conducted using PhyML (Guindon *et al.*, 2010) run with 1,000 bootstrap. The GTR+I+ Γ model
94 was used for 16S rRNA gene sequences. The Rtrév+I+ Γ (Dimmic *et al.*, 2002) model identified
95 by MrBayes as the model that best fits these data was used for amino acid sequences.

96

97 **Cultivation.** In our cultivation attempts, we focused on MRE of *Rhizophagus clarus* NB112A,
98 which originated in Namibia and its experimental population is maintained at the International
99 Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi, INVAM (Morton *et al.*, 1993).
100 Unlike many other AMF, *R. clarus* can be readily maintained *in vitro* in association with root-
101 inducing T-DNA-transformed chicory roots grown on MSR medium (Cranenbrouck *et al.*, 2005)
102 at 28°C. In addition, a draft genome sequence is available for its MRE (Naito *et al.*, 2015) to
103 inform media formulations. AMF filtrates containing MRE cells were subjected to different
104 cultivation media, supplements, temperatures, and atmospheres. Media included Brain Heart
105 Infusion, BHI (Bacto), PPLO Broth Base (BBL), 2x BHI, and 2x PPLO. They were
106 supplemented with horse (Sigma), bovine (Sigma), and porcine serum (Sigma) at concentrations
107 of 1 to 20% in 5% increments, yeast extract and TC yeastolate (Bacto) at concentrations of 0.1%,
108 0.25%, 0.5% and 1%, Tween®80 (Sigma) at concentrations of 0.05% and 0.5%, and AMF spore
109 extracts. AMF spore extracts were made by harvesting spores and hyphae of *R. clarus* NB112A
110 grown *in vitro* by manually removing all associated root structures, and dissolving the Phytigel-
111 solidified medium in 10 mM sodium citrate buffer (pH 6; Fisher Scientific) at 30°C for 20 min.
112 Isolated spores and hyphae were then manually crushed, ground, and passed through a 0.22 µm
113 filter. The filtrate was added directly to the MRE cultivation medium. Incubation conditions
114 included ambient temperature, 28°C, and 30°C as well as ambient, microaerophilic, increased
115 CO₂, and anaerobic atmosphere. All factors (cultivation medium, supplement, temperature and
116 atmosphere) were tested combinatorially. Each medium and supplement condition was prepared
117 as a liquid culture and inoculated at day 0 with AMF filtrate containing MRE cells, followed by
118 incubation at every combination of temperature and atmospheric conditions. On day 0, 1, 3, 7,

119 14, 21, and 30, a portion of the liquid culture was subcultured onto a solid medium of the same
120 type, solidified with agar Noble (Difco), and incubated for an additional 14 days, at the same
121 temperature and atmospheric conditions as before. Any colonies that arose were genotyped by
122 16S rRNA gene sequencing, but none were identified as MRE.

123

124 **Transmission Electron Microscopy.** To explore MRE cell ultrastructure, spores of *R. clarus*
125 NB112A were subjected to high-pressure/freeze-substitution in order to preserve fungal and
126 bacterial cytology, processed as described in Desirò *et al.* (2016), and observed under
127 transmission electron microscope.

128

129 **Fluorescent *in situ* hybridization.** Fluorescent *in situ* hybridization (FISH) was performed on
130 fixed and crushed spores of *R. clarus* NB112A. The MRE-specific probe BLOgrBC (5'-
131 GCCAATCCTACCCTTGTC-3') (Naumann *et al.*, 2010) and the universal bacterial probe
132 EUB338I (Amann *et al.*, 1990) were used as described by Naumann *et al.* (2010) with slight
133 modifications. Specifically, AMF spores were immobilized in polyacrylamide pads for the
134 procedure, and probes were hybridized at a stringency of 30% formamide. Cells were visualized
135 using the DeltaVision RT system (Applied Precision).

136

137 **16S rRNA gene sequence diversity.** To explore the extent of MRE diversity across different
138 *Glomeromycota* hosts, we reconstructed the genealogy of MRE using 16S rRNA gene sequences.
139 In these reconstructions, we included MRE diversity from previously published reports
140 (Naumann *et al.*, 2010; Desirò *et al.*, 2014; Naito *et al.*, 2015; Toomer *et al.*, 2015; Torres-Cortés
141 *et al.*, 2015) as well as sequences newly generated from several populations of *R. clarus*

142 representing different geographic locations. We explored MRE diversity in *R. clarus* because
143 this species is one of few AMF hosts that appear to harbour a homogenous MRE population
144 (Naito *et al.*, 2015). Accessions of *R. clarus* AU402B, CL156, KR104, MG104A, ND269B, and
145 WV219A were obtained from INVAM. AMF spores (isolates) were extracted from the
146 cultivation medium by wet-sieving and sucrose centrifugation (Daniels & Skipper, 1982),
147 followed by surface decontamination as described in Mondo *et al.* (2012), and whole genome
148 (WG) amplified using Illustra™ GenomiPhi-V2 kit (GE Healthcare, Piscataway, NJ). WG
149 amplification products were diluted 1:20 in water for subsequent PCR reactions. Bacterial 16S
150 rRNA gene fragments were PCR-amplified using MRE-specific primers 109F1 (5'-
151 ACGGGTGAGTAATRCTTATCT-3), 109F2 (5'-ACGAGTGAGTAATGCTTATCT-3),
152 1184R1 (5'-GACGACCAGACGTCATCCTY-3), 1184R2 (5'-
153 GACGACCAAACCTTGATCCTC-3), and 1184R3 (5'-GATGATCAGACGTCATCCTC-3)
154 (Naumann *et al.*, 2010) and Phusion® High-Fidelity DNA polymerase (New England Biolabs).
155 PCR reactions contained 1 µL diluted WG-amplified product, 0.02 U µL⁻¹ Phusion polymerase,
156 1x Phusion HF Buffer with 1.5 mM MgCl₂, 180 µM each dNTP, and primers added as a 2:1
157 mixture of the two forward primers (0.75 µM and 0.375 µM) and a 2:1:1 mixture of the three
158 reverse primers (0.75 µM, 0.375 µM, and 0.375 µM). Cycling conditions were 5 min initial
159 denaturation at 98°C followed by 15 cycles of 10 sec at 98°C, 30 sec at 50°C, and 1 min at 72°C,
160 followed by a final extension of 10 min at 72°C. The 1063 bp amplicons were purified using
161 QIAquick PCR purification kit (Qiagen), and cloned using the TOPO® TA Cloning® Kit for
162 Sequencing (Invitrogen Life Technologies). Plasmid DNA from 16 recombinant bacterial
163 colonies per sample was amplified using the Illustra TempliPhi 100/500 DNA Amplification Kit
164 (GE Healthcare Life Sciences). Plasmid inserts were cycle-sequenced with the BigDye

165 Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems) using T3 and T7 primers.
166 Sequences were edited in Geneious 9.1.2 (Biomatters Ltd). To facilitate analyses and display of
167 the MRE 16S rRNA gene data, we used MOTHUR (Schloss *et al.*, 2009) to cluster at a 94%
168 similarity level gene fragments cloned and sequenced from each AMF spore (isolate) and to
169 identify a sequence representative for each cluster. The 94% 16S rRNA gene sequence
170 similarity level is recommended for delineation of species in the *Mollicutes* (Brown *et al.*, 2007).
171 The representative MRE sequences were aligned in MUSCLE (Edgar, 2004). Phylogenies were
172 reconstructed under the GTR+I+ Γ (Tavaré, 1986) nucleotide substitution model implemented in
173 MrBayes 3.2 (Ronquist *et al.*, 2012), with analyses conducted for 15,000,000 generations with
174 25% burn-in, and in PhyML (Guindon *et al.*, 2010) with 1,000 bootstrap replications.

175

176 **RESULTS AND DISCUSSION**

177 Phylogeny reconstructions based on 16S rRNA gene sequences cluster MRE with the
178 representatives of the class *Mollicutes*, albeit without resolving their taxonomic position relative
179 to individual mollicute lineages (Figure 1) (Naumann *et al.*, 2010). In contrast, phylogenies
180 derived from amino acid sequences of 19 conserved genes indicate that MRE share ancestry with
181 members of the *Mycoplasma pneumoniae* group in the family *Mycoplasmataceae* (Figure 2).
182 MRE appear to be uncultivable. Therefore, they do not meet the minimal standards for
183 description of a new species of the class *Mollicutes* (Brown *et al.*, 2007). Nevertheless, we
184 recommend that MRE ubiquity and their potential ecological significance warrant a taxonomic
185 proposal in accordance with the guidelines for a designation of a provisional *Candidatus* taxon
186 (Murray & Stackebrandt, 1995).

187

188 **Description of ‘*Candidatus Moeniiplasma*’**

189 *Moeniiplasma* (Moe.ni.i.pla’sma. L. pl. neut. n. moenia, walls/fortifications; Gr. neut. n. plasma,
190 that which is molded/shaped; N.L. neut. n. Moeniiplasma, shape surrounded by
191 walls/fortifications). Representatives of ‘*Ca. Moeniiplasma*’ inhabit hyphae and spores of
192 *Glomeromycota* and are transmitted vertically from one host generation to the next (Naumann *et*
193 *al.*, 2010; Naito, 2014). In addition, phylogenetic data suggest a history of horizontal
194 transmission in ‘*Ca. Moeniiplasma*’ (Toomer *et al.*, 2015). The occurrence of ‘*Ca.*
195 *Moeniiplasma*’ varies among host populations from different geographic locations. For example,
196 in *Cetraspora pellucida*, *Gigaspora margarita*, *Gi. rosea*, and *Rhizophagus clarus*, ‘*Ca.*
197 *Moeniiplasma*’ was detected in some populations but not in others (Naumann *et al.*, 2010; Desirò
198 *et al.*, 2014; Toomer *et al.*, 2015).

199 ‘*Ca. Moeniiplasma*’ resides directly in the cytoplasm of *Glomeromycota* (Figure 3,
200 Naumann *et al.*, 2010, and Desirò *et al.*, 2013). Cells are coccoid (diameter of 460 nm – 610 nm,
201 measured in 8 cells) but may assume different shapes when, for example, compressed between
202 the lipid bodies (not shown). A thin homogenous layer is present outside the cell membrane, an
203 unusual feature for the wall-less *Mollicutes* class (Figure 3, Naumann *et al.*, 2010, and Desirò *et*
204 *al.*, 2013). However, since the organization of such a layer changes depending on the sample
205 preparation (from an electron-dense to a more transparent layer), and none of the draft genomes
206 available for ‘*Ca. Moeniiplasma*’ reveals genes involved in the peptidoglycan synthesis (Naito *et*
207 *al.*, 2015; Torres-Cortés *et al.*, 2015), we suggest that this structure is not a Gram-positive cell
208 wall.

209 The G+C content of ‘*Ca. Moeniiplasma*’ DNA is 32-34% (Naito *et al.*, 2015; Torres-
210 Cortés *et al.*, 2015), which is comparable to the 32% G+C content of the *M. genitalium* genome

211 (Fraser *et al.*, 1995). The draft genome assemblies span from 662,952 bp to 1,227,948 bp (Naito
212 *et al.*, 2015; Torres-Cortés *et al.*, 2015), thus falling within the range of genome sizes exhibited
213 by other members of the *M. pneumoniae* clade, from 580,070 bp in *M. genitalium* (Fraser *et al.*,
214 1995) to 1,358,633 bp in *M. penetrans* (Sasaki *et al.*, 2002). ‘*Ca. Moeniiplasma*’ utilizes the
215 UGA codon to encode tryptophan rather than as a stop codon (Naito *et al.*, 2015), codon usage
216 shared with other SEM (*Spiroplasma*, *Entomoplasma*, and *Mycoplasma*) but not with AAA
217 (*Asteroleplasma*, *Anaeroplasma*, *Acholeplasma*, and *Phytoplasma*) mycoplasmas (Razin *et al.*,
218 1998). Not unlike other *Mycoplasma* genomes (Marenda, 2014), the genomes of ‘*Ca.*
219 *Moeniiplasma*’ are extraordinarily plastic, a phenomenon related to the retention of
220 recombination machinery and mobile genetic elements (Naito *et al.*, 2015; Naito & Pawlowska,
221 2016).

222 FISH experiments with probes specifically targeting ‘*Ca. Moeniiplasma*’ (Naumann *et*
223 *al.*, 2010) indicate that cells of these endobacteria are present in high numbers in the host
224 cytoplasm (Figure 4). Quantitative PCR results support these observations, suggesting that ‘*Ca.*
225 *Moeniiplasma*’ can reach nearly 1000 cells per AMF spore (Desirò *et al.*, 2014), an estimate
226 based on evidence of a single rRNA locus in the MRE genomes (Naito *et al.*, 2015; Torres-
227 Cortés *et al.*, 2015).

228

229 **Description of ‘*Candidatus Moeniiplasma glomeromycotorum*’**

230 ‘*Candidatus Moeniiplasma glomeromycotorum*’ (glo.me.ro.my.co.to’rum L. neut. n.
231 *glomeromycotorum*, inhabitant of *Glomeromycota*). [(*Mollicutes*) NC; NA; C; NAS;
232 oligonucleotide sequences of unique regions of the 16S rRNA gene 5’-
233 GCCAATCCTACCCTTGTC-3’ (Naumann *et al.*, 2010) and 5’-

234 ATCCRTAGACCTTCMTCCTTC-3' (Desirò *et al.*, 2013); S (*Glomeromycota*, cytoplasm of
235 mycelium and spores); M]. The phenotypic description is the same as that given above for the
236 genus. Electron micrographs are shown in Figure 3.

237 Extensive intrahost diversity of 'Ca. Moeniiplasma glomeromycotorum' 16S rRNA gene
238 sequences is one of the most striking features exhibited by these organisms (Naumann *et al.*,
239 2010; Desirò *et al.*, 2014; Toomer *et al.*, 2015). Heritable endobacteria, such as 'Ca.
240 Moeniiplasma glomeromycotorum', are not expected to be diverse within host individuals
241 because transmission bottlenecks limit the number of bacterial cells that are found in each new
242 intrahost population (Moran *et al.*, 2008). In 'Ca. Moeniiplasma glomeromycotorum', two
243 factors appear to contribute to intrahost population diversity: (1) an ultrarapid rate of mutation
244 accumulation (Naito & Pawlowska, 2016), which is likely related to the loss of DNA repair
245 mechanisms (Naito *et al.*, 2015), and (2) recombination evident across DNA sequences sampled
246 from 'Ca. Moeniiplasma glomeromycotorum' populations associated with highly divergent AMF
247 hosts (Toomer *et al.*, 2015; Naito & Pawlowska, 2016), consistent with retention of active
248 recombination machinery in the 'Ca. Moeniiplasma' genomes (Naito *et al.*, 2015).

249 The genealogy of 'Ca. Moeniiplasma glomeromycotorum' reconstructed using 16S
250 rRNA gene sequences (Figure 5) confirmed previous reports that, with few exceptions, 'Ca.
251 Moeniiplasma glomeromycotorum' sequences from a single host are dispersed across divergent
252 clusters comprising 'Ca. Moeniiplasma glomeromycotorum' associated with highly divergent
253 *Glomeromycota* species (Naumann *et al.*, 2010; Desirò *et al.*, 2014; Naito *et al.*, 2015; Toomer *et*
254 *al.*, 2015). Based on 'Ca. Moeniiplasma glomeromycotorum' genome sequences (Naito *et al.*,
255 2015; Torres-Cortés *et al.*, 2015), this pattern appears to reflect the diversity of 'Ca.
256 Moeniiplasma glomeromycotorum' genotypes within a population, with a single rRNA locus per

257 genome, rather than diversity of multiple rRNA loci present in every genome of a genetically
258 uniform population. In addition, no genetic differentiation is apparent among ‘*Ca.*
259 *Moeniiplasma glomeromycotorum*’ populations associated with isolates of a single AMF host
260 from different geographic regions, *e.g.* *R. clarus* (Figure 5). This pattern is not unexpected given
261 low genetic differentiation of AMF from different geographic locations (Rosendahl *et al.*, 2009;
262 den Bakker *et al.*, 2010).

263 While only 10% of the *Glomeromycota* taxonomic diversity has been surveyed for the
264 presence of ‘*Ca. Moeniiplasma glomeromycotorum*’ thus far, the host taxa sampled represent the
265 phylogenetic breadth of the phylum. Consequently, it is likely that a large portion of ‘*Ca.*
266 *Moeniiplasma glomeromycotorum*’ diversity has been discovered already, with the 16S rRNA
267 gene sequences accumulated to date (Figure 5) displaying 79% similarity. While this degree of
268 intraspecific sequence similarity is inconsistent with the recommendation that a 94% sequence
269 similarity at the 16S rRNA gene should be used for separation of species in *Mollicutes* (Brown *et*
270 *al.*, 2007), it reflects the unique biological properties of ‘*Ca. Moeniiplasma glomeromycotorum*’.
271 In particular, all ‘*Ca. Moeniiplasma glomeromycotorum*’ share: (i) the common habitat of the
272 *Glomeromycota* cytoplasm, (ii) an ultrarapid mutation rate, and (iii) the ability to exchange genes
273 across different genotypes. In addition, the present species delineation proposal for ‘*Ca.*
274 *Moeniiplasma glomeromycotorum*’ is consistent with species definitions in other heritable
275 endobacteria, such as *Buchnera aphidicola* (Munson *et al.*, 1991) and *Wolbachia pipientis* (Lo *et*
276 *al.*, 2007). These species share some of the molecular evolution patterns exhibited by ‘*Ca.*
277 *Moeniiplasma glomeromycotorum*’.

278 While no type material designation is necessary for a provisional taxon (Labeda, 1997),
279 we point out that AMF, which are hosts of ‘*Ca. Moenioplasma glomeromycetorum*’, are
280 available at INVAM, <http://invam.wvu.edu/>.

281

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292

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408 **Figure Legends**

409 **Fig. 1.** Phylogenetic placement of ‘*Ca. Moeniiplasma glomeromycotorum*’ based on 16S rRNA
410 gene sequences. Bayesian posterior probabilities greater than 0.90 are indicated above branches.
411 Branches with Maximum Likelihood bootstrap support greater than 70% are thickened. MRc,
412 ‘*Ca. Moeniiplasma glomeromycotorum*’ of *Rhizophagus clarus*; MRv, ‘*Ca. Moeniiplasma*
413 *glomeromycotorum*’ of *Racocetra verrucosa*; MDh, ‘*Ca. Moeniiplasma glomeromycotorum*’ of
414 *Dentiscutata heterogama*.

415 **Fig. 2.** Phylogenetic placement of ‘*Ca. Moeniiplasma glomeromycotorum*’ based on
416 concatenated amino acid sequences of 19 conserved proteins. Bayesian posterior probabilities
417 greater than 0.90 are indicated above branches. Branches with Maximum Likelihood bootstrap
418 support greater than 70% are thickened. MRc, ‘*Ca. Moeniiplasma glomeromycotorum*’ of
419 *Rhizophagus clarus*; MRv, ‘*Ca. Moeniiplasma glomeromycotorum*’ of *Racocetra verrucosa*;
420 MDh, ‘*Ca. Moeniiplasma glomeromycotorum*’ of *Dentiscutata heterogama*.

421 **Fig. 3.** Transmission electron micrographs of ‘*Ca. Moeniiplasma glomeromycotorum*’ in the
422 cytoplasm of *R. clarus* NB112A. **A.** Endobacteria (b) are directly embedded in the fungal
423 cytoplasm (fc), near the fungal nucleus (n) and lipid bodies (lb). **B.** A homogenous electron-
424 dense layer (arrowhead) is consistently present outside the membrane of the endobacteria, while
425 many ribosomes populate their cytoplasm. Scale bars: A, 0.32 μm ; B, 0.10 μm .

426 **Fig. 4.** FISH of ‘*Ca. Moeniiplasma glomeromycotorum*’ within the cytoplasm of a crushed
427 spore of *R. clarus* NB112A. **A.** MRE visualized with the MRE-specific probe, BLOgrBC (red).
428 **B.** MRE visualized with the universal bacterial probe EUB338I (green). **C.** An overlay of A and
429 B. Scale bars, 5 μm .

430 **Fig 5.** Genealogy of ‘*Candidatus Moeniiplasma glomeromycotorum*’ based on 16S rRNA gene
431 sequences. Bayesian posterior probabilities greater than 0.90 are indicated above branches.
432 Branches with Maximum Likelihood bootstrap support greater than 70% are thickened. Each
433 sequence represents ‘*Candidatus Moeniiplasma glomeromycotorum*’ 16S rRNA genes sampled
434 from a distinct *Glomeromycota* isolate and clustered at a 94% sequence similarity level.