Molecular phylogeny, taxonomy and evolution of arbuscular mycorrhizal fungi

DNA-based characterization and identification of *Glomeromycota*

Kumulative Dissertation

der Fakultät für Biologie

an der Ludwig-Maximilians-Universität München

zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. Nat.)

vorgelegt von

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Tag der Einreichung: 1. Februar 2011

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Tag der mündlichen Prüfung: 6. Mai 2011

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List of Abbreviations (except SI units)

~	approximately
AFTOL	Assembling the Fungal Tree of Life
AM	arbuscular mycorrhiza
AMF	arbuscular mycorrhizal fungi
approx.	approximate(ly)
Att	attempt
BEG	International Bank for the *Glomeromycota*
bp	base pair(s)
BS	bootstrap support
BSA	bovine serum albumin
cf.	Latin: confer (English: compare)
comb. nov.	Latin: combinatio nova (English: new combination)
CTAB	cetyltrimethylammonium bromide
DAOM	Agriculture and Agri-Food Canada National Mycological Herbarium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	deoxyribonucleoside triphosphate
e.g.	Latin: exempli gratia (English: for example)
GlGrA	*Glomus* Group A
GlGrB	*Glomus* Group B
GlGrAa	*Glomus* Group Aa
GlGrAb	*Glomus* Group Ab
INVAM	International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi
ITS	internal transcribed spacer
kb	kilo base pair(s)
LB	lysogeny broth (see Bertani, 2004)
LSU	large subunit
ML	maximum likelihood
MOTU	molecular operational taxonomic unit
mt	mitochondrial
MUCL	Mycothèque de l'Universite Catholique de Louvain
Mya	million years ago
OTU	operational taxonomic unit
PCR	polymerase chain reaction
rDNA	ribosomal DNA
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
ROC	root organ culture
rRNA	ribosomal RNA
sensu	English: in the sense of
SB	sodium borate
SDS	sodium dodecyl sulfate
SSU	small subunit
Taq	*Thermus aquaticus*
Tm	melting temperature
Tris	tris(hydroxymethyl)-amino-methane
U	unit (of enzyme activity)
v/v	volume/volume
w/v	weight/volume

1. Abstract

The arbuscular mycorrhizal fungi are exceptionally important mutualists, forming a symbiosis with 70-90% of all terrestrial plants. This root-fungus association is called the arbuscular mycorrhiza (AM). The plant obtains inorganic nutrients (e.g. N, P) *via* their obligate symbiotic fungal partners and the fungus obtains photosynthetically fixed carbon. In the last decade it turned out that morphological identification of AM fungi (AMF) is often misleading, due to few characters and dimorphic spores produced by many species. Furthermore, species recognition in roots based on morphology is not possible. Molecular data gave insights into many new and unexpected phylogenetic relationships, but were still scattered regarding used molecular markers and taxon sampling, which hampers molecular ecological studies.

The focus of this study was to elaborate a robust molecular phylogeny as a base for natural systematics and as data baseline for molecular characterization and detection of AMF. The nuclear small subunit (SSU) rDNA, the internal transcribed spacer (ITS) region and a part of the large subunit (LSU) rDNA region of many described and several undescribed species was amplified with newly designed AMF specific primers, which were successfully tested and used on DNA-extracts from field sampled plant roots. These primers amplify ~250 bp of the SSU, the whole ITS region and ~800-1000 bp of the LSU rDNA (in total ~1.8-1.5 kb). Using the new, specific primers AMF could be detected and resolved down to the species-level from field collected material. The ~1.5 kb sequences were analyzed for their species resolving power and thus as potential DNA barcoding regions for AMF. Only the complete ~1.5 kb fragment allowed robust species resolution and recognition and therefore an extended DNA barcode, covering the ITS and LSU rDNA region, was recommended.

In addition to the ~1.5 kb fragment, a ~1.8 kb fragment of the SSU rDNA region was amplified and analyzed for (sub-)genus-level resolution. Combining these two fragments, which overlap in the SSU by ~250 bp, a ~2.7 kb fragment could be analyzed including the near full length SSU, the ITS-region (ITS1 and ITS2 region excluded) and 800 bp of the LSU rDNA. Combining these three rDNA markers robust phylogenies could be inferred. Based on this data, the phylogenetic placement of the type species of *Glomus* could be defined, supporting the split of the order *Glomerales* into two families (*Glomeraceae*; *Claroideoglomeraceae*) and five genera (*Glomus*, *Funneliformis*, *Rhizophagus*, *Sclerocystis*; *Claroideoglomus*) and several debated changes in the taxonomy of *Glomeromycota* could be supported or rejected.

The baseline data developed in this study will improve future molecular biodiversity and ecological studies and the uncovering of functional diversity and evolutionary aspects of AMF.

2. Zusammenfassung

Die arbuskulären Mykorrhizapilze bilden mit 70-90% aller Landpflanzenarten eine außergewöhnlich wichtige mutualistische Symbiose. Diese Wurzel-Pilzassoziation nennt man die arbuskuläre Mykorrhiza (AM). Hierbei erhält die Pflanze inorganische Nährstoffe (z.B.: N, P) über ihre symbiotischen Pilzpartner, welche im Gegenzug photosynthetisch-fixierten Kohlenstoff bekommen. Innerhalb der letzten 10 Jahre wurde immer deutlicher, dass die morphologische Charakterisierung von AM-Pilzen oftmals unsicher ist, aufgrund weniger Sporenmerkmale und dimorphischer Sporen, welche von vielen Arten gebildet werden. Darüber hinaus ist die morphologische Artbestimmung von AM-Pilzen in Wurzeln nicht möglich. Seitdem wurden mittels molekularer Charakterisierung die Verwandtschaftsverhältnisse der AM-Pilze näher beleuchtet, durch unterschiedlich genutzte molekulare Marker und abweichendes Taxonsampling, werden molekular-ökologische Studien jedoch erschwert.

Das Ziel dieser Arbeit war es eine Datenbasis zu erstellen, für eine robuste molekulare Phylogenie, welche als Grundlage für eine natürliche Systematik, molekulare Charakterisierung sowie Detektierung von AM-Pilzen genutzt werden kann. Hierfür wurde die small subunit (SSU) rDNA, die internal transcribed spacer (ITS)-Region und die large subunit (LSU) rDNA-Region vieler beschriebener sowie einiger unbeschriebener Arten, mittels neu entwickelten AM-Pilz spezifischen Primern, amplifiziert. Diese wurden erfolgreich getestet und an DNA-Extrakten aus Pflanzenwurzeln angewendet. Die Primer amplifizieren ~250 bp der SSU, die gesamte ITS-Region und ~800-1000 bp der LSU rDNA (insgesamt ~1.8-1.5 kb), womit AM-Pilze sequenzbasiert auf Artebene angesprochen werden können. Das ~1.5 kb Fragment wurde auf potentielle DNA-Barcode Regionen und deren damit verbundene Artauflösung für AM-Pilze getestet. Lediglich das ~1.5 kb Fragment erlaubte robuste Artauflösung und -identifizierung, weshalb ein DNA-Barcode empfohlen wurde, der die ITS und die LSU rDNA Region beinhaltet.

Zusätzlich zu dem ~1.5 kb Fragment, wurden ~1.8 kb der SSU rDNA Region amplifiziert, um AM-Pilze auf Gattungsebene aufzulösen. Beide kombiniert zu einem ~2.7 kb Fragment, mit einem Überlapp von ~250 bp in der SSU, decken die gesamte SSU, die ITS (ITS1 und ITS2 ausgenommen) und 800 bp der LSU rDNA ab. Diese drei rDNA-Marker zusammen ermöglichen robuste Phylogenien. Basierend auf diesen Daten konnte die phylogenetische Position der Typart von *Glomus* und darauffolgende Trennung der *Glomerales* in zwei Familien (*Glomeraceae*; *Claroideoglomeraceae*) und fünf Gattungen (*Glomus*, *Funneliformis*, *Rhizophagus*, *Sclerocystis*; *Claroideoglomus*) und einige debattierte Veränderungen innerhalb der Taxonomie der *Glomeromycota* klargestellt werden.

Die in dieser Arbeit erstellte Datengrundlage wird zukünftige ökologische sowie molekulare Biodiversitätsstudien erleichtern und dazu führen funktionelle Diversitätsaspekte sowie die Evolution der AM-Pilze besser zu verstehen.

3. Introduction

3.1 Arbuscular mycorrhizal fungi

The arbuscular mycorrhiza (AM), a symbiosis formed between land plants and arbuscular mycorrhizal fungi (AMF), is widespread. This is indicated by the percentage of land plants forming this symbiosis, which is about 70-90% (Trappe, 1987; Wang & Qiu, 2006; Smith & Read, 2008). The eponymous feature of this symbioses are the arbuscules (Latin: arbuscula = small tree), tree-like structures which are formed during fungal colonization of the plant root and are present in the state of active bidirectional nutrient transfer between the plant and the fungal partner. The fungal partner of this symbiosis provides phosphorus (Sanders & Tinker, 1971; 1973; Jakobsen et al., 1992a,b; Harrison & van Buuren, 1995), nitrogen (Raven et al., 1978; Smith, 1980; Ames et al., 1983; Johansen et al., 1992; Frey & Schüepp, 1993; Johansen et al. 1996; Hodge et al., 2001; Govindarajulu et al., 2005) and other nutrients (e.g. Cooper & Tinker, 1978; Liu et al., 2000) to the host plant. The plant partner, in exchange, supplies up to 20% of the photosynthetically fixated carbon to the fungus (Douds et al., 2000; Graham, 2000). AMF are ecological and economical important as they can improve pathogen resistance (Vigo et al., 2000; de la Pena et al., 2006) as well as biomass production (Smith et al., 2009) of the host plant. In addition, AMF mitigate different kinds of plant stresses such as drought (Michelson & Rosendahl, 1990; Auge et al., 2001; Aroca et al., 2007), or heavy metal toxicity (Hildebrandt et al., 1999) and protect plants against root herbivores (Gange, 2001). The putative asexual AMF (Sanders, 1999) are obligate symbionts, which means they are dependent on the host plant and cannot be cultivated without it. However, some studies raise the question about whether these fungi are able to grow independently of host plants (Hildebrand et al., 2002; 2006). Due to their hidden lifestyle, many aspects of the AMF are not well understood. Fundamental but unanswered questions regarding the evolution and the functional diversity of the multinucleate, asexual AMF are their hetero- (Kuhn et al., 2001) or homokaryotic nature (Pawlowska & Taylor, 2004), and, partly related to that question, how a reasonable species concept can be applied for AMF.

Are AMF homo-or heterokaryotic? Kuhn et al. (2001) showed indications for the heterokaryotic nature of AMF, which were based on two highly variable ITS2 variants of *Scutellospora castanea*, show to be spread on different nuclei by fluorescence *in situ* hybridization (FISH). The heterokaryosis hypothesis was supported by Hijri & Sanders (2005), but Pawlowska & Taylor (2004) doubted it based on the study of *POL*-like sequences from *Glomus etunicatum*, showing that all sequence variants were present in all offspring, concluding this fungus to be homokaryotic. In a recent review Sanders & Croll (2010) state that

AMF are most likely heterokaryotic trying to explain the results of Pawlowska & Taylor (2004). Although, this matter is still debated most evidence points to heterokaryosis which is also indicated by the high ribosomal DNA (rDNA) polymorphism detected in individuals of AMF, e.g. within a single spore (Stockinger et al., 2009; 2010).

For AMF currently there is no existing biological species concept, as AMF are asexual clonal organisms (Sanders, 2002) and it is challenging to explain speciation within these organisms. There are different explanations, for example speciation may occur as adaptation to specific niches, without the need of sexual reproduction (Birky et al., 2005). How could such ancient fungi survive and overcome the resulting deficits (accumulation of detrimental mutations) of asexual recombination? At the moment, the concept to recognize species in AMF is mainly based on the morphology of the resting spores (Mosse & Bowen, 1968; Morton & Benny, 1990), but this morphospecies concept has many difficulties (Morton, 1985) and should be at least combined with phylogenetic analyses (e.g. Walker et al., 2007; Błaszkowski et al., 2008; Gamper et al., 2009), to reduce or prevent mischaracterization and misidentification of AMF species (see chapter 3.3). There may be a species concept feasible based on anastomosis compatibility of AMF (Càrdenas-Flores et al., 2010). However, hyphal fusion differs for the distinct families of AMF, e.g. *Glomus* species increase their capacity of root colonization with anastomosis and built up hyphal networks, whereas in *Gigasporaceae* anastomosis is mostly used for hyphal healing (de la Providencia et al., 2005). Another approach may be the 'phylogenetic species' concept (Taylor et al., 2000), based on definition of gene concordances e.g. distinct mutation rates and selection pressure. But such data are missing for most AMF species. Nevertheless, a multi-gene sequencing approach (Sokolski et al., 2010) showed essentially the same results as based on an SSU-ITS-LSU rDNA amplicon (Stockinger et al., 2009), showing the model fungus of AMF research DAOM197198 to be conspecific with *Glomus irregulare* (Błaszkowski et al., 2008).

3.2 Evolution of AMF

The AMF are an ancient asexual group of eukaryotes, which separated from the other fungal lineages over 600 million years ago (Mya). The earliest reliable evidence for AM in seed plants occurs in the form of non-septate hyphae, vesicles, arbuscules and clamydospores in silicified roots of the Triassic cycad *Antarcticycas schopfii* (Stubblefield et al., 1987; Phipps & Taylor, 1996). The earliest known direct fossil evidence for AMF forming symbiosis with an early vascular land plant *Aglaophyton major* (400 Mya; Remy et al., 1994) stems from the Rhynie chert. *Aglaophyton major* was shown to also contain well preserved *Scutellospora*- and *Acaulospora*-like spores (Dotzler et al., 2006; 2009). The oldest known fossils representing terrestrial fungi are from approx. 460 My old Ordovician dolomite rock of Wisconsin,

and resemble modern AMF (Redecker et al., 2000). It was concluded, based on this indirect evidence that terrestrial AMF already existed at a time when the land flora most likely consisted only of plants on the bryophytic level (Brundrett, 2002) supporting a mycotrophic origin of land plants (Pirozynski & Malloch, 1975).

Molecular clock estimates of the origin of the AMF have varied considerably depending on the fossil record used as calibration points and the molecular clock estimates (Taylor & Berbee, 2006). AMF are assumed to be older than 650 My based on the conserved hypothesis (Berbee & Taylor, 2001) or over 1000 Mya when using the more extreme hypothesis (Heckman et al., 2001; Hedges & Kumar, 2003).

3.3 Morphological characterization and taxonomy of AMF

Based on pure spore morphology new species have to be described following the International Code of Botanical Nomenclature (McNeill et al., 2006), but molecular characterization is not a prerequisite. The identification of AMF based on their morphological characters is subject to few experts in the field, due to sparse spore characters, the ability of species to form dimorphic spores, ambiguous or incomplete species description and possible spontaneous changes of the spore characters (e.g., color, size). The last point was recently exemplified by Morton & Msiska (2010b) based on a *Scutellospora heterogama* culture that produced an unexpected albino mutant, stable for over 15 years and 19 pot culture generations, if this albino mutant would have been found in the field and described based on spore morphology only it may have been mistaken as new species, indicating the importance of molecular characterization.

Currently there are 228 described AMF species (*Glomeromycota* species list at www.amf-phylogeny.com), but only for about 50% sequence data are available and only ~81 spp. are available as cultures from culture collections (e.g. in the International Culture Collection of VA Mycorrhizal Fungi, INVAM; The International Bank for the *Glomeromycota*, BEG; *Glomeromycota* in Vitro Collection, GINCO; cf. Morton, 1993; Declerck et al., 2005; Fortin et al., 2005). Until 2001 it was discussed whether AMF are a non-monophyletic group of fungi (Morton, 2000), but based on phylogenetic analyses of the small subunit (SSU) rRNA gene, it was shown that the AMF are a monophyletic and well separated clade of fungi (Schüßler et al., 2001b). Thus, the AMF were placed in their own fungal phylum, the *Glomeromycota* (Schüßler et al., 2001b), as weakly supported sister group of *Asco*- and *Basidiomycota* (the *Dikarya*). This sister group relationship was also indicated by a six gene phylogeny (James et al., 2006), but questioned by Lee & Young (2009). The latter study was based on sequences of the mitochondrial genome from *Rhizophagus irregularis* (formerly named *Glomus intraradices*, Stockinger et al., 2009), showing the *Mortierellales* – formerly grouped within the *Zygomycota* – as sister group of AMF.

Regarding the four main lineages in the *Glomeromycota* it was known that the *Paraglomerales* and *Archaeosporales* are basal lineages within the phylum, whereas the branching order was not yet resolved, and separate from the phylogenetically younger orders *Diversisporales* and *Glomerales* (Fig. 1).

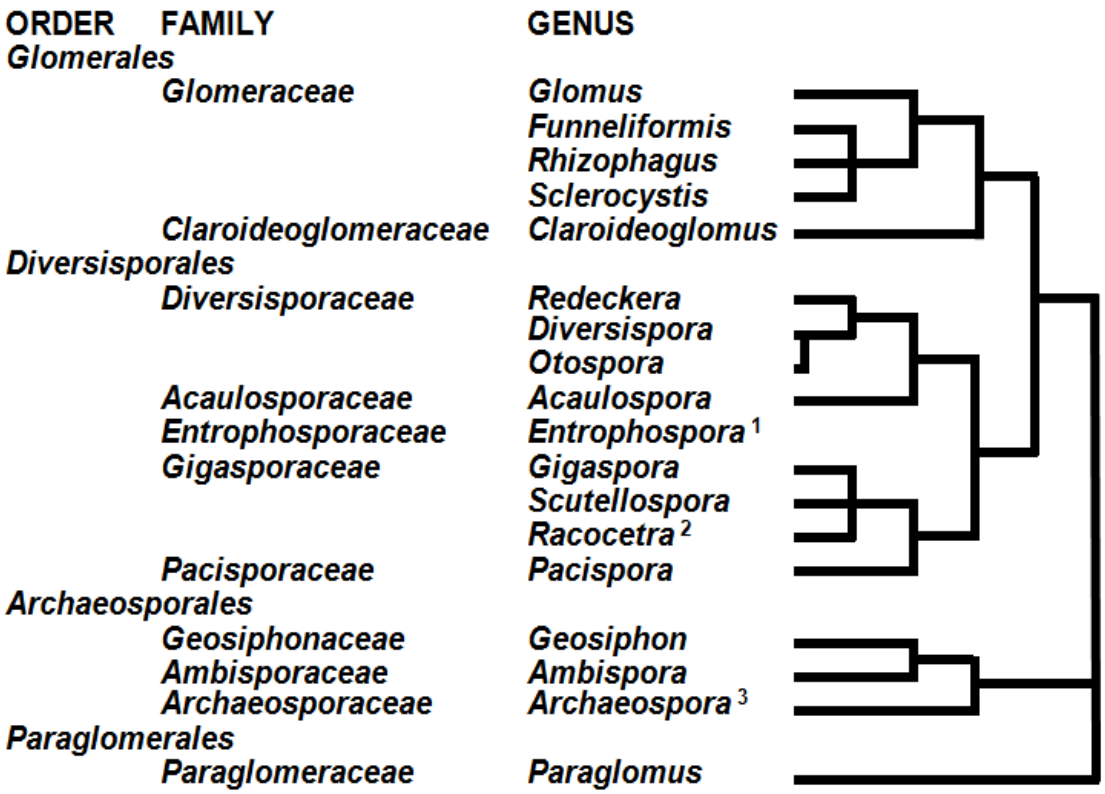

Fig. 1: Schematic phylogenetic relationships of taxa in the *Glomeromycota sensu* Schüßler & Walker (2010). [1] including two phylogenetically uncharacterized species. [2] *Racocetra* now including *Racocetra weresubiae*. [3] the genus *Intraspora* was rejected by Schüßler & Walker (2010) and transferred to *Archaeospora*.

Recently several taxonomic changes within the *Glomeromycota*, mainly in the *Diversisporales*, took place, e.g. the erection of two new (phylogenetically unsupported) genera *Entrophospora* and *Kuklospora* (Sieverding & Oehl, 2006). The latter genus was recently abolished (Kaonongbua et al., 2010). The phylogenetic affiliation of *Entrophospora* still remains unclear as no reliable sequence data are available.

Oehl et al. (2008) published a revision of *Gigasporaceae* and split it into three new families and five new genera, which was controversially debated and recently rejected by Morton & Msiska (2010a) leaving only *Racocetra* as a new genus within the *Gigasporaceae*. A major revision of the *Glomerales* was recently published by Schüßler & Walker (2010). This was so far impossible as the phylogenetic

placement of *Glomus macrocarpum*, the type species of *Glomus*, was unknown and thus the needed evidence for reclassifying of the major clades in the *Glomerales* was lacking. Based on sequences of the SSU rRNA gene of *Glomus macrocarpum*, the order *Glomerales* was now separated into two families (as already proposed by Schwarzott et al., 2001) the *Glomeraceae* (phylogenetically corresponding to the former *Glomus* group [GlGr] A) and *Claroideoglomeraceae* (the former GlGrB). The family *Glomeraceae* now comprises the four genera *Glomus*, *Funneliformis*, *Rhizophagus* and *Sclerocystis*. The family *Claroideoglomeracea* includes one genus, *Claroideoglomus*, based on the former *Glomus claroideum* as generic type.

All these taxonomic changes indicate the difficulties of morphological characterizations without a sound molecular phylogenetic base. The need for reliable molecular markers and the importance of a reliable data baseline for correct identification of AMF on species level is obvious. This was also exemplified by the wrong species affiliation of the model fungus in AMF research, formerly assigned to *Glomus intraradices* (now *Rhizophagus intraradices*) DAOM197198. Based on morphological and molecular characterization, Stockinger et al. (2009) showed that this fungus was misidentified and is conspecific with the recently described *Glomus irregulare* (Błaszkowski et al., 2008), which now is *Rhizophagus irregularis* (Schüßler & Walker, 2010). Sokolski et al. (2010) supported this conspecificity based on the analysis of three protein encoding genes (*elongation factor 1-α*, *V-H$^+$-ATPase VHA5* and *F0F1-ATPase β-subunit*), but for unknown reasons used *Rh. intraradices* KS906 (=DAOM225240) as a reference strain and not the ex-type culture from Florida, *Rhizophagus intraradices* FL208 (Schenck & Smith, 1982). As earlier published KS906 sequences (submitted by Sudarshana et al., 2000) cluster with FL208 sequences (Stockinger et al., 2009) the results seem reasonable. But, as neither SSU, ITS or the LSU rRNA gene was used by Sokolski et al. (2010) as molecular marker, a comparison to existing rDNA data is difficult.

3.4 Molecular characterization of AMF

Systematics based on taxonomy and phylogeny nowadays relies on phylogenetic analyses of molecular data (Bruns et al., 1991; Hibbett et al., 2007) because exclusively using morphological characters is known to be problematic. Recently an increasing number of formal descriptions in the *Glomeromycota* include molecular beside the needed morphological characterization (e.g. Gamper et al., 2009; Kaonongbua et al., 2010). Both are required to place AMF species in their right taxonomic context, therefore, reliable markers are needed, such as the rDNA regions, which are well defined, conserved in function and do not undergo horizontal gene transfer. The largest taxon sampling for AMF is provided for the SSU rDNA marker region, but only allowing phylogenetic resolution down to genus level. This was exemplified for the genus *Ambispora* by Walker et al. (2007), where at least three species (*Ambispora*

leptoticha, *Am. callosa*, *Am. gerdemannii*) were unresolved when using the SSU. Phylogenetic analyses of the ITS and LSU rDNA region could separate these species and these marker regions provide species-level resolution of AMF when combined. Due to the high intraspecific variability of the ITS region, this marker alone is not suited to resolve very closely related species, as for example *Rhizophagus intraradices* (former *Glomus intraradices*) and its close relatives (Stockinger et al., 2009).

Beside the rDNA further molecular markers are available for AMF, such as the genes for the mitochondrial LSU rRNA (Croll et al., 2008; Börstler et al., 2008; Thiéry et al., 2010), β-tubulin (Msiska & Morton, 2009; Morton & Msiska, 2010a,b), elongation factor 1-α (Sokolski et al., 2010), H^+-ATPases (Requena et al., 2003), etc., but they are either inapplicable or data are only available for few closely related AMF.

3.5 In-field detection of AMF and community analyses

Presently, the rDNA region is the most suitable molecular marker region for molecular detection of AMF species in the field and recognition of undescribed species. Furthermore the ITS region will most likely become the DNA-barcoding region for fungi, potentially in combination with the partial LSU rDNA region (see chapter 3.6). Despite the fact that molecular markers have been established and improved during the last years, there are still community analyses of AMF, which are purely based on spore surveys. The problem of these studies is that spores are resting stages and with regard to community analyses this is critical as they do not necessarily reflect the active AMF in the field (Merryweather & Fitter, 1998; Renker et al., 2005; Hempel et al., 2007).

When using a DNA sequence based approach for in-field detection it is important to know the drawbacks, e.g., the SSU rDNA is not suited to resolve species and some frequently used PCR primers are not phylogenetic inclusive or amplify non-target sequences (Schüßler et al., 2001a; Gamper et al., 2009; Krüger et al., 2009 – chapter 4). Therefore the usage of DGGE and T-RFLP methods for in-field community analyses may be problematic. The multiple copies, when using the rDNA as marker region, are a disadvantage as repeats vary considerably. For example, the variability of the ITS region can range from 6% in *Gigaspora margarita* (Lanfranco et al., 1999) to over 15% in species of the genus *Rhizophagus* (containing the former *G. irregulare* and *G. intraradices*, see Stockinger et al., 2009). Thus it is important to define the intraspecific variability for correct interpretation of in-field AMF community studies, as those of Wubet et al. (2003) or Börstler et al. (2006), otherwise sequence variants may lead to mis- or over-interpretations. Especially when using the SSU rDNA region a phylotype may correspond to more than one species or *vice versa* several phylotypes may represent only one species. The diversity of AMF in roots would be nearly unknown without molecular methods. By 1993 about 150 AMF species had

been described (Smith & Read, 1997), today 228 species are known – an increase concerning species numbers of more than 50% within 18 years of research. However, field studies always recover a relatively large number of unknown sequence types, in comparison to sequences which can be assigned to known species (Husband et al., 2002; Wubet et al., 2003; 2004; Haug et al., 2004). Based on the assumption of a similar proportion of 'unknown species' worldwide, Börstler et al. (2006) gave a theoretical estimate of at least 1250 AMF species existing. However, the bottleneck of community studies still is the lack of well-curated reference sequences (Seifert, 2009).

3.6 DNA barcoding

A DNA-barcode is defined as a standardized, short and easy amplifiable DNA fragment allowing recognition of a species (Frézal & Leblois, 2008). Appropriate fungal molecular marker regions are needed, but the SSU rDNA region is not suited as DNA barcode. For fungi the ITS region was proposed as official DNA barcode, which is also frequently used for AMF, but is not robustly resolving very closely related species, e.g. within *Rhizophagus* (former GlGrAb; Stockinger et al., 2009). Therefore a DNA barcode analysis was performed by Stockinger et al. (2010 - chapter 5) based on the 1.5 kb fragment amplified with the AMF specific primers SSUmAf-LSUmAr/SSUmCf-LSUmBr (Krüger et al., 2009 - chapter 4). The ITS2, the LSU-D1 and the LSU-D2 as 400 bp target regions were tested, but individually did not allow robust species-level resolution for closely related *Rhizophagus* species, but when using the 1.5 kb fragment as phylogenetic backbone, species recognition was possible also for such short fragments.

3.7 Deep sequencing of AMF communities

There have been several attempts to detect AMF in the field based on PCR, cloning and sequencing, but this is expensive and time consuming for large scale experiments (Renker et al., 2006). Other ecological studies of AMF communities have been conducted based on massive parallel sequencing approaches (e.g. Öpik et al., 2009, Lumini et al., 2010). Both community analyses were based on the 454 sequencing technology with ~250 bp read lengths, which are too short for reliable phylogenies and the conserved SSU rDNA region is insufficient for species recognition. An improved approach with the recent titanium chemistry for 454 sequencing (read lengths of ~400 bp), AMF specific primers (Krüger et al., 2009 – chapter 4), the results of potential target marker regions (Stockinger et al., 2010) and a comprehensive sequences data baseline, making large scale community analyses, revealing the AMF diversity, are now feasible. In close future tools like the evolutionary placement algorithm (EPA, Stamatakis & Berger, 2009; http://i12k-exelixis3.informatik.tu-muenchen.de/raxml) or the web-based workbench PlutoF (Abarenkov

et al., 2010) will be available for analyses of 400 bp (or longer) 454 reads, which are superior to simple similarity tests using, e.g. BLAST and the public sequence databases.

3.8 Aim of this study

The aims of this study were to provide a phylogenetic framework for AMF as a foundation for a natural systematic and, based on such a data baseline, to develop and establish tools for species-level identification of AMF. Due to the lack of AMF specific primers amplifying rDNA of all main phylogenetic lineages of *Glomeromycota*, new primers were designed targeting the 3' SSU rDNA, the whole ITS region and approx. 800 bp of the LSU rDNA (SSU-ITS-LSU fragment). These discriminate non-target organisms, were tested and shown to specifically and efficiently amplify AMF also from plant root extracted DNA (Krüger et al., 2009 - chapter 4). The rDNA amplified provides species-level resolution and therefore is also suited for in-field investigations at this level. A part of this study (chapter 5) was conducted to analyze potential DNA barcoding regions also in regard to use them for deep sequencing of AMF community analyses. Furthermore the baseline for molecular characterization of AMF was improved using the SSU-ITS-LSU fragment in combination with a second, covering the near full length SSU (Schwarzott et al., 2001), resulting in a robust glomeromycotan phylogeny using 2.7 kb (SSUfull-ITS-LSU) sequences for phylogenetic tree computations (chapter 8). With these molecular detection tools and baseline data the phylogenetic relationship of the AMF species described as *Ambispora brasiliensis* (Goto et al., 2008) could be clarified, placing it into *Acaulospora* and it was also detected in plant roots where the trap culture material was sampled (chapter 6). Furthermore some species formerly assigned to *Glomus*, were placed in their correct phylogenetic context in *Diversispora* (chapter 7).

4. DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi

This chapter is identical to the publication:

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist* 183: 212-223.

New Phytologist

DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi

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Received: *12 December 2008*
Accepted: *23 February 2009*

New Phytologist (2009) **183**: 212–223
doi: 10.1111/j.1469-8137.2009.02835.x

Key words: arbuscular mycorrhizal fungi (AMF), DNA barcoding, ITS region, LSU rRNA gene, molecular community analyses, rDNA, species level resolution, specific primers.

Summary

• At present, molecular ecological studies of arbuscular mycorrhizal fungi (AMF) are only possible above species level when targeting entire communities. To improve molecular species characterization and to allow species level community analyses in the field, a set of newly designed AMF specific PCR primers was successfully tested.
• Nuclear rDNA fragments from diverse phylogenetic AMF lineages were sequenced and analysed to design four primer mixtures, each targeting one binding site in the small subunit (SSU) or large subunit (LSU) rDNA. To allow species resolution, they span a fragment covering the partial SSU, whole internal transcribed spacer (ITS) rDNA region and partial LSU.
• The new primers are suitable for specifically amplifying AMF rDNA from material that may be contaminated by other organisms (e.g., samples from pot cultures or the field), characterizing the diversity of AMF species from field samples, and amplifying a SSU-ITS-LSU fragment that allows phylogenetic analyses with species level resolution.
• The PCR primers can be used to monitor entire AMF field communities, based on a single rDNA marker region. Their application will improve the base for deep sequencing approaches; moreover, they can be efficiently used as DNA barcoding primers.

Introduction

Arbuscular mycorrhizal fungi (AMF) are associated with 70–90% of land plants (Smith & Read, 2008) in a symbiosis called arbuscular mycorrhiza (AM), that has existed for > 400 million yr (Parniske, 2008; Schüßler *et al.*, 2009). The economic and ecological importance of these ancient biotrophic plant symbionts is therefore obvious. Arbuscular mycorrhizal fungi transfer inorganic nutrients and water to the plant and receive carbohydrates in exchange. By driving this bidirectional nutrient transport between soil and plants, they are highly relevant for global phosphorus (P), nitrogen (N) and CO_2 cycles. Moreover, they affect directly and indirectly the diversity and productivity of land-plant communities (van der Heijden *et al.*, 1998) by their central role at the soil–plant interface (van der Heijden *et al.*, 2008). They can also improve host plant pathogen resistance (Vigo *et al.*, 2000; de la Pena *et al.*, 2006) and drought stress tolerance (Michelson & Rosendahl, 1990; Aroca *et al.*, 2007).

Despite the enormous role of AMF in the entire terrestrial ecosystem, their biodiversity in relation to functional aspects is little understood. Most of the 214 currently described species (www.amf-phylogeny.com) are characterized only by spore morphology and the majority have not yet been cultured. Moreover, from molecular ecological studies we know that the species described represent only a small fraction of the existing AMF diversity (Kottke *et al.*, 2008; Öpik *et al.*, 2008). Problems with identification of AMF result from their hidden, biotrophic lifestyle in the soil, few morphological characters, and the potential formation of dimorphic spores. This led to many AMF species, phylogenetically belonging to different orders, being placed in one genus (*Glomus*) and, conversely, individual species forming different spore morphs being described as members of different orders.

Another drawback of morphologically monitoring AMF by their resting spores (Oehl *et al.*, 2005; Wang *et al.*, 2008) is that the presence of spores may not reflect a symbiotically active organism community. Furthermore, many species cannot be reliably identified at all from heterogeneous field samples, and when identifying described species (likely to represent less than 5% of the existing species diversity) similar morphotypes may be erroneously determined as a single species.

To reveal functional and ecological aspects of distinct AMF communities associated with different plants and/or under different environmental conditions it is essential to detect AMF communities in the field on the species level. However, there are as yet no unbiased methods for this purpose, not only for morphological identification but also for molecular methods. Principally, DNA sequence based methods are most useful for detecting organisms at different community levels, but for ecological work they also depend on reliable baseline databases and tools. For example, fingerprinting methods such as random amplification of polymorphic DNA (RAPD), inter-simple sequence repeat PCR (ISSR) and amplified fragment length polymorphism (AFLP) are expected to be error prone in uncharacterized environments because of too many 'unknowns' in the background, which hampers interpretation of specificity (Mathimaran *et al.*, 2008). A similar problem exists for DNA array techniques. Nevertheless, suitable molecular methods are crucial to overcome the limitations of morphological identification (Walker & Schüßler, 2004; Walker *et al.*, 2007; Gamper *et al.*, 2009; Stockinger *et al.*, 2009).

But how are DNA or RNA sequence data for community analyses obtained and how can the current limitations of molecular tools be overcome? Molecular characterization of AMF is in most cases achieved by PCR on DNA from roots of host plants, spores or soil samples. Several primers targeting the rDNA regions as molecular marker were claimed to be AMF specific. Most of these amplify only a restricted number of glomeromycotan taxa or DNA of nontarget organisms. The most comprehensive taxon sampling for the *Glomeromycota* covers the small subunit (SSU) rDNA region (Schüßler *et al.*, 2001a,b), for which a new, AMF specific primer pair was recently published (AML1 and AML2; Lee *et al.*, 2008). Unlike the often used AM1 primer (Helgason *et al.*, 1998) it is perhaps suitable to amplify sequences from all AMF taxa, but the SSU rDNA is inadequate for species resolution of AMF. Inclusion of the internal transcribed spacer (ITS) and the large subunit (LSU) rDNA region allows both robust phylogenetic analyses and species level resolution (Gamper *et al.*, 2009; Stockinger *et al.*, 2009).

The available public database sequences are scattered through SSU, ITS and LSU rDNA subsets with varying lengths, often only 500–800 bp. In most cases this does not allow species level analyses, and short sequences obtained with primers that have inaccurately defined specificity may result in errors. For example, some short database sequences labelled as *Gigaspora* (Jansa *et al.*, 2003) cluster with those of *Glomus versiforme* BEG47 (*Diversisporaceae*) (Gamper *et al.*, 2009). Because of the relatively few LSU sequences in the public databases, the design of improved primers is challenging or even impossible. We therefore sequenced the ITS region and the 5′ part of the LSU rDNA of a set of well-characterized, but phylogenetically diverse AMF, and designed new primers from the resulting database. These primers are suited to amplify DNA from members of all known glomeromycotan lineages and, by allowing elaboration of a more accurate baseline dataset, could be a breakthrough for molecular community analyses of AMF.

Materials and Methods

Fungal and plant material for primer tests

We first tested different samples as DNA templates for PCR to confirm the specificity of the newly designed primers. These included plasmid inserts (Table 1), DNA extractions from single AMF spores and root samples from the Andes (Ecuador) and the Spessart Mountains (Germany). Primers were tested for specificity by PCR with plasmids carrying rDNA fragments with known sequences. All these plasmids had been amplified from single spore DNA extracts with the SSU rDNA primer SSUmAf, described here, and the LSU rDNA primer LR4+2 (modified from LR4; www.aftol.org). The specificity of SSUmAf could therefore not be investigated directly.

DNA extraction for primer tests

All vials, tips, beads, solutions, and other equipment used were sterile and DNA free.

From cleaned, single AMF spores DNA was extracted with the Dynabead DNA DIRECT Universal Kit (Invitrogen, Karlsruhe, Germany) as described in Schwarzott & Schüßler (2001).

Roots potentially colonized by AMF were cut into ten 0.5 cm pieces and collected in a single 1.5 ml Eppendorf tube containing one tungsten carbide bead (diameter 3 mm; Qiagen, Hilden, Germany). They were immediately frozen in liquid N_2 within the closed tube, placed in liquid N_2 precooled Teflon holders, and ground to a fine powder in a MM2000 bead-mill (Retsch, Haan, Germany). Extraction was done by either an innuPREP Plant DNA Kit (Analytik Jena, Jena, Germany) following the instructions of the manufacturer, or a cetyltrimethylammonium bromide (CTAB) protocol modified from Allen *et al.* (2006). For the CTAB protocol, prewarmed extraction buffer (750 µl for 75 mg tissue) was added to each sample of frozen, ground tissue, followed by incubation at 60°C for 30 min. Next, one volume of a chloroform–isoamylalcohol mixture (24 : 1) was added. The samples were centrifuged for 5 min at 2570 ***g*** and the upper phase was transferred into a new tube. After addition of 2.5 µl RNase A (10 mg ml^{-1}) this was incubated at 37°C for 30 min. One volume chloroform–isoamylalcohol (24 : 1) was then added and the tube was centrifuged as above. The supernatant was collected and two-thirds volumes of isopropanol added. The samples were incubated at 4°C for 15 min. After centrifugation (10290 ***g*** for 10 min) the pellet was washed in 70% ethanol, air dried, and eluted in 100 µl of molecular biology grade H_2O. Volumes of 2–5 µl of each DNA extract were used as PCR template.

Table 1 Plasmids used to test primer specificity and their origin

Species (order)	Plasmid no.	Spore no.	Attempt number (culture code)	Voucher	Source (collector)	Origin
Glomus luteum (*Glomerales*)	pMK020.1	2	Att 676-5 (SA101)	W3184	INVAM	Saskatchewan, Canada
Glomus intraradices (*Glomerales*)	pHS051.14	283	Att 1102-12 (MUCL49410)	W5070	GINCO (Nemec)	Orlando, USA
Glomus sp. (*Glomerales*)	pMK010.1	11	Att 15-5 (WUM3)	W2940	Walker (Mercer)	Merredin, Australia
Acaulospora sp. (*Diversisporales*)	pMK005.1	19	Att 869-3 (WUM18)	W2941	Walker (Mercer)	Nedlands, Australia
Pacispora scintillans (*Diversisporales*)	pMK027.1	190	Field collected	W4545	Walker (Schüßler)	Griesheim, Germany
Gigaspora sp. (*Diversisporales*)	pMK003.1	14	Field collected	W2992	Walker (Cabello)	Tres Arroyos, Argentina
Scutellospora heterogama (*Diversisporales*)	pMK029.3	72	Att 334-16 (BEG35)	W3214	Walker (Miranda)	exact location unknown, North America
Glomus versiforme (*Diversisporales*)	pHS036.4	262	Att 475-45 (BEG47)	W5165	Walker (Bianciotto)	Corvallis, USA
Kuklospora kentinensis (*Diversisporales*)	pHS098.16	310	Att 1499-9 (TW111A)	W5346	INVAM	Tainan, Taiwan
Geosiphon pyriformis (*Archaeosporales*)	pMK044.1	8	GEO1	W3619	Schüßler	Bieber, Germany

Single spores from which the cloned amplicons (amplified with primers SSUmAf-LR4+2) originated and the geographic origin of the respective arbuscular mycorrhizal fungi (AMF) are shown.

PCR conditions

The Phusion High-Fidelity DNA polymerase 2× mastermix (Finnzymes, Espoo, Finland) was used for PCR with the SSUmAf–LSUmAr or SSUmCf–LSUmBr primer pairs. SSUmCf and LSUmBr were also applied as nested primers (see Fig. 1c). The final concentration of the reaction mix contained 0.02 U μl^{-1} Phusion polymerase, 1× Phusion HF Buffer with 1.5 mM $MgCl_2$, 200 μM of each dNTP and 0.5 μM of each primer. Thermal cycling was done in an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) with the following conditions for the first PCR: 5 min initial denaturation at 99°C; 40 cycles of 10 s denaturation at 99°C, 30 s annealing at 60°C and 1 min elongation at 72°C; and a 10 min final elongation. The same conditions were used for the nested PCR primers except that the annealing temperature was 63°C and only 30 cycles were carried out. The PCR products were loaded on 1% agarose gels (Agarose NEEO; Carl Roth, Karlsruhe, Germany) with 1× sodium borate buffer (Brody & Kern, 2004) at 220 V, and visualized after ethidium bromide staining (1 μg ml^{-1}).

Cloning, restriction fragment length polymorphism (RFLP) and sequencing

Polymerase chain reaction products were cloned with the Zero Blunt TOPO PCR Cloning Kit (Invitrogen) following the instructions of the manufacturer, except that to reduce costs only one-third of the specified volume of all components was used. Only SOC medium for initial bacterial growth after transformation was used in the volume as per the instructions. From each cloning we analysed up to 48 clones for correct length of plasmid inserts. In some instances fewer clones were available because of low cloning efficiency. Colony-PCR was performed with the GoTaq DNA Polymerase (5 U μl^{-1}; Promega, Mannheim, Germany) and modified M13F and M13R primers. To roughly detect intrasporal and intersporal sequence variability in the clones, RFLP was performed in 10 μl reaction volume, containing 5 μl colony-PCR product, one of the restriction enzymes HinfI (1 U), RsaI (1 U), or MboI (0.5 U) and the specific buffer. One or two clones for each restriction pattern were sequenced, using M13 primers, by the LMU Sequencing Service Unit on an ABI capillary sequencer with the BigDye v3.1 (Applied Biosystems, Foster City, CA, USA) sequencing chemistry. The sequences were assembled and edited in SEQASSEM (www.sequentix.de) and deposited in the EMBL/GenBank/DDBJ databases with the accession numbers FM876780 to FM876839.

Primer design

For the design of new AMF specific primers a sequence alignment was established with the programs ALIGN (www.sequentix.de) and ARB (Ludwig *et al.*, 2004). The alignments contained all AMF sequences present in the public databases and our new data. In total > 1000 AMF sequences, covering all known phylogenetic lineages, were analysed to design the SSU and LSU rDNA primers. To allow comparison to the existing SSU rDNA datasets the primers were designed to overlap (approx. 250 bp) with the SSU rDNA. We used BLAST against the public databases and the probe match tool in ARB to test the specificity of the newly designed primers *in silico*. For the alignment in the ARB database a combination of our new dataset and the 94th release version of the SILVA database (Pruesse *et al.*, 2007, www.arb-silva.de) was used. The oligonucleotides were then synthesized as standard primers (25 nmol, desalted) by Invitrogen.

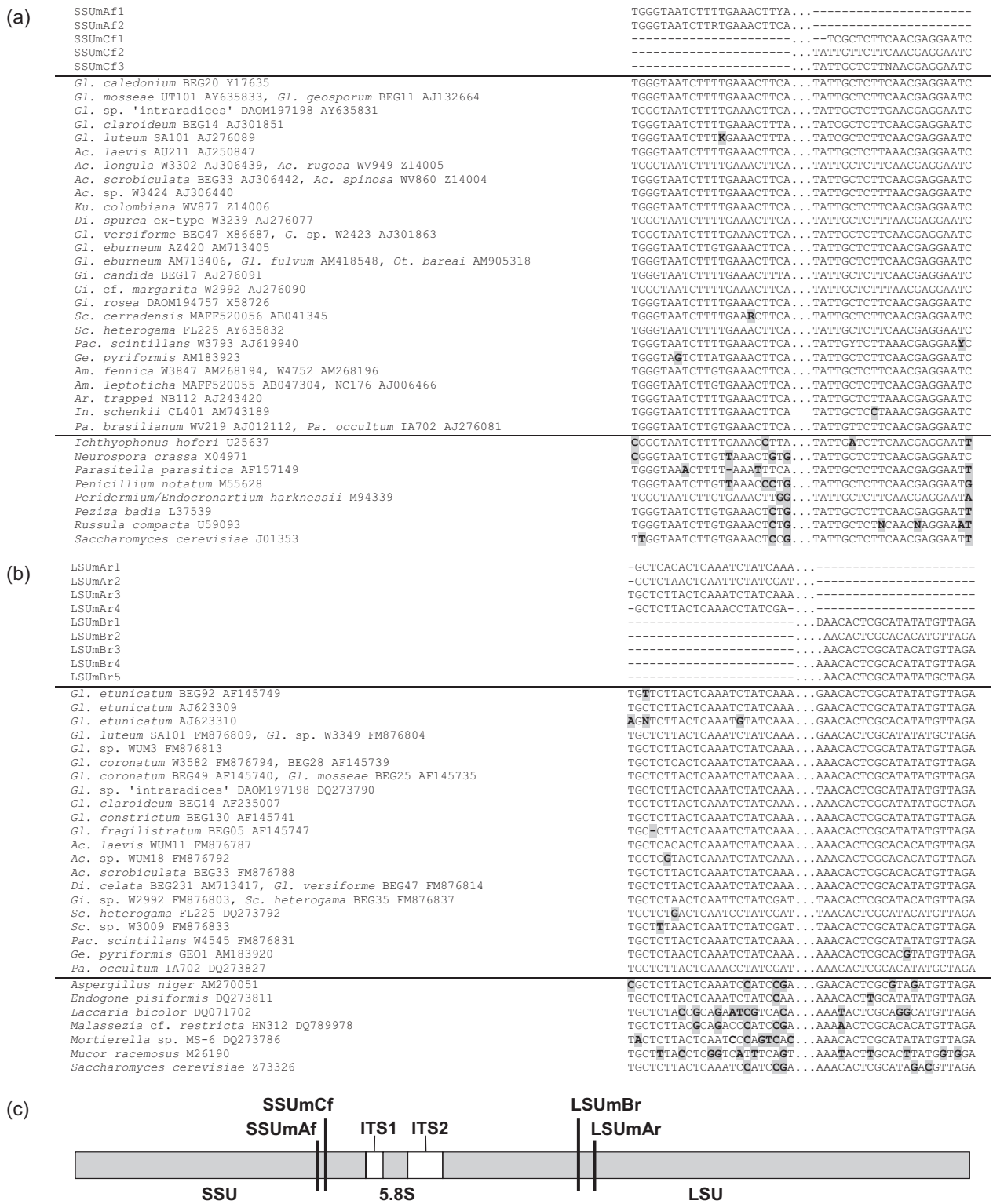

Fig. 1 Forward and reverse primers designed in this study (5′–3′ direction), compared with their annealing sites in sequences from representative members of all main AMF taxa and some non-AMF species. Variable sites not represented in any primer mixture are shaded. When no culture identifiers are known, voucher (W) numbers are given behind the species name. (a) Forward primers SSUmAf (mixture SSUmAf1-2) and SSUmCf (mixture SSUmCf1-3). (b) Reverse primers LSUmAr (mixture LSUmAr1-4) and LSUmBr (mixture LSUmBr1-5). (c) Small subunit (SSU) rDNA, internal transcribed spacer (ITS) region and large subunit (LSU) rDNA (5465 bp) of *Glomus* sp. 'intraradices' DAOM197198 (AFTOL-ID48, other culture/voucher identifiers: MUCL43194, DAOM181602; accession numbers: AY635831, AY997052, DQ273790) showing the binding sites of the newly designed forward and reverse primer mixtures.

Table 2 Polymerase chain reaction primer mixtures designed for amplification of arbuscular mycorrhizal fungi (AMF)

Primer	Nucleotide sequence (5′–3′)	nt	Target taxa (mainly)
SSUmAf1	TGG GTA ATC TTT TGA AAC TTY A	22	*Acaulosporaceae*, *Archaeosporaceae*, *Diversisporaceae*, *Geosiphonaceae*, *Gigasporaceae*, *Glomeraceae* (GlGrA & GlGrB), *Pacisporaceae*
SSUmAf2	TGG GTA ATC TTR TGA AAC TTC A	22	*Ambisporaceae*, *Diversisporaceae*, *Geosiphonaceae*, *Paraglomeraceae*
SSUmAf	Mix SSUmAf1-2 (equimolar)	22	All AMF lineages
SSUmCf1	T CGC TCT TCA ACG AGG AAT C	20	*Archaeosporaceae* (indirect evidence by amplification of *Ambispora fennica*), *Glomeraceae* (mainly GlGrB)
SSUmCf2	TAT TGT TCT TCA ACG AGG AAT C	22	*Paraglomeraceae*
SSUmCf3	TAT TGC TCT TNA ACG AGG AAT C	22	*Acaulosporaceae*, *Ambisporaceae*, *Archaeosporaceae*, *Diversisporaceae*, *Geosiphonaceae*, *Gigasporaceae*, *Glomeracea* (mainly GlGrA), *Pacisporaceae*
SSUmCf	Mix of SSUmCf1-3 (equimolar)	20–22	All AMF lineages
LSUmAr1	GCT CAC ACT CAA ATC TAT CAA A	22	*Acaulosporaceae*
LSUmAr2	GCT CTA ACT CAA TTC TAT CGA T	22	*Gigasporaceae*
LSUmAr3	T GCT CTT ACT CAA ATC TAT CAA A	23	*Acaulosporaceae*, *Diversisporaceae*, *Geosiphonaceae*, *Gigasporaceae*, *Glomeraceae* (GlGrA and GlGrB), *Pacisporaceae*
LSUmAr4	GCT CTT ACT CAA ACC TAT CGA	21	*Paraglomeraceae*
LSUmAr	Mix of LSUmAr1-4 (equimolar)	21–23	All AMF lineages
LSUmBr1	DAA CAC TCG CAT ATA TGT TAG A	22	*Acaulosporaceae*, *Archaeosporaceae*, *Glomeraceae* (GlGrA), *Pacisporaceae*
LSUmBr2	AA CAC TCG CAC ACA TGT TAG A	21	*Acaulosporaceae*
LSUmBr3	AA CAC TCG CAT ACA TGT TAG A	21	*Gigasporaceae*
LSUmBr4	AAA CAC TCG CAC ATA TGT TAG A	22	*Diversisporaceae*, *Geosiphonaceae*, *Glomeraceae*, *Paraglomeraceae*, (primer sequence was also found in amplicons from *Ambispora fennica* and an *Archaeospora* sp.)
LSUmBr5	AA CAC TCG CAT ATA TGC TAG A	21	*Gigasporaceae*, *Glomeraceae* (GlGrB)
LSUmBr	Mix of LSUmBr1-5 (equimolar)	21–22	All AMF lineages

Variable sites among primers of an individual mixture are shaded. Target taxa most likely amplified, according to known binding site sequences, are listed. Comments in parentheses indicate that the primer was successfully used to amplify the given taxon, although the binding site sequences were not known.

Results

Primer design

Potentially suited binding sites for primers that match AMF sequences but discriminate against plant and non-AM fungal (non-AMF) sequences were identified for the SSU rDNA and LSU rDNA. They were located at positions 1484 and 1532 on the SSU, and at positions 827 and 928 on the LSU rDNA (based on *Glomus* sp. 'intraradices' DAOM197198 sequence; Fig. 1c). Sequence variation made it impossible to derive individual primer sequences that specifically amplify all *Glomeromycota*. Thus, a set of four primer mixtures was designed, each targeting one binding site (Table 2, Fig. 1). Certain non-3′ located mismatches that only slightly altered melting temperature and some mismatches (*Glomus etunicatum*) that were perhaps caused by low sequence quality were accepted for primer design (Fig. 1). To discriminate against nontarget organisms mismatches at the 3′ end of the primers were included. BLAST searches indicated high specificity of the new primer pairs for AMF.

Glomeromycota sequences that represent the known variability at the primer binding sites are shown in Fig. 1. We aimed to include as many main phylogenetic lineages (Fig. 2) for primer design as possible. However, the following taxa could not be included for LSU rDNA binding sites analyses: *Entrophosporaceae*, containing only two species lacking sequence data; *Archaeosporaceae*, because available sequences did not cover the LSU rDNA binding sites; *Otospora* for which only two nonoverlapping partial SSU rDNA sequences are known; *Intraspora*, represented by only one SSU rDNA database sequence.

Primer specificity – discrimination against plants

The discrimination of primer SSUmAf1 against 'lower' plants is weak and exemplified by only one mismatch to database sequences from mosses (*Polytrichastrum*, *Leptodontium* and *Pogonatum*), a liverwort (*Trichocoleopsis*), a hornwort (*Phaeoceros*) and a clubmoss (*Selaginella*). *Burmannia*, one *Phaseoleae* sp. and some other plant sequences also showed only one mismatch. All other plant sequences had a minimum

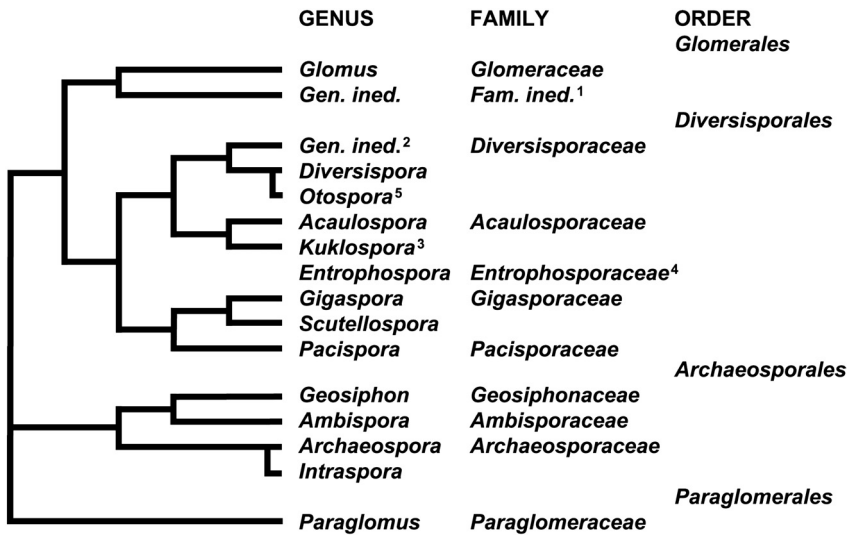

Fig. 2 Phylogenetic relationships of taxa in the *Glomeromycota* (Schüßler *et al*., 2001b; Walker *et al*., 2007). [1]Species currently named *Glomus*. One of the main *Glomus* clades (GlGrA or GlGrB) will represent the *Glomeraceae*, once the phylogenetic affiliation of the type species of *Glomus* is known; [2]contains *Glomus fulvum, Gl. megalocarpum, Gl. pulvinatum*; [3]contains *Kuklospora colombiana* and *Ku. kentinensis* (formerly *Entrophospora*) (Sieverding & Oehl, 2006); [4]contains one genus with two species, *Entrophospora infrequens* and *En. baltica* (Sieverding & Oehl, 2006), neither of which is phylogenetically characterized; [5]*Otospora* (Palenzuela *et al*., 2008) contains one species, *Otospora bareai*. Based on small subunit (SSU) rDNA sequences and from a phylogenetic viewpoint this genus is congeneric with *Diversispora*.

of two mismatches, mainly at the 3′ end of the primer. For SSUmAf2 there were at least two mismatches to all plant sequences, except for a moss (*Archidium*) with only one mismatch. For the nested forward primer SSUmCf1 a minimum of three mismatches for all plants, except for one environmental *Phaseoleae* sequence with two mismatches, were observed. SSUmCf2 mismatched at one site to the same *Phaseoleae* sequence and to liverworts (*Radula*, *Ptilidium* and *Porella*), a hornwort (*Anthoceros*) and a *Taxus* species. Other plant sequences displayed a minimum of two mismatches, at least one at the 3′ end. For SSUmCf3 the above mentioned sequence of *Phaseoleae* showed no mismatch, but all other environmental *Phaseoleae* sequences had at least one mismatch at the 3′ region of the primer. SSUmCf3 also showed only one mismatch for sequences of liverworts (*Radula*, *Ptilidium* and *Porella*), a hornwort (*Anthoceros*) and for one *Liliopsida* and *Taxus* sequence. The remaining BLAST hits displayed two mismatches (several *Taxus* spp., *Pinus* and the liverwort *Haplomitrium*) or more. These results show that for primer mixtures SSUmAf and SSUmCf the discrimination against 'lower' plants is less than for vascular plants.

The LSU rDNA primers had at least two mismatches to plant sequences. The minimum for LSUmAr1 was four mismatches to a *Brassica* sequence. LSUmAr2 and LSUmAr3 showed four mismatches for a *Medicago* sequence, in the case of LSUmAr2 this holds also true for *Vitis vinifera* and *Oryza sativa*. All other plant sequences showed more mismatches to LSUmAr1, LSUmAr2 and LSUmAr3. For LSUmAr4, which was designed to target *Paraglomeraceae*, two mismatches were found for *Solanum lycopersicum* followed by at least three for all other plant sequences. The LSUmBr primer set had a minimum of three mismatches to plant sequences. LSUmBr1 shows more than three mismatches to a *Lotus* and a *Brassica* sequence. At least three mismatches (to *Ephedra* and *Larix*) occurred for LSUmBr2. There were three mismatches for LSUmBr3 to *Selaginella*, followed by a liverwort (*Trichocoleopsis*) and a moss (*Bryum*) species with four. LSUmBr4 had three mismatches for *V. vinifera* and at least five for all other plant sequences. LSUmBr5 displayed more than four mismatches to any plant sequence.

Primer specificity – discrimination against nontarget fungi

The primer mixture SSUmAf should partly exclude amplification of nontarget fungi, whereas SSUmCf poorly discriminates non-AMF (Fig. 1a). Therefore, the highly specific amplification of AMF rDNA results mainly from the LSU primers. The primer mixture LSUmAr discriminates well against most non-AMF. An exception is LSUmAr1 with only one mismatch to a group of sequences from uncultured soil fungi (*Basidiomycota* related) from a Canadian forestry centre. For all other known non-AMF sequences more than four mismatches to LSUmAr1 and three to LSUmAr2 were observed. The primer LSUmAr3 shows only one mismatch with several chytrid sequences. For all other non-AMF LSUmAr3 as well as LSUmAr4 mismatched with at least two sites, mainly at the 3′ end.

For the (nested) LSUmBr primer mixture the specificity is lower; for example, LSUmBr1 showed no mismatch to some fungi in the more ancestral lineages, namely *Endogone lactiflua* and *Mortierellaceae* species, chytrids (*Rhizophlyctis* and *Gonapodya*), an uncultured alpine tundra soil fungus and matched one ascomycete sequence (*Catenulostroma*). For LSUmBr2, no mismatches occurred for sequences of some basidiomycetes (*Bulleribasidium*, *Paullicorticium* and *Russula*) and a zygomycete (*Spiromyces minutus*). Only one mismatch was observed for sequences including basidiomycetes (*Calocera*, *Calostoma* and *Ramaria*) and ascomycetes (*Pyxidiophora*, *Eremithallus* and *Phaeococcus*), and some other fungi. LSUmBr3 discriminates well against other fungi with at least three mismatches, except for one uncultured soil fungus sequence (*Cryptococcus* related) that matched completely. The primer LSUmBr4 showed no mismatch to *Clavulina griseohumicola* and only one to some fungal sequences including ascomycetes (*Pyxidiophora* and *Phaeococcus*) and basidiomycetes (*Cryptococcus* spp.). LSUmBr5 showed only one mismatch to fungal sequences of *Mortierella* spp., a chytrid (*Rhizophlyctis rosea*), and some ascomycetes (*Schizosaccharomyces*, *Verrucocladosporium*, *Passalora* and *Catenulostroma*). In general the LSUmAr primers discriminate better against non-AMF than the nested primers LSUmBr.

Primer efficiency – tests on plasmids and DNA extracts from single spores

The new primer pairs were designed to amplify fragments of approx. 1800 bp (SSUmAf–LSUmAr) and 1500 bp (SSUmCf–LSUmBr). In a first PCR amplification test, samples were chosen to encompass divergent phylogenetic lineages of the *Glomeromycota*. Cloned rDNA of the AMF species *Acaulospora* sp. and *Kuklospora kentinensis* (*Acaulosporaceae*), *Glomus luteum*, *Gl. intraradices* and a *Glomus* sp. (*Glomeraceae*), *Pacispora scintillans* (*Pacisporaceae*), and *Scutellospora heterogama* (*Gigasporaceae*) were used (Table 1, Fig. 3a). In addition, rDNA fragments were amplified from single spore DNA extracts from *Geosiphon pyriformis* (*Geosiphonaceae*), *Gl. mosseae* (*Glomeraceae*), *Gl. eburneum* and *Gl. versiforme* (*Diversisporaceae*), a *Paraglomus* sp. (*Paraglomeraceae*), and a *Gigaspora* sp. (*Gigasporaceae*) (not shown). All tested AMF species were successfully amplified with the new primer set.

To test the potential sensitivity of the new primers, the same plasmids as in the first PCR test and additional plasmids carrying inserts of a *Gigaspora* sp., *Gl. versiforme* and *Ge. pyriformis* (Table 1, Fig. 3b) were used. They were diluted over several magnitudes to contain 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, 1 fg, 0.1 fg and 0.01 fg DNA μl^{-1}. One microlitre was used as template for PCR, whereas the four lowest concentrations correspond with 5000, 500, 50 and 5 plasmid molecules in the 20 µl PCR reaction volume. Both primer sets were tested independently. Differences between specificity of the first and nested primer sets were observed for *Pacispora*, *Kuklospora*, and *Geosiphon*. For *Pacispora* the PCR with SSUmAf and LSUmAr yielded, even with the lowest DNA concentration, a clearly visible band, whereas PCR with SSUmCf and LSUmBr yielded weaker bands, indicating lower specificity. Weaker bands were also observed for the rDNA amplification of *Ku. kentinesis* with the primers SSUmCf-LSUmBr and for *Ge. pyriformis* with SSUmAf-LSUmAr. However, these differences may be within the error-range of photometric DNA concentration measurement of the plasmid stock-solutions. Only slight or no differences occurred between the other plasmid templates, when comparing the intensity of the bands, except for *Gl. versiforme*. Here, clearly visible bands were only found for the higher DNA concentrations, but with the same pattern for both primer pairs. However, this was an artefact caused by low template DNA integrity. Later dilution series with fresh plasmid preparations (also from other *Diversisporaceae*) were indistinguishable from those obtained with the other species shown in Fig. 3(b). For *Ku. kentinensis* no amplicon could be observed after PCR with the primers SSUmAf–LSUmAr, because the cloned fragment was originally amplified with the nested primers. The plasmid therefore serves only as a negative control in the first PCR and as positive control for the PCR with the nested primers.

Primer efficiency – tests on field and nursery sampled roots and spores

To test whether the newly designed primers discriminate against nonglomeromycotan fungi and plants, we used them on DNA extracted from single spores from pot cultures, environmental root samples, and root samples from a tree nursery, in nested PCR approaches. We observed not a single non-AMF contaminant sequence in the 12 environmental root and 40 single spore samples processed. The discrimination against plants was tested with DNA extracts from roots of potential AMF hosts. The species collected comprised *Poa* cf. *annua*, *Ranunculus* cf. *repens*, and *Rumex acetosella* from a field site in Germany, and *Podocarpus* cf. *macrostaqui*, *Heliocarpus americanus* and *Cedrela montana* tree seedlings from a tree nursery in Ecuador. From a large number of nested PCR approaches, on just one occasion, three identical clones carrying a plant sequence (*R. acetosella*) were obtained. The *Rumex* related database sequence (AF189730, 630 bp) covers the ITS region, but not the binding sites for the nested primers. The new primers were also used successfully on DNA extractions from single AMF spores from pot cultures and a root organ culture (ROC). This demonstrates PCR amplification with a broad phylogenetic coverage of AMF, while efficiently discriminating against non-AMF and plants (Table 3).

The results show that the new primers are suitable to amplify DNA from members of the whole *Glomeromycota* and can be used for species level analyses of AMF communities in the field.

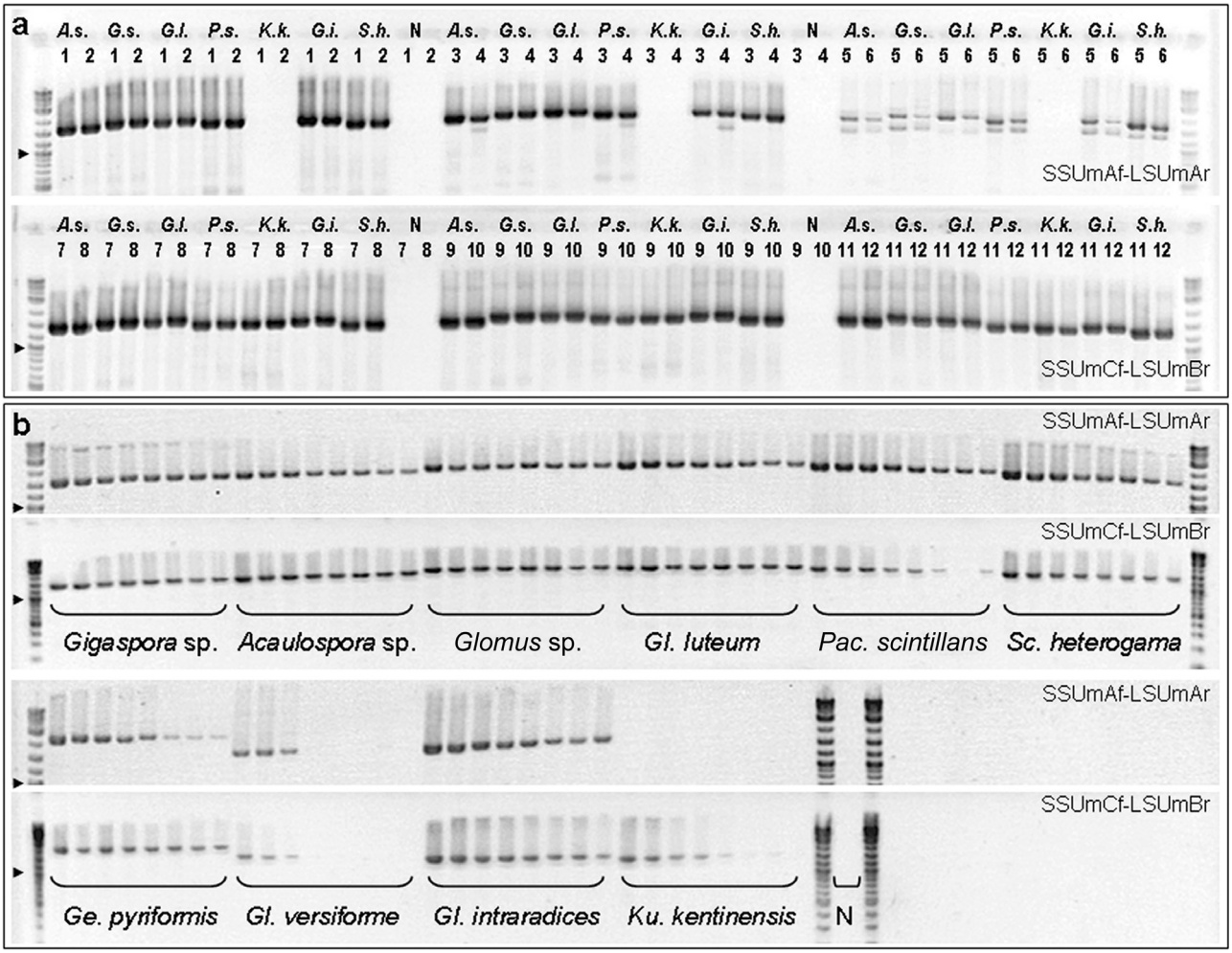

Fig. 3 Polymerase chain reaction amplification with primers SSUmAf–LSUmAr (approx. 1800 bp amplicons) and SSUmCf–LSUmBr (approx. 1500 bp amplicons). (a) PCR on cloned DNA fragments, using different annealing temperatures and a template concentration of 1 ng μl^{-1}. *A.s.*, *Acaulospora* sp.; *G.s.*, *Glomus* sp.; *G.l.*, *Glomus luteum*; *P.s.*, *Pacispora scintillans*; *K.k.*, *Kuklospora kentinensis*; *G.i.*, *Glomus intraradices*; *S.h.*, *Scutellospora heterogama*; N, negative control. Annealing temperatures: 1, 55°C; 2, 55.7°C; 3, 57.8°C; 4, 60.5°C; 5, 63.1°C; 6, 65°C; 7, 55.2°C; 8, 56.6°C; 9, 59.1°C; 10, 61.8°C; 11, 64.2°C; 12, 65.5°C. (b) PCR using 1 µl of a 10-fold plasmid dilution (100 pg – 0.01 fg μl^{-1}) as template, corresponding to 5×10^7 to 5 plasmid molecules in 20 µl PCR reaction volume. Annealing temperatures: SSUmAf–LSUmAr 60°C; SSUmCf-LSUmBr 63°C. N, negative control; Marker, NEB 2-Log DNA Ladder (bp: 10 000, 8000, 6000, 5000, 4000, 3000, 2000, 1500, 1200, 1000 (arrowhead), 900, 800, 700, 600, 500, 400, 300, 200, 100).

Discussion

There have been numerous efforts to design PCR primers generally applicable for detection of the whole group of AMF (Simon *et al.*, 1992; Helgason *et al.*, 1998), but later studies showed that they do not amplify DNA of all *Glomeromycota* or they also amplify ascomycetes, basidiomycetes or plant DNA (Clapp *et al.*, 1995, 1999; Helgason *et al.*, 1999). Other primers were successfully used for certain groups of the *Glomeromycota* (Kjøller & Rosendahl, 2000; Redecker, 2000; Turnau *et al.*, 2001; Wubet *et al.*, 2003, 2006; Gamper & Leuchtmann, 2007).

Many of the approaches require different primer pairs and independent PCR attempts for distinct target taxa. Comparison of such studies can be difficult since the distinct primer binding sites may behave very different in PCR and do not allow semiquantitative approaches. A single primer set for PCR amplification that covers all groups of the *Glomeromycota* and allows the identification of AMF at the species level was not available.

We have chosen the strategy of mixed primer sets to cover the defined sequence variability, instead of using fully degenerated primers. This reduces the degree of degeneration and results in a higher ratio of efficiently binding primers. The approach also allows adjustment of the concentrations of individual primers in future attempts. At the beginning of the study we speculated that the exonuclease activity of the proof-reading DNA polymerase used could hamper discrimination

Table 3 PCR amplification with the new primer pairs; DNA extracted from roots or spores

Environmental samples	Sample or culture	First PCR	Nested PCR	Clones sequenced, most likely genus (BLAST hits for full length and partial sequences)
Cedrela montana roots (tree nursery pot)	N1	–	+	pCK011.1-7 *Ambispora* (uncultured *Archaeospora* LSU)
Cedrela montana roots (tree nursery pot)	N3	+	+	first PCR: pCK009.1-3 *Glomus* (mycorrhizal symbiont of *Marchantia foliacea* SSU, ITS, LSU; *Glomus* sp. MUCL43206 LSU); nested PCR: pCK016.1-3, pCK017.1 *Glomus* (uncultured AMF clone Glom3524.1 SSU; symbiont of *M. foliacea* SSU, ITS, LSU; *Glomus* sp. MUCL43206 LSU, MUCL43194, LSU; *Glomus* sp. 'intraradices' AFTOL-ID845 LSU)
Cedrela montana roots (tree nursery pot)	N8	+	+ (ns)	pCK010.1,2 *Gigaspora* and/or *Scutellospora* (uncultured *Gigasporaceae* clone S2R2 SSU, ITS, LSU; *Gi. rosea* SSU, ITS, LSU; *Sc. heterogama* AFTOL-ID138 LSU)
Heliocarpus americanus roots (tree nursery pot)	N2	–	+	pCK012.2-4 *Archaeospora* and *Glomus* (*Ar. trappei* NB112 SSU, ITS, LSU; *Glomus* sp. 'intraradices' AFTOL-ID845 LSU)
Podocarpus cf. *macrostaqui* root without nodules (seedling from forest)	P0	+	+ (ns)	pCK018.1 *Acaulospora* (*Ac. alpina* clone 1060/33 SSU, ITS; uncultured *Acaulospora* clone: A3-68-c LSU)
Podocarpus cf. *macrostaqui* root with nodules (seedling from forest)	P1	+	+ (ns)	pCK020.1-13 *Acaulospora* (*Ac. alpina* clone 1060/33 SSU, ITS; *Acaulospora* clone: A3-68-c LSU)
Podocarpus cf. *macrostaqui* root nodules only (seedling from forest)	P2	–	+	pCK006.1,2 *Glomus* (*Gl. diaphanum* clone 3.3 SSU, ITS, LSU; *Gl. coronatum* BEG28 LSU; symbiont of *M. foliacea* SSU, ITS1; uncultured *Glomus* LSU)
Podocarpus cf. *macrostaqui* root nodules only (seedling from forest)	P3	–	+	pCK007.1,3,4 *Glomus* (*Glomus* sp. 0171 SSU, ITS; uncultured *Glomus* clone K7-10 SSU, ITS; *Glomus* clone K31-1 LSU; uncultured *Glomus* clone 1298-21 SSU, ITS, LSU; uncultured glomeromycete 2-09 LSU); pCK007.5,6 pCK008.1,3-7 *Glomus* (uncultured *Glomus* clone S1R2 + S2R1/2 SSU, ITS, LSU; *Glomus* sp. MUCL43206 LSU, MUCL43207 LSU; symbiont of *M. foliacea* SSU, ITS1; uncultured *Glomus* clone: A10-28 LSU)
Ranunculus repens roots (field sample)	1A	–	+	pMK078.1-3 *Acaulospora* (uncultured *Acaulospora* SSU; LSU)
Ranunculus repens roots (field sample)	3A	–	+	pMK083.2,3,5 *Acaulospora* (*Acaulospora* sp. ZS2005 SSU, ITS; *Ac. paulinae* clone 2.2 LSU)
Ranunculus repens roots (field sample)	5A	–	+	pMK077.1-5 *Glomus* (uncultured *Glomus* clones S1R2 + 850-23 SSU, ITS; uncultured *Glomus* clone H5-2 LSU)
Ranunculus repens roots (field sample)	7A	–	+	pMK080.1-5 *Diversispora* (*Gl. aurantium* SSU, ITS, LSU; *Gl. versiforme* BEG47 LSU, uncultured *Glomus* LSU); pMK080.6,7 *Glomus* (uncultured *Glomus* clone S1R2 SSU, ITS; uncultured *Glomus* LSU)
Poa annua roots (field sample)	1C	–	+	pMK082.1,4,6,9-17 *Acaulospora* (uncultured *Acaulospora* SSU, ITS, LSU; uncultured *Acaulospora* LSU)
Poa annua roots (field sample)	2C	–	+	pMK081.1,3-5 *Acaulospora* (uncultured *Acaulospora* SSU, ITS, LSU; *Ac. laevis* BEG13 LSU)
Plantago lanceolata roots (pot culture, inoculated with *C. montana* roots)	Att 1451-8	+	+ (ns)	pCK024.1,3,4 *Glomus* (uncultured *Glomus* clone S2R2 SSU, ITS, LSU; uncultured *Glomus* clone S1R2 SSU, ITS, LSU; *Glomus* sp. 'intraradices' AFTOL-ID845 LSU, *Glomus* sp. MUCL43206 LSU; *Glomus* sp. MUCL43203 LSU)
Plantago lanceolata roots (pot culture, inoculated with *H. americanus* roots)	Att 1456-1	–	+	pCK025.1-4 *Glomus* (uncultured *Glomus* clone S1R2 SSU, ITS, LSU; *Glomus* sp. MUCL43203 LSU)
AMF ss (ss pot culture)	Att 1449-5	–	+	pCK022.1-3 *Diversispora* (*Gl. aurantium* SSU, LSU; *Gl. versiforme* BEG47 LSU)
AMF ss (ss pot culture)	Att 1450-1	–	+	pCK023.1-4 *Acaulospora* (*Ac. colossica* clones 15.1+15.4 SSU, ITS, LSU; uncultured *Acaulospora* clone H1-1 LSU)
AMF ss (ss pot culture)	Att 1456-7	–	+	pCK026.1,2-6 *Archaeospora* (uncultured *Archaeospora* clone 1400-71 SSU, ITS, clone R8-37 LSU; *Ar. trappei* SSU, ITS, LSU)
AMF ss (ss pot culture)	Att 1456-11	–	+	pCK027.1-3 *Glomus* (*Gl. claroideum* clone 57.10 SSU, ITS, LSU)
AMF ss (ss pot culture)	Att 1449-10	–	+	pCK028.2-5,7-12 *Glomus* (*Gl. claroideum* clone 57.10 SSU, ITS, LSU)
AMF ss morphotype 1 (ms pot culture)	Att 1451-6	+	+	first PCR: pCK029.1 *Glomus* (*Gl. claroideum* clone 57.10 SSU, ITS, LSU); nested PCR: pCK030.1-6 *Glomus* (uncultured *Glomus* clone Pa127 SSU, ITS, LSU; uncultured *Glomus* clone S1R2 SSU, ITS, LSU; *Gl. etunicatum* LSU; *Glomus* sp. MUCL43203 LSU)
AMF ss morphotype 2 (ms pot culture)	Att 1451-6	–	+	pCK031.1,2 *Gigaspora* (*Gi. rosea* clone Gr8.2 SSU, ITS, LSU; *Sc. heterogama* AFTOL-ID138 LSU)
Glomus intraradices spore cluster (ROC)	Att 4-64 (from FL208)	–	+	pHS099.3,6,8,11,14,16,25,32,36,40,41,47 *Glomus* (uncultured *Glomus* clone S2R2 SSU, ITS, LSU; *Glomus* sp. MUCL43203 LSU, *Glomus* sp. MUCL43206 LSU, MUCL43207 LSU, *Glomus* sp. 'intraradices' AFTOL-ID845 LSU)

First PCR, SSUmAf–LSUmAr; nested PCR, SSUmCf–LSUmBr. PCR reactions are given as positive when a PCR product of the expected size was visible. The closest BLAST hits are shown for the first and/or nested PCR derived sequences. Att, culture attempt; ITS, internal transcribed spacer; LSU, large subunit; ms, multi spore; ns, not sequenced; ROC, root organ culture; ss, single spore; SSU, small subunit.

by terminal 3′ primer mismatches, but no such problems were detected.

Primer specificity

The primers designed show some mismatches to AMF sequences at the 5′ end (Fig. 1), which do not hinder PCR amplification (Bru *et al.*, 2008). Primer mismatches such as C–T, T–C and T–G do not impair amplification strongly even when situated at the 3′ end of the primer (Kwok *et al.*, 1990). The forward primers SSUmAf as well as the reverse primers LSUmBr mismatched once with *Ge. pyriformis*, but did not hamper amplification. The LSU rDNA primers show sufficient sequence similarity to the target organisms, as the mismatches are either in the middle or at the 5′ end. LSUmAr primers displayed individual mismatches to sequences of *Scutellospora* spp., *Gl. etunicatum*, and one *Acaulospora* sp. (Fig. 1). Nevertheless, DNA of these species was successfully amplified from environmental samples and in the primer efficiency test (Fig. 3). *Ambisporaceae* and *Archaeosporaceae* species could not be included in the design of the LSU primers, but *Ambispora fennica* DNA from a single spore extraction (not shown) and *Archaeospora* sp. from single spores and roots of an Ecuadorian tree seedling (Table 3) could be amplified with the new primers, indicating well matching binding sites. Sequences from *Otospora* (*Diversisporaceae*; Palenzuela *et al.*, 2008; matching the SSU primers), *Intraspora* (closely related to *Archaeospora*), and *Entrophospora* (sensu Oehl & Sieverd.; with two species only) are either not or only partly characterized and therefore could not be included in several aspects of primer design. *Otospora* and *Intraspora* are very closely related to their sister genera (maybe congeneric), so the lack of LSU rDNA sequences was therefore interpreted as a minor limitation.

We could successfully amplify all AMF tested with the new primers, but because of the lower number of LSU rDNA sequences available for AMF an optimization of the LSU primers might be reasonable in future. The discrimination against non-AMF and plant DNA is excellent, as shown on DNA extracts from environmental samples and spores from pot cultures. To discriminate against non-AMF, LSUmAr works much better than the nested primers LSUmBr. The cloned plant (*Rumex*) rDNA fragment that originated from root material can be interpreted as an 'outlier'. The primer binding sites could not be investigated for *Rumex*, because of lacking sequence coverage. It should be indicated in this context that we did not use HPLC-purified primers. This means a certain fraction of primers may not be fully synthesized and could result in less specific amplification. All plasmids used in the plasmid test carried inserts that were originally amplified with SSUmAf. Therefore, the efficiency of this primer could not be validated, but because of the high number of SSU rDNA sequences known, it can be stated that the binding sites in the cloned fragments correspond to a realistic situation. The efficient amplification from spore DNA extracts was, moreover, confirmed in numerous former PCR.

Advantages over previously used PCR primer sets

In most former field studies SSU rDNA phylotypes were analysed for molecular detection of AMF. However, this region does not allow species resolution and each defined phylotype, irrespective of the used distance threshold value or phylogenetic analysis method, may hide a number of species (Walker *et al.*, 2007). In general, the LSU rDNA region allows species resolution, and thus the LSU primer pair FLR3–FLR4 (Gollotte *et al.*, 2004) was used for species-level community analyses. However, in particular, FLR4 is not phylogenetically inclusive (Gamper *et al.*, 2009) and discriminates many lineages, including *Diversisporales*, *Archaeosporales* and *Paraglomerales*, which results in a strong bias in community analyses towards the *Glomeraceae*. The primer FLR3 binds to DNA of many nontarget fungi as it shows no mismatch to > 1300 basidiomycete sequences and some ascomycete sequences in the public databases. Such problems obviously may bias tRFLP community analyses (Mummey & Rillig, 2008) and seminested PCR approaches (Pivato *et al.*, 2007) using FLR3 and/or FLR4. The primer pair SSUGlom1–LSUGlom1 (Renker *et al.*, 2003) amplifies many non-AMF and plants. Combined with the primers ITS5–ITS4 in a nested PCR (Hempel *et al.*, 2007) this resulted in a 5.8S rDNA phylogenetic analysis, which resolved only the genus level. Even the ITS region does not always resolve species for AMF (Stockinger *et al.*, 2009).

In some cases, species-specific detection tools are available for individual species or certain well-defined and closely related species. The three closely related AM fungi *Gl. mosseae*, *Gl. caledonium* and *Gl. geosporum* were detected by using LSU primers in field studies (Stukenbrock & Rosendahl, 2005; Rosendahl & Matzen, 2008), but these primers were designed to only amplify subgroups or certain taxa in the *Glomeromycota*. For the well-studied *Gl. intraradices* related AMF (e.g. DAOM197198), which are, however, not conspecific with *Gl. intraradices* (Stockinger *et al.* 2009), microsatellite markers are available for their detection in the field (Croll *et al.*, 2008; Mathimaran *et al.*, 2008). Some mtLSU region markers were also studied (Börstler *et al.*, 2008), but because of the high length variation observed (1070–3935 bp) and the difficulty in amplifying this region it is not very promising for community analyses. Thus, such markers cannot be used for general AMF community analyses.

The new primers described in the present study were used to amplify efficiently and specifically target rDNA from environmental samples of the main phylogenetic groups in the *Glomeromycota*. For the first time, this will allow molecular ecological studies covering all AMF lineages to be carried out with only one primer set. Furthermore, the long sequences allow robust phylogenetic analyses and species level resolution

by inclusion of the variable ITS and LSU rDNA region (Walker *et al.*, 2007; Gamper *et al.*, 2009; Stockinger *et al.* 2009), whereas formerly used primers mainly amplified rDNA fragments of up to 800 bp (Helgason *et al.*, 1999; Redecker, 2000; Lee *et al.*, 2008).

Potential application as DNA barcoding primers

The new primers are suited to amplify the most likely primary DNA barcode region for fungi, the ITS region (already online at the Barcode of Life Data Systems (BOLD) website; www.barcodinglife.org). In general 'barcode primers' should amplify short fragments and for the ITS region the amplicons generated by our primers are in fact too long. However, the main criterion for DNA barcodes is the resolution at species level. Since for *Glomeromycota* this is difficult or impossible to achieve with the ITS region only (Stockinger *et al.*, 2009), the inclusion of the 5′ LSU rDNA fragment is strongly recommended. Our new primer set (SSUmAf, SSUmCf, LSUmAr and LSUmBr) appears to be well suited as barcoding primers for *Glomeromycota*. The primers will be helpful for the molecular characterization of AMF, including species descriptions (Gamper *et al.*, 2009), resulting in a sequence database that allows the design of further primers for the detection of AMF from field samples. LSUmAr and LSUmBr, located approximately at positions 930–950 and 830–850 on the LSU rRNA gene, may be used in combination with new forward LSU primers for amplification of fragments within the variable D1/D2 LSU regions. Based on such amplicons, deep sequencing approaches with the now feasible longer reads of the new 454 FLX-titanium chemistry will allow species level detection of the 'unknown' AMF community, in future molecular ecological studies.

Acknowledgements

The grants for M.K., C.K. and A.S. were financed by the German Research Foundation (DFG). The grant for H.S. was funded by the Marie Curie Early Stage Research Training Fellowship of the European Community's Sixth framework Programme (MEST-CT-2005-021016).

References

Allen GC, Flores-Vergara MA, Krasnyanski S, Kumar S, Thompson WF. 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature Protocols* **1**: 2320–2325.

Aroca R, Porcel R, Ruiz-Lozano JM. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist* **173**: 808–816.

Börstler B, Raab PA, Thiery O, Morton JB, Redecker D. 2008. Genetic diversity of the arbuscular mycorrhizal fungus *Glomus intraradices* as determined by mitochondrial large subunit rRNA gene sequences is considerably higher than previously expected. *New Phytologist* **180**: 452–465.

Brody JR, Kern SE. 2004. Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. *Biotechniques* **36**: 214–215.

Bru D, Martin-Laurent F, Philippot L. 2008. Quantification of the detrimental effect of a single primer–template mismatch by real-time PCR using the 16S rRNA gene as an example. *Applied and Environmental Microbiology* **74**: 1660–1663.

Clapp JP, Fitter AH, Young JPW. 1999. Ribosomal small subunit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp. *Molecular Ecology* **8**: 915–922.

Clapp JP, Young JPW, Merryweather JW, Fitter AH. 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist* **130**: 259–265.

Croll D, Wille L, Gamper HA, Mathimaran N, Lammers PJ, Corradi N, Sanders IR. 2008. Genetic diversity and host plant preferences revealed by simple sequence repeat and mitochondrial markers in a population of the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **178**: 672–687.

De la Pena E, Rodriguez Echeverria S, van der Putten WH, Freitas H, Moens M. 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. *New Phytologist* **169**: 829–840.

Gamper H, Leuchtmann A. 2007. Taxon-specific PCR primers to detect two inconspicuous arbuscular mycorrhizal fungi from temperate agricultural grassland. *Mycorrhiza* **17**: 145–152.

Gamper H, Walker C, Schüßler A. 2009. *Diversispora celata* sp. nov.: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. *New Phytologist* **182**: 495–506.

Gollotte A, van Tuinen D, Atkinson D. 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza* **14**: 111–117.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. 1998. Ploughing up the wood-wide web? *Nature* **394**: 431.

Helgason T, Fitter AH, Young JPW. 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides* nonscripta (bluebell) in a seminatural woodland. *Molecular Ecology* **8**: 659–666.

Hempel S, Renker C, Buscot F. 2007. Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. *Environmental Microbiology* **9**: 1930–1938.

Jansa J, Mozafar A, Kuhn G, Anken T, Ruh R, Sanders IR, Frossard E. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications* **13**: 1164–1176.

Kjøller R, Rosendahl S. 2000. Detection of arbuscular mycorrhizal fungi (*Glomales*) in roots by nested PCR and SSCP (single stranded conformation polymorphism). *Plant and Soil* **226**: 189–196.

Kottke I, Haug I, Setaro S, Suárez JP, Weiß M, Preußing M, Nebel M, Oberwinkler F. 2008. Guilds of mycorrhizal fungi and their relation to trees, ericads, orchids and liverworts in a neotropical mountain rain forest. *Basic and Applied Ecology* **9**: 13–23.

Kwok S, Kellogg DE, McKinney N, Spasic D, Goda L, Levenson C, Sninsky JJ. 1990. Effects of primer–template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. *Nucleic Acids Research* **18**: 999–1005.

Lee J, Lee S, Young JPW. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology* **65**: 339–349.

Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar, Buchner A, Lai T, Steppi S, Jobb G *et al.* 2004. ARB: a software environment for sequence data. *Nucleic Acids Research* **32**: 1363–1371.

Mathimaran N, Falquet L, Ineichen K, Picard C, Redecker D, Boller T, Wiemken A. 2008. Microsatellites for disentangling underground networks: Strain-specific identification of *Glomus intraradices*, an arbuscular mycorrhizal fungus. *Fungal Genetics and Biology* **45**: 812–817.

Michelson A, Rosendahl S. 1990. The effect of VA mycorrhizal fungi, phosphorus and drought stress on the growth of *Acacia nilotica* and *Leucaena leucocephala* seedlings. *Plant and Soil* **124**: 7–13.

Mummey DL, Rillig MC. 2008. Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate grassland. *FEMS Microbiology Ecology* **64**: 260–270.

Oehl F, Sieverding E, Ineichen K, Ris EA, Boller T, Wiemken A. 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytologist* **165**: 273–283.

Öpik M, Moora M, Zobel M, Saks Ü, Wheatley R, Wright F, Daniell T. 2008. High diversity of arbuscular mycorrhizal fungi in a boreal herb-rich coniferous forest. *New Phytologist* **179**: 867–876.

Palenzuela J, Ferrol N, Boller T, Azcon-Aguilar C, Oehl F. 2008. *Otospora bareai*, a new fungal species in the Glomeromycetes from a dolomitic shrub land in Sierra de Baza National Park (Granada, Spain). *Mycologia* **100**: 296–305.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763–775.

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, van Tuinen D. 2007. *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytologist* **176**: 197–210.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* **35**: 7188–7196.

Redecker D. 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. *Mycorrhiza* **10**: 73–80.

Renker C, Heinrichs J, Kaldorf M, Buscot F. 2003. Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. *Mycorrhiza* **13**: 191–198.

Rosendahl S, Matzen HB. 2008. Genetic structure of arbuscular mycorrhizal populations in fallow and cultivated soils. *New Phytologist* **179**: 1154–1161.

Schüßler A, Gehrig H, Schwarzott D, Walker C. 2001a. Analysis of partial *Glomales* SSU rRNA gene sequences: implications for primer design and phylogeny. *Mycological Research* **105**: 5–15.

Schüßler A, Krüger M, Walker C. 2009. Phylogeny, evolution and origin of the 'plant-symbiotic' phylum *Glomeromycota.* In: Wöstemeyer J, Martin W, eds. *The Mycota XIV – evolution of fungi and fungal-like organisms*. Berlin, Germany: Springer-Verlag, in press.

Schüßler A, Schwarzott D, Walker C. 2001b. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.

Schwarzott D, Schüßler A. 2001. A simple and reliable method for SSU rRNA gene DNA extraction, amplification, and cloning from single AM fungal spores. *Mycorrhiza* **10**: 203–207.

Sieverding E, Oehl F. 2006. Revision of *Entrophospora* and description of *Kuklospora* and *Intraspora*, two new genera in the arbuscular mycorrhizal Glomeromycetes. *Journal of Applied Botany and Food Quality* **80**: 69–81.

Simon L, Lalonde M, Bruns TD. 1992. Specific amplification of 18S fungal ribosomal genes from vesicular–arbuscular endomycorrhizal fungi colonizing roots. *Applied Environmental Microbiology* **58**: 291–295.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. Cambridge, UK: Academic Press.

Stockinger H, Walker C, Schüßler A. 2009. '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. New Phytologist, in press.

Stukenbrock EH, Rosendahl S. 2005. Development and amplification of multiple co-dominant genetic markers from single spores of arbuscular mycorrhizal fungi by nested multiplex PCR. *Fungal Genetics and Biology* **42**: 73–80.

Turnau K, Ryszka P, Gianinazzi-Pearson V, van Tuinen D. 2001. Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza* **10**: 169–174.

Van der Heijden MGA, Bardgett RD, van Straalen NM. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**: 296–310.

Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.

Vigo C, Norman JR, Hooker JE. 2000. Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. *Plant Pathology* **49**: 509–514.

Walker C, Schüßler A. 2004. Nomenclatural clarifications and new taxa in the *Glomeromycota*. *Mycological Research* **108**: 981–982.

Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H, Nishmura I, Schüßler A. 2007. Molecular phylogeny and new taxa in the *Archaeosporales* (*Glomeromycota*): *Ambispora fennica* gen. sp nov., *Ambisporaceae* fam. nov., and emendation of *Archaeospora* and *Archaeosporaceae*. *Mycological Research* **111**: 137–153.

Wang YY, Vestberg M, Walker C, Hurme T, Zhang XP, Lindström K. 2008. Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China. *Mycorrhiza* **18**: 59–68.

Wubet T, Weiß M, Kottke I, Oberwinkler F. 2003. Morphology and molecular diversity of arbuscular mycorrhizal fungi in wild and cultivated yew (*Taxus baccata*). The *Canadian Journal of Botany* **81**: 255–266.

Wubet T, Weiß M, Kottke I, Teketay D, Oberwinkler F. 2006. Phylogenetic analysis of nuclear small subunit rDNA sequences suggests that the endangered African Pencil Cedar, *Juniperus procera*, is associated with distinct members of *Glomeraceae*. *Mycological Research* **110**: 1059–1069.

5. DNA barcoding of arbuscular mycorrhizal fungi

This chapter is identical to the publication:

Stockinger H, Krüger M, Schüßler A. **2010**. DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist* 187: 461-474.

New Phytologist

Research

DNA barcoding of arbuscular mycorrhizal fungi

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Received: *14 December 2009*
Accepted: *25 February 2010*

New Phytologist (2010) **187**: 461–474
doi: 10.1111/j.1469-8137.2010.03262.x

Key words: arbuscular mycorrhizal fungi, DNA barcode, ITS rDNA, LSU rDNA, molecular phylogeny.

Summary

- Currently, no official DNA barcode region is defined for the *Fungi*. The *COX1* gene DNA barcode is difficult to apply. The internal transcribed spacer (ITS) region has been suggested as a primary barcode candidate, but for arbuscular mycorrhizal fungi (AMF; *Glomeromycota*) the region is exceptionably variable and does not resolve closely related species.
- DNA barcoding analyses were performed with datasets from several phylogenetic lineages of the *Glomeromycota*. We tested a *c.* 1500 bp fragment spanning small subunit (SSU), ITS region, and large subunit (LSU) nuclear ribosomal DNA for species resolving power. Subfragments covering the complete ITS region, *c.* 800 bp of the LSU rDNA, and three *c.* 400 bp fragments spanning the ITS2, the LSU-D1 or LSU-D2 domains were also analysed.
- Barcode gap analyses did not resolve all species, but neighbour joining analyses, using Kimura two-parameter (K2P) distances, resolved all species when based on the 1500 bp fragment. The shorter fragments failed to separate closely related species.
- We recommend the complete 1500 bp fragment as a basis for AMF DNA barcoding. This will also allow future identification of AMF at species level based on 400 or 1000 bp amplicons in deep sequencing approaches.

Introduction

This study aimed to define a DNA barcoding region for arbuscular mycorrhizal fungi (AMF) that also is useful for molecular in-field community studies. Despite the fact that AMF are perhaps the most important fungi in terrestrial ecosystems, forming mutualistic symbioses with *c.* 80% of land plants (Brundrett, 2009), much of their biology still is enigmatic. One recent example for a new and surprising finding are the *Mycoplasma*-related endobacteria of AMF (Naumann *et al.*, 2010), with completely unknown function. The lack of knowledge about many aspects of AMF biology is partly because of their asexual, obligate symbiotic and subterranean lifestyle. All AMF belong to the phylum *Glomeromycota* (Schüßler *et al.*, 2001) and molecular biological methods revealed cryptic species showing, for example, that spore morphs previously defined as different species in distinct families (e.g. morphs of *Ambispora leptoticha*) are conspecific (Sawaki *et al.*, 1998; Redecker *et al.*, 2000; Walker *et al.*, 2007). However, the asexual reproduction and potentially clonal diversity complicate the interpretation of AMF species boundaries (Stukenbrock & Rosendahl, 2005). Despite this limitation, the present species concept is valuable, congruent with phylogenetic analyses (Walker *et al.*, 2007; Msiska & Morton, 2009; Stockinger *et al.*, 2009) and important for uncovering functional diversity. Unfortunately, the knowledge of preferential associations of AMF with plants under certain environmental conditions is still very limited, although a better understanding of differential AMF–plant associations and symbiotic preferences is of high ecological relevance and will affect sustainable management practices in agriculture and forestry.

Identification of AM fungal species from the field

Community analyses based on morphologically monitoring AMF spore occurrences in the soil reveal some important hints about the species composition in different ecosystems (Oehl *et al.*, 2009; Robinson-Boyer *et al.*, 2009), but spores are resting stages and may not reflect those species that are physiologically active at the time (Sanders, 2004). Moreover, relatively little is known about the influence of environment or host plant on sporulation dynamics over both space and time (Walker *et al.*, 1982).

To overcome such drawbacks, molecular methods were developed to detect AMF directly within roots. The most frequently used markers are one or more of the nuclear rRNA genes, for example the widely used small subunit (SSU) rRNA gene (Helgason *et al.*, 1999; Wubet *et al.*, 2006; Lee *et al.*, 2008), the internal transcribed spacer (ITS) rDNA region including the 5.8S rRNA gene (Wubet *et al.*, 2004; Hempel *et al.*, 2007; Sýkorová *et al.*, 2007), and a part of the large subunit (LSU) rRNA gene (Gollotte *et al.*, 2004; Pivato *et al.*, 2007; Rosendahl *et al.*, 2009). However, many molecular analyses are biased, as some of the primers used detect only parts of the community and the level of taxonomic resolution in most cases is uncertain. Species-level community analyses based on rDNA regions should be feasible (Gamper *et al.*, 2009; Stockinger *et al.*, 2009), but no single molecular marker or DNA barcode is yet suitable for species-level resolution of all AMF.

DNA barcoding for fungal species definition and identification

DNA barcoding in the strict sense is defined as the standardized analysis of an easily amplifiable PCR fragment for sequence-based identification of species. Identifications must be accurate, rapid, cost-effective, culture-independent, universally accessible and usable by nonexperts (Frézal & Leblois, 2008). By DNA barcoding, organisms can be identified in life cycle stages not suited for morphological identification (Gilmore *et al.*, 2009).

In DNA barcoding, species are separated by standardized barcode gap analyses or phylogenetic tree-building methods. A barcode gap exists if the minimum interspecific variation is bigger than the maximum intraspecific variation. Alternatively, phylogenetic neighbour joining analysis based on Kimura two-parameter (K2P = K80) distances is a suggested standard method and in future more sophisticated phylogenetic methods will most likely be applied.

A part of the mitochondrial *cytochrome c oxidase 1* (*COX1*) gene has become the first official animal DNA barcode (Hebert *et al.*, 2004; http://www.barcoding.si.edu/) and for plants an agreed system is based on the plastid loci *rbcL* and *matK* (Hollingsworth *et al.*, 2009), but no official consensus strategy exists for fungi. A standardized DNA-based species identification system for fungi would be extremely useful. There are *c.* 100 000 named fungi (Kirk *et al.*, 2008), and estimates suggest that as many as 1.5–3.5 million species exist (Hawksworth, 2001; O'Brien *et al.*, 2005). Identification of many of these, particularly from their vegetative state, will only be possible by molecular methods.

Primers have long been available for the nuclear ITS rDNA region (White *et al.*, 1990; Gardes & Bruns, 1993) which are now commonly used for fungal identification (Kõljalg *et al.*, 2005; Summerbell *et al.*, 2007). The ITS rDNA region will probably be proposed to the Consortium for the Barcode of Life (CBOL, http://www.barcoding.si.edu) as a fungal barcode (Seifert, 2009). As for many other organism groups, fungal sequence data derived from inaccurately identified material exist in the public databases (Ryberg *et al.*, 2008), and a lack of vouchers often precludes verification of sequences (Agerer *et al.*, 2000). Unfortunately, third party corrections in the GenBank sequence database are prohibited (Bidartondo *et al.*, 2008). Initiatives such as UNITE (http://unite.ut.ee) were established to provide validated and curated data, but such data are still lacking for AMF.

COX1 is not suited as general fungal barcode

Demonstration that the *COX1* region is unsuitable for easy PCR-amplification, sequencing and species identification would preclude its use according to the CBOL standards. Although this region showed promise for *Penicillium* spp. (Seifert *et al.*, 2007), the length of fungal *COX1* is highly variable (1.6–22 kb). The shortest potential barcoding region varies in length from 642 bp to > 12 kb (Seifert, 2009). Moreover fungal species-level discrimination with *COX1* genes may be inaccurate (Chase & Fay, 2009) and in *Fusarium* and the *Aspergillus niger* complex multiple paralogues hinder species-level resolution (Geiser *et al.*, 2007; Gilmore *et al.*, 2009). For the AMF *Glomus* sp. FACE#494, the barcoding region of *COX1* spans 2200 bp and contains several introns (Lee & Young, 2009). Moreover, the mtDNA of *Glomus diaphanum* contains a *COX1* intron with high sequence similarity to a corresponding *COX1* intron in plants and *Rhizopus oryzae* (Lang & Hijri, 2009). The plant intron is thought to have originated by horizontal gene transfer (HGT) from fungi (Vaughn *et al.*, 1995; Lang & Hijri, 2009), further questioning the general usability of *COX1* as a barcode for either fungi or plants.

Defining a DNA barcoding region for AMF

Both potential primary barcoding regions – *COX1* with its large length variation and the ITS rDNA with its lack of discrimination of closely related AMF species (Stockinger *et al.*, 2009) – seem unsuited for AMF. Therefore, we aimed to define a DNA barcoding region for *Glomeromycota* by comparing different nuclear rRNA gene regions and the ITS.

We further on abbreviate the nuclear SSU rRNA gene as SSU, the LSU rRNA gene as LSU, and the 5.8S rRNA gene as 5.8S; the term 'ITS region' is used for the complete ITS1–5.8S–ITS2 rDNA (Fig. 1), for simplicity. A DNA fragment of 1420–1602 bp, amplified with AMF specific primers (Krüger *et al.*, 2009) from species in widely separated AMF clades was sequenced. The fragment covers *c.* 240 bp of the SSU, the 400–526 bp long ITS region, and 776–852 bp of the LSU. We compared the complete

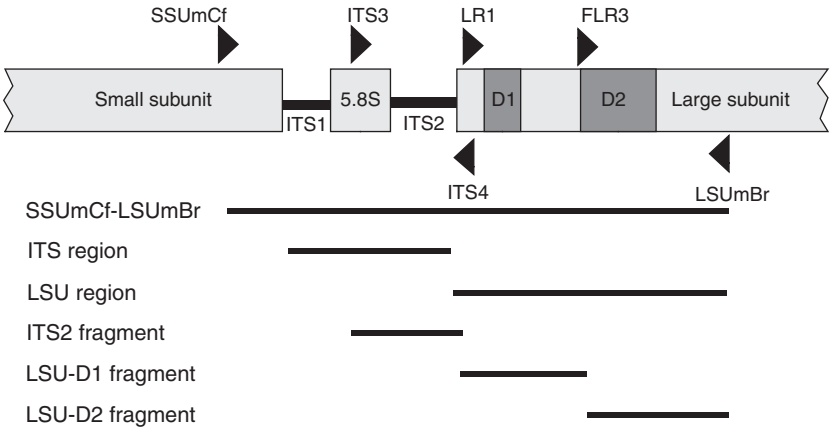

Fig. 1 Schematic representation of the nuclear ribosomal DNA regions studied. Triangles indicate positions of priming sites that were used as borders for *in silico* analyses of the fragments. Lines indicate the fragments analysed.

fragment, the ITS region, the LSU region, and three *c.* 400 bp fragments, covering the 5.8S + ITS2, LSU-D1 or LSU-D2, for species resolving power and suitability as DNA barcode. This corresponds with the resolution level in environmental deep sequencing approaches using the present 454 GS-FLX Titanium system, with *c.* 400 bp average read lengths. The barcode we propose here will also facilitate the identification of species using future deep sequencing systems with > 1000 bp read lengths (http://www.454.com; http://www.pacificbiosciences.com).

Materials and Methods

Taxa and public sequences used for analyses

The 'core dataset' sequences investigated in this study (see the Supporting Information, Table S1) cover the partial SSU, the ITS region and the partial LSU, completely covering a fragment spanning the region amplified with primers SSU-Glom1 (Renker *et al.*, 2003) and NDL22 (van Tuinen *et al.*, 1998). For all AMF analysed, a culture identifier or a voucher deposited in a herbarium (W-numbers) is known; for most, both items of information is available. The attempt (Att) numbers refer to the culture collection of Christopher Walker, BEG identifiers to the 'International bank for the *Glomeromycota*' (http://www.kent.ac.uk/bio/beg), INVAM to the 'International culture collection of (vesicular) arbuscular mycorrhizal fungi' (http://invam.caf.wvu.edu) and MUCL to the '*Glomeromycota in vitro* collection' (GINCO; http://emma.agro.ucl.ac.be/ginco-bel/). Some additional identifiers are listed in Table S1. For analysis of the five AMF species included in the AFTOL (assembling the fungal tree of life) project (James *et al.*, 2006), the individual SSU, ITS and LSU sequences were assembled to a contiguous consensus sequence. For the 'extended dataset', analyses of the *Ambisporaceae*, *Diversisporaceae* and *Glomus* Group Aa additional public database sequences (Tables S2–S6) were included. Sequences probably derived from contaminants (Schüßler *et al.*, 2003) were excluded.

DNA extraction, PCR amplification, cloning and sequencing

Spores were cleaned and DNA was extracted as described in Schwarzott & Schüßler (2001). At first, PCR was performed with the primers SSU-Glom1 combined with NDL22 or LR4+2 (Stockinger *et al.*, 2009). Later, the PCR approach with AMF-specific primers described in Krüger *et al.* (2009) was used, for the majority of the AMF characterized (Table S1). Polymerase chain reactions with the Phusion High Fidelity DNA polymerase (Finnzymes, Espoo, Finland), cloning, restriction fragment length polymorphism (RFLP) analyses and sequencing were performed as described in Krüger *et al.* (2009), except for *Glomus caledonium* BEG20 which was amplified using a *Taq* DNA polymerase (Peqlab, Erlangen, Germany) and some clones that were obtained using the StrataClone Blunt PCR Cloning Kit (Stratagene Agilent Technologies, La Jolla, CA, USA). Sequences were assembled and proofread with SEQASSEM (http://www.sequentix.de) and deposited in the EMBL database with the accession numbers FN547474–FN547681.

Phylogenetic and sequence divergence analyses

The partial SSU, ITS region and the partial LSU sequences from this study and public database sequences covering the same regions were analysed (Table S1). Data were mainly from single-spore DNA extractions or single spore isolates of characterized AMF species. Shorter regions were separated either by the gene borders, or by primer binding sites. The fragments used for analyses were: the ITS region (400–526 bp) including the 5.8S and cut at the gene boundaries to the SSU and LSU; the LSU fragment (776–852 bp) covering the LSU until the binding site of primer LSUmBr (Krüger *et al.*, 2009); the ITS2 fragment (352–430 bp) corresponding to an ITS3–ITS4 (White *et al.*, 1990) amplicon including most of the 5.8S and the complete ITS2 region; the LSU-D1 fragment (281–394 bp) corresponding to a

portion bordered by the LR1 (van Tuinen *et al.*, 1998) and FLR3 (Gollotte *et al.*, 2004) priming sites (whereas FLR3 is a forward primer); the LSU-D2 fragment (370–436 bp) corresponding to an FLR3-LSUmBr amplicon (Fig. 1).

For some analyses, shorter or less well-defined sequences from the database were included and manually aligned to the core dataset with ALIGN (http://www.sequentix.de) or ARB (Ludwig *et al.*, 2004; http://www.arb-home.de). The resulting dataset is referred to as 'extended dataset'. Sequence divergences were calculated based on the K2P model (Kimura, 1980) with pairwise deletion of gaps, using the APE package of R (Paradis *et al.*, 2004). To illustrate the sequence divergences within and between species, TAXONGAP 2.3 (Slabbinck *et al.*, 2008) was used.

The analyses of database sequences included some identical sequences where, from the database entries, it could not be excluded that these possibly originated from different spores or cultures. Phylogenetic analyses were performed with PHYLIP 3.6 (Felsenstein, 2005) with neighbour joining tree-building based on K2P distances. A consensus tree was calculated from 1000-fold bootstrapped analyses with SUMTREES (Sukumaran & Holder, 2008). As an alternative approach, sequences were aligned automatically using the MAFFT online server (MAFFT version 6; http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) before phylogenetic analyses. The iterative refinement option of MAFFT was set to FFT-NS-i (Katoh *et al.*, 2002). Phylogenetic trees were processed with TREEGRAPH2 (treegraph.bioinfweb.info), TREEVIEWJ (Peterson & Colosimo, 2007) and TREEDYN (Chevenet *et al.*, 2006) and refined with Adobe Illustrator CS3.

Results

The phylum *Glomeromycota* presently contains 219 described species. Of these, 81 are available as cultures from the INVAM, BEG and GINCO collections. Only some of these are single-spore isolates and some may be misidentified. Many undescribed or unaffiliated AMF are also hosted in culture collections. In the present work, we analysed a core dataset represented by 28 characterized AMF species from three different orders, with a focus on close relatives. For the *Diversisporaceae*, five of the eight known species could be covered, whereas within the *Gigasporacea*e (*sensu* Morton & Benny, 1990) and the *Acaulosporaceae* five of the 45 and four of the 36 known species, respectively, were studied. For the *Pacisporaceae* (seven species; not available as cultured AMF), one species could be analysed from stored DNA extracts from the study of Walker *et al.* (2004). In the monogeneric *Glomerales* 11 of 102 described *Glomus* species and in the *Ambisporaceae* two of eight could be studied. Further well-defined sequences were used for some groups, such as the *Ambisporaceae* ITS region for five of the eight known species. In general, the availability of well-defined isolates is a major bottleneck for the study of many AMF taxa.

We did not test the AM1-NS31 SSU fragment, used in many environmental studies including a recent 454 GS-FLX sequencing approach (Öpik *et al.*, 2009), because the AM1 primer discriminates many AMF taxa and the amplified region lacks species resolution power.

Intraspecific rDNA sequence variation

No universal intraspecific percentage of sequence variation (K2P distance) could be defined as a threshold to separate AMF species. For the longest DNA fragment studied, SSUmCf-LSUmBr (*c.* 1500 bp, see Table S7, corresponding to the core dataset), the maximum intraspecific variation ranged from 0.47–10.8%. Considering only the seven species for which at least 24 sequence variants are available (*Acaulospora laevis*, *Gigaspora margarita*, *Gigaspora rosea*, *Scutellospora gilmorei*, *Glomus intraradices*, *Glomus* sp. '*irregulare*-like' DAOM197198 and *Glomus versiforme*) the minimum intraspecific variation was 1.55%. The highest value of 10.8% was found in *G. intraradices* (cultures FL208 and MUCL49410).

The ITS region revealed a variation of 0.23–14.6%, or 2.96–14.6% when analysing only the seven species with at least 24 variants of the SSUmCf-LSUmBr fragment available. *Glomus intraradices* (FL208 and MUCL49410) again showed the highest intraspecific variation. The range of variation in the LSU-D2 fragment was 0–15.7% (2.8–15.7% for species with at least 24 sequence variants known), again with *G. intraradices* showing the highest value.

For the LSU-D1 fragment (LR1-FLR3), five species lacked intraspecific variation (number of distinct sequences in parentheses): *Glomus* sp. WUM3 (6), *G. caledonium* (3), *Acaulospora scrobiculata* (4), *Glomus luteum* (5), *Diversispora celata* (3). In general, this region showed the lowest intraspecific variation for most species analysed, with one exception, *Kuklospora kentinensis* (14) where the ITS2 fragment (ITS3–ITS4) showed the lowest variation with only a single basepair insertion in some sequences. Further K2P distance data are shown in the Supporting Information Figs S1, S2.

Barcode gap analyses

A barcode gap is not a prerequisite for DNA barcoding, but may allow easy distinguishing of species (Hebert *et al.*, 2004). Barcode gaps could not be found for all AMF species studied. Comparison of the different regions, regardless of the alignment method used (Table S7, Fig. S1), showed the complete fragment (SSUmCf-LSUmBr) resulting in the lowest number (4) of species without a barcode gap, followed by the complete ITS region (5) and the LSU region

(7). Analysis of the LSU-D2 fragment also resulted in seven species lacking a barcode gap, whereas the LSU-D1 fragment revealed 12 species without a barcode gap. The ITS2 fragment (covering most of the 5.8S) resulted in eight species without a barcode gap. For the complete fragment, the size of the barcode gaps, if they existed, varied from only 0.1% to 22%. Some further analyses of the *Ambisporaceae* and *Diversisporaceae* are shown in Fig. S2.

Phylogenetic analyses of the core dataset

The *Gigasporaceae*, *Acaulosporaceae*, *Diversisporaceae*, *Ambisporaceae*, *Glomus* Group B, *Glomus* Group Aa and *Glomus* Group Ab were analysed separately, as the high variation in the ITS region made it impossible to align across family level groups. For each group, five defined regions covered by the SSUmCf-LSUmBr fragment were analysed (Fig. 1). All positions in the alignment were included in the neighbour joining analyses (Figs 2, S3–S8), as summarized in Table 1 for the core dataset (Figs 2, S3–S8).

The complete fragment (SSUmCf-LSUmBr) provided the best discriminatory power. Each of the analysed species was resolved with bootstrap support of at least 72%, for most species of > 90%. The AFTOL sequences of *Glomus mosseae* and *Scutellospora heterogama* cluster with those of the corresponding species. Sequences of *Glomus* sp. 'irregulare-like' DAOM197198 (= MUCL43194 = DAOM181602, used for the running *Glomus* genome sequencing project) and 'GINCO #4695rac-11G2' cluster with those of *Glomus irregulare*, and together are likely representing one species, confirming the evidence of Stockinger *et al.* (2009).

Almost all species could be separated using the complete ITS region, except *G. intraradices* and its close relatives. The same situation was reported for maximum likelihood analyses of this region (Stockinger *et al.*, 2009) and holds true for analyses of the LSU region only. Using the LSU, *Scutellospora spinosissima* (three sequences) and *Glomus proliferum* (15 sequences) neither were resolved as monophyletic and the *Gigaspora rosea* clade (27 sequences) had bootstrap support below 50%. When the ITS2, LSU-D1 and LSU-D2 fragments were analysed separately, the LSU-D1 fragment performed worst with sequences from 11 of the 25 species not forming monophyletic clades. The ITS2 and LSU-D2 fragments performed better, but still did not separate *G. proliferum* (15 sequences) from *G. intraradices* (47 sequences). *Gigaspora margarita* BEG34 did not form a well-supported clade for either fragment. As for the 800 bp LSU, *S. spinosissima* (three sequences) was not resolved in the LSU-D2 analysis.

Although not included in the CBOL standards or recommendations, a BLAST approach was tested in addition to the phylogenetic analyses. We used the BLASTN default settings of NCBI in both, public database and local BLAST searches, and studied all SSUmCf-LSUmBr fragment sequences for their correct identification. This alternative approach always resulted in first hits corresponding to the correct species (data not shown).

Phylogenetic analyses of the extended dataset

Shorter sequences from the public database, selected according to their assigned name or culture identifier, were included in some analyses. In addition, some environmental sequences were used, predominantly from the *Ambisporaceae*, *Diversisporaceae* and *Glomus* Group Aa.

Analyses of *Ambisporaceae* Only two *Ambisporaceae* species SSUmCf-LSUmBr fragments were available (Table S7, Fig. S1), but five ITS regions and several environmental sequences of *Ambispora* species could be analysed. All were phylogenetically well separated (Fig. S9). The environmental sequences (number in parentheses) from *Taxus baccata* (6), *Prunus africana* (1) or *Plantago lanceolata* (1) roots form branches distant from the characterized species.

Analyses of *Diversisporaceae* The ITS analyses of the *Diversisporaceae* (Fig. S10) did not reveal any fundamental differences from the analyses of the core dataset (Fig. S7). At this point, we draw attention to the fact that several *Glomus* species have not yet been formally transferred to the genus *Diversispora* and therefore carry the 'wrong' genus name. The four ITS database sequences from the INVAM cultures AZ237B from Arizona together with the four sequences of NB101 from Namibia are most likely of conspecific origin. Also, a set of 30 environmental ITS sequences annotated as *G. versiforme* in the database, cluster separately from *G. versiforme* BEG47 and should be annotated as unknown *Diversispora* species. It was already known that *Glomus fulvum* (five sequences), *Glomus megalocarpum* (2) and *Glomus pulvinatum* (2) form a clade much apart from other *Diversisporaceae* species and together probably represent a distinct genus (Redecker *et al.*, 2007).

For the LSU analyses (Fig. S11), the four database sequences (AM947664,65, AY842573,74) from *G. versiforme* BEG47 clustered with the 25 sequences of our BEG47 core dataset sequences, but the sequence EU346868 from a *G. versiforme* culture HDAM-4 was widely separated. All database sequences (EF067886-88) referring to *Glomus eburneum* INVAM AZ420A as well as *D. celata* (Gamper *et al.*, 2009) clustered with those of the respective species in our core dataset. Three *Glomus aurantium* LSU database sequences (EF581860,62,63) are separated from two other sequences (EF581861,64). All five sequences are linked to voucher W4728 and originate from one trap culture setup with material collected near Tel Aviv in Israel (J. Błaszkowski, pers. comm. 21 September, 2009). As trap cultures usually contain several species, it is not certain that

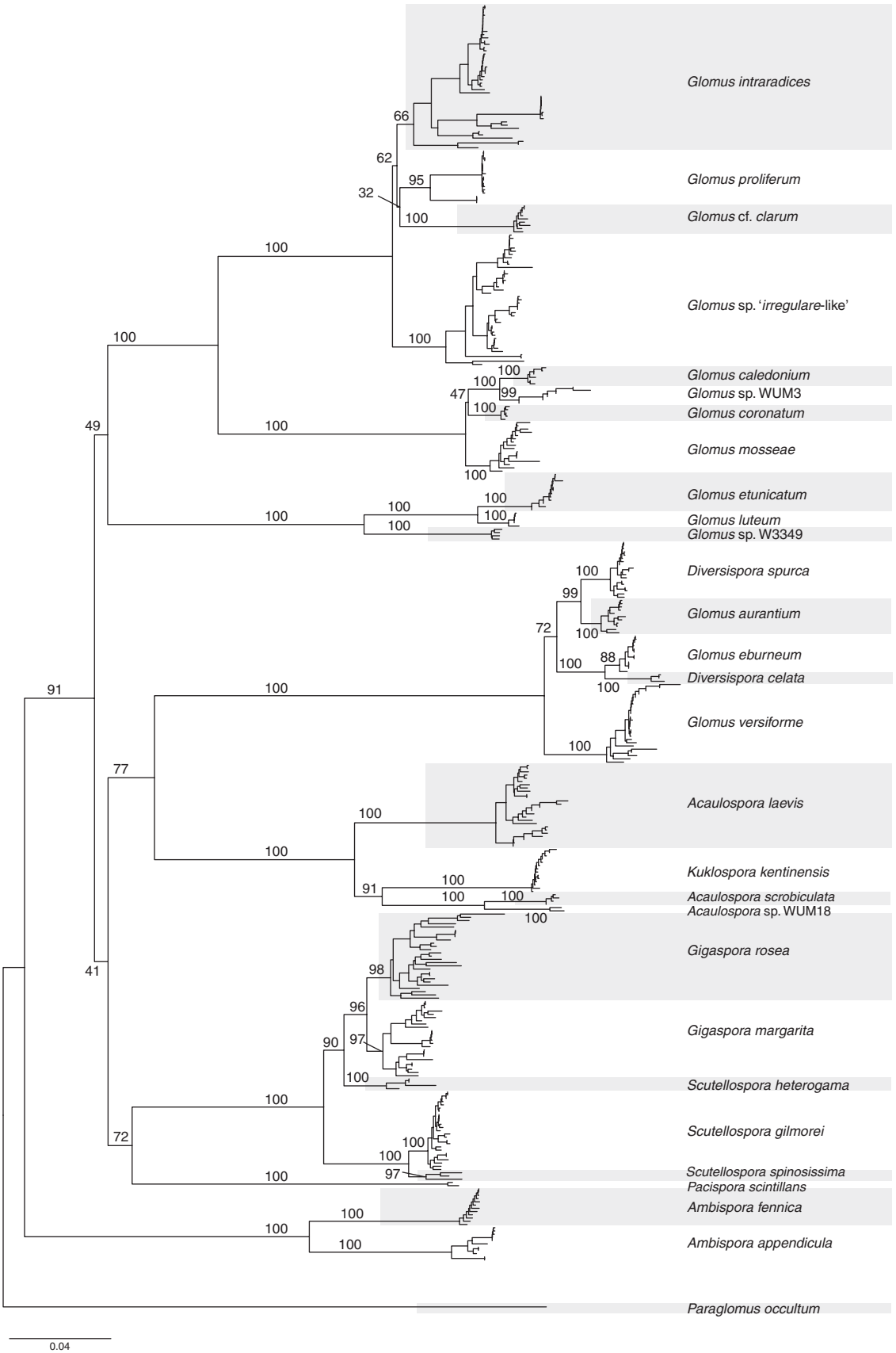

Fig. 2 Phylogenetic tree computed from all *c*. 1500 bp SSUmCf-LSUmBr fragment sequences analysed (core dataset), demonstrating species level resolution. Neighbour joining analyses (1000 bootstraps) with bootstrap (BS) support displayed down to the level of species. Note that the BS support values differ from those given in Table 1, because an unambiguous alignment of internal transcribed spacer 1 (ITS1) and ITS2 sequences between families, as computed here, is impossible. Therefore, the BS values shown here are biased by ambiguously aligned sites in the highly variable regions and for species level comparison the values from Table 1 should be referred to. The corresponding species is written to the right of each cluster; every second cluster is highlighted in grey.

the sequences in the subclades were derived from conspecific organisms.

Analyses of *Glomus* Group Aa ('*Glomus mosseae* group') Analysis of our core dataset of this group showed clear separation of species with the ITS region, the ITS2 fragment, and both LSU fragments analysed. However, the situation changed when including database sequences for the 'extended dataset' (see Figs 3, S4).

For the ITS region, *Glomus* sp. WUM3 (six sequences), *G. caledonium* (10 sequences) and *Glomus geosporum* (31 sequences) formed well-separated clades. *Glomus mosseae* sequences formed two well supported subclades (Fig. 3), which were rendered paraphyletic by the clustering of the ex-type of *Glomus coronatum* BEG28 (16 sequences) in between. However, the minor *G. mosseae* clade (only seven sequences) consists exclusively of sequences derived from field sampled spores with identifiers GMO2 and GMO3. From spore GMO2 one sequence (AF161058) clusters in the minor clade while the other entire ones (AF161055-57, AF166276) cluster within the major clade.

The ITS sequences in *Glomus* Group Aa reveal more discrepancies. *Glomus monosporum* (IT102: AF004689; FR115: AF004690, AF125195), *Glomus dimorphicum* (BEG59: X96838-41) and '*Glomus fasciculatum*' BEG58 (X96842,43; but see following text) sequences cluster in the major *G. mosseae* clade.

For the *G. mosseae* major clade (excluding the GMO2 and GMO3 sequences), the intraspecific variation of the complete ITS region is 12.1% (100 sequences). When

Table 1 Respective bootstrap values supporting species as monophyletic after neighbour joining analyses (based on K2P distances, 1000 bootstraps) of six different regions (complete SSUmCf-LSUmBr fragment, complete internal transcribed spacer (ITS) region, ITS2, large subunit (LSU), LSU-D1 and LSU-D2 fragments)

	SSUmCf-LSUmBr	ITS region	LSU	ITS2 (ITS3-ITS4)	LSU-D1 (LR1-FLR3)	LSU-D2 (FLR3-LSUmBr)
Gigaspora margarita	88	75	55	47		34
Gigaspora rosea	100	90	48	90		59
Scutellospora gilmorei	100	99	88	93		69
Scutellospora spinosissima	92	98		95		
Scutellospora heterogama	100	99	100	100	97	98
Length of alignment (positions)	**1505**	**468**	**795**	**394**	**398**	**376**
Acaulospora laevis	100	100	100	100	100	100
Acaulospora scrobiculata	100	100	100	100	100	100
Acaulospora sp. WUM18	100	100	100	100	100	100
Kuklospora kentinensis	100	100	100	100	100	100
Length of alignment (positions)	**1591**	**525**	**826**	**436**	**403**	**401**
Diversispora celata	100	95	100	70	99	100
Diversispora spurca	100	96	100	97		100
Glomus aurantium	100	94	94	95		94
Glomus eburneum	100	75	100	72	99	93
Glomus versiforme	100	100	100	100	100	100
Length of alignment (positions)	**1600**	**497**	**860**	**407**	**398**	**440**
Glomus cf. *clarum*	100	100	100	100	100	100
Glomus intraradices	72					
Glomus sp. '*irregulare*-like'	100	96	99	53		95
Glomus proliferum	94	80				
Length of alignment (positions)	**1644**	**540**	**863**	**437**	**400**	**440**
Glomus mosseae	100	97	100	93	98	99
Glomus sp. WUM3	100	97	100	98		100
Glomus caledonium	100	100	96	99		97
Glomus coronatum	100	100	100	100	99	99
Length of alignment (positions)	**1664**	**565**	**862**	**448**	**397**	**442**
Glomus etunicatum	100	99	100	90	96	100
Glomus sp. W3349	100	100	100	100	100	100
Glomus luteum	100	100	100	100	96	93
Length of alignment (positions)	1624	539	843	433	392	430

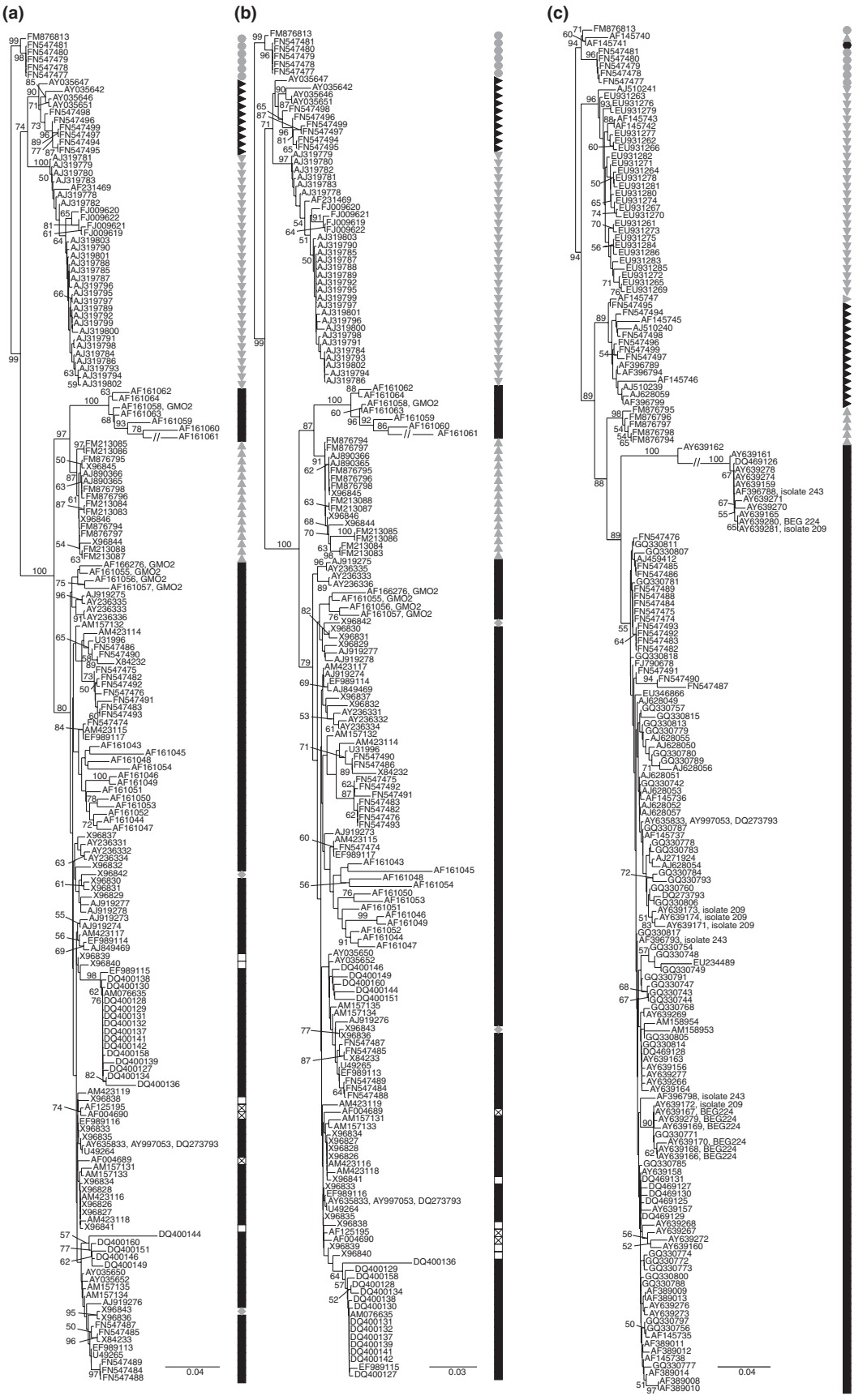

Fig. 3 Internal transcribed spacer (ITS) region (a), ITS2 fragment (b) and the large subunit (LSU)-D2 fragment (c) neighbour joining analyses (1000 bootstraps) of *Glomus* Group Aa. Analysis (c) is performed with a different dataset than (a) and (b) (for details see the Supporting Information, Tables S5, S6). Some long branches were reduced in length to 50% (//). 'AY635833, AY997053, DQ273793' represents the consensus sequences of these sequences. *Glomus mosseae* (closed square), *Glomus* sp. WUM3 (grey circle), *Glomus coronatum* (grey triangle, apex up), *Glomus caledonium* (black triangle, apex right), *Glomus monosporum* (open square with cross), *Glomus fasciculatum* (diamond), *Glomus geosporum* (grey triangle, apex down), *Glomus dimorphicum* (open square), *Glomus constrictum* (black circle), *Glomus fragilistratum* (grey triangle, apex right).

adding the *G. monosporum, G. fasciculatum* BEG58 and *G. dimorphicum* sequences clustering in this clade the variation increased only marginally to 12.2% (109 sequences). The intraspecific variation of the other characterized species within *Glomus* Group Aa varied between 0.8 and 2.8%.

The LSU-D2 fragment analysis resulted in clear separation into several well-supported clades (Fig. 3), but some contain sequences from more than one species. One *Glomus fragilistratum* sequence clusters within the *G. caledonium* clade. One *G. coronatum* BEG49 sequence is distant from those of the ex-type culture *G. coronatum* BEG28 (=Att108). BEG49 clusters with *Glomus* sp. WUM3, but a *Glomus constrictum* BEG130 sequence also falls in this clade. The intraspecific variation of the LSU-D2 fragment is 19.4% (170 sequences). The major *G. mosseae* clade had a variation of 15.8% (158 sequences) and the smaller clade of 11.2% (12 sequences). The other species in this group showed an intraspecific variation between 1.2–5.0% (5–28 sequences, respectively).

Discussion

In this study, we analysed several regions of the nuclear rDNA region as possible candidates for DNA barcoding of AMF, including the ITS region which is widely used for identification of fungi. Because it was demonstrated that the ITS region alone is unsuitable to resolve closely related AMF species (Stockinger *et al.*, 2009), whereas a longer, 1500 bp fragment could be successfully applied, we used this longer rDNA fragment as a baseline. Moreover, *c.* 400 bp fragments were analysed for their power to resolve species and suitability for community analyses using the 454 GS-FLX Titanium pyrosequencing method (Valentini *et al.*, 2009).

Intraspecific rDNA variation and its definition

In the present study, we calculated intrasporal and intraspecific rDNA variability for several species. However, the determination of species in the *Glomeromycota* is largely based on a morphological species concept and the apparent asexual lifestyle may complicate the interpretation of species borders, though asexual speciation is found in diverse organism groups. For AMF, perhaps the best-studied clade, *Glomus* Group Ab, may exemplify the problems. A very high intraspecific variation was found in *G. intraradices* (Stockinger *et al.*, 2009). This was characterized from two isolates and the parent culture of one of the isolates (the 'ex-type culture' of this species, FL208, derived from a root trap culture). The 1500 bp rDNA from a single spore, interestingly, roughly encompassed the amount rDNA variation and moreover also the pattern of sequence types found in the entirety of samples analysed, which were derived from two isolates and the FL208 culture. Both isolates originated from the same field site, but from material sampled 20 yr apart. The results raise questions such as whether one AMF spore contains most of the existing intraspecific rDNA variation, or whether the similarity in the sequence type patterns reflects, for example, the sampling of two recent descendents of a clonal lineage. These are open questions, but the closely related '*G. irregulare*-clade' (likely representing a single species) contains a huge number of sequences derived from diverse ecosystems and many continents. *Glomus intraradices* sequences have never been detected in these ecosystems, but are up to now only known from *Citrus* sp. in Florida. We interpret these data as most likely reflecting a biologically meaningful genetic separation of different organisms. Although we can currently separate all morphospecies studied, and take this as support for the applicability of DNA barcoding for AMF, it must be noted that the species concept used to define these asexual organisms may change.

The intraspecific and intrasporal variation varied considerably among the studied AMF, for all regions analysed (Figs S1, S2). Here, we followed the CBOL barcoding standards (http://www.barcoding.si.edu) and used K2P distances. We stress this because the numbers for sequence variation differ significantly, depending on the method used for estimation; for example, the *G. intraradices* ITS region (47 sequences) 14.6% K2P distances correspond to > 23% uncorrected distances including gaps as a fifth character (Stockinger *et al.*, 2009). Similarly high K2P distances occur for the ITS region of *G. mosseae* (12.2%, 109 sequences). The intrasporal ITS variation we found in the *G. mosseae* sequences was 4.6% (16 sequences) and only slightly increased to 5.3% when adding 45 database sequences from cultures with geographically widespread origin published in Avio *et al.* (2009). An example for high ITS variation is *G. fulvum* (*Diversisporaceae*), where the addition of one sequence raises the variability from < 10% to 15% (five sequences in total). The 'outlier' sequence is derived from a different geographical location and might also represent a closely related, but distinct species.

In general, for AMF the simple use of a percentage variation value as threshold to define and cluster molecular operational taxonomic units (MOTUs) for species identification must be considered inapplicable.

Barcode gap and phylogenetic analyses

The comparison of the maximum intraspecific and the minimum interspecific variation revealed that none of the studied DNA fragments allowed absolute AMF species separation by barcode gap analyses. Evidently, when based on the rDNA regions studied, this method cannot be applied to AMF. In general, barcode gaps may often be an artefact of insufficient taxon sampling (Wiemers & Fiedler, 2007). The likely existence of a large number of undescribed and uncharacterized species (Sýkorová *et al.*, 2007; Öpik *et al.*, 2009) adds further complexity to the topic. Moreover, there are several inaccurate species determinations in the public sequence databases and contaminant sequences cannot be ruled out when using spores from mixed species cultures (Schüßler *et al.*, 2003). Examples of inconsistencies are *G. fasciculatum* BEG53 and BEG58 sequences that cluster in *Glomus* Group Ab and in *Glomus* Group Aa, respectively. Morphologically interpreted, it is very unlikely that the BEG58 sequences belong to *G. fasciculatum* (Lloyd-Macglip *et al.*, 1996).

DNA barcode-based identification of species can also be derived from phylogenetic inference. The simple neighbour joining analysis based on K2P distances of the complete fragment (SSUmCf-LSUmBr) resulted in support for all species investigated here. It allowed a distinction between all closely related species in *Glomus* Group Ab. The species concept in this difficult group is also supported by the fact that the mitochondrial LSU rDNA as a marker (Börstler *et al.*, 2008) distinguishes *G. intraradices* from the genome sequenced *Glomus* species DAOM197198 that is represented by the '*G. irregulare* clade'.

For the 1500 bp fragment BLAST searches performed well and could be an alternative tool for identification, but this may be problematic for unknown species. It should be kept in mind that similarity-based comparisons can be misleading and phylogenetic methods generally perform better. Therefore, we recommend a phylogenetic approach, but BLAST surely is an alternative for fast data screening or to select sequences to be analysed more in detail.

The ITS region

The ITS region resolved many of the known species, but not the closely related members within *Glomus* Groups Ab and Aa, respectively. However, the ITS region was suited to resolve relatively closely related species in the *Ambisporaceae* (Walker *et al.*, 2007), and also shows, for example, that a set of environmental ITS sequences labelled as *G. versiforme* does not cluster with those of *G. versiforme* BEG47 and probably represent distinct species. The ITS region might be useful for species delineation, but with some limitations.

Other problems with species resolution might be caused by synonyms. For example, in *Glomus* Group Aa several sequences with uncertain assignment to species are from *G. dimorphicum* and *G. monosporum*, which were, on morphological grounds, discussed as possibly conspecific with *G. mosseae* (Walker, 1992). However, the difficulties might also result from the use of mixed species cultures. The fungus identified as *G. monosporum* INVAM FR115 was in a culture that also contained spores of *G. mosseae* and *Paraglomus occultum* (http://invam.caf.wvu.edu/cultures/accessionculturedetails.cfm?ID=6356, 12.02.2010). The *G. monosporum* culture INVAM IT102 also contained *G. mosseae* and *Glomus etunicatum* spores (from http://invam.caf.wvu.edu/cultures/accessionculturedetails.cfm?ID=6895, 12 Feb 2010). It can therefore not be ruled out that the spores identified as *G. mosseae* and *G. monosporum* are of conspecific origin, or that contaminant sequences gave rise to incorrect assignation.

The *G. mosseae* ITS sequences formed two distinct clades, with the minor clade consisting only of sequences from two field sampled spores (GMO2 and GMO3). As already discussed in Antoniolli *et al.* (2000) spore GMO3 could be an unidentified species, and the 'outlier' sequence AF161058 from spore GMO2 might be interpreted as a contaminant originating from GMO3. Currently, when including the database ITS sequences, it seems impossible to state whether the *G. mosseae* clade consists of one species or several species that cannot be separated or have been misdetermined. Analysing the complete fragment (SSUmCf-LSUmBr) for more and well-defined isolates may solve such questions.

The LSU region

Using the 800 bp LSU region of the core dataset resulted in more unresolved species than using the ITS region, but the LSU-D2 region alone showed about the same species resolution power as the ITS region. The LSU-D1 fragment behaved worst with both extended and core datasets. It seems unsuited for obtaining good resolution and this may explain why the 800 bp LSU region resolution is not better than that of the shorter LSU-D2. The *G. mosseae* sequences analysed by Rosendahl *et al.* (2009), from cultures with geographically widespread origin, all fell into the main *G. mosseae* LSU subclade (Fig. 3, lower clade). The authors proposed, based on the genetic variability found in the LSU and in *FOX2* and *TOR* gene introns, that these cultures are closely related and the panglobal distribution likely was caused by anthropogenic dispersal. It should also be mentioned that three single-spore isolates (HG isolate 209, BEG224, JJ isolate 243) each gave rise to divergent

sequence variants located in both *G. mosseae* LSU subclades. This indicates that the rDNA variation reported in some other studies is an underestimate, caused by a lack of detection of less frequent sequence types (represented by the upper LSU-D2 subclade in Fig. 3).

DNA fragments for deep sequencing technologies

The 454 GS-FLX Titanium pyrosequencing technology currently allows an average read length of *c.* 350–450 bp and offers great potential for ecological studies. Our data demonstrate that a read length of 400 bp will not be sufficient to identify all AMF species with certainty, based on neighbour joining analyses using such a short fragment only. However, there are alternative phylogenetic approaches that may overcome this lack of resolution when taking an alignment based on longer sequences as a 'backbone' for the phylogenetic inference. For example, the program RAXML 7.2.6 (http://arxiv.org/abs/0911.2852v1; Stamatakis *et al.*, 2010) includes a novel likelihood-based algorithm for evolutionary placement of short reads into a given reference tree of full length sequences. We show the LSU-D2 and ITS2 fragments to be good candidates for species identification by 454 pyrosequencing. The LSU-D2 region may be preferred if AMF sequences are specifically amplified from roots or soil (Krüger *et al.*, 2009). In studies where the diversity of other groups of fungi is also investigated, the ITS2 fragment is a good alternative and can be amplified with established primers for fungi. Although most such published ITS and LSU region primers do not match all AMF sequence variants, many do not strictly discriminate AMF taxa, as they match at least 50% of the known intraspecific sequence variants. These primers are ITS1 (White *et al.*, 1990) with a ratio of total number of sequences analysed : total mismatches : 3′-end mismatches in the last four sites of 1250 : 56 : 5, ITS4 with 1271 : 23 : 5, ITS5 (White *et al.*, 1990) with 1217 : 36 : 4, LR3 (http://www.biology.duke.edu/fungi/mycolab/primers.htm) with 929 : 24 : 15 and ITS1F (Gardes & Bruns, 1993) with 1250 : 75 : 4. ITS1F shows mismatches to a number of AMF, such as most *Ambispora* species, some *Glomus* species, *Scutellospora projecturata* and many members of the *Diversisporaceae* and *Acaulosporaceae*, but at positions that should not hamper amplification if PCR conditions are not too stringent. Conversely, the following primers must be interpreted as not suited to amplify all AMF: the LSU forward primer FLR3 (1239 : 128 : 64) discriminates, for example some *Scutellospora* and *Paraglomus* species; ITS3 (1219 : 604 : 577) mismatches at the 3′-end to most *Glomus* Group Ab, *Ambisporaceae* and an unidentified *Acaulospora* species. Moreover, it has up to five 5′-end mismatches to the *Geosiphon pyriformis* sequences.

New developments in 454 pyrosequencing methods will soon allow a read length of 1000 bp. For this, new primers could be designed targeting a fragment consisting of the ITS2-LSU region (complete ITS2 and LSU until primer LSUmBr), with a length of *c.* 960–1117 bp. This fragment allowed resolution of all species investigated by NJ analyses (data not shown), although with lower bootstrap support when compared with the 1500 bp fragment.

Conclusion

We have shown that barcode gap analyses based on the rDNA regions are not suited for AMF barcoding. The intraspecific variation seems heterogeneous and exceptionally high in some groups. Phylogenetic analyses of the *c.* 1500 bp SSUmCf-LSUmBr rDNA fragment distinguished all species investigated, whereas shorter rDNA fragments did not allow a separation of very closely related species. The LSU-D2 and ITS2 fragments appear most suitable for high-throughput 454 GS-FLX Titanium pyrosequencing technology with 400 bp read length,

However, in addition to methodological aspects, species recognition is mainly hampered by the lack of a comprehensive and accurate baseline dataset and accessibility of biological material. To overcome this and to avoid problems using mixed or cross-contaminated cultures it would be desirable to establish, provide and use single-spore isolates. Many open questions could be answered by studying more defined cultures and isolates, or sometimes by more in-depth characterization of field material. Surprisingly, for many very recently described AMF species no biological material seems to be available at all, except for the voucher that is needed for the formal description. Consequently these species are not available from culture collections, making any proof or improvement of concepts very difficult.

From the molecular biological point of view, the use of proof reading polymerases under optimal PCR conditions is highly recommended, as it considerably reduces PCR errors and sequence chimaera, as discussed in Lahr & Katz (2009) for example, although it should be noted that the Phusion-PCR conditions used in that paper are unsuitable (see http://www.finnzymes.com). To mark errors in the public databases, a third party annotation facility in GenBank (as proposed by many mycologists, such as Bidartondo *et al.*, 2008) would help, but unfortunately is not allowed. Therefore, curated databases such as UNITE currently seem to be the only option to provide reliable data.

For future analyses, a 'quantitative world of community analysis' beyond the current limit of 400 bp read length will be feasible, as 1000 bp 454-reads are possible (http://www.454.com) and new high throughput (and possibly low-cost) sequencing technologies may allow even longer reads, soon (e.g. Pacific Biosciences, http://www.pacificbiosciences.com; Eid *et al.*, 2009). This may be taken

as another argument in favour of using longer DNA barcodes for better species resolution, as suggested here.

As a baseline for *Glomeromycota* DNA barcoding, we propose the sequencing of variants of the easily PCR amplifiable SSUmCf-LSUmBr 1500 bp fragment. We also recommend that such a molecular characterization should be included in AMF species descriptions whenever possible. The sequence data will be very important for future molecular ecological studies of AMF–plant associations and preferences in the field, which are still mostly hidden.

Acknowledgements

The grant for H.S. was funded by the Marie Curie Early Stage Research Training Fellowship of the European Community's Sixth Framework Programme (MEST-CT-2005-021016, 'TRACEAM'). The grants for M.K. and A.S. were financed by the German Research Foundation (DFG). Thanks to all who supplied samples. We thank Chris Walker for discussion and proofreading of the manuscript.

References

Agerer R, Ammirati J, Blanz P, Courtecuisse R, Desjardin DE, Gams W, Hallenberg N, Halling R, Hawksworth DL, Horak E *et al.* 2000. Always deposit vouchers. *Mycological Research* **104**: 642–644.

Antoniolli Z, Schachtman D, Ophel-Keller K, Smith S. 2000. Variation in rDNA ITS sequences in *Glomus mosseae* and *Gigaspora margarita* spores from a permanent pasture. *Mycological Research* **104**: 708–715.

Avio L, Cristani C, Strani P, Giovannetti M. 2009. Genetic and phenotypic diversity of geographically different isolates of *Glomus mosseae*. *Canadian Journal of Microbiology* **55**: 242–253.

Bidartondo MI, Bruns TD, Blackwell M, Edwards I, Taylor AFS, Horton T, Zhang N, Kõljalg U, May G, Kuyper TW *et al.* 2008. Preserving accuracy in GenBank. *Science* **319**: 1616.

Börstler B, Raab PA, Thiéry O, Morton JB, Redecker D. 2008. Genetic diversity of the arbuscular mycorrhizal fungus *Glomus intraradices* as determined by mitochondrial large subunit rRNA gene sequences is considerably higher than previously expected. *New Phytologist* **180**: 452–465.

Brundrett M. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* **320**: 37–77.

Chase MW, Fay MF. 2009. Barcoding of plants and fungi. *Science* **325**: 682–683.

Chevenet F, Brun C, Banuls A, Jacq B, Christen R. 2006. TreeDyn: towards dynamic graphics and annotations for analyses of trees. *BMC Bioinformatics* **7**: 439.

Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B *et al.* 2009. Real-Time DNA sequencing from single polymerase molecules. *Science* **323**: 133–138.

Felsenstein J. 2005. *PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author*. Department of Genome Sciences, University of Washington, Seattle, WA, USA.

Frézal L, Leblois R. 2008. Four years of DNA barcoding: current advances and prospects. *Infection, Genetics and Evolution* **8**: 727–736.

Gamper HA, Walker C, Schüßler A. 2009. *Diversispora celata* sp. nov: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. *New Phytologist* **182**: 495–506.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for *Basidiomycetes* – application to the identification of mycorrhizae and rusts. *Molecular Ecology Notes* **2**: 113–118.

Geiser DM, Klich M, Frisvad J, Peterson S, Varga J, Samson R. 2007. The current status of species recognition and identification in *Aspergillus*. *Studies in Mycology* **59**: 1–10.

Gilmore SR, Gräfenhahn T, Louis-Seize G, Seifert KA. 2009. Multiple copies of cytochrome oxidase 1 in species of the fungal genus *Fusarium*. *Molecular Ecology Resources* **9**: 90–98.

Gollotte A, van Tuinen D, Atkinson D. 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza* **14**: 111–117.

Hawksworth DL. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* **105**: 1422–1432.

Hebert PD, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of birds through DNA barcodes. *PLoS Biology* **2**: e312.

Helgason T, Fitter AH, Young JPW. 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. *Molecular Ecology* **8**: 659–666.

Hempel S, Renker C, Buscot F. 2007. Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. *Environmental Microbiology* **9**: 1930–1938.

Hollingsworth PM, Forrest LL, Spouge JL, Hajibabaei M, Ratnasingham S, van der Bank M, Chase MW, Cowan RS, Erickson DL, Fazekas AJ *et al.* 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences, USA* **106**: 12794–12797.

James TY, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E *et al.* 2006. Reconstructing the early evolution of *Fungi* using a six-gene phylogeny. *Nature* **443**: 818–822.

Katoh K, Misawa K, Kuma KI, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111–120.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. *Dictionary of the fungi, 10th edn*. Wallingford, UK: CABI.

Kõljalg U, Larsson K, Abarenkov K, Nilsson R, Alexander I, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E *et al.* 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist* **166**: 1063–1068.

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist* **183**: 212–223.

Lahr DJG, Katz LA. 2009. Reducing the impact of PCR-mediated recombination in molecular evolution and environmental studies using a new-generation high-fidelity DNA polymerase. *BioTechniques* **47**: 857–866.

Lang BF, Hijri M. 2009. The complete *Glomus intraradices* mitochondrial genome sequence – a milestone in mycorrhizal research. *New Phytologist* **183**: 3–6.

Lee J, Lee S, Young JPW. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology* **65**: 339–349.

Lee J, Young JPW. 2009. The mitochondrial genome sequence of the arbuscular mycorrhizal fungus *Glomus intraradices* isolate 494 and implications for the phylogenetic placement of *Glomus*. *New Phytologist* **183**: 200–211.

Lloyd-Macglip SA, Chambers SM, Dodd J, Fitter AH, Walker C, Young JW. 1996. Diversity of the ribosomal internal transcribed spacers within and among isolates of *Glomus mosseae* and related mycorrhizal fungi. *New Phytologist* **133**: 103–111.

Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar AB, Buchner A, Lai T, Steppi S, Jobb G *et al.* 2004. ARB: a software environment for sequence data. *Nucleic Acids Research* **32**: 1363–1371.

Morton JB, Benny G. 1990. Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of *Glomaceae*. *Mycotaxon* **37**: 471–491.

Msiska Z, Morton J. 2009. Phylogenetic analysis of the Glomeromycota by partial β-tubulin gene sequences. *Mycorrhiza* **19**: 1432–1890.

Naumann M, Schüßler A, Bonfante P. 2010. The obligate endobacteria of arbuscular mycorrhizal fungi are ancient heritable components related to the Mollicutes. *The ISME Journal*, doi:10.1038/ismej.2010.21.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. 2005. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**: 5544–5550.

Oehl F, Sieverding E, Ineichen K, Mäder P, Wiemken A, Boller T. 2009. Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. *Agriculture, Ecosystems and Environment* **134**: 257–268.

Öpik M, Metsis M, Daniell TJ, Zobel M, Moora M. 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist* **184**: 424–437.

Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**: 289–290.

Peterson MW, Colosimo ME. 2007. TREEVIEWJ: an application for viewing and analyzing phylogenetic trees. *Source Code for Biology and Medicine* **2**: 7.

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel G, van Tuinen D. 2007. *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytologist* **176**: 197–210.

Redecker D, Morton JB, Bruns TD. 2000. Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). *Molecular Phylogenetics and Evolution* **14**: 276–284.

Redecker D, Raab PA, Oehl F, Camacho FJ, Court R. 2007. A novel clade of sporocarp-forming species of glomeromycotan fungi in the *Diversisporales* lineage. *Mycological Progress* **6**: 35–44.

Renker C, Heinrichs J, Kaldorf M, Buscot F. 2003. Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. *Mycorrhiza* **13**: 191–198.

Robinson-Boyer L, Grzyb I, Jeffries P. 2009. Shifting the balance from qualitative to quantitative analysis of arbuscular mycorrhizal communities in field soils. *Fungal Ecology* **2**: 1–9.

Rosendahl S, McGee P, Morton JB. 2009. Lack of global population genetic differentiation in the arbuscular mycorrhizal fungus *Glomus mosseae* suggests a recent range expansion which may have coincided with the spread of agriculture. *Molecular Ecology* **18**: 4316–4329.

Ryberg M, Nilsson RH, Kristiansson E, Topel M, Jacobsson S, Larsson E. 2008. Mining metadata from unidentified ITS sequences in GenBank: a case study in *Inocybe* (Basidiomycota). *BMC Evolutionary Biology* **8**: 50.

Sanders IR. 2004. Plant and arbuscular mycorrhizal fungal diversity: are we looking at the relevant levels of diversity and are we using the right techniques? *New Phytologist* **164**: 415–418.

Sawaki H, Sugawara K, Saito M. 1998. Phylogenetic position of an arbuscular mycorrhizal fungus, *Acaulospora gerdemannii*, and its synanamorph *Glomus leptotichum*, based upon 18S rRNA gene sequence. *Mycoscience* **39**: 477–480.

Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.

Schüßler A, Schwarzott D, Walker C. 2003. Glomeromycota rRNA genes – the diversity of myths? *Mycorrhiza* **13**: 233–236.

Schwarzott D, Schüßler A. 2001. A simple and reliable method for SSU rRNA gene DNA extraction, amplification, and cloning from single AM fungal spores. *Mycorrhiza* **10**: 203–207.

Seifert KA. 2009. Progress towards DNA barcoding of fungi. *Molecular Ecology Resources* **9**: 83–89.

Seifert KA, Samson RA, deWaard JR, Houbraken J, Levesque C, Moncalvo JM, Louis-Seize G, Hebert PD. 2007. Prospects for fungus identification using CO1 DNA barcodes, with *Penicillium* as a test case. *Proceedings of the National Academy of Sciences, USA* **104**: 3901–3906.

Slabbinck B, Dawyndt P, Martens M, De Vos P, De Baets B. 2008. TaxonGap: a visualization tool for intra- and inter-species variation among individual biomarkers. *Bioinformatics* **24**: 866–867.

Stamatakis A, Komornik Z, Berger SA. 2010. *Evolutionary placement of short sequence reads on multi-core architectures*. Accepted for publication at 8th ACS/IEEE International Conference on Computer Systems and Applications (AICCSA-10), Hammamet, Tunisia, May 2010.

Stockinger H, Walker C, Schüßler A. 2009. '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. *New Phytologist* **183**: 1176–1187.

Stukenbrock EH, Rosendahl S. 2005. Clonal diversity and population genetic structure of arbuscular mycorrhizal fungi (*Glomus* spp.) studied by multilocus genotyping of single spores. *Molecular Ecology* **14**: 743–752.

Sukumaran J, Holder MT. 2008. SumTrees: summarization of split support on phylogenetic trees. Version 1.0.2. Part of the DendroPy Phylogenetic Computation Library Version 2.1.3 (http://pypi.python.org/pypi/DendroPy).

Summerbell RC, Moore MK, Starink-Willemse M, Van Iperen A. 2007. ITS barcodes for *Trichophyton tonsurans* and *T. equinum*. *Medical Mycology* **45**: 193–200.

Sýkorová Z, Wiemken A, Redecker D. 2007. Cooccurring *Gentiana verna* and *Gentiana acaulis* and their neighboring plants in two swiss upper montane meadows harbor distinct arbuscular mycorrhizal fungal communities. *Applied and Environmental Microbiology* **73**: 5426–5434.

van Tuinen D, Zhao B, Gianinazzi-Pearson V. 1998. *PCR in studies of AM fungi: from primers to application. Mycorrhiza manual*. Berlin, Heidelberg, Germany: Springer, 387–400.

Valentini A, Pompanon F, Taberlet P. 2009. DNA barcoding for ecologists. *Trends in Ecology and Evolution* **24**: 110–117.

Vaughn JC, Mason MT, Sper-Whitis GL, Kuhlman P, Palmer JD. 1995. Fungal origin by horizontal transfer of a plant mitochondrial group I intron in the chimeric COXI gene of *Peperomia*. *Journal of Molecular Evolution* **41**: 563–572.

Walker C. 1992. Systematics and taxonomy of the arbuscular endomycorrhizal fungi (*Glomales*) – a possible way forward. *Agronomie* **12**: 887–897.

Walker C, Blaszkowski J, Schwarzott D, Schussler A. 2004. *Gerdemannia* gen. nov., a genus separated from *Glomus*, and *Gerdemanniaceae* fam. nov., a new family in the *Glomeromycota*. *Mycological Research* **108**: 707–718.

Walker C, Mize CW, McNabb HS. 1982. Populations of endogonaceous fungi at two locations in central Iowa. *Canadian Journal of Botany* **60**: 2518–2519.

Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H, Nishmura I, Schüßler A. 2007. Molecular phylogeny and new taxa in the *Archaeosporales* (*Glomeromycota*): *Ambispora fennica* gen. sp. nov., *Ambisporaceae* fam. nov., and emendation of *Archaeospora* and *Archaeosporaceae*. *Mycological Research* **111**: 137–153.

White T, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. San Diego, CA, USA: Academic Press, 315–322.

Wiemers M, Fiedler K. 2007. Does the DNA barcoding gap exist? – a case study in blue butterflies (*Lepidoptera*: *Lycaenidae*). *Frontiers in Zoology* **4**: 8.

Wubet T, Weiß M, Kottke I, Teketay D, Oberwinkler F. 2004. Molecular diversity of arbuscular mycorrhizal fungi in *Prunus africana*, an endangered medicinal tree species in dry Afromontane forests of Ethiopia. *New Phytologist* **161**: 517–528.

Wubet T, Weiß M, Kottke I, Teketay D, Oberwinkler F. 2006. Phylogenetic analysis of nuclear small subunit rDNA sequences suggests that the endangered African Pencil Cedar, *Juniperus procera*, is associated with distinct members of *Glomeraceae*. *Mycological Research* **110**: 1059–1069.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Barcode gap analyses of the rDNA regions studied.

Fig. S2 Barcode gap analyses of the *Ambisporaceae* and the *Diversisporaceae*, including database sequences.

Fig. S3 SSUmCf-LSUmBr (A), internal transcribed spacer (ITS) region (B), large subunit (LSU) region (C), ITS2 fragment (D), LSU-D1 fragment (E), or LSU-D2 fragment (F) neighbour joining (NJ) analyses, 1000 bootstraps (BS), of *Glomus* Group Ab from the core dataset.

Fig. S4 SSUmCf-LSUmBr (A), internal transcribed spacer (ITS) region (B), large subunit (LSU) region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) neighbour joining (NJ) analyses (1000 BS) of *Glomus* Group Aa from the core dataset.

Fig. S5 SSUmCf-LSUmBr (A), internal transcribed spacer (ITS) region (B), large subunit (LSU) region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) neighbour joining (NJ) analyses (1000 BS) of *Acaulosporaceae* from the core dataset.

Fig. S6 SSUmCf-LSUmBr (A), internal transcribed spacer (ITS) region (B), large subunit (LSU) region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) neighbour joining (NJ) analyses (1000 BS) of *Glomus* Group B from the core dataset.

Fig. S7 SSUmCf-LSUmBr (A), internal transcribed spacer (ITS) region (B), large subunit (LSU) region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) neighbour joining (NJ) analyses (1000 BS) of *Diversisporaceae* from the core dataset.

Fig. S8 SSUmCf-LSUmBr (A), internal transcribed spacer (ITS) region (B), large subunit (LSU) region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) neighbour joining (NJ) analyses (1000 BS) of *Gigasporaceae* from core dataset.

Fig. S9 Internal transcribed spacer (ITS) region (A) and ITS2 fragment (B) neighbour joining (NJ) analyses (1000 BS) of the *Ambisporaceae*.

Fig. S10 Internal transcribed spacer (ITS) region (A) and ITS2 fragment (B) neighbour joining (NJ) analyses (1000 BS) of the *Diversisporaceae*.

Fig. S11 Large subunit (LSU) region (A), LSU-D1 fragment (B) and LSU-D2 fragment (C) neighbour joining (NJ) analyses (1000 BS) of the *Diversisporaceae*.

Table S1 Sequences used to assemble the core dataset.

Table S2 Sequences used for analysing the *Ambisporaceae* internal transcribed spacer (ITS) region.

Table S3 Sequences used for analysing the *Diversisporaceae* internal transcribed spacer (ITS) region.

Table S4 Sequences used for analysing the *Diversisporaceae* large subunit (LSU) region.

Table S5 Sequences used for analysing the *Glomus* Group Aa internal transcribed spacer (ITS) region.

Table S6 Sequences used for analysing the *Glomus* Group Aa large subunit (LSU)-D2 fragment.

Table S7 Barcode gap analyses with TAXONGAP 2.3 using pairwise comparison of K2P distances based on a manual or automated alignment (MAFFT) of the large SSUmCf-LSUmBr fragment.

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6. *Acaulospora brasiliensis* comb. nov. and *Acaulospora alpina* (*Glomeromycota*) from upland Scotland: morphology, molecular phylogeny and DNA-based detection in roots

This chapter is identical to the publication:

Krüger M, Walker C, Schüßler A. **2011**. *Acaulospora brasiliensis* comb. nov. and *Acaulospora alpina* (*Glomeromycota*) from upland Scotland: morphology, molecular phylogeny and DNA based detection in roots. *Mycorrhiza* 21: 577-587.

Acaulospora brasiliensis comb. nov. and *Acaulospora alpina* (*Glomeromycota*) from upland Scotland: morphology, molecular phylogeny and DNA-based detection in roots

Krüger M, Walker C, Schüßler A. 2011.

Abstract

Spores of two supposedly arbuscular mycorrhizal fungal species, new to the United Kingdom and recently described as *Acaulospora alpina* and *Ambispora brasiliensis* (*Glomeromycota*), were discovered in soil samples from moorland in upland Scotland. Soil and plant trap pot cultures were established, but attempts to establish these fungi in single-species pot cultures with *Plantago lanceolata* as host were unsuccessful. Nevertheless, based on a 1.5-kb DNA fragment spanning part of the small subunit rRNA gene, the internal transcribed spacer region and part of the large subunit rRNA gene, both these species could be detected directly in field-sampled roots, together with one uncultured species each of *Scutellospora*, *Rhizophagus* (former *Glomus* group Ab, or '*Glomus intraradices* clade') and *Acaulospora*. Whereas *A. alpina* has characteristic morphological similarities to other species in its genus, *A. brasiliensis* morphologically has little in common with any other species in *Ambispora*. The molecular phylogeny, DNA barcoding and morphological evidence clearly place *A. brasiliensis* in the genus *Acaulospora*. We therefore rename the species, reported from Brazil and Scotland, as *Acaulospora brasiliensis* comb. nov., and discuss ecological aspects of the very different environments from which *A. brasiliensis* and *A. alpina* have been reported.

Introduction

This study was initiated during an investigation of the mycorrhizal colonisation potential of Scottish upland soils for *Salix lapponum* cuttings (Milne et al. 2006). Natural *S. lapponum* and *S. herbacea* were sampled and examined for the occurrence of arbuscular mycorrhiza (AM). The presence of vesicles confirmed that AM fungi (*Glomeromycota*; Schüßler et al. 2001) were present, and samples were examined for the presence of glomeromycotan spores for morphological identification. Abundant spores that resembled *Acaulospora alpina* (from high altitude in Switzerland; Oehl et al. 2006) and *Ambispora brasiliensis* (from Minas Gerais State, Brazil; Goto et al. 2008) were recovered from trap cultures. The specimens of '*A. brasiliensis*' appeared to be more like an *Acaulospora* species (*Diversisporales*), than a member of *Ambispora* (*Archaeosporales*), thus conflicting with the published description. Therefore, we re-examined and expanded our data and studied the taxonomic, phylogenetic and systematic position of the Scottish organism and *A. brasiliensis* with a view to reconciling this apparent conflict. There is no

DNA sequence data for the Brazilian organism, but a morphological study was undertaken to compare it with the Scottish collections. The holotype of *A. brasiliensis*, consisting of spores preserved on microscope slides, was examined and compared with similar preparations of the Scottish specimens. The Scottish *A. brasiliensis*-like fungus was also characterised by DNA sequences providing species-level resolution, including a region that probably will cover the official DNA barcode for fungi (see also Stockinger et al. 2010). This allowed a direct detection of the fungus in the roots of plants from the Scottish upland moorland, together with *A. alpina* and additional uncultured species, one each of *Scutellospora*, *Rhizophagus* and *Acaulospora*. The discovery of the same species of arbuscular mycorrhizal fungi (AMF) in very different ecological conditions is discussed.

Materials and Methods

Origin of plant and fungal material

On the 23rd of September 2003 an excursion was made to Meall nan Tarmachan (approximately 900 m altitude, UK national grid coordinates NN 58789 38612: 56° 31′ 5.82″ N 4° 17′ 48.29″ W), an upland site in Scotland, to collect fruiting bodies of ectomycorrhizal fungi associated with *Salix herbacea* along with samples of the acidic soil (pH 4.0–5.0, measurements west of Lochan na Lairige; Stevens and Wilson 1970) and vegetation. Samples were collected by removing a small patch of turf and attached soil with a hand trowel to a depth of about 10 cm. These samples came from a mainly grassy area supporting a mixed plant population of *Festuca vivipara, Nardus stricta, Salix herbacea, Alchemilla alpina, Vaccinium myrtillus, Vaccinium vitis-idea, Galium rotundifolium, Carex* spp. and *Rhacomitrium lanuginosum*. On 16 April 2010, six new samples were collected from Meall nan Tarmachan by National Trust for Scotland staff. Spore extractions from these yielded the same species with acaulosporoid spores as had been found in the earlier samples. Mixed plant species root samples were taken for DNA extraction. More new samples were taken from a nearby location (close to Lochan na Lairige) at a slightly lower altitude (56°31′14.20″N 4°16′ 47.60″W at approximately 500 m amsl) on 6 September 2010. The soil was thin and peaty, with a pH (in water) of 4.9, and these also contained both species.

Culture attempts

Subsamples of the soil (approximately 15 ml) were subjected to centrifugation and sucrose floatation to extract spores (Walker et al. 1982). Attempts were made to establish multi-spore pot cultures with *Plantago lanceolata* in Sunbags (Sigma-Aldrich, UK) by pipetting spores onto seedling roots in the planting hole in 10 cm diameter pots containing a heat-disinfested mixture (3:1, v/v) of horticultural sand and Terragreen™ (expanded attapulgite clay, Oil Dry Corp., USA) (Walker 1999). Further culture

attempts, as ‘soil plus plant traps’ were established by mixing the soil with equal parts of Terragreen™ and replanting the sward sample to establish closed pot cultures in Sunbags (Walker and Vestberg 1994).

Morphological analyses

The holotype of *A. brasiliensis* consists of a single microscope slide, labelled ‘*Ambispora brasiliensis* 15 08 06 Serra do Cipó’. The slide was contained in a cardboard slide holder upon which was written ‘URM78879 *Ambispora brasiliensis* (typus)’. No other information was provided with the specimen except a note from URM saying ‘URM78880, also requested by Dr. Chris Walker, is not available.’

The spores on the slide were studied in detail through a Zeiss Axioskop research microscope. Digital images were captured with a Canon EOS5D camera and size measurements were made with a calibrated eyepiece reticle. For the Scottish material, extracted spores were examined initially in water under a dissecting microscope, followed by study of spores in polyvinyl alcohol lactoglycerol (PVLG) without or with Melzer’s reagent (1:4, v/v; PVLG-M) under the compound microscope as described above. Some specimens were also examined in glycerol. Spain (1990) suggested including unmodified wall structure observations from water immersed specimens, but without special objective lenses water has poor optical properties for compound microscopy, and dries rapidly in unsealed mounts. Glycerol does not affect the wall structure and gives a satisfactory refractive index. Comparisons with other glomeromycotan fungi were made from original species descriptions (e.g. Walker and Trappe 1981; Walker et al. 1993; Walker et al. 2004) and from herbarium specimens collected by Walker since 1974. Spore colour descriptions were from spores in water, either by comparison with a chart (Anon 1969; Anon 1990) or, when unmatched, by use of vernacular colour names The purely morphological terms ‘acaulosporoid’ or ‘acaulospore’ refer to a spore produced in the stalk or neck of a sporiferous saccule and do not imply homology with similar spores of *Ambispora* or *Archaeospora* spp. We do not use the term ‘glomerospore’ (Goto and Maia 2006) used in the protologue of *A. brasiliensis* because there are several different kinds of spores produced by glomeromycotan fungi, and they are likely not to be homologues (Morton and Msiska 2010). Glomoid spores are found amongst widely separated systematic groups, and are unlikely to be homologous either amongst glomeromycotan higher taxa or with either acaulosporoid or gigasporoid spore morphs.

Molecular characterisation

DNA extractions from single spores, polymerase chain reaction (PCR), cloning, sequencing and sequence editing were as described in Schwarzott et al. (2001) and Krüger et al. (2009). The near full-length small subunit (SSU) rRNA gene was analysed together with the complete internal transcribed spacer (ITS) region, including the 5.8S rRNA gene and ~800-bp of the large subunit (LSU) rRNA gene.

For the SSU rDNA three clones revealing slightly different sequence variants were sequenced from sample W4699/Att1211-0, taken 19th September 2004 to obtain robust evidence on the genus level. For

the ITS and LSU rDNA regions a ~1.5-kb fragment was cloned and analysed, to achieve species-level resolution (Stockinger et al. 2010) and to cover the potential official fungal primary DNA barcode (the ITS region or a combination of the ITS and the 5′ LSU regions). Part of the sequence data (clones pMK062-3; pMK064-4, 6; pMK065-4, 5, 6, 7; pMK109-1, 2) was derived from the same, stored material as the SSU rDNA (W4699/Att1211-0). The remaining clones sequenced (pCK032-1, 2, 4) came from a subculture (W5473/Att1210-5) sampled on the 5th of July 2008. DNA was extracted from 10 cm (20 randomly taken root fragments of 0.5 cm length; approximately 150 mg fresh weight) of field-sampled mixed plant roots. To cover a fraction of the intraspecific sequence variability, ten distinct sequences from two separate attempts (W4699/Att1211-0 and W5473/Att1210-5) were characterised and used for phylogenetic analyses of the ~1.5-kb SSU-ITS-LSU rDNA fragment.

The SSU rDNA sequences were submitted to the EMBL database with the accession numbers FN825898-900, those of the SSU-ITS-LSU rDNA regions with the accession numbers FN825901–912 and those for the DNA directly amplified from the roots with FR681926–936 and FR772326–334.

Phylogenetic analyses were performed with RAxML 7.2. (Stamatakis et al. 2008) hosted at the CIPRES Portal 2.2 (http://www.phylo.org/portal2/) using the GTRGAMMA model for the bootstrapping phase and for the final tree inference model, with 1,000 bootstraps. Analyses of the SSU rDNA, using sequences covering all main phylogenetic lineages in the *Glomeromycota*, clearly showed the new sequences obtained to be *Acaulospora*-related. Further phylogenetic analyses of the 1.5-kb fragment were then restricted to sequences from the *Acaulosporaceae* only incorporating all well-characterised sequences from the public databases and *Diversispora* sequences as outgroup.

The taxonomy and the sequence annotations used are adopted from the most recent systematic treatment of the *Glomeromycota* published by Schüßler and Walker (2010).

Results

The two dominantly sporulating species found in all three samplings from the upland moorland in Scotland possessed small, ornamented acaulosporoid spores. They were accompanied by a few spores of other glomeromycotan fungi. The trap cultures, in contrast, initially yielded only the two putative *Acaulospora* spp., later described as *A. alpina* by Oehl et al. (2006) and *A. brasiliensis* by Goto et al. (2008). Several unsuccessful attempts were made to isolate both these organisms in pot culture. Sporulation continued in these pots until March 2006, but when sampled again in October 2006 and in January 2008, no spore of either species was found. In November 2009, further sampling of the pot cultures revealed an *Ambispora* sp. (probably undescribed) and *Glomus ambisporum*, but all attempts at establishing subcultures of these species failed. The morphology of the spores of *A. alpina* was substantially as in the description of Oehl et al. (2006) and thus will not be discussed further herein.

Morphology of the Ambispora brasiliensis holotype

The holotype consists of a number of specimens mounted under two 22-mm square cover slips in what appears to be PVLG. There were 15 spores of the species concerned, as well as one spore of an undetermined species of *Scutellospora*, and two small, globose spores of an undetermined *Rhizophagus* sp. There were also a few other inclusions, but these were not glomeromycotan. All but four of the specimens were crushed, and only one had a short 'pedicel' at the point of origin. It was not possible to observe a scar or pedicel on any of the remaining spores. Because the spore base could not be identified, shortest by longest dimension of the four uncrushed specimens were measured. The resultant measurements were 72 × 88, 78 × 80, 75 × 83 and 69 × 75 μm. The crushed spores were also measured and their approximate original, uncrushed size was estimated to have been 64–88 × 64–88 μm. There was no saccule on the type slide, and thus no observations could be made for comparison with the original species description.

The wall structure of the type specimens was difficult to assess because, although they were crushed, in most specimens such detail was obscured and satisfactory observations were impossible. We interpret the most likely structure to be A(UoL)B(F)C(FF), where U refers to a 'unit component', L to a laminated component, and F to a flexible component.

Morphology of the Scottish fungus

The appearance of the specimens (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) did not differ in glycerol, PVLG or PVLG-M. Because of the particularly small size of the spores, there is inevitably some doubt when interpreting the wall structure. Some components are flexible in nature, and because they wrinkle on crushing, it is often difficult to distinguish real components from artefactual ones resulting from folding. The outer component of the acaulospore wall of this species is also very difficult to see because of the ornamentation which usually obscures its origin.

The acaulospores have a sparkling brownish yellow appearance in water under reflected light (Fig. 1). The colour of the spores varied depending on the collection. A few were more or less colourless (hyaline), but most were various shades of yellow to brown (Figs. 2, 3; Table S1). Some specimens were found with the sporiferous saccule still attached, though in all of these, it was collapsed and devoid of contents (Figs. 1, 3). The saccule wall appears to consist of just one component, about 1 μm thick (Fig. 4, arrow). The majority of spores had become detached in the manner typical of most species in the genus *Acaulospora*. We did not find a saccule with content or with young or developing spores attached.

The wall structure followed the expected pattern for members of the genus *Acaulospora* in that it consisted of a continuation of the saccule wall (Fig. 6), overlaying a laminated, pigmented, and relatively rigid, main structural component up to 4 μm thick, but mostly between 1 and 2 μm. These constitute a single wall group, A. This outer wall group is brittle and it fragments readily upon heavy crushing.

Although the saccule wall itself and the mycelium from which it is formed, are smooth, component 1 is ornamented to varying degrees with large, colourless collicles (more or less rounded elevations, Fig. 2) up to 10 μm high, and in length and width up to 20 × 30 μm, seemingly developed from the saccule wall component (Fig. 8). In outline, the collicles may be smooth or irregular. They vary considerably in size, and their outlines in plain view also is variable, from circular to oval to irregular with smooth to jagged boundaries. Their height, even on the same specimen, can vary from about 1 to 10 μm. On some spores, they are low and quite difficult to see, whereas on others, they are immediately evident, even under the dissecting microscope. Occasional specimens are almost smooth with only a few collicles remaining attached to the structural component, indicating that perhaps this outer component may break down over time.

Inside the main structural wall group there sometimes appears to be a second group, B that is very difficult to observe. It is a single very thin flexible component up to, but normally considerably less than, 1 μm thick (Fig. 7). On most spores, it cannot be seen at all and might be an artefact of microscopy. It is more likely to be an ontogenetic character, as a similar group occurs in spores of some *Acaulospora* spp. that have been studied developmentally (e.g., Stürmer and Morton 1999). If it is part of a developmental sequence, it either is delicate, disintegrating when the spore is crushed, or it is ephemeral, disappearing at spore maturity. We could not resolve which is correct. Surrounded by this is a third wall group, C, consisting of a pair of apparently adherent thin components (Fig. 7). The outermost of these is very thin (<1 μm) and flexible, detaching on crushing from an innermost component (up to 1 μm thick) which encloses the spore contents.

There is either a short pedunculate stalk (Fig. 5) formed from the proximal part of the sporiferous saccule wall or a distinct caldera-shaped scar resulting from a slightly raised collar at the point of formation of the laminated wall component (Fig. 9). There was no reaction to Melzer's reagent. Glomoid spores were not found in either field samples or pot cultures. Germination was observed in one specimen (Fig. 10), but it was not possible to distinguish any pregermination structure such as a germination shield on this spore.

Spore size comparison of holotype and Scottish material

Fungal spore size measurements should be quoted as 'length by width' (Hawksworth et al. 1983). Ours are made by taking the length as normal to the spore base (origin of spore) and the width at right angles to this. By following this convention (see e.g. Thaxter 1922; Gerdemann and Trappe 1974) it is possible to determine if spores are broader than they are long, and to compare shapes by using terms such as ovoid *versus* obovoid and pyriform *versus* obpyriform. The dimensions given by Goto et al. (2008) in the protologue of *A. brasiliensis* seem to be simply shortest dimension (presumably width) by longest dimensions (presumably length) without reference to the spore base. We have combined the dimensions given in the protologue with our own measurements for the description of the new combination.

The size range of the Scottish spores is somewhat smaller than that given in the protologue of *A. brasiliensis.* We consider the difference between 48–91 × 51–96 μm, mean 66 × 67 μm (n = 215) for the Scottish material, and 59–88 × 69–100 (−118) μm (mean and number of specimens measured unstated in the protologue) given for the Brazilian specimens to be within the intraspecific range of glomeromycotan spores. Measurements of the images in the protologue give one complete spore at 74 × 84 μm, and two for which only a single dimension could be measured at 88 and 93 μm, respectively. All these values are within the range of the Scottish material as well as our measurements of the spores in the holotype (64–88 × 64–88, mean 75 × 78 μm, n =15).

Phylogenetic analyses

The phylogenetic analysis of the SSU rRNA gene sequences (Fig. 1) clearly showed that the species described as *A. brasiliensis* (Goto et al. 2008) clusters with *Acaulospora* (*Acaulosporaceae*, *Diversisporales*) and not with *Ambispora* (*Ambisporaceae*, *Archaeosporales*). Thus, the species not only belongs in a different genus from that proposed in the protologue, but consequentially it must also be placed in a different order. For achievement of species-level resolution, we analysed an approximately 1.5 kb rDNA fragment and we also characterised part of the intraspecific variability for this fragment (Krüger et al. 2009; Stockinger et al. 2010). When compared with the species for which sequence information is available, the Scottish fungus appeared most closely related to the recently published species *Acaulospora colliculosa* (Kaonongbua et al. 2010), followed by *A. alpina* (Fig. 12). We also detected the *A. brasiliensis*-like fungus in plant roots from the Scottish sampling site (sample no. 1518, Meall nan Tarmachan, 16 April 2010). Sequences representing *A. alpina* (Fig. 12), a *Scutellospora* sp. closely related to, but not conspecific with *S. gilmorei* (not shown), an unknown *Rhizophagus* sp. (not shown), and a further, unknown *Acaulospora* species also were obtained from the same plant root sample. Both the phylogenetic trees computed from the SSU rDNA and the ITS-LSU rDNA fragments, unquestionable show that the Scottish fungus, morphologically appearing conspecific with *A. brasiliensis*, clusters within *Acaulospora* (*Acaulosporaceae*) and does not belong in the *Ambisporaceae*.

Formal transfer of Ambispora brasiliensis to Acaulospora

Acaulospora brasiliensis (B.T. Goto, L.C. Maia & Oehl) C. Walker, M. Krüger & A. Schüßler **comb. nov.** Figs 1-12.

MycoBank no. MB 518748

Basionym: *Ambispora brasiliensis* B.T. Goto, L.C. Maia & Oehl, Mycotaxon **105**: 13 (2008) (MycoBank no. 511612).

Acaulosporoid spores (acaulospores) borne singly in the soil, laterally in the neck of a hyaline sporiferous saccule, almost colourless to yellow to olive yellow to very pale brown to brownish yellow to yellowish brown to reddish yellow to yellowish red, globose to subglobose to broadly ellipsoid (rarely irregular), 48–91 × 51–100 µm (rarely up to 118 µm in the longest dimension). Spore wall structure of five components 1–5 in three groups, A–C. Group A of two components; outer component hyaline, originating from the neck of the sporiferous saccule, forming a collicular ornamentation of variable size, apparently arising from a continuous basal layer approximately 1 µm thick, tightly adherent to a laminated, pigmented structural component, its point of origin appearing as a slightly raised collar or occasionally as a pedicel of variable length. Wall group B of one thin, flexible, hyaline, component, <1 µm thick. Wall group C, of two components, the outermost very thin and elastic, up to 1 µm thick, juxtaposed with a more robust component, approximately 1 µm thick enclosing the spore contents. No reaction to Melzer's reagent.

Distribution and habitat: Known from the Cerrado biome of Serra do Cipó, Minas Gerais State, Brazil (Goto et al. 2008) from a site described as 'mainly consisting of *Velozzia caruncularis*', and from an upland heathland in Scotland in which the dominant vegetation consists of *Festuca vivipara* and *Nardus stricta*, with *Salix herbacea*, *Alchemilla alpina*, *Vaccinium myrtillus*, *V. vitis-idea*, *Galium rotundifolium*, *G. saxatile*, *Carex* spp., and *Rhacomitrium lanuginosum*. From sequence analyses, it is known to be a member of a glomeromycotan community among the roots of these plants, including *A. alpina*, another *Acaulospora* sp., a *Scutellospora* sp. closely related to *S. gilmorei* and an undetermined *Rhizophagus* sp.

Mycorrhizal associations are unknown, but root colonisation shown by DNA-based detection in plant roots that were sampled from the field site.

Specimens examined

Typus: Brazil. Minas Gerais. Serra do Cipó, beneath cerrado vegetation (dominated by Velozzia caruncularis). Microscope slide (URM78879) dated 15 Aug. 2006. In the protologue, the collection date is given as 'July 2004'.

United Kingdom, Scotland, Perthshire, Ben Lawers National Nature Reserve, Meall nan Tarmachan (Hill of the Ptarmigan), approximately 900 m amsl, from within 200 m of UK National Grid Reference: NN58789 38612 (latitude, 56.518284N; longitude, 4.296748W) from soil beneath heathland vegetation or from subsequent pot cultures. C. Walker (voucher numbers preceded by W). W4514 from sample 1136 on 23 Sep 2003; W5748 from Sample 1517; W5751 from sample 1518; W5755 from sample 1519; W5759 from sample 1520; W5762 from sample 1521; W5765 from sample 1522, all collected 16 April 2010. W5827 from sample 1527, close to Lochan na Lairige (56°31′14.20″N 4°16′47.60″W) at approximately 500 m amsl, collected 6 September 2010. From trap pot cultures from containing Festuca vivipara, Nardus stricta and Galium rotundifolium: W4699 from Att1211-0 from sample 1136 on 19 September 2004; W4786 from Att1210-0 from sample 1136 on 6 February 2006; W4796 from Att1210-0 from sample 1136 on 21 February 2006; W4833 from Att1210-0 from sample 1136 on 15 July 2006.

Discussion

We showed first records of two *Acaulospora* spp., *A. alpina* and *A. brasiliensis* from a Scottish upland. The latter species was initially described as *Ambispora brasiliensis* (Goto et al. 2008) and is transferred to *Acaulospora* (*Acaulosporaceae*) based on molecular evidence and morphological characterisation.

To study its morphology, isotypes of *A. brasiliensis* were requested as a loan from the herbaria OSC and Z+ZT (Oregon State University and Zurich), but neither of them could locate the specimens concerned. Nevertheless, it is clear from the holotype and the protologue of *A. brasiliensis* that there are no significant differences between spores of the Brazilian and Scottish organisms, and we conclude they are conspecific. Goto et al. (2008) described, but did not illustrate, one glomoid spore of 25–30 μm in diameter attached to a germinating hypha from a single acaulosporoid spore. The Scottish collections contained glomoid spores of an *Ambispora* sp., but these were very large (~300 μm in diameter) in comparison with those of *A. brasiliensis*, and corresponded with the descriptions given for members of *Ambispora* (Walker et al. 2007). No glomoid spores have been found linked to the Scottish acaulospores. Therefore, more evidence is needed before the asserted dimorphic nature of this organism can be verified.

The Brazilian acaulospores have a slightly larger maximum dimension than those from Scotland, but similar differences even occur among subcultures of single-spore AMF isolates (Walker and Vestberg 1998). Though the Brazilian spores are described as being 'hyaline to light yellow', images in the protologue show them to be yellow to brown. The range of colour for the Scottish collections is almost colourless to yellow to pale yellow brown or reddish brown. Such differences are likely to result from different perceptions and methods of comparison and, as the slight size differences, are not sufficient to separate species. The 'pedicel' used to place the organism in *Ambispora* is not a feature confined to that genus being present on members of *Acaulospora* and *Entrophospora infrequens* (Hall 1977). Some specimens of *A. brasiliensis* from Scotland had a short stalk although most had only a circular or oval scar as seen in most *Acaulospora* spores. The illustration of a 'collar' in the Brazilian species description (Goto et al. 2008) is similar to those typical of spores in the genus *Acaulospora*, showing that both scars and short 'pedicels' may be present.

We could not reconcile the wall structure in the species description with either the holotype specimens or those in our own collections. Even with large-spored species, it usually is impossible to follow spore development from field-collected material. In our collections and trap cultures, we have so far found spores either completely sessile or attached only to empty and collapsed saccules. Thus, it was impossible to follow the development of the saccules or spore wall structure. The thickened and uneven ornamentation on the acaulospore surface makes it difficult to determine wall structure or to see internal structures such as a germination shield.

The sporiferous saccule wall is described by Goto et al. (2008) as being two-layered, but their illustrations do not convincingly illustrate more than one layer, and saccules are completely lacking from the holotype material available to us. Their 'evanescent outer layer' appears to be soil particles adherent to the collapsed and decaying saccule. We have been unable to see more than a single wall component in our specimens, and from the images in the protologue, the wall structure seems the same as that observed in the Scottish material. In our interpretation, the main structural wall group of the spore consists of two components. The first is colourless and seems to be continuous with the wall of the saccule. It is ornamented to varying degrees with pustule-like collicles which occur only around the spore and not on the saccule itself. However, the limitations of light microscopy on such small specimens must be considered. The illustration of the pedicel in Goto et al. (2008) as continuous with the main structural spore wall ('outer wall') does not adequately illustrate such a feature. Although one specimen on the holotype slide does have a short pedicel, it is presented in such a way that its structure and relationship to the wall components of the acaulospore could not be determined. We interpret it as part of the outermost component (the saccule wall). Tightly adherent to it is the coloured outer component of the spore itself. This is probably 'laminated', though in many specimens it is so thin that layers cannot be seen. Many spores of glomeromycotan species seem to have such a laminated component as the main structural component or layer. We, therefore, interpret the wall structure of wall group 1 as consisting of one component originating from the saccule wall and a second component, the structural wall of the acaulospore, that is probably produced de novo within a lateral swelling in the saccule neck. Goto et al. (2008), however, consider that the saccule has two components (layers) that later differentiate into two separate 'walls', the outermost having three layers and the innermost having two layers. From examination of many specimens, it is clear that the inner wall groups lack any attachment to either the saccule wall or the main structural wall group of the acaulospore. Spores of both *Acaulospora* spp. and *Ambispora* spp. develop their main structural wall de novo within the saccule wall (Kaonongbua et al. 2010; Stürmer and Morton 1999; Walker et al. 2007).

Moving towards the interior of the spore, Goto et al. (2008) describe a 'middle wall' that consists of two layers (formed by differentiation from the saccule wall). Such a development has not been recorded for any species in the *Glomeromycota*, and in particular is different from the structure of either *Ambispora* or *Acaulospora* (Kaonongbua et al. 2010; Walker et al. 2007). We could see only a very thin flexible component that we consider to be a second wall group because sometimes, upon crushing the spore, it remains close to wall group 1, and sometimes to the innermost group (group 3). Goto et al. (2008) illustrate a third 'wall' consisting of three layers. We interpret the third wall group as having two distinct components of more or less equal thickness, though sometimes only a single one could be seen. We were able to see what we thought might be a germination shield from a lateral view on one specimen (not

shown), but we were not certain that we were interpreting it correctly. Goto et al. (2008) described (but did not illustrate) a germination shield on one spore only as being a lobed structure similar to that present in spores of species in *Scutellospora* or *Racocetra* (Morton and Msiska 2010). We could not find a germination shield on any of the holotype specimens.

With the exception of *A. colliculosa*, no other member of the *Acaulosporaceae* has small, yellow to brownish yellow acaulosporoid spores possessing collicular ornamentation. The spores of *A. brasiliensis* lack reaction to Melzer's reagent, even after the most vigorous crushing on a microscope slide with PVLG/Melzer's (4:1, v/v) and in pure Melzer's reagent. Although most *Acaulospora* species react to this reagent, producing a pale purple to dark purple colour associated with at least one internal component, a few species, such as *A. laevis*, and *A. colliculosa* (Kaonongbua et al. 2010) lack such a reaction. However, *A. alpina*, which is a close relative of *A. brasiliensis*, possesses an inner wall component that becomes purple when spores are crushed in PVLG/Melzer's (Oehl et al. 2006; C. Walker unpublished). This provides support for the opinion that the reaction to Melzer's reagent may not be a phylogenetically informative character (Kaonongbua et al. 2010).

Neither ourselves nor Goto et al. (2008) have been able to establish the fungus in pure culture or to isolate it by single-spore culturing attempts. Spores of *A. brasiliensis* have been produced only in pot cultures established from field soil and natural plants, but these could not be maintained even by moving entire plants to a new pot of sterilised substrate. However, we could directly detect the presence of *A. brasiliensis* in field-collected roots from the Scottish location by molecular biological methods, together with *A. alpina* and one undetermined AMF species each of *Scutellospora* (closely related to *S. gilmorei*), *Rhizophagus* (different from any other species yet sequenced from this genus), and *Acaulospora* (clustering in a monophyletic clade with *A. colliculosa*, *A. brasiliensis* and *A. alpina*). It will still be necessary to establish it in pure culture before its mycorrhizal nature can be confirmed through the application of Koch's postulates.

Acaulospora alpina was previously known only from altitudes above 1,300 m amsl in the alpine region of mainland Europe. Although the Scottish locations are at much lower altitude (500–900 m amsl), the climatic conditions in Scotland are also very severe, but soil conditions and plant communities clearly are very different in these ecosystems. The Scottish samples came from a thin, peaty soil of approximately pH 5, overlaying a 'Ben Lawers schist'. In contrast, the bedrock in the alpine areas from which *A. alpina* is known seems to be very variable. Spores of *A. alpina* were found in '...acidic sandstones, siliceous gneiss and granite rocks, up to ultrabasic serpentinite and calcareous "Bündner Schiefer" schists and carbonatic and dolomitic limestones ...' (Oehl et al. 2006). The pH value given is five for the sample from which the type material came. However, it is much more unexpected to find a fungus, *A. brasiliensis*,

reported from a dry, cerrado ecosystem with predominantly summer rainfall (Minas Gerais State, Brazil) on almost permanently wet, cold, peaty Scottish moorland. Nevertheless, the bedrock in the Serra do Cipó also seems to be igneous, and has a low pH of 4.7 (Goto et al. 2008), as does the Scottish site (pH 4–5). Low pH has been shown as a likely key factor in affecting populations of glomeromycotan fungi in agricultural conditions (Wang et al. 1985).

The distribution of some species in the *Glomeromycota* is known to be very wide with respect to different site conditions (Börstler et al. 2010), even to the point of speculation that humans have been responsible for spread through agricultural practices (Rosendahl et al. 2009). *A. brasiliensis* to date is known only from two sites that are not so heavily influenced by humans and its occurrence in such widely different ecosystems could lead to suggestions that it may be very widespread. On the other hand from two records, it is certainly too early to draw conclusions about its ecological preferences as a species, and it is not too far from the truth that the known distribution of organism may reflect the distribution of people interested in them rather than their true spread. As far as we can discover, the only common factor seems to be igneous bedrock with low soil pH, and this might be one of the problems in relation to establishing pot cultures. Molecular tools with species-level resolution should soon provide a better basis for interpreting such ecological and biogeographical information at the level of species on a secure foundation.

Acknowledgements

We thank Jeremy Milne, Royal Botanic Garden Edinburgh, for collecting the original samples, Helen Cole & Clare Rickerby, Naturalist, National Trust for Scotland, Killin, Perthshire for taking the second batch of samples; and Claudia Krüger for PCR amplifying and cloning DNA from some of the samples. We also thank Maria Leonora Maia, Curator, URM, for the loan of the holotype. Some minor funding was obtained from the DFG (German Research Foundation, Schu 1203/8).

References

Anon (1969) Royal Botanic Garden Edinburgh. Flora of the British fungi. Colour identification chart. Her Majesty's Stationery Office, Edinburgh

Anon (1990) Munsell soil color charts, 1990 edition, revised. Munsell Color, Baltimore

Börstler B, Thiéry O, Sýkorová Z, Berner A, Redecker D (2010) Diversity of mitochondrial large subunit rDNA haplotypes of *Glomus intraradices* in two agricultural field experiments and two semi-natural grasslands. Mol Ecol 19:1497–1511. doi:10.1111/j.1365-294X.2010.04590.x

Gerdemann JW, Trappe JM (1974) The Endogonaceae in the Pacific Northwest. Mycologia Memoir No. 5

Goto BT, Maia LC (2006) Glomerospores: a new denomination for the spores of Glomeromycota, a group molecularly distinct from Zygomycota. Mycotaxon 96:129–132

Goto BT, Maia LC, Oehl F (2008) *Ambispora brasiliensis*, a new ornamented species in the arbuscular mycorrhiza-forming Glomeromycetes. Mycotaxon 105:11–18

Hall IR (1977) Species and mycorrhizal infections of New Zealand Endogonaceae. T Brit Mycol Soc 68:341–356. doi:10.1016/ S0007-1536(77)80186-1

Hawksworth DL, Sutton BC, Ainsworth GC (1983) Ainsworth & Bisby's dictionary of the fungi, 7th edn. CAB, Kew

Kaonongbua W, Morton JB, Bever JD (2010) Taxonomic revision transferring species in *Kuklospora* to *Acaulospora* (*Glomeromycota*) and a description of *Acaulospora colliculosa* sp. nov. from field collected spores. Mycologia 102:1497–1509. doi:10.3852/10-011

Krüger M, Stockinger H, Krüger C, Schüßler A (2009) DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol 183: 212-223. doi:10.1111/j.1469-8137.2009.02835.x

Milne JM, Ennos RA, Hollingsworth PM (2006) Vegetation influence on ectomycorrhizal inoculum available to sub-arctic willow (Salix lapponum L.) planted in an upland site. Bot J Scot 58:19–34. doi:10.1080/03746600608685104

Morton JB, Msiska Z (2010) Phylogenies from genetic and morphological characters do not support a revision of *Gigasporaceae* (*Glomeromycota*) into four families and five genera. Mycorrhiza 20:483–496. doi:10.1007/s00572-010-0303-9

Oehl F, Sýkorová Z, Redecker D, Wiemken A (2006) *Acaulospora alpina*, a new arbuscular mycorrhizal fungal species characteristic for high mountainous and alpine regions of the Swiss Alps. Mycologia 98:286–294. doi:10.3852/mycologia.98.2.286

Rosendahl S, McGee P, Morton JB (2009) Lack of global population genetic differentiation in the arbuscular mycorrhizal fungus *Glomus mosseae* suggests a recent range expansion which may have coincided with the spread of agriculture. Mol Ecol 18:4316–4329. doi:10.1111/j.1365-294X.2009.04359.x

Schüßler A, Walker C (2010) The *Glomeromycota*: a species list with new families and new genera. Schüßler A, Walker C, Gloucester, Published in libraries at Royal Botanic Garden Edinburgh, Kew, Botanische Staatssammlung Munich, and Oregon State Univer- sity; freely available online at www.amf-phylogeny.com

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the *Glomeromycota*: evolution and phylogeny. Mycol Res 105:1413–1421. doi:10.1017/S0953756201005196

Schwarzott D, Walker C, Schüßler A (2001) *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (*Glomales*), is nonmonophyletic. Mol Phylogenet Evol 21: 190-197. doi:10.1006/mpev.2001.1007

Spain JL (1990) Arguments for diagnoses based on unaltered wall structures. Mycotaxon 38:71–76

Stamatakis A, Hoover P, Rougemont J (2008) A fast bootstrapping algorithm for the RAxML web-servers. Syst Biol 57:758–771. doi:10.1080/10635150802429642

Stevens JH, Wilson MJ (1970) Alpine podzol soils on the Ben Lawers Massif Perthshire. J Soil Sci 21:85–95. doi:10.1111/j.1365-2389.1970.tb01155.x

Stockinger H, Krüger M, Schüßler A (2010) DNA barcoding of arbuscular mycorrhizal fungi. New Phytol 187: 461-474. doi:10.1111/j.1469-8137.2010.03262.x

Stürmer SL, Morton JB (1999) Taxonomic reinterpretation of morphological characters in *Acaulosporaceae* based on develop- mental patterns. Mycologia 91:849–857

Thaxter R (1922) A revision of the *Endogoneae*. Proc AAAS 57:290–351

Walker C (1999) Methods for culturing and isolating arbuscular mycorrhizal fungi. Mycorrhiza News 11:2–3

Walker C, Trappe JM (1981) *Acaulospora spinosa* sp. nov. with a key to the species of Acaulospora. Mycotaxon 12:515–521

Walker C, Vestberg M (1994) A simple and inexpensive method for producing and maintaining closed pot cultures of arbuscular mycorrhizal fungi. Agr Sci Finland 3:233–240

Walker C, Vestberg M (1998) Synonymy amongst the arbuscular mycorrhizal fungi: *Glomus claroideum, G. maculosum, G. multisubstensum* and *G. fistulosum*. Ann Bot Lond 82:601–624

Walker C, Mize CW, McNabb HS (1982) Populations of endogonaceous fungi at two localities in central Iowa. Can J Bot 60:2518–2529

Walker C, Gianinazzi-Pearson V, Marion-Espinasse H (1993) *Scutellospora castanea*, a newly described arbuscular mycorrhizal fungus. Cryptogam Mycol 14:279–286

Walker C, Błazkowski J, Schwarzott D, Schüßler A (2004) *Gerdemannia* gen. nov., a genus separated from *Glomus* and *Gerdemanniaceae* fam. nov., a new family in the *Glomeromycota*. Mycol Res 108:707–718. doi:10.1017/S0953756204000346

Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H, Nishmura I, Schüßler A (2007) Molecular phylogeny and new taxa in the *Archaeosporales* (*Glomeromycota*): *Ambispora fennica* gen. sp nov., *Ambisporaceae* fam. nov., and emendation of *Archaeospora* and *Archaeosporaceae*. Mycol Res 111:137–153. doi:10.1016/j.mycres.2006.11.008

Wang GM, Stribley DT, Tinker PB, Walker C (1985) Soil pH and vesicular-arbuscular mycorrhiza. In: Fitter AH (ed) British ecological society special symposium-ecological interactions in the soil environment: plants microbes and animals. Blackwell, Oxford, pp 219–224

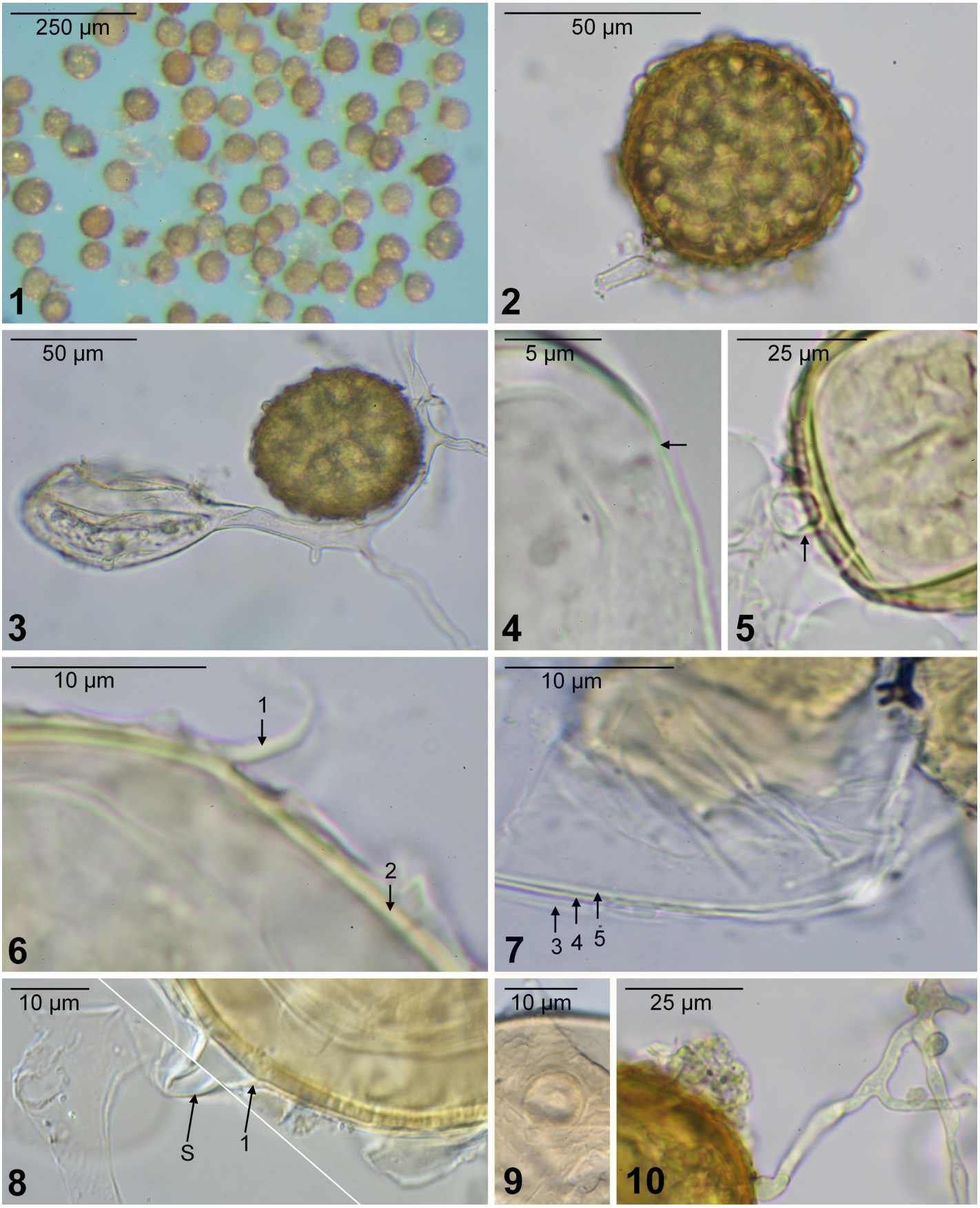
Legends to figures

Fig. 1 *Acaulospora brasiliensis* comb. nov. Several acaulospores, some with attached saccules, extracted from substrate by swirling and decanting. **Fig. 2** *Acaulospora brasiliensis* comb. nov. Individual spore, detached from the saccule, showing the collicular ornamentation on the outermost surface. **Fig. 3** *Acaulospora brasiliensis* comb. nov. Spore still attached to the colourless, transparent collapsed sporiferous saccule. **Fig. 4** *Acaulospora brasiliensis* comb. nov. Detail of saccule wall, showing a single component (indicated with an arrow). **Fig. 5** *Acaulospora brasiliensis* comb. nov. Pedicel-like spore base (indicated with an arrow) formed by the thickened saccule neck at the point of spore development. **Fig. 6** *Acaulospora brasiliensis* comb. nov. Point at which the spore has detached from the saccule showing a short 'pedicel' and the components of the main structural wall group (indicated with 1 & 2, respectively). **Fig. 7** *Acaulospora brasiliensis* comb. nov. Structure of the apparent middle (3), and paired innermost wall components (4 & 5). **Fig. 8** *Acaulospora brasiliensis* comb. nov. Composite image at two depths of focus (joined at the white diagonal line), showing the continuous nature of the saccule wall (S) and the outermost component of the acaulospore (1). **Fig. 9** *Acaulospora brasiliensis* comb. nov. The caldera-shaped scar at the point of detachment of the spore from the saccule. **Fig. 10** *Acaulospora brasiliensis* comb. nov. Germinating acaulospore; the thick, coloured outer wall components obscure the contents, and it is not possible to see if a germination shield is formed.

Fig. 11 Phylogenetic maximum likelihood tree computed with RAxML from individual or consensus sequences of near full-length SSU rRNA gene sequences, including all main lineages of the *Glomeromycota*. New taxa are adopted from Schüßler and Walker (2010). Support values derived from a 1,000-fold bootstrapped analysis are shown on the branches; values below 60% were considered as unresolved and the respective topologies were collapsed to polytomies. *Paraglomus* sequences were used as outgroup.

Fig. 12 Phylogenetic maximum likelihood tree computed with RAxML from approx. 1500 bp sequences covering approx. 250 bp of the SSU rRNA gene, the whole ITS region and an approx. 800 bp of the LSU rRNA gene. Some shorter sequences from the public databases were also included for comparison and are marked as follows: #, covering partial SSU and whole ITS region; *, covering partial LSU. Support values derived from a 1000-fold bootstrapped analysis are shown on the branches; values below 60% were considered as unresolved and the respective topologies were collapsed to polytomies. The tree was rooted with *Diversispora* sequences as outgroup; the root was shortened by 50%, as indicated by diagonal slashes.

Figures 1-10


250 µm
1
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2
50 µm
3
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4
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5
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Fig. 11

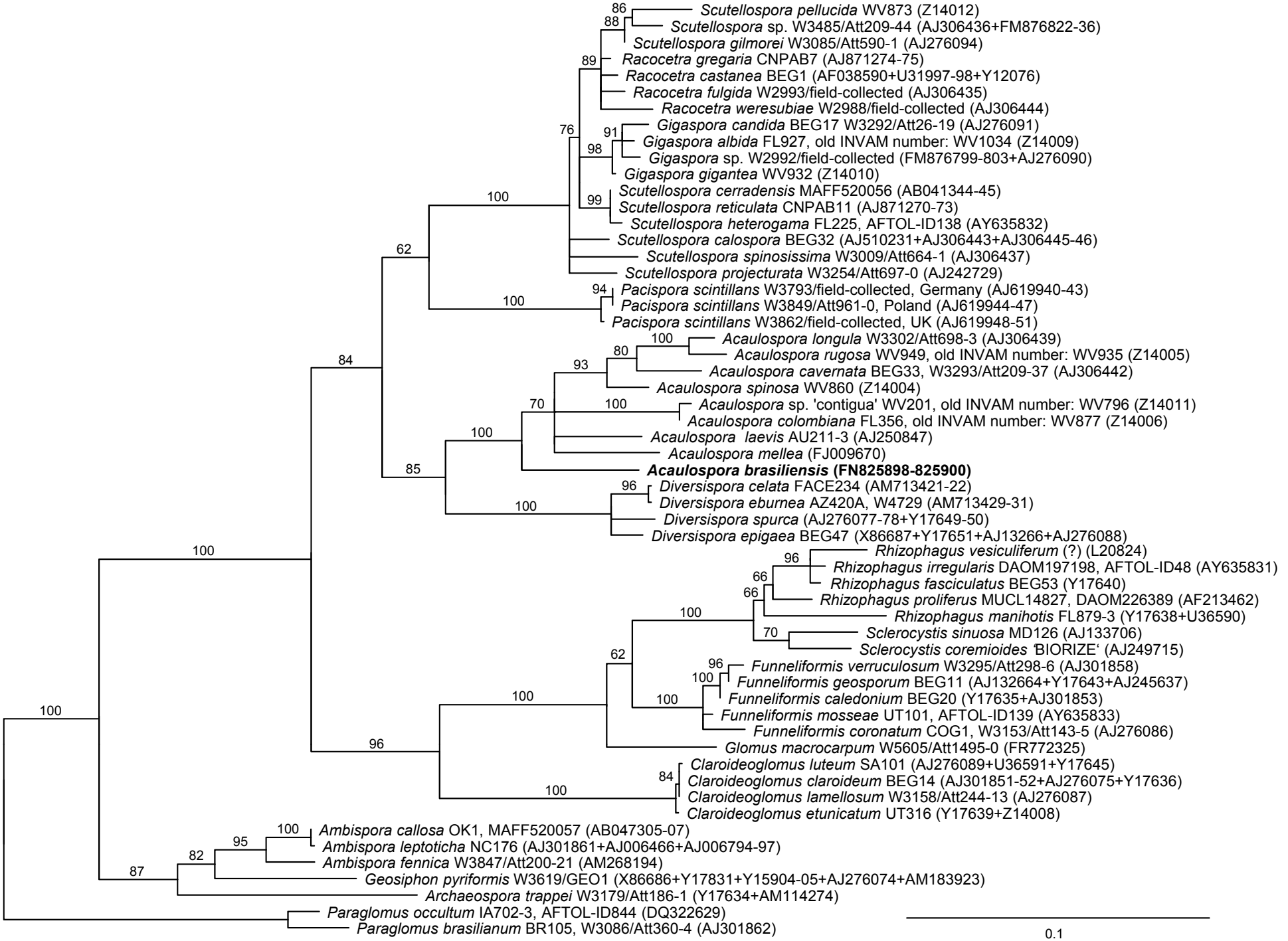
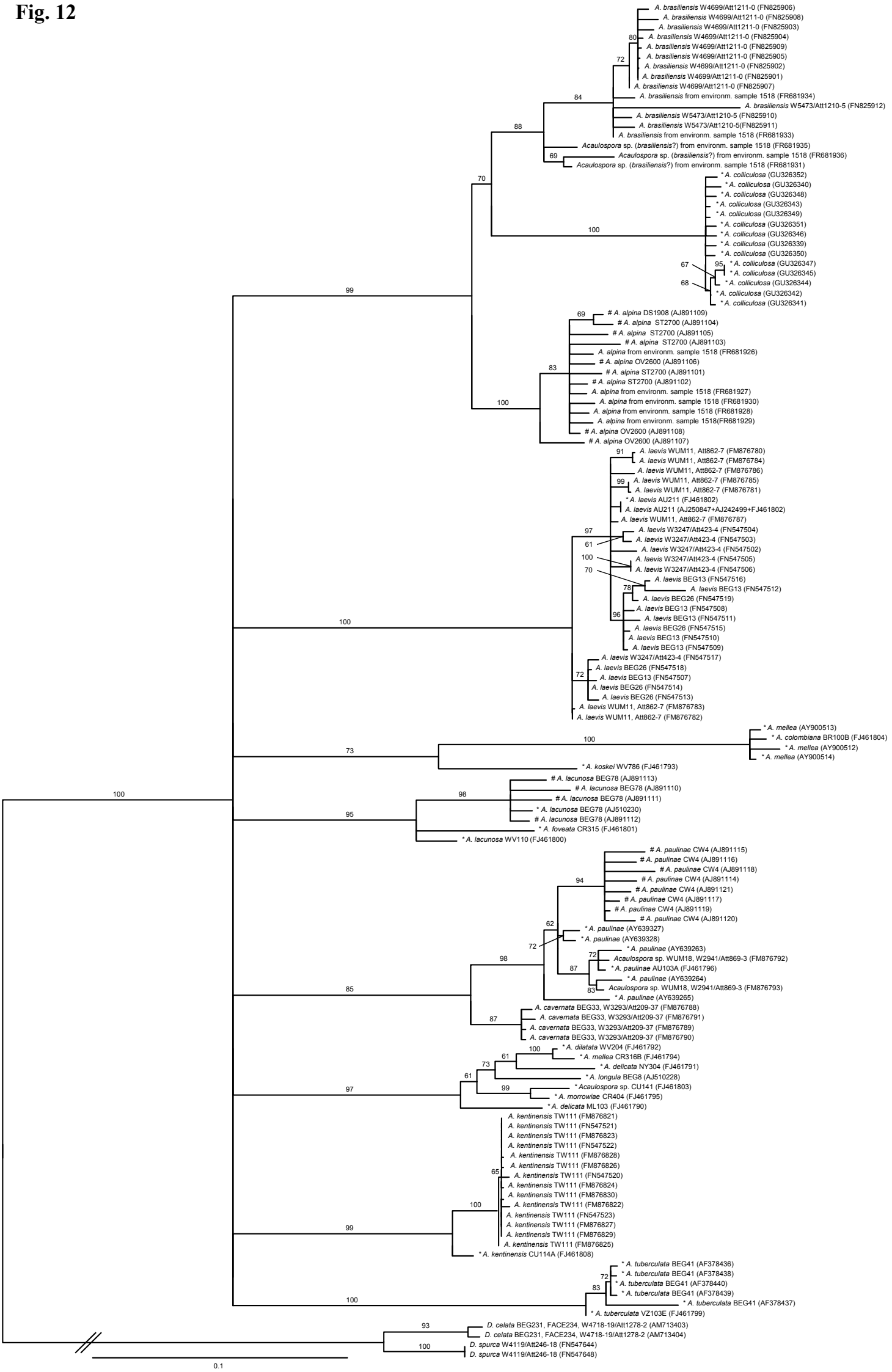

Scutellospora pellucida WV873 (Z14012)
Scutellospora sp. W3485/Att209-44 (AJ306436+FM876822-36)
Scutellospora gilmorei W3085/Att590-1 (AJ276094)
Racocetra gregaria CNPAB7 (AJ871274-75)
Racocetra castanea BEG1 (AF038590+U31997-98+Y12076)
Racocetra fulgida W2993/field-collected (AJ306435)
Racocetra weresubiae W2988/field-collected (AJ306444)
Gigaspora candida BEG17 W3292/Att26-19 (AJ276091)
Gigaspora albida FL927, old INVAM number: WV1034 (Z14009)
Gigaspora sp. W2992/field-collected (FM876799-803+AJ276090)
Gigaspora gigantea WV932 (Z14010)
Scutellospora cerradensis MAFF520056 (AB041344-45)
Scutellospora reticulata CNPAB11 (AJ871270-73)
Scutellospora heterogama FL225, AFTOL-ID138 (AY635832)
Scutellospora calospora BEG32 (AJ510231+AJ306443+AJ306445-46)
Scutellospora spinosissima W3009/Att664-1 (AJ306437)
Scutellospora projecturata W3254/Att697-0 (AJ242729)
Pacispora scintillans W3793/field-collected, Germany (AJ619940-43)
Pacispora scintillans W3849/Att961-0, Poland (AJ619944-47)
Pacispora scintillans W3862/field-collected, UK (AJ619948-51)
Acaulospora longula W3302/Att698-3 (AJ306439)
Acaulospora rugosa WV949, old INVAM number: WV935 (Z14005)
Acaulospora cavernata BEG33, W3293/Att209-37 (AJ306442)
Acaulospora spinosa WV860 (Z14004)
Acaulospora sp. 'contigua' WV201, old INVAM number: WV796 (Z14011)
Acaulospora colombiana FL356, old INVAM number: WV877 (Z14006)
Acaulospora laevis AU211-3 (AJ250847)
Acaulospora mellea (FJ009670)
Acaulospora brasiliensis (FN825898-825900)
Diversispora celata FACE234 (AM713421-22)
Diversispora eburnea AZ420A, W4729 (AM713429-31)
Diversispora spurca (AJ276077-78+Y17649-50)
Diversispora epigaea BEG47 (X86687+Y17651+AJ13266+AJ276088)
Rhizophagus vesiculiferum (?) (L20824)
Rhizophagus irregularis DAOM197198, AFTOL-ID48 (AY635831)
Rhizophagus fasciculatus BEG53 (Y17640)
Rhizophagus proliferus MUCL14827, DAOM226389 (AF213462)
Rhizophagus manihotis FL879-3 (Y17638+U36590)
Sclerocystis sinuosa MD126 (AJ133706)
Sclerocystis coremioides 'BIORIZE' (AJ249715)
Funneliformis verruculosum W3295/Att298-6 (AJ301858)
Funneliformis geosporum BEG11 (AJ132664+Y17643+AJ245637)
Funneliformis caledonium BEG20 (Y17635+AJ301853)
Funneliformis mosseae UT101, AFTOL-ID139 (AY635833)
Funneliformis coronatum COG1, W3153/Att143-5 (AJ276086)
Glomus macrocarpum W5605/Att1495-0 (FR772325)
Claroideoglomus luteum SA101 (AJ276089+U36591+Y17645)
Claroideoglomus claroideum BEG14 (AJ301851-52+AJ276075+Y17636)
Claroideoglomus lamellosum W3158/Att244-13 (AJ276087)
Claroideoglomus etunicatum UT316 (Y17639+Z14008)
Ambispora callosa OK1, MAFF520057 (AB047305-07)
Ambispora leptoticha NC176 (AJ301861+AJ006466+AJ006794-97)
Ambispora fennica W3847/Att200-21 (AM268194)
Geosiphon pyriformis W3619/GEO1 (X86686+Y17831+Y15904-05+AJ276074+AM183923)
Archaeospora trappei W3179/Att186-1 (Y17634+AM114274)
Paraglomus occultum IA702-3, AFTOL-ID844 (DQ322629)
Paraglomus brasilianum BR105, W3086/Att360-4 (AJ301862)
0.1

Fig. 12


A. brasiliensis W4699/Att1211-0 (FN825906)
A. brasiliensis W4699/Att1211-0 (FN825908)
A. brasiliensis W4699/Att1211-0 (FN825903)
A. brasiliensis W4699/Att1211-0 (FN825904)
A. brasiliensis W4699/Att1211-0 (FN825909)
A. brasiliensis W4699/Att1211-0 (FN825905)
A. brasiliensis W4699/Att1211-0 (FN825902)
A. brasiliensis W4699/Att1211-0 (FN825901)
A. brasiliensis W4699/Att1211-0 (FN825907)
A. brasiliensis from environm. sample 1518 (FR681934)
A. brasiliensis W5473/Att1210-5 (FN825912)
A. brasiliensis W5473/Att1210-5 (FN825910)
A. brasiliensis W5473/Att1210-5(FN825911)
A. brasiliensis from environm. sample 1518 (FR681933)
Acaulospora sp. (brasiliensis?) from environm. sample 1518 (FR681935)
Acaulospora sp. (brasiliensis?) from environm. sample 1518 (FR681936)
Acaulospora sp. (brasiliensis?) from environm. sample 1518 (FR681931)
* A. colliculosa (GU326352)
* A. colliculosa (GU326340)
* A. colliculosa (GU326348)
* A. colliculosa (GU326343)
* A. colliculosa (GU326349)
* A. colliculosa (GU326351)
* A. colliculosa (GU326346)
* A. colliculosa (GU326339)
* A. colliculosa (GU326350)
* A. colliculosa (GU326347)
* A. colliculosa (GU326345)
* A. colliculosa (GU326344)
* A. colliculosa (GU326342)
* A. colliculosa (GU326341)
# A. alpina DS1908 (AJ891109)
# A. alpina ST2700 (AJ891104)
# A. alpina ST2700 (AJ891105)
# A. alpina ST2700 (AJ891103)
A. alpina from environm. sample 1518 (FR681926)
A. alpina OV2600 (AJ891106)
A. alpina ST2700 (AJ891101)
A. alpina ST2700 (AJ891102)
A. alpina from environm. sample 1518 (FR681927)
A. alpina from environm. sample 1518 (FR681930)
A. alpina from environm. sample 1518 (FR681928)
A. alpina from environm. sample 1518(FR681929)
A. alpina OV2600 (AJ891108)
A. alpina OV2600 (AJ891107)
A. laevis WUM11, Att862-7 (FM876780)
A. laevis WUM11, Att862-7 (FM876784)
A. laevis WUM11, Att862-7 (FM876786)
A. laevis WUM11, Att862-7 (FM876785)
A. laevis WUM11, Att862-7 (FM876781)
* A. laevis AU211 (FJ461802)
A. laevis AU211 (AJ250847+AJ242499+FJ461802)
A. laevis WUM11, Att862-7 (FM876787)
A. laevis W3247/Att423-4 (FN547504)
A. laevis W3247/Att423-4 (FN547503)
A. laevis W3247/Att423-4 (FN547502)
A. laevis W3247/Att423-4 (FN547505)
A. laevis W3247/Att423-4 (FN547506)
A. laevis BEG13 (FN547516)
A. laevis BEG13 (FN547512)
A. laevis BEG26 (FN547519)
A. laevis BEG13 (FN547508)
A. laevis BEG13 (FN547511)
A. laevis BEG26 (FN547515)
A. laevis BEG13 (FN547510)
A. laevis BEG13 (FN547509)
A. laevis W3247/Att423-4 (FN547517)
A. laevis BEG26 (FN547518)
A. laevis BEG13 (FN547507)
A. laevis BEG26 (FN547514)
A. laevis BEG26 (FN547513)
A. laevis WUM11, Att862-7 (FM876783)
A. laevis WUM11, Att862-7 (FM876782)
* A. mellea (AY900513)
* A. colombiana BR100B (FJ461804)
* A. mellea (AY900512)
* A. mellea (AY900514)
* A. koskei WV786 (FJ461793)
# A. lacunosa BEG78 (AJ891113)
# A. lacunosa BEG78 (AJ891110)
# A. lacunosa BEG78 (AJ891111)
* A. lacunosa BEG78 (AJ510230)
# A. lacunosa BEG78 (AJ891112)
* A. foveata CR315 (FJ461801)
* A. lacunosa WV110 (FJ461800)
# A. paulinae CW4 (AJ891115)
# A. paulinae CW4 (AJ891116)
# A. paulinae CW4 (AJ891118)
# A. paulinae CW4 (AJ891114)
# A. paulinae CW4 (AJ891121)
# A. paulinae CW4 (AJ891117)
# A. paulinae CW4 (AJ891119)
# A. paulinae CW4 (AJ891120)
* A. paulinae (AY639327)
* A. paulinae (AY639328)
* A. paulinae (AY639263)
Acaulospora sp. WUM18, W2941/Att869-3 (FM876792)
* A. paulinae AU103A (FJ461796)
* A. paulinae (AY639264)
Acaulospora sp. WUM18, W2941/Att869-3 (FM876793)
* A. paulinae (AY639265)
A. cavernata BEG33, W3293/Att209-37 (FM876788)
A. cavernata BEG33, W3293/Att209-37 (FM876791)
A. cavernata BEG33, W3293/Att209-37 (FM876789)
A. cavernata BEG33, W3293/Att209-37 (FM876790)
* A. dilatata WV204 (FJ461792)
* A. mellea CR316B (FJ461794)
* A. delicata NY304 (FJ461791)
* A. longula BEG8 (AJ510228)
* Acaulospora sp. CU141 (FJ461803)
* A. morrowiae CR404 (FJ461795)
* A. delicata ML103 (FJ461790)
A. kentinensis TW111 (FM876821)
A. kentinensis TW111 (FN547521)
A. kentinensis TW111 (FM876823)
A. kentinensis TW111 (FN547522)
A. kentinensis TW111 (FM876828)
A. kentinensis TW111 (FM876826)
A. kentinensis TW111 (FN547520)
A. kentinensis TW111 (FM876824)
A. kentinensis TW111 (FM876830)
A. kentinensis TW111 (FM876822)
A. kentinensis TW111 (FN547523)
A. kentinensis TW111 (FM876827)
A. kentinensis TW111 (FM876829)
A. kentinensis TW111 (FM876825)
* A. kentinensis CU114A (FJ461808)
* A. tuberculata BEG41 (AF378436)
* A. tuberculata BEG41 (AF378438)
* A. tuberculata BEG41 (AF378440)
* A. tuberculata BEG41 (AF378439)
* A. tuberculata BEG41 (AF378437)
* A. tuberculata VZ103E (FJ461799)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AM713403)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AM713404)
D. spurca W4119/Att246-18 (FN547644)
D. spurca W4119/Att246-18 (FN547648)
0.1

7. Revealing natural relationships among arbuscular mycorrhizal fungi: culture line BEG47 represents *Diversispora epigaea*, not *Glomus versiforme*

This chapter is identical to the publication:

Schüßler A, Krüger M, Walker C. 2011. Revealing natural relationships among arbuscular mycorrhizal fungi: culture line BEG47 represents *Diversispora epigaea*, not *Glomus versiforme*. PLoS ONE 6: e23333.

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Revealing Natural Relationships among Arbuscular Mycorrhizal Fungi: Culture Line BEG47 Represents *Diversispora epigaea*, Not *Glomus versiforme*

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Abstract

Background: Understanding the mechanisms underlying biological phenomena, such as evolutionarily conservative trait inheritance, is predicated on knowledge of the natural relationships among organisms. However, despite their enormous ecological significance, many of the ubiquitous soil inhabiting and plant symbiotic arbuscular mycorrhizal fungi (AMF, phylum *Glomeromycota*) are incorrectly classified.

Methodology/Principal Findings: Here, we focused on a frequently used model AMF registered as culture BEG47. This fungus is a descendent of the ex-type culture-lineage of *Glomus epigaeum*, which in 1983 was synonymised with *Glomus versiforme*. It has since then been used as '*G. versiforme* BEG47'. We show by morphological comparisons, based on type material, collected 1860–61, of *G. versiforme* and on type material and living ex-type cultures of *G. epigaeum*, that these two AMF species cannot be conspecific, and by molecular phylogenetics that BEG47 is a member of the genus *Diversispora*.

Conclusions: This study highlights that experimental works published during the last >25 years on an AMF named '*G. versiforme*' or 'BEG47' refer to *D. epigaea*, a species that is actually evolutionarily separated by hundreds of millions of years from all members of the genera in the *Glomerales* and thus from most other commonly used AMF 'laboratory strains'. Detailed redescriptions substantiate the renaming of *G. epigaeum* (BEG47) as *D. epigaea*, positioning it systematically in the order *Diversisporales*, thus enabling an evolutionary understanding of genetical, physiological, and ecological traits, relative to those of other AMF. *Diversispora epigaea* is widely cultured as a laboratory strain of AMF, whereas *G. versiforme* appears not to have been cultured nor found in the field since its original description.

Citation: Schüßler A, Krüger M, Walker C (2011) Revealing Natural Relationships among Arbuscular Mycorrhizal Fungi: Culture Line BEG47 Represents *Diversispora epigaea*, Not *Glomus versiforme*. PLoS ONE 6(8): e23333. doi:10.1371/journal.pone.0023333

Editor: Vishnu Chaturvedi, New York State Health Department and University at Albany, United States of America

Received March 28, 2011; **Accepted** July 14, 2011; **Published** August 11, 2011

Funding: Part of the work was funded by the DFG (German Research foundation) under grant number Schu1203/8 (was running until end 2007). There is no current external funding sources for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

A solid phylogeny is the basis for natural systematics and the understanding of hierarchical levels in taxonomy and functional diversity of organisms. This is particularly important for those organisms that are widely used in basic research and are commonly known as model species. Here, we clarify and rectify the systematic classification of an experimentally frequently used arbuscular mycorrhizal fungus (AMF). This fungus, catalogued as BEG47, is phylogenetically distinct from most other laboratory strains affiliated with the genus *Glomus*, but since the early 1980s has erroneously been known as *Glomus versiforme*.

Fungi forming arbuscular mycorrhiza (AM) are main drivers of most terrestrial ecosystems, living in intimate mutualistic symbiosis with the majority of vascular land plants, which they provide with water and inorganic nutrients, mainly phosphorus (P). Because most crop plants form AM, and global P deposits are on the verge of depletion, AMF can be considered indispensable for sustainable agriculture. It will thus become very important to better understand the biology and ecology of individual AMF species. The fact that they are asexual, multikaryotic, and obligately biotrophic, however, makes their study complicated and difficult. All AMF are placed in the monophyletic fungal phylum, *Glomeromycota* [1]. In the past, morphological classification often yielded taxonomic groupings that did not reflect natural relationships. Fortunately, such misclassifications are now less frequent as DNA based characterisation becomes more common.

Many AMF formerly assigned to the genus *Glomus*, based on a limited number of morphological characters, have now been shown to belong to any one clade of the four presently described orders of the *Glomeromycota*, separated by hundreds of millions of years of evolution. For example, the former *G. occultum* and its relatives were shown to belong to an ancient lineage [2] and consequently transferred to *Paraglomus* in the *Paraglomeraceae* [3], which later was assigned to a separate order, the *Paraglomerales* [1]. Likewise, *G. callosum* and *G. gerdemannii* are now placed in the genus *Ambispora* [4–5] (*Archaeosporales*), another basal glomeromycotan lineage. Many systematically misplaced species were thus trans-

ferred from *Glomus* to other genera, in agreement with a natural classification [6], and recently several species from the phyloclade *Glomus* Group C (GlGrC, [7]) have been transferred to the genus *Diversispora* (*Diversisporales*) [8]. Nonetheless, there are many species still called *Glomus*, which remain to be correctly placed once their phylogenetic affiliation is known.

A natural classification system is crucial for the description and understanding of phylogenetic, functional and trait diversity that influence patterns of plant and AMF community productivity. Plant phylogenetic diversity is possibly correlated with community productivity through functional diversity, and high AMF diversity has been shown to promote plant diversity and also plant community productivity [9–10]. Functional differences of AMF and plants must impact upon each other and order- or family-level phylogenetic relations, or both, have been shown to determine AMF community assemblies and mycorrhizal symbiotic functioning [11]. Phylogenetic affiliation may also be important for understanding functioning at the molecular level, as might, for example, be indicated by differential gene expression and pathogen resistance upon colonization by either culture DAOM197198 (as *G. intraradices*, *Glomerales*), BEG47 (as *G. versiforme*) or *Gigaspora gigantea* (*Diversisporales*) [12]. In this instance, BEG47, although named '*Glomus*', is a species from the *Diversisporales* and thus more closely related to *Gigaspora* than to '*G. intraradices*' DAOM197198.

As previously presented for the 'model fungus' in AM research, DAOM197198 [13] (now *Rhizophagus irregularis*: synonym *G. irregulare*, [8][14]), we here present a detailed review of the phylogenetic position of BEG47, which is probably the second most often used AMF culture in basic research and molecular biological studies (e.g. [15–17]). The type material of both, *G. epigaeum* and *G. versiforme* (synonym *Endogone versiformis*) and the synonymisation [18] of BEG47 with *G. versiforme* were re-examined.

The species under consideration in relation to BEG47 are:

i) *Endogone versiformis*, named from combined collections (November 1860 to January 1861) [19] and deposited in the Helsingfor Botanic Garden, Helsinki (H) by W. Nylander. The species was later transferred to the genus *Glomus* as a heterotypic synonym of *G. macrocarpus* var. *macrocarpus* [20] and then recognised as not conspecific with *G. macrocarpum*, and classified as *G. versiforme* [18].

ii) *Glomus epigaeum* (described as *G. epigaeus*) [21], synonymised as a later heterotypic synonym of *G. versiforme* [18]. The species was described from a pot culture at Oregon State University, numerous subcultures of which have been extensively used for research, as *G. epigaeus* [22], as *G. epigaeum* [23] and, most commonly, as *G. versiforme* (e.g., [15–17][24–25]). The culture-line used in basic research, which includes BEG47, stems from the original multi-spore culture from which *G. epigaeum* was described in 1979 [21].

This study aimed at substantiating the phylotaxonomic affiliation of BEG47 and clarifying its phylogenetic relationship within the *Diversisporaceae*. We also included some other species recently transferred from *Glomus* to *Diversispora* and *Redeckera* [8] and considered, in addition, the environmental sequences of *Diversisporaceae* from public databases to analyse the global distribution of species from the *Diversisporaceae*. These data will also facilitate future molecular ecological, evolutionary and taxonomic studies, as they are currently implemented in a third party annotated, web-accessible database [26] for reliable analyses based on well-annotated fungal sequences.

Results

The culture-line represented by BEG47, which was already known to be phylogenetically distinct from most other species in *Glomus* [27–28], produces both pale (e.g. W5167/Att475-45) and darkly coloured (e.g. W5165/Att475-45) spores. The pale spores (which are considerably larger than the size range given for *E. versiformis* [= *G. versiforme*] and may darken with age) are characterized by the same rDNA sequence types as the darker ones and thus are doubtless conspecific.

Molecular phylogeny of *Diversispora epigaea* BEG47 and *Diversisporaceae*

To study the phylogenetic relationships in greater detail, a core sequence dataset was analysed consisting of all *Diversisporaceae* sequences available, except environmental sequences lacking species assignment. The internal transcribed spacer (ITS) and partial large subunit (LSU) rDNA regions of the generic type species, *D. spurca*, were also characterised. The phylogenetic analysis (Figure 1) clearly shows that *G. epigaea* (= *G. versiforme* BEG47), *G. aurantium*, *G. eburneum*, and *G. trimurales* all belong to *Diversispora*, in the *Diversisporaceae*, in agreement with the recent major taxonomic revision of *Glomeromycota* [8]. *Redeckera* is well separated from *Diversispora*, justifying its generic status as already suggested by Redecker and colleagues [29].

The extended dataset contained environmental sequences carrying sufficient phylogenetic information for analysis below genus level (Figure 2), although the sequences that vary greatly in length did not always overlap in the multiple alignment. From non-monophyletic clustering of such non- or partly-overlapping sequences it is impossible to prove whether or not they are of conspecific origin. A couple of short environmental database SSU rDNA sequences were omitted from the analysis shown in Figure 2 because they lowered phylogenetic resolution and disturbed tree-topologies. They all clustered within *Diversispora* at the generic level (Figure S1), except one environmental sequence (DQ357079) from *Ammophila arenaria* rhizosphere soil from Portugal, which clusters basally in the *Diversisporaceae*. The geographical annotations of sequences falling within the phylogenetic lineage of *Diversispora* indicate a panglobal distribution of the genus, through Europe, Africa, Asia, Hawaii, the Middle East, North America and Central America (Figure 2; Figure S1).

Morphology of the spores in the type material of *Endogone versiformis* (*G. versiforme*)

The herbarium packet was annotated 'Type of *Endogone versiforme* Karst. DET: S. M. BERCH DATE: AUG 25, 1983'. The sample was accompanied by a note with sketches in ink, dated 'nov.1860'. The note is expanded with additional drawings and further annotation in pencil, indicating that it was originally in the hand of W. Nylander; however, the additional drawings are unsigned and it could not be established when or by whom they were made. The original notes on the type material, together with the translation into English of the Latin descriptions and annotations, are shown in Figure S2 and the spore dimensions are given in Figure 3. The type consisted of two small packets, each containing a very small quantity of dried substrate incorporating a few very small fragments of sporocarps (Figure S3). No prepared microscope slides or other preserved material were included. Examination of the holotype material of *G. versiforme* (Figure 4) shows that it contained two rather distinctive kinds of spores (Figure 4A–C,I), found either individually in the substrate or as fragments of sporocarps (Figure 4A–E). One morph consists of small, pale spores (Figure 4D,F) with relatively thin walls (Figure 4J). The second morph (Figure 4K) has large, thick-walled darkly coloured spores. Both morphs are directly compared in Figure 4C and Figure 4I. The type was fractionated but it is difficult to determine

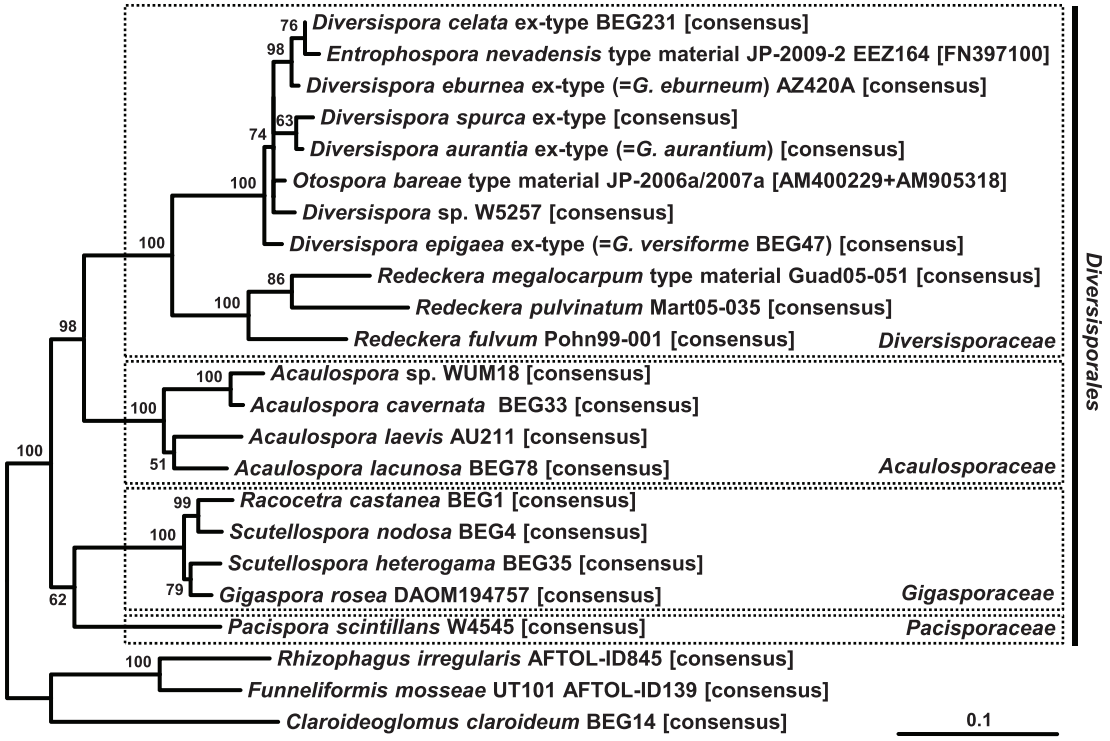

Figure 1. Phylogenetic tree of *Diversisporales* computed from the core dataset of nuclear SSU-ITS-LSU rDNA sequences. RAxML maximum likelihood analysis with bootstrap support shown at the branches; topologies with support below 50% were collapsed to polytomies. The most recent synonyms for species in *Diversispora* are given in brackets. The published '*Entrophospora nevadensis*' sequence (SSU rDNA) is short and does not allow species resolution, but clusters with high support within the *Diversispora celata - D. eburnea* clade. The two short, concatenated '*Otospora bareae*' sequences (SSU rDNA) also cluster within the genus *Diversispora*. The genus *Redeckera* comprises the species formerly published as *Glomus fulvum*, *G. megalocarpum* and *G. pulvinatum*. The tree is rooted with three representative sequences of the sister order *Glomerales*. The scale bar indicates proportional substitutions per site.
doi:10.1371/journal.pone.0023333.g001

if the individual spores result from disintegration of the sporocarps during almost one-and-a-half centuries of storage and handling, or if they actually were produced ectocarpically in the substrate. Nevertheless, for both morphs, spores in the sporocarps and substrate are morphologically identical.

Pale coloured spores form epigeously in sporocarps that are up to 1 cm wide (information from the protologue), though only minute fragments remain in the type collection. The sporocarp peridium has a whitish, matted appearance and consists of tightly tangled thin-walled (<1 μm thick) somewhat squamous aseptate hyphae, 3-6 μm in diameter (Figure 4G). The glebal hyphae appear tangled and are colourless, up to 15 μm wide, with very thin (<1 μm) walls.

The spores (Figure 4D,F,J) are very pale in colour (Methuen 3A3, yellow) and translucent. For 27 of 85 measured spores, it was impossible to determine the point of detachment from the subtending hypha (spore origin) and thus also to determine their lengths and widths. The dimensions of these, by simply taking the longest and shortest dimensions, were 70–104×64–91 (mean 85×77) μm. There is little variation in spore shape, and no spore was noted that exceeded the broadly ellipsoid category, defined by a maximum ratio of length to width of 1:1.3 [30]. Of the remaining 58 spores that could be measured conventionally, 16 were broader than long. Their dimensions were 64–109×64–99 (mean, 83×82) μm. Spore shape varied little; 26 were globose, 29 subglobose, and three broadly ellipsoidal. No truly ellipsoidal (elongate, see [30]) spores were found. The structural spore wall most probably consists of two colourless components in a single group (Figure 4P). Component 1 is persistent and found on all specimens. It is up to 1 μm thick and tightly adherent to component 2 which is 2–5 μm thick. In some specimens, there appears to be a third component, <1 μm thick, but this might be an artefact caused by congealing of spore contents in these very old dried specimens. Most spores were completely detached from their subtending hypha. However, where the subtending hypha could be seen (Figure 4L,M) it was very short (no more than a few μm, but rarely up to 15 μm long), with a very thin (≤1 μm) wall, up to 7 μm wide distally, and usually tapered sharply proximally to a width of ~1 μm. Hyphal attachments appear to be occluded by fusing of the spore wall internally.

Redescription of **Glomus versiforme** (P. Karst.) S. M. Berch (MycoBank **MB106567**) ≡ *Endogone versiformis* P. Karst (MycoBank **MB372848**) (Figure 4A,D,F,G,J,L,M,P).

Sporocarps of indeterminate size and irregular shape, with a pale, felty peridium; protruding through, or on the surface of substrate. Spores globose to subglobose to broadly ellipsoid, 64–109×64–99 (mean, 83×82) μm, with a subtending hypha, often truncated proximally and difficult or impossible to locate. Sealed by a septum-like structure apparently formed from the inner layers of the main structural wall component. Wall structure of an outer, unit wall component (up to 1 μm thick) adherent to an inner, laminated main structural component, 2–5 μm thick, both being continuous with the wall of the subtending hypha, and thus presumably of the sporogenous mycelium. Spores in sporocarps accompanied by thin-walled (<1 μm), balloon-shaped vesicles, 41–92×61–196 μm.

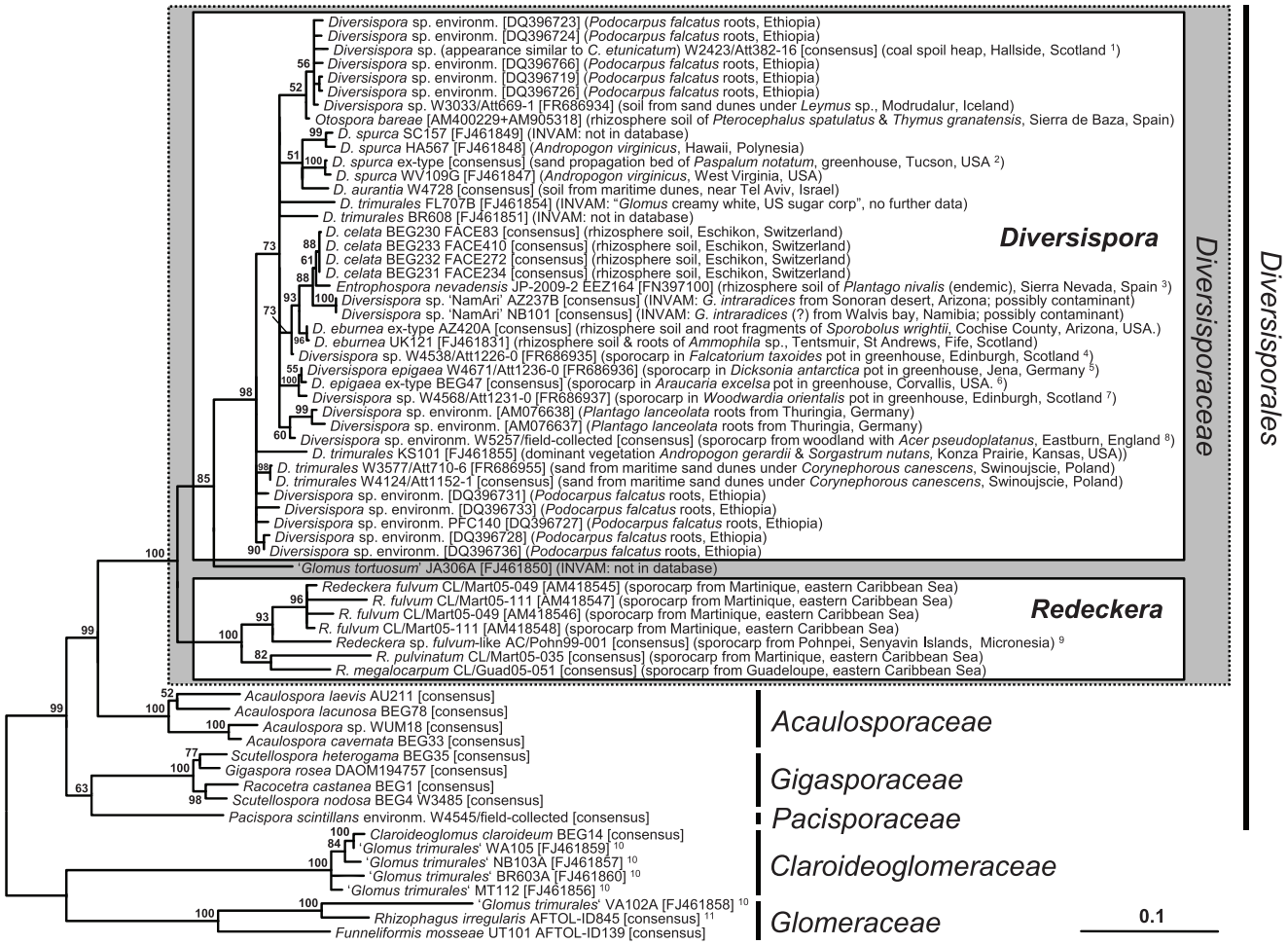

Figure 2. Phylogenetic tree of *Diversisporaceae* computed from the extended dataset, including environmental nuclear rDNA sequences. RAxML maximum likelihood analysis with bootstrap support shown at the branches; topologies with support below 50% were collapsed to polytomies. The tree is rooted with representatives of the *Glomerales*. The scale bar indicates proportional substitutions per site. Except for very short environmental SSU rDNA sequences that distorted the tree topology, all *Diversisporaceae* sequences which were available from the public databases were used and have the following origins: [1] the specimen from which this sequence was derived has *Claroideoglomus etunicatum*-like spore morphology; soil from a re-vegetated coal spoil heap, beneath *Salix* sp. and associated weeds, which included *Plantago major*, *P. lanceolata*, *Fragaria vesca* and various grasses; [2] Fazio's Greenhouse, from M. Pfeiffer's pot culture no. 157, Building 42-2R, University of Arizona; [3] other plants reported at the soil sampling location were *Alchemilla fontqueri* and *Senecio elodes* (both endemic) and *Sorbus* hybrid (non-endemic); [4] fungus with an appearance similar to a 'large-spored *D. epigaea*', from a temperate greenhouse of Royal Botanical Garden Edinburgh, Plant No. 842581 H; [5] immature spores; from fern house of Botanical Garden Jena (the plant was transferred to Jena from the botanical garden of the Wilhelma, Stuttgart, Germany); [7] *Diversispora epigaea*-like spores; temperate greenhouse of Royal Botanical Garden Edinburgh, the pot also contained an *Oxalis* sp. as a weed; [6] tropical greenhouse at the USDA-ARS horticultural research station; [8] sporocarp from litter layer of semi natural woodland, with associated understory, including an *Allium* sp.; [9] this sequence most likely represents a species distinct from *Redeckera fulvum*, therefore it is annotated here as '*R. fulvum*-like'; [10] sequences annotated as '*D. trimurales*', from the same submission as the three sequences (FJ461851,54,55) that cluster in *Diversispora*, but clearly falling in distinct families; [11] culture published as GINCO4695rac-11G2 from the AFTOL project, but lacking further information.

doi:10.1371/journal.pone.0023333.g002

Mycorrhizal status unknown, but by analogy with other members of the *Glomeromycota*, and considering that the specimens came from potted plants in a greenhouse, it is likely that *G. versiforme* forms AM.

Specimens examined: **Finland**, Nylandia, Helsingfors (Helsinki). Spores and fragments of sporocarps from the potting substrate of *Cercocarpus ledifolia* grown in a cold glasshouse, '23. XI. 1860 – I. 1861' [*sic*], leg. W. Nylander (Mus. Bot. Univ., Helsinki 3936 p.p. H – Lectotype [Voucher W4551 (H, isolectotype E)]).

Dark coloured spores form in sporocarps, embedded in coarse, reddish yellow glebal hyphae, and ectocarpically in the substrate (Figure 4B). Because the type sample is fragmented, it is impossible to determine the original size of the sporocarps. The spores are abundant in the substrate as individual spores and also found embedded in substrate aggregates. Therefore it appears that they can be formed ectocarpically and hypogeously. The peridium is reddish yellow (Methuen 4A6) in colour and has a woolly appearance, consisting of angular, thin-walled anastomosing coenocytic mycelium ~3–18 µm diameter (Figure 4H). The spores (Figure 4C,E,K) are coloured variably in shades of orange to brown (Methuen 5D8–5D8), and are opaque due to their thick coloured wall (Figure 4K). Of the 121 measured spores, for 52 it was impossible to determine the location of the attachment to the subtending hypha, and thus impossible to distinguish lengths from

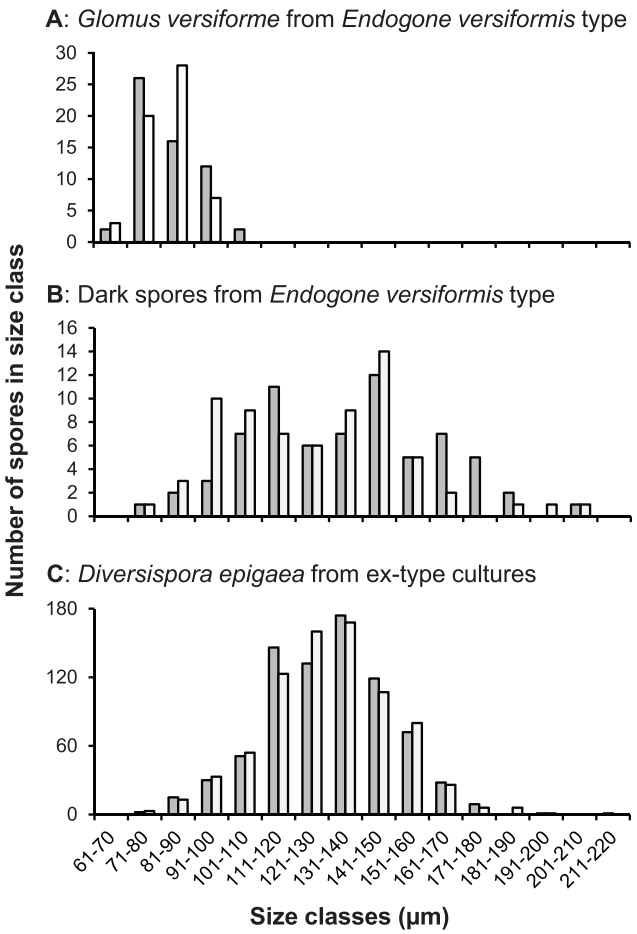

Figure 3. Dimensions of spores from *Glomus versiforme* type collection and *of Diversispora epigaea* (grey: lengths and white: width). A. Spores of the lectotype of *Glomus versiforme* (W4551) prepared from the *Endogone versiformis* type material. **B**. Large spore type (W4550) of an unknown species in the *E. versiformis* type material. **C**. *Diversispora epigaea* BEG47 (combined measurements of specimens from 49 voucher collections sampled from among 29 ex-type sub-cultures).
doi:10.1371/journal.pone.0023333.g003

breadth. By simply taking longest and shortest dimensions, the resulting size range was 73–208×73–208 µm (mean 137×128 µm). There is considerable variation in spore shape, and many spores exceeded the broadly ellipsoid category and were ellipsoidal. Of the remaining 69 spores, 15 were broader than long and 47 were longer than broad. The shape of the spores varied considerably. Seven were globose, 32 subglobose, 20 were broadly ellipsoidal, and 10 were ellipsoidal (elongate).

The spore wall consists of three, possibly four, components (Figure 4Q,R). Component 1 at first is thin, ~1 µm thick. It appears to expand to become as much as 4 µm thick, and eventually to disintegrate and disappear, and thus can be classified as evanescent as defined by Walker [31]. It tightly adheres to component 2, a unit component that varies in thickness from 1–5 µm. Wall component 3 is 5–12 µm thick and very finely laminated, though the laminations often are difficult to distinguish. In many specimens, there seems to be a fourth thin flexible inner component 4 (Figure 4R), though on others it was not detectable (Figure 4Q). It is not clear if this is an artefact of specimen preparation such as a loose lamina of component 3, but it is evident in both glycerol and PVLG-based preparations. The wall thins at the spore base to produce a bowl-shaped lumen 3–10 µm diameter internally, tapering to ~1 µm externally where the subtending hypha is attached (Figure 4N,O). The majority of spores have their subtending hypha detached close to the spore base. When it is retained, it is very difficult to see because it often is extremely thin-walled (normally <1 µm). It can be up to 37 µm long and as much as 15 µm wide distally, tapering to become constricted proximally to about 1 µm in diameter, where it usually becomes detached. On a few specimens, the subtending hypha is thickened to ~2 µm proximally (Figure 4O) and sometimes it appears to be occluded by a plug of amorphous material.

Morphology of *Glomus epigaeum* from the holotype and ex-type culture-lines, including BEG47

The spores are produced in dense masses, lacking a peridium (Figure 5A–C) and with or without varying amounts of brownish contextual hyphae, or singly (Figure 5D), or in loose clusters in the substrate. The spore masses (referred to in the protologue as 'sporocarps') were originally recorded as being 2–8×3–15 mm [21], but they are very variable in size and shape. The colour of the spores is variable (Figure 5B–G). They are colourless at first, soon becoming pale yellow, gradually becoming orange at maturity to dark reddish brown (Methuen 8E8) when moribund. The spore wall components do not react to Melzer's reagent, although the pale spores may become overall slightly yellow.

Seven-hundred and eighty spores were measured from among 29 ex-type cultures (Table S1; Figure 3C); 346 were broader than long, 158 were equal in length and width, and 276 were longer than broad. Spore shape was not very variable, 497 spores being globose, 212 subglobose, 56 broadly ellipsoidal, and only 15 ellipsoidal. Some of these spores were ovoid (8) or obovoid (28), two were flattened somewhat on one side, six were pyriform, and two were subtriangular. The spore dimensions were 78–213×78–192 µm (mean = 131×131 µm, n = 780). The protologue gives spore measurements for the epigeous spores as (60−)75–140(−165)×95–140 µm. In one sample, 100 dark epigeous spores and 100 pale hypogeous spores were measured separately, yielding dimensions of 82–146×85–146 µm (mean = 115×116 µm) and 85–194×96–192 µm (mean = 135×134 µm).

In some spores, the spore wall appears to have a unit outer component (Figure 5J), but on others, it breaks down in patches (Figure 5K), and thus must be considered to be evanescent. The coloured main structural component sometimes seems laminated (Figure 5H,J,K), and at other times the laminae cannot be seen by light microscopy (Figure 5I). Finally there is an innermost component (Figure 5J,K) that is often difficult to discern under the light microscope, but was described as clearly visible in transmission electron micrographs [23]. By light microscopy, the wall structure of spores in PVLG is of three components as follows: component 1 unit or more or less evanescent, colourless, up to 1 µm thick; component 2 laminated, pigmented, 1–10 µm thick depending on age; component 3 <1 µm thick, lightly pigmented, often tightly adherent to component 2 and difficult to discern, sometimes appearing flexible due to shrinkage after immersion in the mounting medium (Figure 5J,K). In a few spores the inner wall component appears to form a septum (Figure 5O). The subtending hypha is variable (Figure 5H,I,M,N,O), very narrow, not more than 10 µm at the base of the spore; straight (Figure 5I) or slightly curved (Figure 5H), or often constricted at the base (Figure 5M). Usually the subtending hyphal wall is thin (1–2 µm), tapering little in most (though not all) of the pale spores. On some mainly darkly coloured spores, the wall of the subtending hypha tapers quite

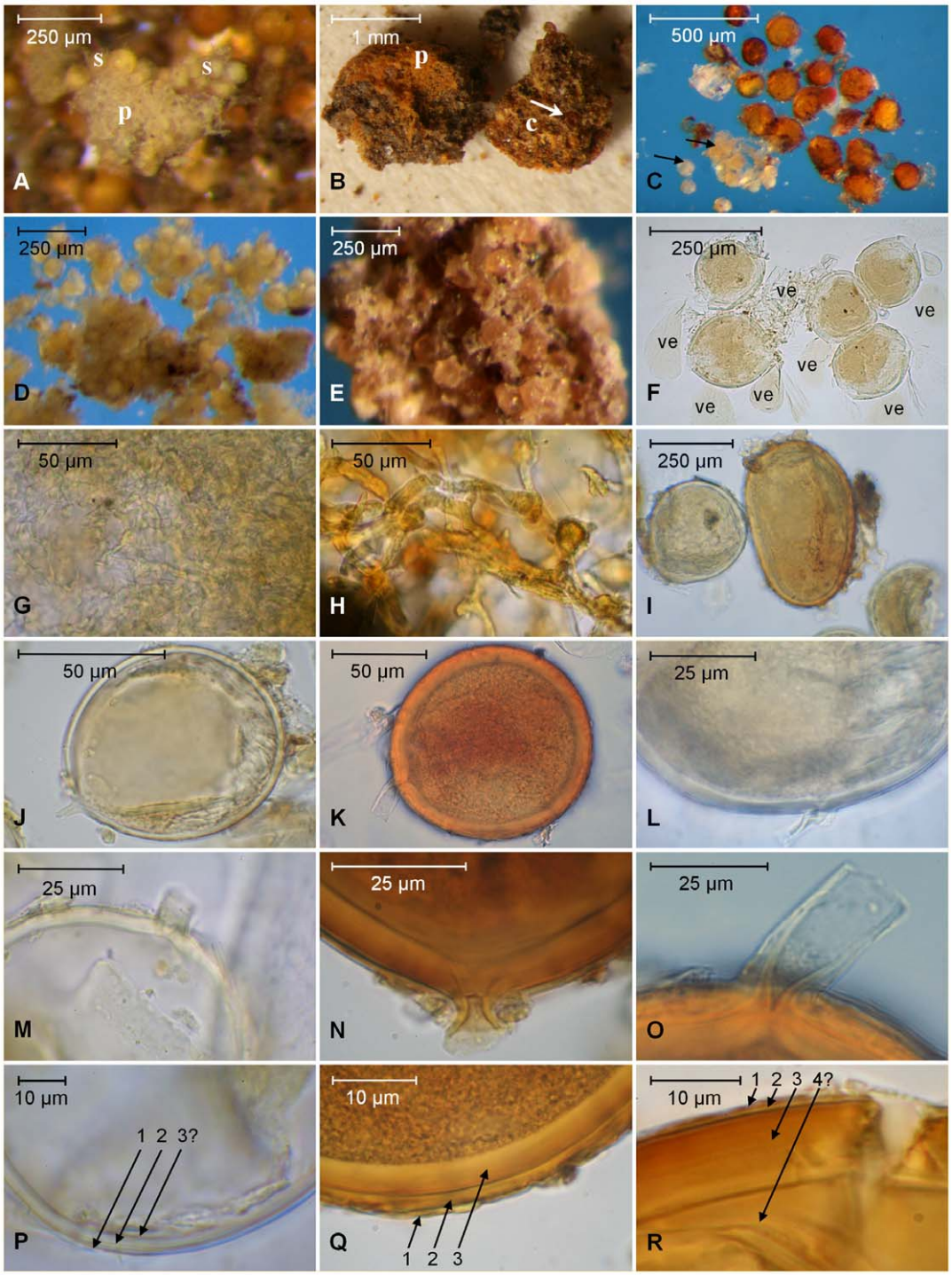

Figure 4. Photomicrographs of specimens from the holotype collection of *Glomus versiforme* (basionym *Endogone versiformis*). Pale spores (*G. versiforme*) of W4551, dark spores (undetermined *Glomus* sp.) of W4550. **A**. Sporocarp portion of *G. versiforme* showing pale spores (s) and a felted, pale-coloured peridium (p). Larger, dark coloured spores of an unknown *Glomus* sp. can be seen out of focus in the background. **B**. Part of a sporocarp of *Glomus* sp. showing the pigmented peridial (p) and contextual (c) hyphae and embedded spores (arrows). **C**. The two different spore morphs in water (*G. versiforme* indicated by arrows), illustrating the difference in spore size and colour. **D**. The pale-coloured spores of *G. versiforme* showing clustered spores from a sporocarp. **E**. Sporocarp portion of the dark spored unknown *Glomus* sp. **F**. Five clustered spores of *G. versiforme* from a sporocarp with accompanying vesicles (ve). **G**. Peridial hyphae of *G. versiforme* showing size and colour. **H**. Peridial hyphae of the dark spored *Glomus* sp. **I**. Spores of *G. versiforme* (left) and of the dark spored *Glomus* sp. (centre), allowing comparison of size, shape and pigmentation. **J**. Thin-walled pale-coloured spore of *G. versiforme*. **K**. A thick-walled darkly coloured spore of *Glomus* sp. **L** and **M**. Subtending hyphae of *G. versiforme*. Most specimens are sessile because of breakage of the very thin subtending hyphal wall at the spore base. **N** and **O**. Subtending hyphae of the dark spored *Glomus* sp., broken close to the spore base and occluded by an amorphous plug in the bowl-shaped lumen (N) or persistent and occluded by spore wall thickening (O). **P**. Wall detail of a spore of *G. versiforme* showing two components in the structural spore wall (1, 2) and a questionable third component internally (3?). **Q** and **R**. Wall detail of a spore of the dark spored *Glomus* sp. showing three components in the structural spore wall (Q), and a possible fourth (4?) separate component (R) internally.
doi:10.1371/journal.pone.0023333.g004

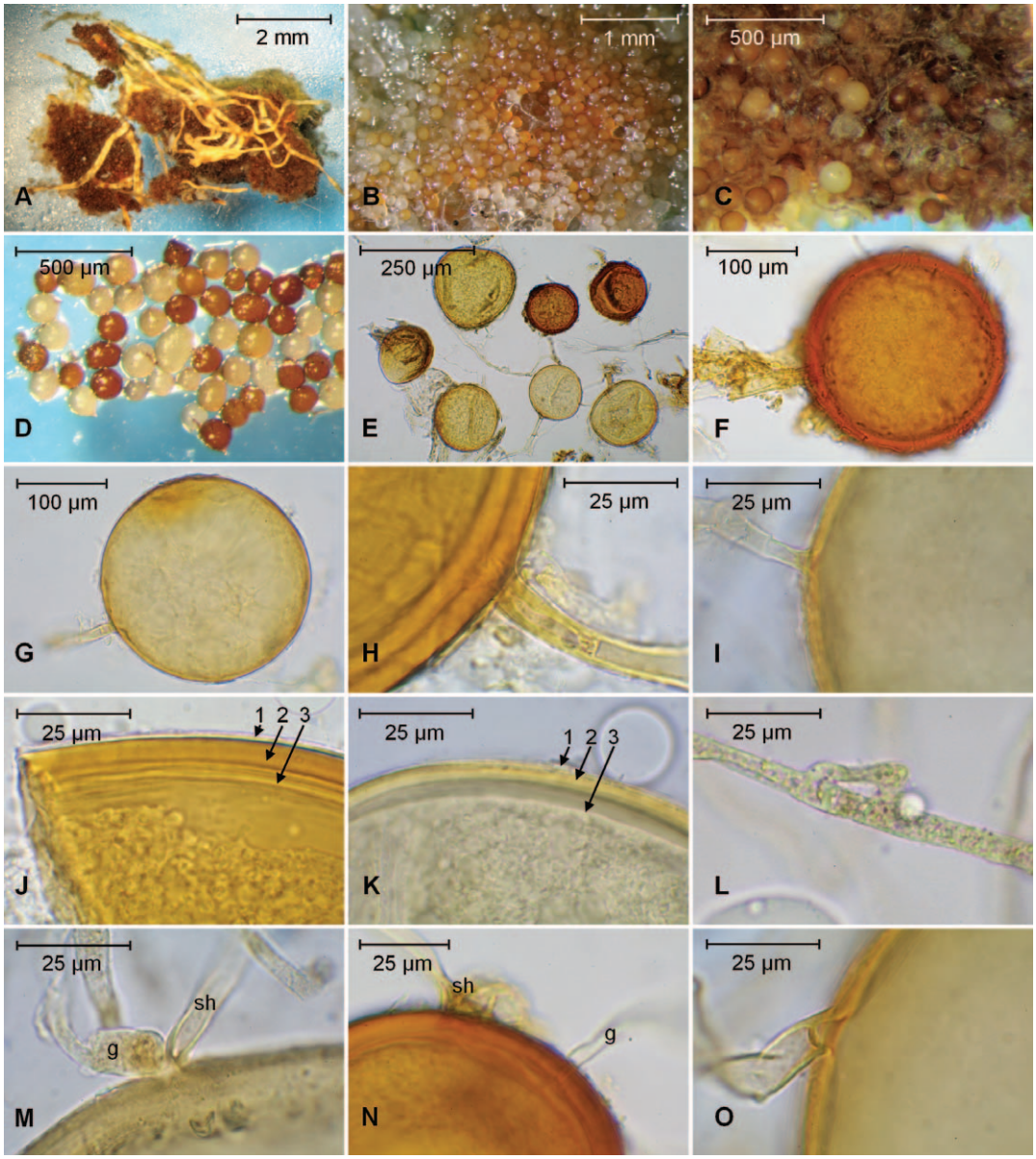

Figure 5. Photomicrographs of specimens from *Diversispora epigaea* ex-type pot cultures (including culture line BEG47). Dark spores of W5165, pale spores of W5167 except Figure 5L, which is from W4565. **A**. Spore cluster, formed on roots near the surface of a pot. **B**. View of a spore cluster showing the undifferentiated aggregation of pale coloured and orange spores. **C**. Spore mass, showing pale and dark spores. **D**. Spores photographed in water, uncovered on a glass microscope slide. **E**. Spores of both colours, showing variation in size, shape and pigmentation. **F**. A thick-walled pigmented spore of the dark morph. **G**. Thin-walled, immature pale-coloured spore. **H** and **I**. Subtending hyphae of dark (H) and pale (I) spores showing occlusion by spore wall thickening and a distal septum in the dark morph. Note the difference in wall thickness. **J** and **K**. Wall structure of dark (J) and pale (K), spores showing thin outer (1), thick laminated (2), and thin inner (3) components. **L**. Hyphal bridging, also known as wound healing, in the somatic mycelium. **M**. Spore germination (g) at the base of the subtending hypha (sh). **N**. Germination directly through the wall. **O**. A septum occluding the hyphal attachment of a thin-walled spore of the pale morph close to the spore base.
doi:10.1371/journal.pone.0023333.g005

sharply from up to 5 µm thick proximally (Figure 5H) to <1 µm distally where detached from the mycelium.

Germination is by emergence of a germ tube through the remnant subtending hypha or directly through the spore wall (Figure 5M,N). This species exhibits the type of self-anastomosis known as hyphal bridging (Figure 5L) or wound healing [32], also found in *D. celata* [33] and *D. spurca* [34]. This phenomenon has also been observed for members of *Ambispora*, *Gigaspora*, and *Scutellospora*, but differs from the formation of interhyphal anastomoses in hyphal networks of members of the *Glomeraceae* [35–36].

Redescription of **Diversispora epigaea** (B.A. Daniels and Trappe) C. Walker and A. Schüßler (MycoBank **MB542916**) ≡ *Glomus epigaeum* (MycoBank **MB314591**) (Figure 5).

Two spore morphs (overall size range 60–213×78–192 µm), depending upon whether formed epigeously or hypogeously. Epigeous dense spore clusters, sometimes called sporocarps, irregular, known to be 2–8×3–15 mm, but seemingly indeterminate in size and shape, formed on substrate surface: peridium lacking, sometimes with a basal hyphal mat extending around the lower sides of the spore cluster. Spores globose to subglobose to

broadly ellipsoid 60–170×85–174 µm, pale cream when young, becoming dull brownish yellow to orange at maturity or, at senescence, brown. Spore wall structure of three components in two groups. Wall group 1 of an evanescent component up to 1 µm thick overlaying a laminated component up to 10 µm thick. Wall group 2 of a thin (<1 µm) flexible component. Subtending hypha variable, straight or slightly curved, up to 10 µm in diameter and often constricted proximally, to 4–6 µm in diameter; subtending hyphal wall proximally up to 5 µm thick, tapering to 1 µm distally, the continuous inner wall component appearing to form an internal septum. Hypogeous spores formed singly, or in loose clusters in the soil; rarely as single spores, bursting through the root cortex; formed on colourless mycelium; colourless at first, soon becoming orange-white to light orange; globose to subglobose or broadly ellipsoid 85–213×96–192 µm. Wall structure and subtending hypha as for epigeous spores. Neither hypogeous nor epigeous spores react in PVLG-Melzer's or pure Melzer's reagent except to become slightly yellowish (contents sometimes becoming orange). Anastomosis of the type known as hyphal bridging (wound healing) present in extraradical somatic mycelium.

Forming arbuscular mycorrhiza with numerous hosts including Araucaria excelsa [21], Asparagus officinalis, Sorghum bicolor, Allium porrum, Plantago lanceolata, Trifolium repens, Lotus japonicus and Festuca ovina (see Table S1).

Specimens examined: Spores and spore clusters from the type material and 29 other ex-type collections from cultures maintained in the USA, UK, Italy, France, Belgium, Finland and Germany (see Table S1).

Discussion

Glomus versiforme (= *Endogone versiformis*)

The epithet given by Karsten [19], *versiforme,* indicates variability although in the protologue there is no mention of extreme variation or of the presence of two morphs in the type material. Obviously, only the paler morph was included in the species circumscription of W. Nylander (Figure S2), and this has been followed by Karsten [19] in his species description, which is brief, but specific. It describes the spores as globose and white, and gives spore dimensions (65–95 µm) that fit only with the smaller of the two morphs. The size range we measured for the pale-coloured spores in the type material of *G. versiforme* corresponds well with that of the protologue of that species. Both the size and appearance of these are very different from those of the larger, orange and more ovoid spores in the substrate comprising the type material. The smaller paler-coloured spores were produced in sporocarps with a pale coloured peridium with white woolly elements, specified in the protologue as a feature of *E. versiformis*. The larger, darkly-coloured spore clusters come from sporocarps with darkly coloured peridial hyphae. With the description of spore colour, size and shape [19] this confirms the opinion that the author's intention was to apply the epithet *versiformis* only to the pale spores. The notes left by W. Nylander and the pencilled annotations (Figure S2) thereon also support this view. Drawings show only globose spores with a rather thin wall, relative to the spore dimensions, unlike the more darkly pigmented spores which have relatively thick walls and received no particular attention by either authority.

Diversispora epigaea (= *Glomus epigaeum*)

The species defined as *G. epigaeum* by Daniels and Trappe [21] and the monospecific type material lodged at OSC required little emendation with respect to its morphology. The junction of the subtending hypha is somewhat more varied than the description implies, and the statement that the subtending hypha is 'inserted into the spore wall' is misleading, because it is continuous with both spore components. In addition, the weak orange reaction to Melzer's reagent is in the cytoplasm, and not in the wall. Spore colour changes considerably with spore development, from nearly colourless for young spores to light orange (hypogeous spores) or dark orange for old epigeous spores. The wall structure of the spores was difficult to assess, sometimes the main structural wall appeared laminated, and other times laminations could not be detected. Because transmission electron microscopy of *D. epigaea* spores [23] showed fine laminae as twisted microfibril layers, the light microscopically visible lamination is considered not to be artefactual. Molecular phylogenetic evidence (Figure 1, Figure S1) clearly shows that BEG47 is not a member of the genus *Glomus* but belongs to *Diversispora*.

Glomus versiforme (= *E. versiformis*) is a fungal species neither cultured nor re-discovered since its original description

The size, colour and nature of the peridium of the two different kinds of sporocarps in the *E. versiformis* (= *G. versiforme*) type collection already indicate that they are unlikely to be conspecific, as indicated by differences in colour, size, form of the subtending hypha and wall structure of the smaller pale and the larger dark-spored morphs. For the pale morph most spores are more or less globose or broader than long, whereas for the dark morph most were longer than broad (we considered this significant, because the ratio of length to width has been used as a species-specific characteristic [37][20]). The pale sporocarps of *E. versiformis* have balloon-shaped saccules amongst their spores, a feature lacking in the larger, darkly pigmented spores, which are morphologically similar to mature spores of *D. epigaea*.

Although spore size of the dark spores in the *E. versiformis* type material is not very different from those of *D. epigaea* BEG47, there are some morphological differences. In the former, hyphal attachments are rare; 68% of spores were broader than long; and there appears to be a complete peridium although only fragments of it were preserved. In contrast, for BEG47, hyphal attachments are easily found; only 44% of spores were broader than long. They are produced in large naked masses of ectocarpic epigeous spores on the surface of the substrate. Whilst it is possible that peridial development may depend on environmental conditions, true sporocarps with peridia have never been reported from cultures of BEG47 over decades of propagation in different laboratories and with different plant hosts and substrates. This further supports the distinctiveness of *D. epigaea* and both *G. versiforme* and the accompanying dark-spored fungus.

Berch and Fortin noted [18] that spores of *G. epigaeum* were much darker and larger than the description in the protologue and concluded that the spores used for the protologue were 'probably immature'. Based on this assumption both the small, pale spores and the large, coloured spores were incorporated within a single combined description [18]. From our microscopic examination of the type material, however, we conclude that the different spore types in the type collection of *E. versiformis* most likely represent different organisms mixed in the same herbarium packet. The use of the plural (glasshouses, plants), and the dating of the collection (23.XI.1860-I.1861) in W. Nylander's notes and P. A. Karsten's protologue indicates that the type material is composed of several collections from different glasshouses and plants and thus is most likely to be mixed. The current Botanical Code dictates that type material must come from only one collection, but no such requirement applied at the time of Karsten's description.

The Botanical Code, Articles 9.9, 9.12, requires that the spore morph selected to represent *G. versiforme* from the mixed collection must be that which most closely conforms to the original diagnosis. The pale spores, presence of a 'white-woolly' peridium with fine hyphae and the narrow hyphal attachments therefore preclude *G. epigaeum* (= *D. epigaea* BEG47) as a potential synonym of *G. versiforme*. Nevertheless, given that we could not obtain glomeromycotan DNA sequences from the type material of *G. versiforme*, we cannot completely exclude the possibility that the small pale and large pigmented spores in the type collection originate from a single dimorphic species, although this seems extremely unlikely. As a consequence of this notion that the original species description of *G. versiforme* was based on more than one species, a lectotype (W4551) was designated to define precisely the species [8] and to provide an emended description, based only on the pale spores (W4551). It should be noted that the new species description of *G. versiforme* is made from a combination of the original protologue and a limited number of dead spores from a mixed collection preserved in air-dried substrate for about 150 years, during which time the spores have deteriorated. To date we have not found any other conspecific specimens, nor can we find evidence that similar spores have been collected by anybody else since the original description of the species. If a representative of *G. versiforme* were to be found, it would be advantageous to define an epitype and to resolve its phylogenetic position. Without molecular evidence, the natural systematic position of *G. versiforme* must remain uncertain but morphologically, it is not conspecific with *D. epigaea*.

BEG47 represents *Diversispora epigaea* (= *G. epigaeum*) and not *Glomus versiforme* (= *E. versiformis*)

Based on the present investigation, we must conclude that BEG47 is not synonymous with *G. versiforme* in the strict sense because:

a) two distinct spore morphs from more than one collection were included in the type material of *E. versiformis* (= *G. versiforme*), most likely from two different AMF species, whereas the species description of *E. versiformis* clearly refers only to the smaller spore morph and does not mention the *D. epigaea*-like spore morph;

b) BEG47 and other *D. epigaea* (= *G. epigaeum*) ex-type cultures do not form spores similar to the small pale spore morph in the type collection of *E. versiformis*, which represent *G. versiforme*.

Molecular evidence presented here shows BEG47 to belong to the genus *Diversispora*, and consequently, under the rules of the Botanical Code, it has to be placed in that genus as *D. epigaea*. *Diversispora epigaea* is widely cultured and frequently used as a laboratory strain for molecular, physiological and ultrastructural research, whereas *G. versiforme* appears not to have been cultured nor found in the field since its original description.

DNA sequence annotation in the public databases

Based on previous phylogenetic analyses [6][33] and additional data gathered during this study, *D. aurantium*, *D. eburnea*, and *D. trimurales* were also transferred from *Glomus* to *Diversispora* [8]. Several of these sequences are still annotated as '*Glomus*', in the public databases. Another database sequence ascribed to *G. tortuosum* culture accession JA306A clusters basal to *Diversispora* but has to be considered of uncertain phylogenetic affiliation. No entry with the identifier JA306 could be found in the INVAM culture collection database and the sequence was included in a sequence deposition (FJ461790-FJ461888) to Genbank that likely contains mis-annotations or contaminant sequences, as for example, those attributed to '*G. trimurales*' which are derived from at least three divergent AMF lineages (Figure 2). There are many sequences in the public databases that probably are incorrectly named. This problem will soon be overcome by third party annotation using the PlutoF workbench [26], through which environmental sequences from the ITS region, such as those earlier annotated as 'uncultured *Glomus versiforme*' from Thuringia (AM076638, AM076637), will be accessible. Species identity of these environmental sequences is not known, but is unlikely to be conspecific with *D. epigaea* (BEG47) [33], and thus should be annotated as '*Diversispora* sp.'. The *Diversispora* sp. sequences annotated as 'NamAri' from the INVAM cultures NB101 (AF185682,90-91, AF185693-95; from Namibia) and AZ237B (AF185677-81; from Arizona) are most likely of conspecific origin and are very closely related to, or perhaps conspecific with, *D. celata*. Also the short SSU rDNA sequence FN397100 ascribed to *Entrophospora nevadensis* from Sierra Nevada, Spain, is very closely related to those of *D. celata*. For the INVAM cultures NB101 and AZ237B, we suspect that the sequences could be derived from culture contaminants, wrongly determined species, or that there was a mistake made during sequence annotation, because the cultures themselves are named as '*G. intraradices*' in the INVAM database. The taxonomic assignment of the sequence for *E. nevadensis* is difficult to explain. Perhaps it has been derived from a contaminant and not from the fungus morphologically described in its protologue [38], which does not share morphological characteristics with any other species in *Diversispora*.

Biogeography of the genera *Diversispora* and *Redeckera* (*Diversisporaceae*)

Members of the genus *Diversispora* appear to occur worldwide, with sequence-based records from Europe (England, Scotland, Spain, Switzerland, Germany, Poland, Estonia, Iceland), North America (California), Central America (Panama), Africa (Ethiopia), Asia (South Korea), Hawaii, and the Middle East (Israel). One sequence from Portugal (DQ357079) might be derived from another as yet undescribed genus in the *Diversisporaceae*. Habitats and hosts of *Diversispora* spp. are diverse and include some from natural and disturbed temporal and tropical ecosystems. So far, members of the genus *Redeckera* have been recorded from Guadeloupe (Caribbean Sea) and Micronesia, and one environmental sequence representing this genus originated from South Korea. Regarding the biogeography of the species in the *Diversisporaceae*, present data do not yet provide a distinct picture of global biogeography, and in some instances (e.g. for *Diversispora* sp. 'NamAri') the origin of the sequences seems questionable. Nevertheless, members of the genus *Diversispora* are widely distributed, reinforcing the notion that species of this genus are much overlooked although integral parts of many ecosystems [33][45]. Improved molecular characterisation and in-field identification, in future will lead to better understanding of this ecologically and perhaps also economically significant group of AMF.

Materials and Methods

Generation of sequences and gathering of reference sequences

To study the phylogenetic relationships of BEG47 with other members of the *Diversisporaceae*, a core dataset was analysed that contained all available sequences of *Diversisporaceae*, except environmental sequences lacking species assignment. For the generic type species, *D. spurca*, the nuclear internal transcribed spacer (ITS) and large subunit (LSU) rDNA sequences were also characterised in this study.

For BEG47, DNA was extracted from single spores (see Table S2). PCR amplification of the near full length nuclear small subunit (SSU) rRNA gene was carried out with the primer pairs NS1/Geo10 and

GeoA2/Geo11. Cloning, sequencing and sequence editing were carried out as described previously [6]. Some shorter fragments were amplified with different primer pairs, which are noted in the corresponding sequence database entries. The ITS region of nuclear rDNA was initially amplified with the primers SSU-Glom1 [39] and LSU-Glom1b (TCGTTTCCCTTTCAACAATTTCAC; [5]) or the reverse primer LR4+2 [13]. PCR was run with the Phusion High-Fidelity DNA polymerase with the following thermocycling program: 99°C denaturation for 2 min; 35 cycles of: 99°C for 10 s, 65°C for 30 s, 72°C for 60 s; final elongation at 72°C for 5 min. Later, the ITS region was amplified together with a part of the LSU rRNA gene as previously described [40]. The resulting SSU-ITS-LSU fragment covers ~250 bp (3′ end) of the SSU rDNA, the complete ITS region including the 5.8S rRNA gene, and ~800 bp (5′ end) of the LSU rDNA. After cloning and plasmid isolation, fragments were sequenced on an ABI automated capillary sequencer (Applied Biosystems, Forsters City, CA, USA). Electropherograms were proof-read, trimmed and assembled with SeqAssem and sequences manually aligned to a seed-alignment by using Align (both programs from Sequentix, Klein Raden, Germany; http://www.sequentix.de). The nucleotide basic local alignment search tool (nBLAST [41]) at NCBI was used to compare the new nucleotide sequences against entries in public databases and to identify diversisporacean public database sequences.

The core alignment comprised the near full-length SSU rRNA gene sequences from this study as well as such of the *Diversisporaceae* from public databases. These SSU rDNA sequences were condensed to one strict consensus sequence (coding any variable site as a degenerate base, according to IUPAC ambiguity code) if from the same fungal isolate or culture, or in one instance (*Redeckera fulvum*; synonym *G. fulvum*) from field-collected material. Details about how the strict consensus sequences were calculated are given in Table S2. The term 'ex-type' is used in a broad sense to indicate that the studied material is derived from a descendent of the type culture. Besides culture-derived sequences also environmental public database sequences of *Diversisporaceae* were included. An extended alignment was created for a second, broader phylogenetic analysis containing those additional short environmental sequences that did not completely disturb tree topology at the below genus level. A third dataset, used to compute the tree shown in Figure S1, additionally comprised all short environmental sequences available from the databases, including very short ones.

Computation of phylogenetic trees

Phylogenetic maximum likelihood (ML) analyses were performed with the software RAxML through the CIPRES science gateway (http://www.phylo.org/portal2/) with the GTRGAMMA model for 1000-fold bootstrapping as well as for final tree construction. The analyses, with species from the *Glomerales* as outgroup, were based on 3043 sites from an alignment of 23 sequences (core dataset, Figure 1) or 3023 sites from an alignment of 86 sequences (extended dataset, Figure 2). Neighbour joining and parsimony analyses gave essentially the same results as the ML method (results not shown). Resulting trees were drawn in FigTree 1.3.1 (http://tree.bio.ed.ac.uk//) and edited with Microsoft PowerPoint 2007 and Adobe Illustrator CS3. New rDNA sequences were deposited in the EMBL database with the accession numbers AM713428, AM713432, and FR686934-FR686958.

Morphology of spores, spore masses and sporocarps

Spores from pot culture substrate were extracted by centrifugation and sugar floatation [42] or by agitating and swirling in water and decanting through sieves with 35 or 50 µm openings. Selected spores were mounted in polyvinyl alcohol lactophenol (PVL) or polyvinyl alcohol lacto-glycerol (PVLG) with (PVLG/M) or without the addition of Melzer's reagent (4:1 PVLG:Melzer's v/v) and observed through a compound microscope, with or without Nomarski differential interference contrast optics. Vouchers were stored as colonised, dried potting substrate containing roots and spores, or as semi-permanent microscope slides with specimens mounted in PVL, PVLG or PVLG/M. Vouchers, other than types, are deposited in the herbarium of the Royal Botanic Garden Edinburgh (E), along with an isolectotype of *G. versiforme* consisting of a prepared microscope slide in PVLG (Slide W4551-8). The terminology for defining spore shapes and the convention of giving spore dimensions as length by breadth, including ornamentation but excluding appendages, follows Hawksworth and colleagues [30]. Length was always taken as a perpendicular from the spore base (point of subtending hypha). Consequently, spores can be 'broader than long'. Spore dimensions were measured on selected samples with a calibrated eyepiece graticule under a compound microscope and colours were matched with the Methuen Handbook of Colour [43]. Specimens were indexed by referring to pot cultures as Attempts (Att) and giving herbarium voucher specimens a number with a 'W' prefix [44], which from our own work always include microscope-slide preparations, but that may be any preserved material.

The culture tracking and specimen vouchering system allows the addition of cultures and vouchers from other sources. Thus in this study, we notionally numbered the original *Araucaria* plant, part of the plant collection in the tropical glasshouse at Oregon State University, as Att475-0 even though it was not a deliberate attempt to create a mycorrhizal pot culture. The subsequent pot culture, established by B. Daniels on asparagus with spores taken from Att475-0, was given the notional number Att475-1. The holotype of *Glomus epigaeum* (now *Diversispora epigaea*) came from this type culture pot. It was given the voucher number W90, and an authenticated sample from this pot culture, provided to C. Walker on 12 Apr 1979 by B. Daniels, was numbered W100.

The holotype of *Endogone versiformis* (now *Glomus versiforme*), loaned by the herbarium in Helsinki (H), consisted of two small packets of dried spore masses or fragments of spore masses in a gritty substrate. It included no prepared slides or other evidence of microscopic preparations, though there were some annotations by previous workers (Figures S2, S3). Type specimens were examined first dry, and then, as small subsamples, in a dish of water. Where the spore masses were sufficiently large, they were illuminated by reflected light and examined through a dissecting microscope. Colour determinations were made in comparison with standard charts, illuminated with the same light as the specimens through a split fibre optic light source at its full working voltage (colour temperature, ~3100 K). Individual spores or very small spore clusters were selected with fine forceps and suspended in water for detailed examination.

For *G. epigaeum* we examined type or authenticated material and living ex-type subcultures such as BEG47. The type material (OSC39475) consisted of a herbarium packet that included a slide holder, labelled 'TYPE *Glomus epigaeum* B. Daniels', The slide mailer also has 'Pot217' (or 'Pot2,7') and '7/7/78' hand printed on the upper right corner. There was also a small unlabelled vial about half full of lactophenol containing spores and spore masses. In addition, a plastic slide holder with two slides made by J. Spain, one with spores in lactophenol and one with spores in PVLG+lactophenol, was included. The former had dried out, and was re-constituted with acidified glycerol. There was also a slide (spores in what seems to be PVLG) made by S. M. Berch in 1983. The original lactophenol mounted slide (Trappe 5174) was missing. Three new slides were made by mounting spores and

small fragments of spore masses in PVLG, and given the voucher numbers W90-2, W90-3, and W90-4. By deduction from the protologue and from personal communication with Barbara Hetrick (née Daniels), we determined that the type culture of *G. epigaeum* (now named *D. epigaea*) was established with *Asparagus officinalis* between autumn 1976 and an unknown date in 1977, with a single spore mass removed from a greenhouse pot with *Araucaria excelsa*. No further details of the culturing history and origin of the species are available. Thirty nine vouchers, collected from among 29 ex-type subcultures between 1979 and the present, are available from the herbarium of the Royal Botanic Garden Edinburgh (E) (C. Walker collection; see Table S1).

Supporting Information

Figure S1 Phylogenetic tree of *Diversisporaceae* with additional environmental nuclear rDNA sequences. Owing to the short length of most environmental sequences several branches lack statistical support and phylogenetic resolution. RAxML maximum likelihood tree with bootstrap support shown at the branches; topologies with support below 50% were collapsed to polytomies. Sequences that were not included in the analysis shown in Figure 2 all cluster in the *Diversispora* clade, except one (DQ357079 from rhizosphere soil from Portugal), which clusters basally in the *Diversisporaceae*. The other short sequences not shown in Figure 2 originated from Great Britain, from colonised roots of *Agrostis capillaries* and *Trifolium repens* (annotated as 'phylotype Glo12', AF437656, AF437657) and from roots, probably of *Acer pseudoplatanus*, from an urban environment (indirect evidence, no definitive source given in database, AJ716004); from Estonia, from roots of *Fragaria vesca* (AM849266, AM849271F) sampled in a boreo-nemoral forest in Koeru and from roots of *Oxalis acetosella* (AM849285) and *Hepatica nobilis* (AM849295, AM849296, AM849307); from South Korea, Chungbuk, from *Panax japonicus* roots (EU332718, EU332719, EU332707); from U.S.A., California, from a grassland (EU123386, EU123387, EU123390, EU123394, EU123465, EU123391, EU123392); from Panama, Barro Colorado Island, from *Faramea occidentalis* seedling roots (AY129577).
(PDF)

Figure S2 Information accompanying the *Endogone versiformis* type material. Transcription of the handwritten labels and notes of W. Nylander (23 Nov 1860 – Jan 1861), and annotations included in the herbarium packet containing the holotype of *Glomus versiforme* (basionym *Endogone versiformis*), and their translation into English. Protologue of *E. versiformis* (Karsten 1884) and its translation into English.
(PDF)

Figure S3 Type collection of *Endogone versiformis*. Open herbarium packet of the type of *E. versiformis*, containing dried substrate from potted plants, with spores and fragments of sporocarps and a Petri dish (5 cm diameter) containing sporocarp fragments from the dried substrate.
(PDF)

Table S1 List of studied samples of the *Diversispora epigaea* (= *Glomus epigaeum*) ex-type culture-line. The culture that was registered as BEG47 is part of the ex-type culture-line of *D. epigaea*.
(PDF)

Table S2 Composition of the strict consensus sequences used in the phylogenetic analyses. In strict consensus sequences, site variations are coded by the IUPAC ambiguity code, thus retaining information of the source sequences as degenerate bases, unlike majority rule consensus sequences.
(PDF)

Acknowledgments

We are grateful to the curators at H for loan of the *E. versiformis* holotype and for permission to remove parts of the type for study, and at OSC for the loan of the *G. epigaeum* holotype. We thank Herbert Stockinger for PCR amplifying and cloning DNA from some BEG47 samples. We also thank Barbara Hetrick, John Menge, Denise Egel, Carol Grace, Francis Sanders, Paola Bonfante, Alice Broome, Valeria Bianciotto, and Bachar Blal for supplying specimens of *D. epigaea*, and we are grateful to James Trappe and Max Ellerbeck for their help with transcribing and translating the Latin of W. Nylander's notes and the protologue of *E. versiformis* and to James Trappe for his helpful comments on early versions of the manuscript.

Author Contributions

Conceived and designed the experiments: AS CW. Performed the experiments: AS CW MK. Analyzed the data: AS CW. Contributed reagents/materials/analysis tools: AS CW MK. Wrote the paper: AS CW.

References

1. Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the *Glomeromycota*: evolution and phylogeny. Mycol Res 105: 1413–1421.
2. Redecker D, Morton JB, Bruns TD (2000) Ancestral lineages of arbuscular mycorrhizal fungi (*Glomales*). Mol Phylogenet Evol 14: 276–284.
3. Morton JB, Redecker D (2001) Two new families of *Glomales*, *Archaeosporaceae* and *Paraglomaceae*, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. Mycologia 93: 181–195.
4. Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, et al. (2007) Molecular phylogeny and new taxa in the *Archaeosporales* (*Glomeromycota*): *Ambispora fennica* gen. sp. nov., *Ambisporaceae* fam. nov., and emendation of *Archaeospora* and *Archaeosporaceae*. Mycol Res 11: 137–153.
5. Walker C, Vestberg M, Schüßler A (2007) Nomenclatural clarifications in *Glomeromycota*. Mycol Res 11: 253–256.
6. Schwarzott D, Walker C, Schüßler A (2001) *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (*Glomales*), is non-monophyletic. Mol Phylogenet Evol 21: 190–197.
7. Schüßler A, Gehrig H, Schwarzott D, Walker C (2001) Analysis of partial *Glomales* SSU rRNA gene sequences: implications for primer design and phylogeny. Mycol Res 105: 5–15.
8. Schüßler A, Walker C (2010) The *Glomeromycota*: a species list with new families and new genera. Schüßler A, Walker C, Gloucester, Published in libraries at The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University; electronic version freely available online at www.amf-phylogeny.com.
9. Cadotte MW, Cavender-Bares J, Tilman D, Oakley TH (2009) Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. PLoS ONE 4(5): e5695.
10. van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, et al. (1998) Mycorrhizal fungi diversity determines plant biodiversity, ecosystem variability and productivity. Nature. pp 69–72.
11. Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316: 1746–1748.
12. Liu JY, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant J 50: 529–544.
13. Stockinger H, Walker C, Schüßler A (2009) '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. New Phytol 183: 1176–1187.
14. Sokolski S, Dalpé Y, Séguin S, Khasa D, Lévesque CA, et al. (2010) Conspecificity of DAOM197198, the model arbuscular mycorrhizal fungus, with *Glomus irregulare*: molecular evidence with three protein-encoding genes. Botany 88: 829–838.
15. Gomez SK, Harrison MJ (2009) Laser microdissection and its application to analyze gene expression in arbuscular mycorrhizal symbiosis. Pest Manag Sci 65: 1526–4998.
16. Naumann M, Schüßler A, Bonfante P (2010) The obligate endobacteria of arbuscular mycorrhizal fungi are ancient heritable components related to the *Mollicutes*. ISME J 4: 862–871.
17. Zhang Q, Blaylock LA, Harrison MJ (2010) Two *Medicago truncatula* half-ABC transporters are essential for arbuscule development in arbuscular mycorrhizal symbiosis. Plant Cell 22: 1483–1497.
18. Berch SM, Fortin JA (1983) Lectotypification of *Glomus macrocarpum* and proposal of new combinations: *Glomus australe*, *Glomus versiforme*, and *Glomus tenebrosum* (*Endogonaceae*). Can J Botany 61: 2608–2617.

19. Karsten PA (1884) Fragmentia mycologica XII. Hedwigia 23: 39–40.
20. Gerdemann JW, Trappe JM (1974) The *Endogonaceae* in the Pacific Northwest. Mycologia Memoir No. 5. New York: New York Botanical Garden. 76 p.
21. Daniels BA, Trappe JM (1979) *Glomus epigaeus* sp. nov., a useful fungus for vesicular-arbuscular mycorrhizal research. Can J Botany 57: 539–542.
22. Daniels BA, Menge JA (1980) Hyperparasitization of vesicular-arbuscular mycorrhizal fungi. Phytopathology 70: 584–588.
23. Bonfante-Fasolo P, Vian B (1984) Wall texture in the spore of a vesicular-arbuscular mycorrhizal fungus. Protoplasma 120: 51–60.
24. Harrison MJ, van Burren ML (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378: 626–629.
25. Lanfranco L, Garnero L, Bonfante P (1999) Chitin synthase genes in the arbuscular mycorrhizal fungus *Glomus versiforme*: full sequence of a gene encoding a class IV chitin synthase. FEMS Microbiol Lett 170: 59–67.
26. Abarenkov K, Tedersoo L, Nilsson RH, Vellak K, Saar I, et al. (2010) PlutoF – a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. Evol Bioinform: 6: 189–196.
27. Gehrig H, Schüßler A, Kluge M (1996) *Geosiphon pyriforme*, a fungus forming endocytobiosis with *Nostoc* (*Cyanobacteria*), is an ancestral member of the *Glomales*: evidence by SSU rRNA analysis. J Mol Evol 43: 71–81.
28. Walker C, Schüßler A (2004) Nomenclatural clarifications and new taxa in the *Glomeromycota*. Mycol Res 108: 981–982.
29. Redecker D, Raab P, Oehl F, Camacho FJ, Courtecuisse R (2007) A novel clade of sporocarp-forming fungi in the *Diversisporales* lineage. Mycol Prog 6: 35–44.
30. Hawksworth DL, Sutton BC, Ainsworth GC (1983) Ainsworth and Bisby's Dictionary of the *Fungi*, 7th edn. Kew, UK: CMI. 445 p.
31. Walker C (1983) Taxonomic concepts in the *Endogonaceae*: spore wall characteristics in species descriptions. Mycotaxon 18: 443–455.
32. Gerdemann JW (1955) Wound-healing of hyphae in a phycomycetous mycorrhizal fungus. Mycologia 47: 916–918.
33. Gamper HA, Walker C, Schüßler A (2009) *Diversispora celata* sp. nov: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. New Phytol 182: 495–506.
34. Pfeiffer CM, Walker C, Bloss HE (1996) *Glomus spurcum*: a new endomycorrhizal fungus from Arizona. Mycotaxon 59: 373–382.
35. Voets L, de la Providencia IE, Declerck S (2006) *Glomeraceae* and *Gigasporaceae* differ in their ability to form hyphal networks. New Phytol 172: 185–188.
36. de la Providencia IE, de Souza FA, Fernández F, Delmas NS, Declerck S (2005) Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. New Phytol 165: 261–271.
37. Thaxter R (1922) A revision of the *Endogoneae*. P Am Acad Arts Sci 57: 291–351.
38. Palenzuela J, Barea JM, Miguel J, Ferrol N, Azcón-Aguilar C, et al. (2010) *Entrophospora nevadensis*, a new arbuscular mycorrhizal fungus from Sierra Nevada National Park (southeastern Spain). Mycologia 102: 624–632.
39. Renker C, Heinrichs J, Kaldorf M, Buscot F (2003) Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. Mycorrhiza 13: 191–198.
40. Krüger M, Stockinger H, Krüger C, Schüßler A (2009) DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol 183: 212–223.
41. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402.
42. Walker C, Mize CW, McNabb JrHS (1982) Populations of endogonaceous fungi at two locations in central Iowa. Can J Botany 60: 2518–2529.
43. Kornerup A, Wanscher JH (1978) Methuen Handbook of Colour, 3rd edn. London: Methuen. 252 p.
44. Walker C, Vestberg M (1988) Synonymy amongst the arbuscular mycorrhizal fungi: *Glomus claroideum, G. maculosum, G. multisubstensum* and *G. fistulosum*. Ann Bot-London 82: 601–624.
45. Gamper H, Leuchtmann A (2007) Taxon-specific PCR primers to detect two inconspicuous arbuscular mycorrhizal fungi from temperate agricultural grassland. Mycorrhiza 17: 145–152.

8. A phylogenetic framework for the natural systematics of arbuscular mycorrhizal fungi: from phylum to species-level resolution and environmental deep sequencing

The content of this chapter is identical to the manuscript resubmitted for publication at 19. August 2011.

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. **2011**. A phylogenetic framework for the natural systematics of arbuscular mycorrhizal fungi: from phylum to species-level resolution and environmental deep sequencing. *New Phytologist*, resubmitted.

A phylogenetic framework for the natural systematics of arbuscular mycorrhizal fungi: from phylum to species-level resolution and environmental deep sequencing

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. 2011.

Summary

- Although the molecular phylogeny, evolution and biodiversity of the arbuscular mycorrhizal fungi (AMF) are becoming clearer, reliable sequence data are still limited. Therefore, a dataset allowing resolution and environmental tracing across all major taxonomic levels, including species, is provided.
- Two overlapping nuclear DNA regions, totalling ~3 kb were analysed: the small subunit (SSU) rRNA gene (up to 1800 bp) and a fragment spanning ~250 bp of the SSU rDNA, the internal transcribed spacer region (ITS region, ~475-520 bp) and ~800 bp of the large subunit (LSU) rRNA gene. The entire range could be analysed for 34 species, the SSU rDNA for ~76 unnamed and 18 undefined species, and the ITS or LSU rDNA or a combination of both of ~87 named and 17 yet undefined species were analysed.
- Phylogenetic analyses of the three rDNA markers provide a reliable and robust resolution from Phylum to species level. Altogether 105 named and 28 cultures ascribed to yet undefined species were analysed.
- With this study we provide a baseline dataset for molecular systematics and community analyses of AMF in the field, including analyses based on deep sequencing.

Introduction

The arbuscular mycorrhizal (AM) fungi (*Glomeromycota*; Schüßler *et al*., 2001) form symbioses with most land plants, in almost any terrestrial ecosystem (Smith & Read, 2008). Despite the considerable ecological importance of these fungi, their biology and ecology is still not well understood. This is partly because of their obligately symbiotic, asexual and hidden lifestyle in soil and roots.

The characterization and identification of AM fungi (AMF) has been mainly based on the structure of their spores. However, conclusions may be flawed because many taxa show limited morphological characters. Some species form more than one spore morph, and cryptic species can be determined only through molecular evidence. Such problems are reflected by several recent taxonomic revisions (Kaonongbua *et al*., 2010; Morton & Msiska, 2010a; Schüßler & Walker, 2010). Irrespective of difficulties in AMF classification, in many studies it is important to know the fungal identities and species. However, even 'model fungi' in AM research were shown to be misclassified (Stockinger *et al*., 2009; Sokolski *et al*., 2010; Schüßler *et al.*, 2011).

Correct affiliations are crucial for AMF community studies, which are increasingly performed solely based on molecular genetic markers. Most commonly used is the nuclear small subunit (SSU) rRNA gene, hereafter referred to as SSU. Several SSU-targeting PCR primers (e.g. Simon *et al.*, 1992; Helgason *et al*., 1998; Lee *et al.*, 2008) that amplify fragments of ~500-800 bp have been widely applied in ecological studies (Öpik *et al*., 2008; Zhang *et al*., 2010). However, even the full length SSU does not resolve closely related species (Walker *et al*., 2007; Gamper *et al*., 2009). In SSU datasets, one phylotype may represent several different species and, conversely, different phylotypes may belong to one species. We therefore eschew terms like 'virtual taxa' (Öpik *et al*., 2010) for taxonomically undefined phylotypes, as 'taxon' in mycology is clearly defined (Botanical Code, Article 1.1). A more appropriate term is molecular operational taxonomic unit (MOTU). Standardised MOTUs are a goal for the classification of unknown fungal species from environmental samples (Hibbett *et al*., 2011), but care has to be taken that the units indeed are based on coherent taxonomic levels (Hawksworth *et al*., 2011).

The more variable region covering the nuclear internal transcribed spacer (ITS) 1, the 5.8S rRNA gene and ITS2 rDNA (hereafter referred to as ITS region) has also been used for detecting AMF (Redecker *et al*., 2000; Renker *et al*., 2003; Hempel *et al*., 2007), but is often inadequate for discriminating closely related species (Stockinger *et al*., 2010). As a marker with intermediate sequence variability the nuclear large subunit rRNA gene (hereafter referred to as LSU) has proved useful for AMF detection (Gollotte *et al.*, 2004; Pivato *et al.*, 2007), although many of the primers used do not amplify particular AMF lineages (Krüger *et al.*, 2009). Other markers such as the mitochondrial LSU rRNA gene (Börstler *et al.*, 2010; Sýkorová *et al.*, 2011), *β-tubulin* (Msiska & Morton, 2009), *RPB1* and *RPB2* (James *et al*., 2006; Redecker & Raab, 2006) or H^+-*ATPase* (Corradi *et al.*, 2004; Sokolski *et al.*, 2010) have been used, but either they are

inapplicable for AMF identification, only studied for few species, or unsuitable for phylogenetic species resolution.

The nuclear rDNA region sequence dataset is taxonomically sufficiently broad to permit molecular ecological field studies of AMF communities. However, comparisons among studies are often difficult because of inconsistency in the use and coverage of the different loci. The variable ITS region sequences are often used to determine fungal species (e.g., Tedersoo *et al.*, 2008), but for AMF most environmental phylotypes based on this region are not determined, and often are not determinable (Stockinger *et al.*, 2010), to species-level. Thus, neither the SSU nor the highly variable ITS region alone resolve closely related AMF, but reliable species identification is possible based on a ~1.5 kb rDNA fragment (Stockinger *et al.*, 2009), easily amplifiable with AMF specific primers (Krüger *et al.*, 2009). This SSU-ITS-LSU fragment covers ~250 bp of the SSU, the complete ITS region and ~800 bp of the LSU. Shorter fragments, such as the ~400 or soon 800 bp reads, provided by 454 sequencing, can provide species resolution if analysed together with a 'phylogenetic backbone' based on longer sequences (Stockinger *et al*., 2010).

In this further effort to establish a solid reference database, we (re-)analysed the nuclear rDNA regions that i) can be specifically and easily PCR-amplified for AMF (Krüger et al., 2009), ii) resolve closely related species to allow DNA barcoding (Stockinger et al., 2009, 2010), and iii) facilitate the application of deep sequencing technologies for in-field detection of AMF (Stockinger et al., 2010).

Materials and Methods

AMF material, DNA-extraction, PCR, cloning and sequencing

The identities of the AMF subjected to molecular analyses were determined from morphological characters. For most of them, vouchers were deposited in the C. Walker collection and are available from the Royal Botanic Garden Edinburgh (Table S1).

Cleaned AMF spores were used for DNA extraction or stored as described in Schwarzott & Schüßler (2001). For some extractions, a simplified PCR-buffer protocol was followed (Naumann *et al*., 2010). DNA was extracted from individual spores, except for some isolates (derived from one single spore) for which up to 10 spores were pooled. PCR amplification of the near full

length SSU was as described in Schwarzott & Schüßler (2001). Some SSU fragments, from earlier studies, were amplified with the primers AML1-AML2, NS1-NS2, NS1-Geo10 and GeoA1-ITS1Frc (ITS1F reverse complementary, 5'-TTACTTCCTCTAAATGACCAAG-3').

For amplification of a ~1.8 kb SSU-ITS-LSU fragment, the primers SSUmAf-LSUmAr (in some cases with LR4+2 as reverse primer; Stockinger *et al*., 2009) were used, mostly followed by a nested PCR with the primers SSUmCf-LSUmBr or, in some earlier attempts, SSU-Glom1-NDL22 (Krüger *et al*., 2009; Stockinger *et al*., 2010), resulting in an ~1.5 kb amplicon covering ~250 bp of the SSU, the whole ITS region and ~800 bp of the LSU. PCR products were cloned and analysed as described in Krüger *et al.* (2009).

New sequences were deposited in the EMBL database under the accession numbers AM114274, AM713432, FR750012-FR750095, FR750101-FR750117, FR750126-FR750127, FR750134-FR750217, FR750219-FR750228, FR750363-FR750376, FR750526-FR750544, FR772325, FR773142-FR773152 and FR774917.

Sequence data and Glomeromycota *taxonomy used*

Sequences in the public databases were reviewed to establish if they were from defined cultures or environmental samples. Environmental sequences not identified to species were excluded. Defined sequences of >650 bp and some shorter sequences were included or assembled to 'contiguous' sequences if they were the only ones available for a particular taxon, or culture. For several database sequences it is unclear if they refer to an AMF single spore isolate, multi spore culture, or simply to a recombinant DNA *E. coli* clone number. Our annotations follow the most recent systematics of the *Glomeromycota* (Schüßler & Walker, 2010), including the suggestions of Morton & Msiska (2010a) and Kaonongbua *et al.* (2010). Detailed sequence origin information is listed in Table S1.

Phylogenetic analyses

For the SSU sequences, one strict (with variable sites coded according to IUPAC as degenerated bases) consensus sequence was deduced from up to 10 sequence variants for each isolate or culture. The PCR primer binding sites were excluded, when known. Three different datasets were then analysed:

i) For the phylogenetic tree computed from ~2.7 kb sequences (Fig. 1) we concatenated the above noted strict SSU consensus sequence with one strict consensus sequence made from all SSU-ITS-LSU sequence variants of the same fungus (defined by culture identifier), whereas the unalignable ITS1 and ITS2 were excluded. Such SSUfull-5.8S-LSU sequences could be assembled for 34 species from 38 cultures. Since there were no corresponding SSU and SSU-ITS-LSU sequences available for an individual *Archaeospora schenckii* culture, sequences from two different cultures (Att58-6, Att212-4; sequences identical in the 250 bp SSU overlap) had to be concatenated to cover the genus *Archaeospora*. *Batrachochytrium dendrobatidis* (*Chytridiomycota*) was used as outgroup and the following members of basal fungal lineages and *Dikarya* were also included: *Ascomycota* (*Exophiala dermatitidis*, *Schizosaccharomyces pombe*), *Basidiomycota* (*Henningsomyces candidus*, *Rhodotorula hordea*), *Kickxellomycotina* (*Orphella haysii*, *Smittium culisetae*), *Mucoromycotina* (*Endogone pisiformis*, *Mortierella verticillata*, *Phycomyces blakesleeanus*, *Rhizopus oryzae*) and *Blastocladiales* (*Allomyces arbusculus*, *Coelomomyces stegomyiae*).

ii) Near full length SSU strict consensus sequences (≤1.8 kb) were used to compute a SSU tree (Fig. 2) for 76 AMF species from 145 cultures (including shorter fragments of 500-1300 bp for 18 species from 26 cultures).

iii) All individual SSU-ITS-LSU sequences (up to 24 variants; ~1.5 kb) available from a culture were analysed. To 'anchor' phylogenetically the variable ITS and LSU sequences by the more conserved SSU, each variant was concatenated at the 5' end with one SSU strict consensus sequence of the same culture, if available. This allows a more robust resolution of deeper (above genus) topologies and avoids artificial clustering resulting from misalignment or convergent characters due to mutational saturation in the highly variable regions. Subtrees at order and family level could be computed for 87 defined and 17 unnamed species (Figs 3-9), representing all main lineages in the *Glomeromycota*. For the model fungus *Rhizophagus irregularis* DAOM197198, a reduced sequence set, still representing the breadth of rDNA variability, was used, as a detailed analysis was already published in Stockinger *et al.* (2009). For *Gigasporaceae*, *Paraglomerales* and *Archaeosporales*, the composite dataset also included short database sequences (≥500 bp) if their inclusion did not reduce the topological support too much (Figs 3, 4). For the genera in the *Glomerales* (except *Rhizophagus*) separate analyses were conducted for long sequences (Figs 7, 9), and after inclusion of short sequences (Figs S1, S2).

All maximum likelihood phylogenetic analyses were computed through the CIPRES web-portal with RAxML ver. 7.2.7 (Stamatakis *et al*., 2008) using the GTRGAMMA model and 1000 bootstraps for both the bootstrapping phase and the final tree inference model.

Results

For phylogenetic analyses, a ~1.8 kb SSU fragment and a ~1.5 kb SSU-ITS-LSU fragment, both overlapping by ~250 bp in the 3' SSU, were analysed (Table S1) together with public database sequences. Altogether, sequences derived from 105 AMF annotated to species and 28 undescribed species could be analysed phylogenetically.

SSUfull-5.8S-LSU phylogeny of the Glomeromycota (Fig. 1)

The phylogenetic tree was computed from 39 assembled 2.7 kb consensus sequences representing 34 species. The highly variable ITS1 and ITS2 regions were excluded because alignment is impossible among higher taxa. However, their inclusion did not alter tree topology (data not shown), demonstrating robust phylogenetic anchoring by the more conserved regions (that receive more weight in RAxML analyses). The topology of the SSUfull-5.8S-LSU tree is congruent with previously published rDNA trees, but with higher bootstrap support (BS). The *Glomeromycota* are supported as monophyletic, with the *Paraglomerales* as the most ancestral lineage (separated with 85% BS from all other AMF lineages). The next basal lineage, the *Archaeosporales* (including *Geosiphonaceae*, *Archaeosporaceae* and *Ambisporaceae*) resolves as monophyletic (88% BS) and the following sister clades *Diversisporales* and *Glomerales* cluster together with 100% BS. The *Diversisporales* appears monophyletic (94% BS), with all its families well supported (except *Entrophosporaceae* which had to be excluded for lack of reliable sequence data).

Members of the *Glomerales* (63% BS) separate into the *Glomeraceae* (former *Glomus* Group [GlGr] A) and *Claroideoglomeraceae* (former GlGrB). The *Glomeraceae* contains the four genera *Funneliformis* (former GlGrAa), *Rhizophagus* and *Sclerocystis* (former GlGrAb), and *Glomus* (former GlGrAc). *Glomus* is represented by the generic type species *Glomus macrocarpum* (epitype W5581/Att1495-0) and *Funneliformis* by *F. mosseae*, *F. coronatum*, *F. caledonium* and *Funneliformis* sp. WUM3. In *Rhizophagus*, the 'model fungus' *Rh. irregularis* DAOM197198 clusters with two other cultures of this species, GINCO4695rac11G2 and a root

organ culture (ROC) annotated as DAOM212349. However, the last number is the voucher number also used for the type material of *Claroideoglomus lamellosum* (from a field collection) and moreover for an 'isotype' pot culture of that species. The sequences of *Rh. intraradices* ex-type culture FL208 cluster as sister to *Rh. proliferus* (DAOM226389).

SSU phylogeny of the Glomeromycota (Fig. 2)

The available sequences of 76 species (145 cultures) were analysed. For the basal lineages *Archaeosporales* and *Paraglomerales* relatively few are characterised. Sequences of the former *Intraspora schenckii* cluster among those of *Archaeospora*.

In the *Diversisporales*, the SSU tree shows 100% BS for the *Gigasporaceae*. *Gigaspora* appears monophyletic, but *Racocetra* and *Scutellospora* are not convincingly resolved. *Scutellospora gilmorei*, *S. nodosa* and *S. pellucida* cluster on a branch together with *Racocetra* species. *Scutellospora cerradensis*, *S. reticulata*, *S. heterogama* and the recently described *Dentiscutata colliculosa* form a monophyletic clade (80% BS), and the remaining *Scutellospora* species fall close to the type species *S. calospora*, in an unresolved basal polytomy. The family *Acaulosporaceae* is well supported (100% BS), but not the deeper branching order within the family. For *Otospora bareae* (Palenzuela *et al*., 2008) the concatenation of two short non-overlapping partial SSU sequences (AM400229, AM905318) clusters among *Diversispora* sequences, as does the only sequence (FN397100) published for *Entrophospora nevadensis* (Palenzuela *et al*., 2010). *Redeckera*, a genus based on data from Redecker *et al.* (2007), clearly separates from *Diversispora*. The *Pacisporaceae* are sister to *Gigasporaceae* with 79% BS.

The *Glomeraceae* and *Claroideoglomeraceae* are both supported by 100% BS. *Glomus iranicum* and *G. indicum* (Błaszkowski *et al*., 2010a,b) fall basally into a polytomy in the *Glomeraceae*. *Funneliformis* is composed of *F. mosseae* (9 cultures), *F. coronatum* (W3582/Att108-7, COG1), *F. geosporum* (BEG11), *Funneliformis* sp. DAOM225952, *F. caledonium* (BEG15, BEG20), *Funneliformis* sp. WUM3, *F. fragilistratum* and *F. verruculosum*. *Funneliformis constrictum*, together with *F. africanum*, clusters basally. *Glomus* clusters with low BS (61%) sister to *Funneliformis*, comprising sequences of *G. macrocarpum* (W5293, W5605/Att1495-0) and *Glomus* sp. W3347/Att565-7. *Rhizophagus* comprises *Rh. irregularis* (DAOM197198, AFTOL-ID845, W4533/Att1225-1, and the above noted DAOM212349), *Rhizophagus* sp. W3563, *F. vesiculiferum* (W2857/Att14-8; erroneously placed in *Funneliformis* in Schüßler & Walker, 2010,

to be classified as *Rhizophagus*), *Rh. fasciculatus* BEG53, *Rh. intraradices* FL208, *Rh. clarus* (BR147B, W3776/Att894-7) and *Rh. manihotis* (FL879, W3224/Att575-9). The genus *Sclerocystis* is represented by two sequences, one each from *Sc. sinuosa* (MD126) and *Sc. coremioides* (BIORIZE), forming a lineage basal to *Rhizophagus*. *Claroideoglomus* separates into two clades, one comprising *Claroideoglomus* sp. W3349/Att565-11 and *C. viscosum* BEG27 (possibly incorrectly annotated, see discussion) sequences, and the other containing sequences of *C. lamellosum* (W3161/Att672-13, W3158/Att244-7 (ex-'isotype' culture, corresponding to DAOM212349), W3814/Att756-1, W3816/Att844-2), *C. etunicatum* (UT316, W3815/Att843-1, W3808/Att367-3), *C. luteum* SA101, *C. claroideum* (BEG14, BEG23, BEG31), and *Claroideoglomus* spp. (BR212, W3234/Att13-7, DAOM215235).

SSU-ITS-LSU phylogeny of the basal AMF lineages - Paraglomerales *and* Archaeosporales (Fig. 3)

Sequence data are available for all three described *Paraglomus* species. *Paraglomus occultum* sequences from four cultures cluster together with 95% BS, including two of three sequences from *P. occultum* CL383. The third short CL383 sequence and one from *P. occultum* FL703 group with *P. laccatum*, but with low support. One sequence (FJ461809) of W5141 and one annotated as *Archaeospora schenckii* (FJ461809), submitted to the database by Amarasinghe & Morton in 2010, tightly group with *P. laccatum*. The latter must be misannotated. All sequences from this submission are marked below with '◄' (see also Figs 3-6, S1-S2). Sequence FJ461884◄ of the INVAM culture NI116B clusters basally to these sub-clades, and U81987◄ ascribed to *P. occultum* GR582 falls in the *P. brasilianum* clade, implying a possible misannotation.

The *Archaeosporales* are represented by sequences from 15 *Ambispora*, five *Archaeospora* and one *Geosiphon* cultures. *Archaeospora trappei* was analysed using concatenated sequences for cultures AU219 (=WUM19) and NB112, respectively. *Ar. schenckii* sequences cluster with those assigned to *Ar. trappei*. For *Ar. schenckii* CL401 the two short sequences available could not be concatenated, because sequence AM743189 (3'-SSUpartial-ITS) clustered close to *Ar. trappei* NB112, but a partial LSU sequence (FJ461809◄) clusters in *Paraglomus*. According to personal communication (J. Morton, 8 Apr 2011) regarding this sequence submission set (◄), it later was discovered that the CL401 culture also contains *P. occultum*; therefore FJ461809◄ must be considered as contaminant-derived. *Ambispora leptoticha* (85% BS), *Am. callosa* (79% BS), *Am.*

fennica (98% BS), and *Am. granatensis* (Palenzuela *et al*., 2011; 100% BS) are well resolved, but when including the short NC169-3 sequences, which cluster unresolved, BS decreases. NC169-3 was recently named *Am. appendicula* (Kaonongbua *et al*., 2010) based on conspecificity with the former *Acaulospora appendicula* (Morton *et al*., 1997). The concatenated sequence of *Am. gerdemannii* AU215 clusters with *Am. callosa* (BS 85%). Another sequence annotated as *Am. gerdemannii* MT106 (FJ461885◄) clusters with *Am. fennica* (BS 100%), pointing to misannotation or a contaminant.

SSU-ITS-LSU phylogeny of the Diversisporales – Gigasporaceae (Fig. 4)

After two recent revisions (Oehl *et al*., 2008; Morton & Msiska, 2010a), the family *Gigasporaceae* currently contains *Gigaspora, Scutellospora* and *Racocetra*. *Gigaspora* and *Racocetra* are supported without conflict. From the nine described *Gigaspora* species, five could be analysed and separated into two subclades. One comprises *Gi. rosea* (DAOM194757, BEG9) along with sequences of putatively conspecific field-collected yellowish *Gigaspora* spores (W2992), and one shorter sequence each of *Gi. albida* BR235◄ (listed as '*Gi. rosea*?' in INVAM) and *Gi. gigantea* MA401◄. The other clade comprises *Gi. margarita* BEG34 sequences from two independent cultures and shorter sequences, one from *Gi. decipiens* AU102◄, three from '*Gi. gigantea* isolates' and two *Gi. margarita* sequences (Gigmar58, Gigmar60).

In *Scutellospora*, comprising 23 described species including *Dentiscutata colliculosa*, sequences are available for 11 species. *Scutellospora* divides in three groups, one (*Scutellospora* sensu Oehl *et al*., 2008) clusters basally within the *Gigasporaceae* and is represented by *S. spinosissima* W3009/Att664-1, four *S. calospora* (generic type) cultures, and *S. dipurpurescens* WV930◄. A second clade (90% BS; corresponding to *Cetraspora* sensu Oehl *et al*., 2008) clusters with high support sister to *Racocetra* and comprises *S. gilmorei* (99% BS when short sequences were excluded; not shown) and *S. nodosa* BEG4 (100% BS when short sequences excluded; not shown). When including short *S. pellucida* sequences (AY639261, AY639309, AY639313, AY639323; Gamper & Leuchtmann, 2004), the BS for *S. nodosa* BEG4 decreased to 60% and *S. gilmorei* is no longer supported, and the short *S. pellucida* NC155C◄ sequence clusters among sequences of *S. nodosa* BEG4 (Fig. 4). The third clade of *Scutellospora* (85% BS), corresponding to *Dentiscutata* and *Quatunica* sensu Oehl *et al.* (2008), is basal to *Gigaspora.* It comprises sequences from several *S. heterogama* cultures (BR155, NY320, WV858B, SN722, FL225,

CL157, BEG35, FL654=W5611/Att1577-4 originally determined by Schenck as *S. dipapillosa*), *S. cerradensis* MAFF520056, *S. reticulata* CNPAB11 and some short sequences of *S. reticulata* (annotated as *S. nigra*, but re-determined by C. Walker as *S. reticulata* from stored specimens provided by J. Jansa, Dec 2010) and *S. erythropa*. Short sequences of two *S. erythropa* cultures (Sen, MA453B) cluster together with reasonable support while a third one (HA150◄) is unresolved. The well supported genus *Racocetra* (96% BS) comprises sequences from six species. *Racocetra fulgida* (W2993) is well supported (not shown), but becomes unresolved when including shorter sequences of *R. verrucosa*, *R. gregaria*, *R. persica* and *R. coralloidea*. *Racocetra weresubiae* was transferred back to *Scutellospora* by Morton & Msiska (2010a), but returned to *Racocetra* (Schüßler & Walker, 2010) because of its phylogenetic position (Fig. 4).

SSU-ITS-LSU phylogeny of the Diversisporales – Acaulosporaceae (Fig. 5)

Presently there are sequences from 38 described *Acaulospora* species, 21 of which could be analysed. The phylogenetic tree clearly supports the transfer of the former *Kuklospora kentinensis* TW110 and *K. colombiana* to *Acaulospora* (Kaonongbua *et al*., 2010).

Acaulospora alpina, *A. brasiliensis*, *A. colliculosa*, *A. lacunosa*, *A. kentinensis* and *A. laevis* are well resolved. The species concept for *A. entreriana* is questionable as it appears morphologically indistinguishable from *A. laevis*. Sequences of cultures from both species could be separated in the analyses if the variable ITS region was included (Fig. 5). For *A. paulinae* two sister-clades appear, one comprising eight sequences of CW4 and a second clade containing one *A. paulinae* AU103A◄ and two *Acaulospora* sp. WUM18 sequences. *Acaulospora cavernata* BEG33 and *A. denticulata* cluster monophyletically with *A. paulinae* (note: BEG33 was earlier mis-determined as *A. scrobiculata* by C. Walker, the error has been communicated to the BEG for correction). The only available partial LSU sequence of *A. scrobiculata* AU303◄ clusters much apart, sister to *A. tuberculata* (VZ103E) in a clade together with *A. spinosa* W3574/Att165-9 (ex-type culture) and MN405B◄. For several short sequences the results are rather unclear, as they are only represented by one sequence or by sequences from different cultures that cluster apart from each other.

SSU-ITS-LSU phylogeny of the Diversisporales – Diversisporaceae (Fig. 6)

All data available for *Pacispora* were already shown in Figs 1 and 2 and are therefore omitted here. For *Diversispora*, there are six described species (Schüßler & Walker, 2010), all

characterised by rDNA sequences. The relatively short sequences of *Diversispora* sp. NB101 and *Diversispora* sp. AZ237B with stated origin from Namibia and Arizona, respectively, are very closely related. Including these short sequences decreases the BS for *D. celata* as a monophyletic clade from 99% (not shown) to 62% (Fig. 6). The *Diversispora* species are well supported, but for both *D. spurca* and *D. aurantia*, two distinct clades appear in the phylogenetic analysis. One *D. spurca* clade is well defined by sequences from an ex-type culture (W4119/Att246-18) and contains a sequence of *D. spurca* WV109◄. The second clade is composed of two sequences (FJ461848◄, FJ461849◄) from other cultures, and might represent another species. Despite the reasonable support of the *D. aurantia* clade, comprising sequences derived from the holotype trap culture (W4728/Att1296-0), two sequences from the same culture (EF581864, EF581861) form a separated clade. The only sequence published for *G. tortuosum* JA306A (FJ461850◄) clusters in a basal polytomy. Three diverse '*D. trimurales*' sequences from the cultures KS101◄, FL707◄ and BR608◄ cluster at different positions throughout *Diversispora* and require further validation. The three species in *Redeckera* form a separated, well supported clade (99% BS).

Entrophosporaceae *is phylogenetically undefined*

There are only two described species, *E. baltica* and *E. infrequens* (generic type), in the *Entrophosporaceae.* Additionally *E. nevadensis* was recently described (Palenzuela *et al*., 2010), but its sequence clusters in the *Diversispora* clade (Fig. 2). Other database sequences annotated as *Entrophospora* species are often shorter than 450 bp (e.g., AF378456-523), environmental, uncharacterised, or should be annotated as *Acaulospora* (Kaonongbua *et al*., 2010). We excluded all *E. infrequens* sequences from the analyses as they were very short or showed high similarity with *Claroideoglomus*, *Gigaspora* or *Rhizopus oryzae* sequences (see Schüßler *et al*., 2003). Sequences from the cultures CA203◄ and IN215◄, all of which are of doubtful identity, also cluster within *Claroideoglomus* (not shown).

SSU-ITS-LSU phylogeny of the Glomerales – Glomeraceae (Funneliformis *and* Glomus, Fig. 7)

Glomus in its strict sense currently comprises only *Glomus macrocarpum* (W5581/Att1495-0, W5293/field-collected) and *Glomus* sp. W3347/Att565-7, morphologically similar to *G. macrocarpum*, but distinct because of a darker spore color. One sequence attributed to *Glomus hoi* (BEG104) clusters with *Glomus* sp. W3347 and one of *G. aggregatum* (OR212◄) clusters

basally to *G. macrocarpum* (Fig. S1). *Funneliformis* is well supported and represented by *F. mosseae* (75% BS), *F. coronatum* W3582/Att108-7 (100% BS), *Funneliformis* sp. WUM3 (100%) and *F. caledonium* BEG20 (97%), agreeing with Stockinger *et al.* (2010).

When including short sequences (Fig. S1), *F. coronatum* ZTL clusters with cultures W3582/Att108-7, BEG28, and IMA3. A BEG49 sequence clusters apart, together with *F. constrictum* BEG130. *Funneliformis multiforum* DAOM240256 is well supported; *F. geosporum* separates in two clades. For culture MD124 one ITS sequence annotated as *G. geosporum* (AF197918) clusters within *Claroideoglomus* (Fig. S2) and one LSU sequence (FJ461841◄) annotated as *G. macrocarpum* clusters with *F. geosporum* (Fig. S1). Examination of MD124 (C. Walker W2843 in 1996, W5729 in 2010) showed it to be *F. geosporum*. *Funelliformis caledonium* sequences (BEG86, BEG20, DAOM234210, SC658, RMC658, RWC658, JJ45) cluster unresolved. Several such discrepancies (e.g. for *F. monosporum, F. dimorphicum*) were already revealed by Stockinger *et al.* (2010). Sequences of *G. deserticola*, represented by an ex-type culture (BEG73, AJ746249), *F. xanthium*, and *F. constrictum* (NE202◄, UT188◄) cluster in a separated clade, and a sequence from IN214A◄ forms another, basal and very long branch (Fig. S1). This also holds true for *G. globiferum* FL327B◄ and *G. insculptum* PL121◄, which were excluded from our analyses.

SSU-ITS-LSU phylogeny of the Glomerales – Glomeraceae (Rhizophagus *and* Sclerocystis, Fig. 8)

For *Rh. irregularis* and *Rh. intraradices*, Stockinger *et al.* (2009) already published detailed analyses. Here, we add new sequences from '*G. cerebriforme*' MUCL43208 (not formally placed in *Rhizophagus*, because of uncertain identification), *Rhizophagus* sp. MUCL46100, and several *Rh. irregularis* cultures (W4682/Att857-12, BEG195, DAOM197198, DAOM233750, MUCL46240, MUCL43205, FTRS203). *Rhizophagus irregularis*, *Rhizophagus* sp. MUCL46100, *Rh. intraradices* (FL208, MUCL49410), *Rh. clarus* W3776/Att894-7 and *Glomus cerebriforme* DAOM227022 (species identification needs further study), which clusters basally to all studied *Rhizophagus* species, are very well supported (96-100% BS). The weaker support for *Rh. proliferus* DAOM226389 (68% BS) is caused by the short sequence GQ205079 that most likely is of chimeric origin. When including short sequences, one from *G. microaggregatum* DAOM212150 clusters close to *Rhizophagus* sp. MUCL46100 (not shown), and one from *G. microaggregatum* UT216B◄ on a long branch within *Claroideoglomus* (Fig. S2). All three

available *Rh. custos* DAOM236381 sequence variants cluster among sequences of *Rh. irregularis* and one '*Glomus trimurales*' VA102A◄ sequence clusters with those of *Rh. irregularis* (not shown). One of ML110◄ and two sequences annotated as '*Glomus intraradices*' (Gamper & Leuchtmann, 2004) apparently are neither *Rh. intraradices* nor *Rh. irregularis* (Stockinger *et al*., 2009; 2010). *Rhizophagus clarus* sequences from 10 cultures cluster in a well resolved monophyletic clade together with *Rh. manihotis* sequences. *Sclerocystis sinuosa* MD126 falls basal to *Rhizophagus* and *G. achrum* (FM253379-81). *Glomus bistratum* (FM253382-84) and *G. indicum* (GU059544-49) cluster basally within *Glomeraceae* (formerly GlGrAb) in a polytomy (not shown).

SSU-ITS-LSU phylogeny of the Glomerales - Claroideoglomeraceae (Fig. 9)

Claroideoglomus walkeri, C. drummondi and *C. etunicatum* are well supported, but *C. claroideum* is rendered paraphyletic by *C. luteum* SA101 sequences. The supplementary analysis including shorter sequences (Fig. S2) shows a number of sequences from additional *C. etunicatum* cultures (AU401, NB119, CA-OT-126-3-2, KE118, etc.) clustering together (66% BS). Sequences of *C. drummondi* also form a well supported clade. *Claroideoglomus luteum*, *C. claroideum* and a sequence annotated as *G. microaggregatum* UT126B◄ cluster unresolved.

Discussion

By publishing further sequences produced over the recent years and re-analyses of available phylotaxonomic reference sequences, we established what we consider could serve as a phylogenetic backbone for a natural systematics of *Glomeromycota* and a basis for future environmental (deep) sequencing projects. For some analyses we use consensus sequences, which are theoretical constructs that may cause problems in some instances (Lindner & Banik, 2011). However, in our AMF analyses the use of strict (all variations represented by degenerate base symbols) SSU consensus sequences anchors taxa by conserved sequences and thus reduces the risks of coincidential phylogenetic attraction by shared characters at highly variable sites and of potential problems by inhomogenous sequence or taxon sampling. We analysed the available nuclear rDNA data of ~105 described species and ~28 unnamed AMF cultures and samples ascribed to undescribed species (approximate numbers, because determinations may not always be correct). More than 50% (118 species) of the currently 228 described AMF are covered by sequences deposited in the public databases, but only 81 (~36%) are propagated in the culture

collections INVAM (http://invam.caf.wvu.edu), BEG (http://www.kent.ac.uk/bio/beg), and GINCO (http://emma.agro.ucl.ac.be/ginco-bel), making reanalyses or improvements of the sequence database difficult.

The need for a solid molecular genetic base for the systematics of Glomeromycota

SSU analyses (Schüßler *et al*., 2001) and the six-gene phylogeny of James *et al.* (2006) indicated a likely sister-grouping of the *Glomeromycota* to *Dikarya*. By including basal fungal lineages as well as members of *Dikarya*, we again found the same sister grouping (Fig. 1). In contrast, analyses of the mitochondrial genome of *Rh. irregularis* isolate 494 (Lee & Young, 2009) and of nucleus-encoded proteins (Liu *et al*., 2009) questioned this sister relationship and indicate a possible common ancestry of AMF with *Mortierellales*. However, tree topologies in the latter study varied dependent on taxon sampling. At present resolving these differences must await more data from phylogenetically basal AMF, but clearly the *Glomeromycota* are a monophyletic and basal group of terrestrial fungi.

The dataset and analyses presented here provided one of the foundations for major taxonomic reclassifications in the *Glomeromycota* (Schüßler & Walker, 2010). Such data are also important as reference for new species descriptions. For example, the sole use of morphology for the description of *Ambispora brasiliensis* (Goto *et al*., 2008) placed an *Acaulospora* species incorrectly at generic, familial and even ordinal level (Krüger *et al*., 2011). Similar instances of species descriptions only based on morphology were discussed by Morton & Msiska (2010b) who reported an albino mutant of *S. heterogama* WV859, which would have been considered as a new morphospecies if found in the field. Another example was the description of *G. irregulare* (Błaszkowski *et al*., 2008), now *Rh. irregularis*, based on a restricted analysis of intraspecific morphological plasticity. Therefore, the quality of formal species descriptions should be improved by including an appropriate phylogenetic characterization whenever possible. Obviously, this is particularly important for newly described species not represented by publicly available isolates.

The phylogenetically basal lineages, Paraglomerales *and* Archaeosporales

Only relatively few data are available for evolutionarily ancient phylogenetic lineages of *Glomeromycota*. Presently there are only three recognized or described species in the *Paraglomerales* and 11 in the *Archaeosporales* (www.amf-phylogeny.com), but most likely this

is only a small proportion of the existing species. Our study is the first to yield reasonable branch support for *Paraglomerales* as the most ancient lineage of the *Glomeromycota* (Fig. 1). It also supports the genus *Intraspora* (Sieverding & Oehl, 2006) as congeneric with *Archaeospora* (Schüßler & Walker, 2010).

Diversisporales

There has been considerable nomenclatural change among the *Diversisporales* recently. Oehl *et al.* (2008) split the genus *Scutellospora* into three new families containing six genera (*Scutellospora* in the *Scutellosporaceae*; *Racocetra* and *Cetraspora* in the *Racocetraceae*; *Dentiscutata*, *Fuscutata*, and *Quatunica* in the *Dentiscutataceae*). Except for *Racocetra*, these new taxa were all rejected by Morton & Msiska (2010a). Nevertheless, it has long been indicated that *Scutellospora* is non-monophyletic (e.g., Kramadibrata *et al*., 2000; da Silva *et al*., 2006). Although we support the notion of Morton & Msiska (2010a) that a robust taxon sampling and phylogenetic analysis should be the base of taxonomic changes, the phylogeny of *Gigasporaceae* presented herein may provide support for some of the genera proposed by Oehl *et al.* (2008), but certainly not for erecting new families in this clade.

The case of two different *D. aurantia* clades exemplifies problems in interpretation of data from trap cultures seemingly producing spores of one species (often called single species cultures). It seems possible, but cannot be proven, that the trap culture contained more than one species. For the monospecific genus *Otospora* (Palenzuela *et al*., 2008), the assembled two short, non-overlapping *O. bareae* sequences cluster within *Diversispora*. This could support the view that *O. bareae* is a morphologically exceptional member of the *Diversisporaceae*, but might as readily be the result of a contamination. The sequence of the recently described *Entrophospora nevadensis* (Palenzuela *et al*., 2010) also clusters unexpectedly, in regard to its morphology, among those of *Diversispora*. A detailed analysis of *Diversisporaceae*, with focus on *D. epigaea*, often named '*Glomus versiforme* BEG47', and including biogeographical aspects, is given in Schüßler *et al.* (2011).

Kuklospora sensu Oehl & Sieverding (2006) was described based solely on spore morphology. The recent transfer of all *Kuklospora* species to *Acaulospora* (Kaonongbua *et al.*, 2010) is congruent with our analyses. In our opinion the species, *A. laevis* and *A. entreriana* are

morphologically indistinguishable. They could not be separated in analyses when excluding the ITS1 and ITS2, but more data are needed to confirm conspecificity.

Glomerales

A decade ago, it was proposed that *Glomus* should be split into several families (Schwarzott *et al*., 2001). These were named as phylogenetic groups, *Glomus* Group (GlGr) A and B, until it was clear where the generic type of *Glomus*, *G. macrocarpum*, belongs phylogenetically (Schüßler & Walker, 2010). Now, the family *Glomeraceae* represents the former GlGrA, separated into four genera: *Glomus* (GlGrAc), *Funneliformis* (GlGrAa), *Rhizophagus* and *Sclerocystis* (both GlGrAb). In addition, *G. iranicum* and *G. indicum* sequences form a basal clade in this family, and *G. bistratum* and *G. achrum* cluster in a basal polytomy in the *Glomeraceae*. However, the correct phylogenetic placements may require additional data. The family *Claroideoglomeraceae* corresponds to the former GlGrB.

For *Claroideoglomus, Funneliformis* and *Rhizophagus*, detailed analyses were already conducted by Stockinger *et al.* (2010), under the previous generic name *Glomus*. The uncovered inconsistencies discussed in that study are also recognizable from the phylogenetic trees of the present study, but are not further discussed here. *Rhizophagus irregularis* was defined (Błaszkowski *et al.*, 2008), as *G. irregulare*, mainly based on perceived morphological differences from *G. intraradices* in a former sense, which included DAOM197198. The analysis of corresponding sequences is implemented in Fig. 8 and show that the organisms interpreted as different, based on morphology, in fact belong to the same species. *Glomus irregulare* (now *Rh. irregularis*) is conspecific with DAOM197198 (and other cultures of '*G. intraradices*' in the former sense), and not with *G. intraradices* (now *Rh. intraradices*) (Sokolski *et al*., 2010; Stockinger *et al*., 2009, 2010). The molecular data suggest that *Rh. clarus* and *Rh. manihotis* are conspecific, but this issue requires further morphological work before the species can be synonymized.

Putative errors in public sequence databases

As discussed repeatedly (e.g. Schüßler *et al.*, 2003; Bidartondo *et al*., 2008), annotation of sequence entries in public databases is often inadequate or incorrect. There are different types of errors; some sequences are obviously based on wrong identification or undiscovered species synonymy, some on contaminants, and others perhaps on accidental or simple misannotation. For

example, a batch of LSU sequences submitted by Amarasinghe & Morton (FJ461790-FJ461888◄) caused numerous problems in our initial analyses, until we realized that many of the contained sequences seem to be either misannotated or derived from contaminants and must be interpreted with caution. For example, sequences from '*Glomus trimurales*', originally annotated as *Glomus* sp., fall among three different orders, in the genera *Diversispora*, *Claroideoglomus* and *Rhizophagus*. Several entries will be updated (personal communication J. Morton, 8 Apr 2011). The failure to update public database sequences with taxonomic changes can result in confusion and for accurate analyses many of the database entries cannot be accepted as provided. Our own past errors include the annotation of *A. cavernata* BEG33 as *A. scrobiculata*, and mixing up two samples resulting in mistakenly naming the corresponding sequences of *S. spinosissima* W3009/Att664-1 as *S. nodosa* BEG4 and *vice versa*. Moreover, we doubt our own annotation of a sequence (Y17652) attributed to *G. viscosum* BEG27 because morphologically, an affiliation to *Claroideoglomus* is surprising and the culture used for sequencing later turned out also to contain a contaminant *C. claroideum*-like fungus. A revived culture of *G. viscosum* has been established and will be used to clarify this matter. An example for putative culture mis-annotation is DAOM212349. The number originally refers, as a voucher number, to both the *C. lamellosum* holotype (field collected) and, additionally, a pot culture from which specimens designated as 'isotype' (which cannot be correct, as, by definition, an isotype has to be from the original type-collection) were derived (Dalpé *et al.*, 1992). A later ROC established from this pot culture was given the same number in the GINCO database, but it contains *Rh. irregularis*. DAOM212349 therefore must represent either an initially mixed culture, or a later contamination, but certainly one identifier is used for fungi from two distinct genera. To facilitate the correct interpretation of AMF sequence data, third party annotations are currently implemented in a PlutoF (Abarenkov *et al*., 2010) based metadatabase.

Conclusions

Systematics and molecular phylogenetics influence more scientific disciplines than often is realised. It is therefore important to correct misclassifications of organisms as soon as possible after discovery. This is particularly true for those used as model organisms, to allow correct interpretation of studies on functional, genetic and ecological traits.

Besides providing a solid phylogenetic backbone, the dataset presented here covers the most likely future primary DNA barcode for fungi, namely the ITS region, and the 5' portion of the

LSU for use as an extended barcode. Such data will assist the detection of species in the field (Stockinger *et al*., 2010). However, the database must be further improved by filling the gaps in relation to sequence and taxon coverage. The latter relates to described species, but also to environmental MOTUs, for which affiliation to species is feasible by the use of the SSU-ITS-LSU fragment. Using such data will facilitate more accurate molecular ecological and, for example, biosafety analyses based on next generation sequencing of fungal communities. For AMF the lack of available well characterised biological material from described species is a problem, which partly lies in the nature of the organisms (many have so far proved impossible or difficult to establish or maintain in culture). This problem could be alleviated by contributing more isolates (single spore cultures) to public culture collections. Maintenance of non-commercial living culture collections seems, however, hampered by inadequate funding.

AMF are integral components of nearly all terrestrial ecosystems. To ascertain more about AMF-plant preferences and the functional roles of AMF a solid systematic classification is indispensable, the foundation for which may have been laid with the dataset and analyses described herein. More sequences with sufficient lengths would morover facilitate improved understanding of biogeography and evolution of AMF, and research in practical aspects, such as biosafety assessments and AMF species traceability in field applications.

Acknowledgements

We thank all the people who have provided material, three anonymous reviewers for detailed comments, and Milagros Lovera for pointing out the problem with the *S. nodosa* and *S. spinosissima* sequences. Part of this work was financed by the German Research Foundation (DFG grant Schu1203/8) and the Marie Curie Early Stage Research Training Fellowship of the European Community's Sixth framework Programme (MEST-CT-2005-021016).

References

Abarenkov K, Tedersoo L, Nilsson HR, Vellak K, Saar I, Veldre V, Parmasto E, Prous M, Aan A, Ots M *et al*. 2010. PlutoF - a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. *Evolutionary Bioinformatics* **6**: 189–196.

Bidartondo MI, Bruns TD, Blackwell M, Edwards I, Taylor AFS, Horton T, Zhang N, Kõljalg U, May G, Kuyper TW *et al*. 2008. Preserving accuracy in GenBank. Science 319: 1616.

Błaszkowski J, Czerniawska B, Wubet T, Schäfer T, Buscot F, Renker C. 2008. *Glomus irregulare*, a new arbuscular mycorrhizal fungus in the *Glomeromycota*. *Mycotaxon* **106**: 247–267.

Błaszkowski J, Kovács GM, Balázs TK, Orłowska E, Sadravi M, Wubet T, Buscot F. 2010a. *Glomus africanum* and *G. iranicum*, two new species of arbuscular mycorrhizal fungi (*Glomeromycota*). *Mycologia* **102**: 1450–1462.

Błaszkowski J, Wubet T, Harikumar VS, Ryszka P, Buscot F. 2010b. *Glomus indicum*, a new arbuscular mycorrhizal fungus. *Botany* **88**:132–143.

Börstler B, Thiéry O, Sýkorová Z, Berner A, Redecker D. 2010. Diversity of mitochondrial large subunit rDNA haplotypes of *Glomus intraradices* in two agricultural field experiments and two semi-natural grasslands. *Molecular Ecology* **19**: 1497–1511.

Corradi N, Kuhn G, Sanders IR. 2004. Monophyly of β-*tubulin* and H^+-*ATPase* gene variants in *Glomus intraradices*: consequences for molecular evolutionary studies of AM fungal genes. *Fungal Genetics and Biology* **41**: 262–273.

Dalpé Y, Koske RE, Tews LL. 1992. *Glomus lamellosum* sp. nov.: a new *Glomaceae* associated with beach grass. *Mycotaxon* **63**: 289-293.

da Silva GA, Lumini E, Maia LC, Bonfante PB. 2006. Phylogenetic analysis of *Glomeromycota* by partial LSU rDNA sequences. *Mycorrhiza* **16**: 183–189.

Gamper HA, Walker C, Schüßler A. 2009. *Diversispora celata* sp. nov: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. *New Phytologist* **182**: 495–506.

Gollotte A, van Tuinen D, Atkinson D. 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza* **14**: 111–117.

Goto BT, Maia LC, Oehl F. 2008. *Ambispora brasiliensis*, a new ornamented species in the arbuscular mycorrhiza-forming *Glomeromycetes*. *Mycotaxon* **105**: 11–18.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ, Abaci Ö, Aime C, *et al*. 2011. The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* **2**: 105–112.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. 1998. Ploughing up the wood-wide web? *Nature* **394**: 431.

Hempel S, Renker C, Buscot F. 2007. Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. *Environmental Microbiology* **9**: 1930–1938.

Hibbett DS, Ohman A, Glotzer D, Nuhn M, Kirk P, Nilsson RH. 2011. Progress in molecular and morphological taxon discovery in *Fungi* and options for formal classification of environmental sequences. *Fungal Biology Reviews* **25**: 38–47.

James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox C, Celio G, Gueidan C, Fraker E, Miadlikowska J, *et al.* 2006. Reconstructing the early evolution of the *Fungi* using a six gene phylogeny. *Nature* **443**:818–822.

Kaonongbua W, Morton JB, Bever JD. 2010. Taxonomic revision transferring species in *Kuklospora* to *Acaulospora* (*Glomeromycota*) and a description of *Acaulospora colliculosa* sp. nov. from field collected spores. *Mycologia* **102**: 1497–1509.

Kramadibrata K, Walker C, Schwarzott D, Schüßler A. 2000. A new species of *Scutellospora* with a coiled germination shield. *Annals of Botany* **86**: 21–27.

Krüger M, Walker C, Schüßler A. 2011. *Acaulospora brasiliensis* comb. nov. and *Acaulospora alpina* (*Glomeromycota*) from upland Scotland: morphology, molecular phylogeny and DNA-based detection in roots. *Mycorrhiza* **21**:577–587.

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist* **183**: 212–223.

Lee J, Young JPW. 2009. The mitochondrial genome sequence of the arbuscular mycorrhizal fungus *Glomus intraradices* isolate 494 and implications for the phylogenetic placement of *Glomus*. *New Phytologist* **183**: 200–211.

Lee J, Lee S, Young JPW. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology* **65**: 339–349.

Lindner DL, Banik MT. 2011. Intra-genomic variation in the ITS rDNA region obscures phylogenetic relationships and inflates estimates of operational taxonomic units in genus *Laetiporus*. *Mycologia* **103**: 731-740.

Liu Y, Leigh JW, Brinkmann H, Cushion MT, Rodriguez-Ezpeleta N, Philippe H, Lang BF. 2009. Phylogenomic analyses support the monophyly of *Taphrinomycotina*, including *Schizosaccharomyces* fission yeasts. *Molecular Biology and Evolution* **26**: 27–34.

Morton JB, Msiska Z. 2010a. Phylogenies from genetic and morphological characters do not support a revision of *Gigasporaceae* (*Glomeromycota*) into four families and five genera. *Mycorrhiza* **20**: 483–496.

Morton JB, Msiska Z. 2010b. Ontogeny and phylogeny of a *Scutellospora heterogama* mutant, with implications for morphological recognition of species in *Glomeromycota*. *Fungal Biology* **114**: 410–420.

Morton JB, Bever JD, Pfleger FL. 1997. Taxonomy of *Acaulospora gerdemannii* and *Glomus leptotichum*, synanamorphs of an arbuscular mycorrhizal fungus in the *Glomales*. *Mycological Research* **101**: 625–631.

Msiska Z, Morton J. 2009. Phylogenetic analysis of the *Glomeromycota* by partial β-tubulin gene sequences. *Mycorrhiza* **19**: 247–254.

Mummey DL, Rillig MC, Holben WE. 2005. Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. *Plant and Soil* **271**: 83–90.

Naumann M, Schüßler A, Bonfante P. 2010. The obligate endobacteria of arbuscular mycorrhizal fungi are ancient heritable components related to the *Mollicutes*. *The ISME Journal* **4**: 862–871.

Oehl F, de Souza FA, Sieverding E. 2008. Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming *Glomeromycetes*. *Mycotaxon* **106**: 311–360.

Öpik M, Moora M, Zobel M, Saks Ü, Wheatley R, Wright F, Daniell T. 2008. High diversity of arbuscular mycorrhizal fungi in a boreal herb-rich coniferous forest. *New Phytologist* **179:** 867–876.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (*Glomeromycota*). *New Phytologist* **188**: 223–241.

Palenzuela J, Barea JM, Ferrol N, Oehl F. 2011. *Ambispora granatensis*, a new arbuscular mycorrhizal fungus, associated with *Asparagus officinalis* in Andalucia (Spain). *Mycologia* **103**: 333–340

Palenzuela J, Barea JM, Ferrol N, Azcon-Aguilar C, Oehl F. 2010. *Entrophospora nevadensis*, a new arbuscular mycorrhizal fungus from Sierra Nevada National Park (southeastern Spain). *Mycologia* **102**: 624–632.

Palenzuela J, Ferrol N, Boller T, Azcón-Aguilar C, Oehl F. 2008. *Otospora bareai*, a new fungal species in the *Glomeromycetes* from a dolomitic shrub land in Sierra de Baza National Park (Granada, Spain). *Mycologia* **100**: 296–305.

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, van Tuinen D. 2007. *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytologist* **176**: 197–210.

Redecker D, Raab P, Oehl F, Camacho F, Courtecuisse R. 2007. A novel clade of sporocarp-forming species of glomeromycotan fungi in the *Diversisporales* lineage. *Mycological Progress* **6**: 35–44.

Redecker D, Raab P. 2006. Phylogeny of the *Glomeromycota* (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* **98**: 885–895.

Redecker D, Morton JB, Bruns TD. 2000. Molecular phylogeny of the arbuscular mycorrhizal fungi *Glomus sinuosum* and *Sclerocystis coremioides*. ***Mycologia*** **92**: 282–285.

Renker C, Heinrichs J, Kaldorf M, Buscot F. 2003. Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. *Mycorrhiza* **13**: 191–198.

Schüßler A, Krüger M, Walker C. 2011. Revealing natural relationships among arbuscular mycorrhizal fungi: culture line BEG47 represents *Diversispora epigaea*, not *Glomus versiforme*. PLoS ONE **6**: e23333.

Schüßler A, Walker C. 2010. The *Glomeromycota*: a species list with new families and genera. Arthur Schüßler & Christopher Walker, Gloucester. Published in December 2010 in libraries at The Royal Botanic Garden Edinburgh, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University. Electronic version freely available online at www.amf-phylogeny.com.

Schüßler A, Schwarzott D, Walker C. 2003. *Glomeromycota* rRNA genes–the diversity of myths? *Mycorrhiza* **13**: 233–236.

Schüßler A, Schwarzott D, Walker C. 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* **105**: 1413–1421.

Schwarzott D, Schüßler A. 2001. A simple and reliable method for SSU rRNA gene DNA extraction, amplification, and cloning from single AM fungal spores. *Mycorrhiza* **10**: 203–207.

Schwarzott D, Walker C, Schüßler A. 2001. *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (*Glomales*), is nonmonophyletic. *Molecular Phylogenetics and Evolution* **21**: 190–197.

Sieverding E, Oehl F. 2006. Revision of *Entrophospora* and description of *Kuklospora* and *Intraspora*, two new genera in the arbuscular mycorrhizal *Glomeromycetes*. *Journal of Applied Botany and Food Quality* **80**: 69–81.

Simon L, Lalonde M, Bruns TD. 1992. Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied and Environmental Microbiology* **58**: 291–295.

Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. Amsterdam; Boston: Academic Press.

Sokolski S, Dalpé Y, Séguin S, Khasa D, Lévesque CA, Piché Y. 2010. Conspecificity of DAOM197198, the model arbuscular mycorrhizal fungus, with *Glomus irregulare*: molecular evidence with three protein-encoding genes. *Botany* **88**: 829–838.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**: 758–771.

Stockinger H, Krüger M, Schüßler A. 2010. DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist* **187**: 461–474.

Stockinger H, Walker C, Schüßler A. 2009. '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. *New Phytologist* **183**: 1176–1187.

Sýkorová Z, Börstler B, Zvolenská S, Fehrer J, Gryndler M, Vosátka M, Redecker D. 2011. Long-term tracing of *Rhizophagus irregularis* isolate BEG140 inoculated on *Phalaris arundinacea* in a coal mine spoil bank, using mitochondrial large subunit rDNA markers. *Mycorrhiza* online first, doi: 10.1007/s00572-011-0375-1.

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U. 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist* **180**: 479–490.

Turnau K, Ryszka P, Gianinazzi-Pearson V, van Tuinen D. 2001. Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza* **10**: 169–174.

Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H, Nishmura I, Schüßler A. 2007. Molecular phylogeny and new taxa in the *Archaeosporales* (*Glomeromycota*): *Ambispora fennica* gen. sp nov., *Ambisporaceae* fam. nov., and emendation of *Archaeospora* and *Archaeosporaceae*. *Mycological Research* **111**: 137–153.

Zhang H, Tang M, Chen H, Tian Z, Xue Y, Feng Y. 2010. Communities of arbuscular mycorrhizal fungi and bacteria in the rhizosphere of *Caragana korshinkii* and *Hippophae rhamnoides* in Zhifanggou watershed. *Plant and Soil* **326**: 415–424.

Figure Legends

Fig. 1 Maximum likelihood phylogenetic tree based on concatenated nuclear SSUfull-5.8S-LSU rDNA strict consensus sequences (~2700 bp) of the *Glomeromycota* and other fungal lineages that were used as outgroups. Branches receiving less than 60% bootstrap support (1000 bootstraps) were collapsed to polytomies, long branches were shortened by 50% as indicated with the diagonal slashes. Terminal nodes marked with (consensus #) represent strict consensus sequences of sequences with the accession numbers listed in Supporting Information S3. Scale bar, number of substitutions per site. The following culture identifiers are not shown in the tree for space reasons: *Acaulospora brasiliensis* (consensus 5) is derived from W4699/Att1211-0 and W5473/Att1210-5, *Diversispora spurca* (consensus 7) from W2396/Att246-4 and W4119/Att246-18, *Diversispora aurantia* (consensus 8) from W4728/Att1296-0, *Glomus macrocarpum* (consensus 22) from a field collected sporocarp (W5288) and Att1495-0 (two independent samplings W5581 and W5605), *Ambispora fennica* (consensus 36) from W4752/Att200-23 and W3569/Att200-11, and *Archaeospora schenckii* is derived from W3571/Att58-6 and W5673/Att212-4.

Fig. 2 Maximum likelihood phylogenetic tree based on concatenated nuclear SSU rDNA strict consensus sequences (~1.8 kb). *Paraglomus* was used as outgroup as it represents the most basal glomeromycotan branch (see Fig. 1). Branches receiving less than 60% bootstrap support (1000 bootstraps) were collapsed to polytomies. Terminal nodes marked with (consensus #) represent strict consensus sequences of sequences with the accession numbers listed in Supporting Information S4. Scale bar, number of substitutions per site. Sequences ≤1300 bp are indicated with *. The generic type species, when included in the analysis, is shown in bold and underlined.

Figs 3, 4. Maximum likelihood phylogenetic tree based on individual SSU-ITS-LSU rDNA sequence variants assembled with, when available, the corresponding SSU strict consensus sequence. Branches receiving less than 60% bootstrap support (1000 bootstraps) were collapsed to polytomies, long branches were shortened by 50% as indicated with two diagonal slashes or by 75% indicated with three slashes. Bootstrap values are given for branches among but not within different cultures. Scale bar, number of substitutions per site. Sequences submitted by Amarasinge & Morton, 2010 are marked with ◄, potential contaminant or wrongly annotated sequences are indicated with ●, the respective sequence length of all sequences shorter than 1 kb is shown within the taxon labels. **Fig. 3** *Paraglomerales* and *Archaeosporales*, *Ascomycota* and *Basidiomycota* were used as outgroup. Terminal nodes marked with (consensus #) represent strict consensus sequences of sequences with the accession numbers listed in Supporting Information S5. **Fig. 4** *Gigasporaceae*, including public database sequences of >700 bp; *Acaulospora*

species were used as outgroup. Consensus 10 is a strict consensus sequence of the sequences AY635832, AY997088, DQ273792 and consensus 11 from sequences AJ871270-73.

Figs 5, 6. Maximum likelihood phylogenetic tree based on SSU-ITS-LSU rDNA sequence variants assembled with, when available, the corresponding SSU strict consensus sequence. Branches receiving less than 60% bootstrap support (1000 bootstraps) were collapsed to polytomies, long branches were shortened by 50% as indicated with two diagonal slashes or by 75% indicated with three slashes. Bootstrap values are given for branches among but not within different cultures. Scale bar, number of substitutions per site. Sequences submitted by Amarasinge & Morton, 2010 are marked with ◄, potential contaminant or wrongly annotated sequences are indicated with ●, the respective sequence length of all sequences shorter than 1 kb is noted. **Fig. 5** *Acaulosporaceae*, with *Diversispora* as outgroup. Consensus 1 is a strict consensus sequence of sequences AJ250847, AJ242499, FJ461802. **Fig. 6** *Diversisporaceae*, *Acaulospora* species were used as outgroup. Consensus 2 is a strict consensus sequence of sequences DQ350448-53 and consensus 3 of sequences AM418543-44.

Fig. 7-9. Maximum likelihood phylogenetic tree based on SSU-ITS-LSU rDNA sequence variants of the *Glomerales* assembled with, when available, the corresponding SSU strict consensus sequence. Branches receiving less than 60% bootstrap support (1000 bootstraps) were collapsed to polytomies, long branches were shortened by 50% as indicated with two diagonal slashes or by 75% indicated with three slashes. Bootstrap values are given for branches among but not within different cultures. Scale bar, number of substitutions per site. Sequences submitted by Amarasinge & Morton, 2010 are marked with ◄, potential contaminant or wrongly annotated sequences are indicated with ●, the respective sequence length of all sequences shorter than 1 kb is shown within the taxon labels. **Fig. 7** *Funneliformis* and *Glomus*. Consensus 1 is a strict consensus sequence of sequences AY635833, AY997053, DQ273793; **Fig. 8** *Rhizophagus* and *Sclerocystis*; consensus 2 is a strict consensus sequence of sequences DQ322630, AY997054, DQ273828 and consensus 3 of AY635831, AY997052, DQ273790; **Fig. 9** *Claroideoglomus*; consensus 4 is a strict consensus sequence of Y17639, Z14008, AJ239125.

Acaulospora cavernata BEG33 epitype (consensus 1)
Acaulospora sp. WUM18 (consensus 2)
Acaulospora spinosa W3574 ex-type (consensus 3)
Acaulospora laevis AU211 (consensus 4)
Acaulospora brasiliensis (consensus 5)
Acaulospora lacunosa BEG78 (consensus 6)
Diversisporales
Acaulosporaceae
Diversispora spurca ex-type (consensus 7)
Diversispora aurantia ex-type (consensus 8)
Diversispora celata BEG231 ex-type (consensus 9)
Diversispora eburnea AZ420A (consensus 10)
Diversispora epigaea BEG47 ex-type (consensus 11)
Diversisporaceae
Scutellospora heterogama BEG35 (consensus 12)
Scutellospora heterogama FL225 (consensus 13)
Gigaspora sp. W2992/field collected (consensus 14)
Gigaspora rosea DAOM194757 (consensus 15)
Scutellospora nodosa BEG4 ex-epitype (consensus 16)
Racocetra castanea BEG1 ex-type (consensus 17)
Scutellospora spinosissima W3009/Att664-1 type (cons. 18)
Gigasporaceae
Pacispora scintillans W4545/field collected (consensus 19)
Pacisporaceae
Glomus sp. W3347/Att565-7 (consensus 20)
Glomus macrocarpum W5293 (consensus 21)
Glomus macrocarpum epitype (consensus 22)
Funneliformis mosseae UT101 (consensus 23)
Funneliformis mosseae BEG12 epitype (consensus 24)
Funneliformis coronatum W3582/Att108-7 (consensus 25)
Funneliformis sp. WUM3 (consensus 26)
Funneliformis caledonium BEG20 (consensus 27)
Rhizophagus irregularis MUCL43195 (consensus 28)
Rhizophagus irregularis DAOM197198 (consensus 29)
Rhizophagus irregularis AFTOL-ID845 (consensus 30)
Rhizophagus proliferus MUCL41827 (consensus 31)
Rhizophagus intraradices FL208 ex-type (consensus 32)
Sclerocystis sinuosa MD126 (consensus 33)
Glomeraceae
Claroideoglomus sp. W3349/Att565-11 (consensus 34)
Claroideoglomus luteum SA101 (consensus 35)
Claroideoglomeraceae
Glomerales
Ambispora fennica ex-type (consensus 36)
Ambisporaceae
Geosiphon pyriformis GEO1 (consensus 37)
Geosiphonaceae
Archaeospora schenckii (consensus 38)
Archaeosporaceae
Archaeosporales
Paraglomus occultum IA702 (consensus 39)
Paraglomeraceae
Paraglomerales
Glomeromycota
Henningsomyces candidus AFTOL-ID468
Rhodotorula hordea AFTOL-ID674
Exophiala dermatitidis AFTOL-ID668
Schizosaccharomyces pombe AFTOL-ID1199
Basidiomycota
Ascomycota
Dikarya
Phycomyces blakesleeanus AFTOL-ID184
Rhizopus oryzae AFTOL-ID1241
Endogone pisiformis AFTOL-ID539
Mortierella verticillata AFTOL-ID141
Mucoromycotina
Coelomomyces stegomyiae AFTOL-ID18
Allomyces arbusculus AFTOL-ID300
Blastocladiomycota
Orphella haysii AFTOL-ID1062
Smittium culisetae AFTOL-ID29
Kickxellomycotina
Batrachochytrium dendrobatidis AFTOL-ID21
Chytridiomycota
0.5

Scutellospora heterogama WV858B (Z14013)
Scutellospora heterogama UFPE19 (AJ852609)
Scutellospora heterogama BR154-5 (U36593) *
Scutellospora heterogama BEG35 (consensus 1) *
Scutellospora heterogama W4733/Att1283-1 (consensus 2)
Scutellospora heterogama FL225, AFTOL-ID138 (consensus 3)
Scutellospora cerradensis MAFF520056 (consensus 4)
Scutellospora reticulata CNPAB11 (consensus 5)
Dentiscutata colliculosa (GQ376067)
Scutellospora (*Dentiscutata*, *Fuscutata* and *Quatunica* sensu Oehl *et al.,* 2008)

Gigaspora rosea BEG9 (consensus 6) *
Gigaspora candida BEG17 epitype (consensus 7)
Gigaspora rosea DAOM194757 (consensus 8)
Gigaspora albida FL927 (Z14009)
Gigaspora sp. W2992/field collected (consensus 9)
Gigaspora gigantea WV932 (Z14010)
Gigaspora decipiens BEG45 ex-type (U96146) *
Gigaspora margarita BEG34 (consensus 10) *
Gigaspora

Scutellospora pellucida WV873 (Z14012)
Scutellospora pellucida CL750A (FR750215) *
Scutellospora nodosa BEG4 ex-epitype (consensus 11)
Scutellospora gilmorei FCPC1145 (consensus 12)
Scutellospora (*Cetraspora* sensu Oehl *et al.,* 2008)

Racocetra gregaria CNPAB7 (consensus 13)
Racocetra weresubiae W2988/field collected (consensus 14)
Racocetra castanea BEG1 ex-type (consensus 15)
Racocetra fulgida W2993/field collected (consensus 16)
Racocetra

Scutellospora spinosissima W3009/Att664-1 type (consensus 17)
Scutellospora calospora BEG32 epitype (consensus 18)
Scutellospora dipurpurescens (AF074342) *
Scutellospora auriglobа WUM53 (consensus 19)
Scutellospora projecturata W3254/Att697-0 (AJ242729)
Scutellospora

Pacispora franciscana W3850/Att961-1 (FR750224)
Pacispora franciscana W3251/Att599-7 (FR750375)
Pacispora scintillans W3793 (consensus 20)
Pacispora scintillans W3849/Att961-0 (consensus 21)
Pacispora scintillans W3862 (consensus 22)
Pacispora scintillans W4545 (consensus 23)
Pacispora

Diversispora celata BEG230 (consensus 24)
Diversispora celata BEG231 ex-type (consensus 25)
Diversispora celata BEG232 (consensus 26)
Entrophospora nevadensis JP2009-2, EEZ164 from protologue (FN397100) *
Diversispora eburnea AZ420A ex-type (consensus 27)
Diversispora sp. W4538/Att1226-0 (FR686935)
Diversispora sp. W3033/Att669-1 (FR686934)
Diversispora sp. W2423/Att382-16 (consensus 28)
Otospora bareae JP-2006a/2007a from protologue (consensus 29) *
Diversispora aurantia W4728 (consensus 30)
Diversispora spurca ex-type (consensus 31)
Diversispora sp. W4568/Att1231-0 (FR686937)
Diversispora epigaea W4671/Att1236-0 (FR686936)
Diversispora epigaea BEG47 ex-type (consensus 32)
Diversispora trimurales W4124/Att1152-1 (consensus 33)
Diversispora trimurales W3577/Att710-6 (FR686955)
Diversispora

Redeckera fulvum AC/Pohn99-001 (consensus 34)
Redeckera

Acaulospora sp. WUM46 (Y17633)
Acaulospora cavernata BEG33 epitype (consensus 35)
Acaulospora sp. WUM18 (consensus 36)
Acaulospora rugosa WV949 (Z14005)
Acaulospora longula W3302/Att698-3 (AJ306439)
Acaulospora spinosa WV860 (Z14004)
Acaulospora spinosa W3574/Att165-9 ex-type (consensus 37)
Acaulospora colombiana FL356 (Z14006)
Acaulospora sp. WV201 (Z14011)
Acaulospora lacunosa BEG78 (consensus 38)
Acaulospora mellea Wuy104 (FJ009670)
Acaulospora laevis AU211 (consensus 39) *
Acaulospora laevis W3247/Att423-4 (consensus 40) *
Acaulospora capsicula W4681/Att1186-5 (FR750213)
Acaulospora brasiliensis W4699/Att1211-0, W5473/Att1210-5 (consensus 41)
Acaulospora sp. W3424/Att729-0 (AJ306440)
Acaulospora

Funneliformis mosseae DAOM212595 (U96143) *
Funneliformis mosseae DAOM221475 (U96145) *
Funneliformis mosseae BEG25 (consensus 42) *
Funneliformis mosseae BEG69 (U96141) *
Funneliformis mosseae DAOM198394 (U96142) *
Funneliformis mosseae UT101 (consensus 43)
Funneliformis mosseae W3528/Att867-10 (AJ306438)
Funneliformis mosseae BEG12 epitype (consensus 44)
Funneliformis mosseae FL156 (consensus 45)
Funneliformis geosporum BEG11 (consensus 46)
Funneliformis verruculosum W3295/Att298-6 (AJ301858)
Funneliformis sp. WUM3 (consensus 47)
Funneliformis sp. DAOM225952, W2538/Att599-0 (AF139733) *
Funneliformis fragilistratum W3238/Att112-6 ex-type (AJ276085)
Funneliformis caledonium BEG20 (consensus 48)
Funneliformis caledonium BEG15 (consensus 49)
Funneliformis coronatum W3582/Att108-7 ex-type (consensus 50)
Funneliformis coronatum COG1, W3153/Att143-5 (AJ276086)
Funneliformis constrictum W3809/Att756-1 (FR750212)
Funneliformis africanum (consensus 51) *
Funneliformis

Glomus macrocarpum W5293 (consensus 52)
Glomus macrocarpum W5288, W5581, W5605 epitype (consensus 53)
Glomus sp. W3347/Att565-7 (consensus 54)
Glomus

Rhizophagus irregularis DAOM197198 (consensus 55)
Rhizophagus irregularis MUCL43195 (consensus 56)
Rhizophagus irregularis W4533/Att1225-1 (FR750223)
Rhizophagus sp. W3563/field collected (FR750373)
Rhizophagus irregularis (consensus 57)
Rhizophagus vesiculiferus W2857/Att14-8 (FR750374)
Rhizophagus irregularis AFTOL-ID845 (consensus 58)
Rhizophagus fasciculatus BEG53 (Y17640)
Rhizophagus intraradices FL208 ex-type (consensus 59)
Rhizophagus proliferus MUCL41827 ex-type (consensus 60)
Rhizophagus manihotis W3224/Att575-9 ex-type (Y17648)
Rhizophagus clarus W3776/Att894-7 (consensus 61)
Rhizophagus manihotis FL879 (consensus 62)
Rhizophagus clarus BR147B (AJ276084)
Rhizophagus

Sclerocystis sinuosa MD126 (consensus 63)
***Sclerocystis coremioides* BIORIZE (AJ249715)**
Sclerocystis

Glomus indicum (consensus 64) *
Glomus iranicum (consensus 65) *
uncertain position

Claroideoglomus sp. W3234/Att13-7 (AJ301855)
Claroideoglomus etunicatum W3808/Att367-3 (FR750216)
Claroideoglomus etunicatum W3815/Att843-1 (FR750217)
Claroideoglomus lamellosum W3816/Att844-2 (FR750221)
Claroideoglomus etunicatum UT316 (consensus 66)
Claroideoglomus lamellosum W3814/Att756-1 (FR750220)
Claroideoglomus lamellosum W3158/Att244-7 (consensus 67)
Claroideoglomus sp. DAOM215235 (U96144) *
Claroideoglomus lamellosum W3161/Att672-13 (AJ276083)
Claroideoglomus claroideum BEG31 (consensus 68)
Claroideoglomus claroideum BEG14 (consensus 69)
Claroideoglomus claroideum BEG23 (consensus 70)
Claroideoglomus luteum SA101 (consensus 71)
Claroideoglomus sp. BR212 (U36592) *
Claroideoglomus sp. W3349/Att565-11 (consensus 72)
Claroideoglomus viscosum (?) BEG27 (Y17652)
Claroideoglomus

Ambispora leptoticha FL130 (consensus 73) *
Ambispora leptoticha NC176 (consensus 74)
Ambispora leptoticha F3b, MAFF520055 (consensus 75)
Ambispora callosa OK1, MAFF520057 (consensus 76)
Ambispora callosa V1, MAFF520058 (consensus 77)
Ambispora leptoticha WL1 (AB220172) *
Ambispora fennica W4752/Att200-23, W3569/Att200-11 ex-type (consensus 78)
Ambispora granatensis JEP-2010 (consensus 79)
Ambispora

Geosiphon pyriformis GEO1 (consensus 80)
Geosiphon

Archaeospora trappei AU219 (consensus 81) *
Archaeospora trappei NB112 (consensus 82)
Archaeospora schenckii W3571/Att58-6, W5673/Att212-4 (consensus 83)
Archaeospora trappei W3179/Att186-1 (consensus 84)
Archaeospora

Paraglomus brasilianum WV219 (consensus 85) *
Paraglomus brasilianum BR105, W3086/Att260-4 ex-type (AJ301862)
Paraglomus occultum IA702, AFTOL-ID844 (consensus 86)
Paraglomus occultum HA771 (AJ006799)
Paraglomus

0.04

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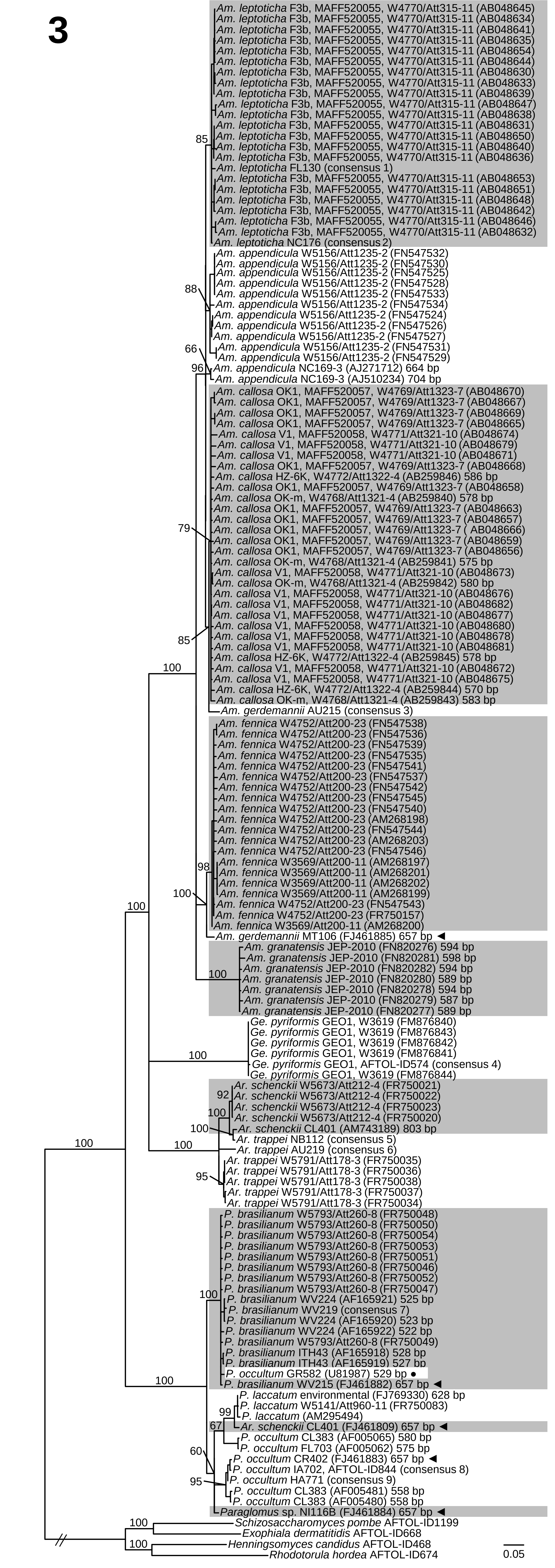
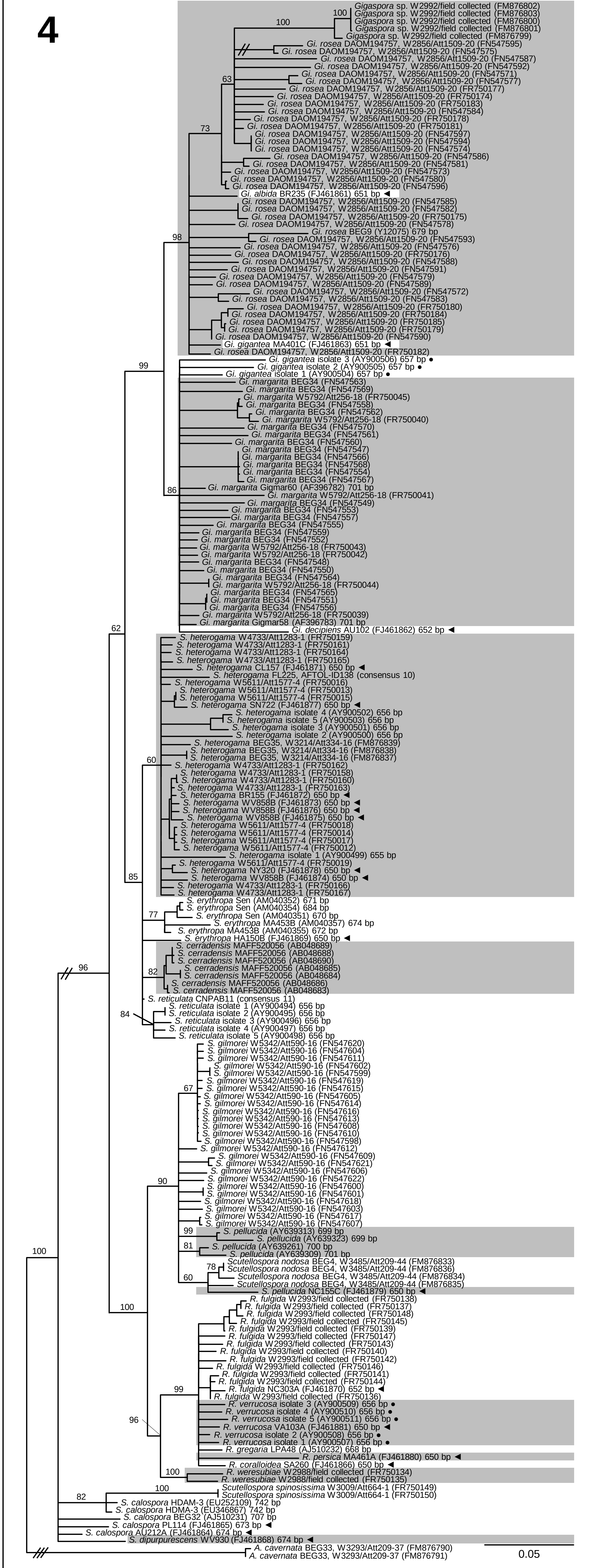


Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048645)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048634)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048641)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048635)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048654)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048644)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048630)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048633)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048639)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048647)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048638)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048631)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048650)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048640)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048636)
Am. leptoticha FL130 (consensus 1)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048653)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048651)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048648)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048642)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048646)
Am. leptoticha F3b, MAFF520055, W4770/Att315-11 (AB048632)
Am. leptoticha NC176 (consensus 2)
Am. appendicula W5156/Att1235-2 (FN547532)
Am. appendicula W5156/Att1235-2 (FN547530)
Am. appendicula W5156/Att1235-2 (FN547525)
Am. appendicula W5156/Att1235-2 (FN547528)
Am. appendicula W5156/Att1235-2 (FN547533)
Am. appendicula W5156/Att1235-2 (FN547534)
Am. appendicula W5156/Att1235-2 (FN547524)
Am. appendicula W5156/Att1235-2 (FN547526)
Am. appendicula W5156/Att1235-2 (FN547527)
Am. appendicula W5156/Att1235-2 (FN547531)
Am. appendicula W5156/Att1235-2 (FN547529)
Am. appendicula NC169-3 (AJ271712) 664 bp
Am. appendicula NC169-3 (AJ510234) 704 bp
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048670)
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048667)
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048669)
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048665)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048674)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048679)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048671)
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048668)
Am. callosa HZ-6K, W4772/Att1322-4 (AB259846) 586 bp
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048658)
Am. callosa OK-m, W4768/Att1321-4 (AB259840) 578 bp
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048663)
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048657)
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048666)
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048659)
Am. callosa OK1, MAFF520057, W4769/Att1323-7 (AB048656)
Am. callosa OK-m, W4768/Att1321-4 (AB259841) 575 bp
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048673)
Am. callosa OK-m, W4768/Att1321-4 (AB259842) 580 bp
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048676)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048682)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048677)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048680)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048678)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048681)
Am. callosa HZ-6K, W4772/Att1322-4 (AB259845) 578 bp
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048672)
Am. callosa V1, MAFF520058, W4771/Att321-10 (AB048675)
Am. callosa HZ-6K, W4772/Att1322-4 (AB259844) 570 bp
Am. callosa OK-m, W4768/Att1321-4 (AB259843) 583 bp
Am. gerdemannii AU215 (consensus 3)
Am. fennica W4752/Att200-23 (FN547538)
Am. fennica W4752/Att200-23 (FN547536)
Am. fennica W4752/Att200-23 (FN547539)
Am. fennica W4752/Att200-23 (FN547535)
Am. fennica W4752/Att200-23 (FN547541)
Am. fennica W4752/Att200-23 (FN547537)
Am. fennica W4752/Att200-23 (FN547542)
Am. fennica W4752/Att200-23 (FN547545)
Am. fennica W4752/Att200-23 (FN547540)
Am. fennica W4752/Att200-23 (AM268198)
Am. fennica W4752/Att200-23 (FN547544)
Am. fennica W4752/Att200-23 (AM268203)
Am. fennica W4752/Att200-23 (FN547546)
Am. fennica W3569/Att200-11 (AM268197)
Am. fennica W3569/Att200-11 (AM268201)
Am. fennica W3569/Att200-11 (AM268202)
Am. fennica W3569/Att200-11 (AM268199)
Am. fennica W4752/Att200-23 (FN547543)
Am. fennica W4752/Att200-23 (FR750157)
Am. fennica W3569/Att200-11 (AM268200)
Am. gerdemannii MT106 (FJ461885) 657 bp ◄
Am. granatensis JEP-2010 (FN820276) 594 bp
Am. granatensis JEP-2010 (FN820281) 598 bp
Am. granatensis JEP-2010 (FN820282) 594 bp
Am. granatensis JEP-2010 (FN820280) 589 bp
Am. granatensis JEP-2010 (FN820278) 594 bp
Am. granatensis JEP-2010 (FN820279) 587 bp
Am. granatensis JEP-2010 (FN820277) 589 bp
Ge. pyriformis GEO1, W3619 (FM876840)
Ge. pyriformis GEO1, W3619 (FM876843)
Ge. pyriformis GEO1, W3619 (FM876842)
Ge. pyriformis GEO1, W3619 (FM876841)
Ge. pyriformis GEO1, AFTOL-ID574 (consensus 4)
Ge. pyriformis GEO1, W3619 (FM876844)
Ar. schenckii W5673/Att212-4 (FR750021)
Ar. schenckii W5673/Att212-4 (FR750022)
Ar. schenckii W5673/Att212-4 (FR750023)
Ar. schenckii W5673/Att212-4 (FR750020)
Ar. schenckii CL401 (AM743189) 803 bp
Ar. trappei NB112 (consensus 5)
Ar. trappei AU219 (consensus 6)
Ar. trappei W5791/Att178-3 (FR750035)
Ar. trappei W5791/Att178-3 (FR750036)
Ar. trappei W5791/Att178-3 (FR750038)
Ar. trappei W5791/Att178-3 (FR750037)
Ar. trappei W5791/Att178-3 (FR750034)
P. brasilianum W5793/Att260-8 (FR750048)
P. brasilianum W5793/Att260-8 (FR750050)
P. brasilianum W5793/Att260-8 (FR750054)
P. brasilianum W5793/Att260-8 (FR750053)
P. brasilianum W5793/Att260-8 (FR750051)
P. brasilianum W5793/Att260-8 (FR750046)
P. brasilianum W5793/Att260-8 (FR750052)
P. brasilianum W5793/Att260-8 (FR750047)
P. brasilianum WV224 (AF165921) 525 bp
P. brasilianum WV219 (consensus 7)
P. brasilianum WV224 (AF165920) 523 bp
P. brasilianum WV224 (AF165922) 522 bp
P. brasilianum W5793/Att260-8 (FR750049)
P. brasilianum ITH43 (AF165918) 528 bp
P. brasilianum ITH43 (AF165919) 527 bp
P. occultum GR582 (U81987) 529 bp ●
P. brasilianum WV215 (FJ461882) 657 bp ◄
P. laccatum environmental (FJ769330) 628 bp
P. laccatum W5141/Att960-11 (FR750083)
P. laccatum (AM295494)
Ar. schenckii CL401 (FJ461809) 657 bp ◄
P. occultum CL383 (AF005065) 580 bp
P. occultum FL703 (AF005062) 575 bp
P. occultum CR402 (FJ461883) 657 bp ◄
P. occultum IA702, AFTOL-ID844 (consensus 8)
P. occultum HA771 (consensus 9)
P. occultum CL383 (AF005481) 558 bp
P. occultum CL383 (AF005480) 558 bp
Paraglomus sp. NI116B (FJ461884) 657 bp ◄
Schizosaccharomyces pombe AFTOL-ID1199
Exophiala dermatitidis AFTOL-ID668
Henningsomyces candidus AFTOL-ID468
Rhodotorula hordea AFTOL-ID674
0.05

4



Gigaspora sp. W2992/field collected (FM876802)
Gigaspora sp. W2992/field collected (FM876803)
Gigaspora sp. W2992/field collected (FM876800)
Gigaspora sp. W2992/field collected (FM876801)
Gigaspora sp. W2992/field collected (FM876799)
Gi. rosea DAOM194757, W2856/Att1509-20 (FN547595)
Gi. albida BR235 (FJ461861) 651 bp ◄
Gi. rosea BEG9 (Y12075) 679 bp
Gi. gigantea MA401C (FJ461863) 651 bp ◄
Gi. gigantea isolate 3 (AY900506) 657 bp ●
Gi. gigantea isolate 2 (AY900505) 657 bp ●
Gi. gigantea isolate 1 (AY900504) 657 bp ●
Gi. margarita BEG34 (FN547563)
Gi. margarita W5792/Att256-18 (FR750045)
Gi. margarita Gigmar60 (AF396782) 701 bp
Gi. margarita Gigmar58 (AF396783) 701 bp
Gi. decipiens AU102 (FJ461862) 652 bp ◄
S. heterogama W4733/Att1283-1 (FR750159)
S. heterogama CL157 (FJ461871) 650 bp ◄
S. heterogama FL225, AFTOL-ID138 (consensus 10)
S. heterogama W5611/Att1577-4 (FR750016)
S. heterogama SN722 (FJ461877) 650 bp ◄
S. heterogama isolate 4 (AY900502) 656 bp
S. heterogama isolate 5 (AY900503) 656 bp
S. heterogama isolate 3 (AY900501) 656 bp
S. heterogama isolate 2 (AY900500) 656 bp
S. heterogama BEG35, W3214/Att334-16 (FM876839)
S. heterogama BEG35, W3214/Att334-16 (FM876838)
S. heterogama BEG35, W3214/Att334-16 (FM876837)
S. heterogama BR155 (FJ461872) 650 bp ◄
S. heterogama WV858B (FJ461873) 650 bp ◄
S. heterogama WV858B (FJ461876) 650 bp ◄
S. heterogama WV858B (FJ461875) 650 bp ◄
S. heterogama isolate 1 (AY900499) 656 bp
S. heterogama NY320 (FJ461878) 650 bp ◄
S. heterogama WV858B (FJ461874) 650 bp ◄
S. erythropa Sen (AM040352) 671 bp
S. erythropa Sen (AM040354) 684 bp
S. erythropa Sen (AM040351) 670 bp
S. erythropa MA453B (AM040357) 674 bp
S. erythropa MA453B (AM040355) 672 bp
S. erythropa HA150B (FJ461869) 650 bp ◄
S. cerradensis MAFF520056 (AB048689)
S. cerradensis MAFF520056 (AB048688)
S. cerradensis MAFF520056 (AB048690)
S. cerradensis MAFF520056 (AB048685)
S. cerradensis MAFF520056 (AB048684)
S. cerradensis MAFF520056 (AB048686)
S. cerradensis MAFF520056 (AB048683)
S. reticulata CNPAB11 (consensus 11)
S. reticulata isolate 1 (AY900494) 656 bp
S. reticulata isolate 2 (AY900495) 656 bp
S. reticulata isolate 3 (AY900496) 656 bp
S. reticulata isolate 4 (AY900497) 656 bp
S. reticulata isolate 5 (AY900498) 656 bp
S. gilmorei W5342/Att590-16 (FN547620)
S. pellucida (AY639313) 699 bp
S. pellucida (AY639323) 699 bp
S. pellucida (AY639261) 700 bp
S. pellucida (AY639309) 701 bp
Scutellospora nodosa BEG4, W3485/Att209-44 (FM876833)
Scutellospora nodosa BEG4, W3485/Att209-44 (FM876836)
Scutellospora nodosa BEG4, W3485/Att209-44 (FM876834)
Scutellospora nodosa BEG4, W3485/Att209-44 (FM876835)
S. pellucida NC155C (FJ461879) 650 bp ◄
R. fulgida W2993/field collected (FR750138)
R. fulgida NC303A (FJ461870) 652 bp ◄
R. verrucosa isolate 3 (AY900509) 656 bp ●
R. verrucosa isolate 4 (AY900510) 656 bp ●
R. verrucosa isolate 5 (AY900511) 656 bp ●
R. verrucosa VA103A (FJ461881) 650 bp ◄
R. verrucosa isolate 2 (AY900508) 656 bp ●
R. verrucosa isolate 1 (AY900507) 656 bp ●
R. gregaria LPA48 (AJ510232) 668 bp
R. persica MA461A (FJ461880) 650 bp ◄
R. coralloidea SA260 (FJ461866) 650 bp ◄
R. weresubiae W2988/field collected (FR750134)
R. weresubiae W2988/field collected (FR750135)
Scutellospora spinosissima W3009/Att664-1 (FR750149)
Scutellospora spinosissima W3009/Att664-1 (FR750150)
S. calospora HDAM-3 (EU252109) 742 bp
S. calospora HDMA-3 (EU346867) 742 bp
S. calospora BEG32 (AJ510231) 707 bp
S. calospora PL114 (FJ461865) 673 bp ◄
S. calospora AU212A (FJ461864) 674 bp ◄
S. dipurpurescens WV930 (FJ461868) 674 bp ◄
A. cavernata BEG33, W3293/Att209-37 (FM876790)
A. cavernata BEG33, W3293/Att209-37 (FM876791)
0.05

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A. brasiliensis W4699/Att1211-0 (FN825905)
A. brasiliensis W4699/Att1211-0 (FN825909)
A. brasiliensis W4699/Att1211-0 (FN825904)
A. brasiliensis W4699/Att1211-0 (FN825907)
A. brasiliensis W4699/Att1211-0 (FN825901)
A. brasiliensis W4699/Att1211-0 (FN825902)
A. brasiliensis W5473/Att1210-5 (FN825911)
A. brasiliensis W5473/Att1210-5 (FN825910)
A. brasiliensis W4699/Att1211-0 (FN825903)
A. brasiliensis W4699/Att1211-0 (FN825906)
A. brasiliensis W4699/Att1211-0 (FN825908)
A. alpina ST2700 (AJ891103) 551 bp
A. alpina OV2600 (AJ891106) 572 bp
A. alpina DS1908 (AJ891109) 571 bp
A. alpina ST2700 (AJ891104) 552 bp
A. alpina ST2700 (AJ891102) 561 bp
A. alpina ST2700 (AJ891101) 562 bp
A. alpina ST2700 (AJ891105) 552 bp
A. alpina OV2600 (AJ891108) 564 bp
A. alpina OV2600 (AJ891107) 547 bp
A. colliculosa (GU326352) 662 bp
A. colliculosa (GU326340) 662 bp
A. colliculosa (GU326348) 662 bp
A. colliculosa (GU326349) 662 bp
A. colliculosa (GU326343) 662 bp
A. colliculosa (GU326339) 662 bp
A. colliculosa (GU326351) 662 bp
A. colliculosa (GU326346) 662 bp
A. colliculosa (GU326350) 662 bp
A. colliculosa (GU326347) 662 bp
A. colliculosa (GU326345) 662 bp
A. colliculosa (GU326344) 662 bp
A. colliculosa (GU326341) 662 bp
A. colliculosa (GU326342) 662 bp
A. laevis BEG13 (FN547507)
A. laevis BEG26 (FN547518)
A. laevis BEG26 (FN547514)
A. laevis BEG26 (FN547513)
A. laevis W3247/Att423-4 (FN547517)
A. laevis W3247/Att423-4 (FN547504)
A. laevis W3247/Att423-4 (FN547503)
A. laevis W3247/Att423-4 (FN547502)
A. laevis W3247/Att423-4 (FN547506)
A. laevis W3247/Att423-4 (FN547505)
A. laevis BEG13 (FN547510)
A. laevis BEG13 (FN547509)
A. laevis BEG13 (FN547511)
A. laevis BEG26 (FN547515)
A. laevis BEG13 (FN547512)
A. laevis BEG13 (FN547516)
A. laevis BEG26 (FN547519)
A. laevis BEG13 (FN547508)
A. laevis BEG13 (AJ510229) 705 bp
A. laevis AU211 (consensus 1)
A. entreriana W5469 (FR750172)
A. entreriana W5469 (FR750168)
A. entreriana W5469 (FR750173)
A. entreriana W5469 (FR750171)
A. entreriana W5469 (FR750169)
A. entreriana W5469 (FR750170)
A. colombiana BR100B (FJ461804) 657 bp ◄
A. colombiana W5795/Att1476-8 (FR750063)
A. colombiana C-18-3 (AJ239117) 523 bp
Acaulospora sp. CL283, FL709 (FJ461810) 658 bp ◄
Acaulospora sp. BHRRA-2009a, VA105E (FJ461805) 657 bp ◄
A. mellea isolate 2 (AY900513) 663 bp ●
A. mellea isolate 1 (AY900512) 662 bp ●
A. mellea isolate 3 (AY900514) 663 bp ●
A. koskei WV786 (FJ461793) 658 bp ◄
A. lacunosa BEG78 (AJ891113)
A. lacunosa BEG78 (AJ891110)
A. lacunosa BEG78 (AJ510230)
A. lacunosa BEG78 (AJ891111)
A. lacunosa BEG78 (AJ891112)
A. lacunosa WV110 (FJ461800) 653 bp ◄
A. foveata CR315 (FJ461801) 645 bp ◄
A. paulinae CW4 (AJ891118) 535 bp
A. paulinae CW4 (AJ891116) 548 bp
A. paulinae CW4 (AJ891115) 525 bp
A. paulinae CW4 (AJ891114) 549 bp
A. paulinae CW4 (AJ891117) 532 bp
A. paulinae CW4 (AJ891120) 535 bp
A. paulinae CW4 (AJ891121) 539 bp
A. paulinae CW4 (AJ891119) 536 bp
A. paulinae AU103A (FJ461796) 674 bp ◄
Acaulospora sp. WUM18, W2941/Att869-3 (FM876792)
Acaulospora sp. WUM18, W2941/Att869-3 (FM876793)
A. denticulata CL139-3 (AJ239115) 497 bp
A. cavernata BEG33, W3293/Att209-37 (FM876791)
A. cavernata BEG33, W3293/Att209-37 (FM876788)
A. cavernata BEG33, W3293/Att209-37 (FM876789)
A. cavernata BEG33, W3293/Att209-37 (FM876790)
A. longula BEG8 (AJ510228) 709 bp
A. longula AcS (AM040292) 709 bp
A. longula AcS (AM040291) 687 bp
A. longula AcS (AM040294) 689 bp
A. longula AcS (AM040293) 689 bp
A. mellea CR316B (FJ461794) 675 bp ◄
A. dilatata WV204 (FJ461792) 674 bp ◄
A. delicata NY304 (FJ461791) 675 bp ◄
A. delicata ML103 (FJ461790) 675 bp ◄
Acaulospora sp. CU141 (FJ461803) 674 bp ◄
A. morrowiae CR404 (FJ461795) 674 bp ◄
A. spinosa W3574/Att-none (FR750152)
A. spinosa W3574/Att-none (FR750155)
A. spinosa W3574/Att-none (FR750154)
A. spinosa W3574/Att-none (FR750153)
A. spinosa W3574/Att-none (FR750156)
A. spinosa W3574/Att-none (FR750151)
A. spinosa MN405B (FJ461798) 675 bp ◄
A. scrobiculata AU303 (FJ461797) 673 bp ◄
A. tuberculata VZ103E (FJ461799) 673 bp ◄
A. kentinensis TW111 (FN547522)
A. kentinensis TW111 (FM876821)
A. kentinensis TW111 (FN547521)
A. kentinensis TW111 (FM876823)
A. kentinensis TW111 (FM876828)
A. kentinensis TW111 (FM876826)
A. kentinensis TW111 (FN547520)
A. kentinensis TW111 (FM876824)
A. kentinensis TW111 (FM876830)
A. kentinensis TW111 (FM876822)
A. kentinensis TW111 (FM876827)
A. kentinensis TW111 (FN547523)
A. kentinensis TW111 (FM876829)
A. kentinensis TW111 (FM876825)
A. kentinensis CU114A (FJ461808) 679 bp ◄
Acaulospora sp. CL283, FL709 (FJ461811) 678 bp ◄
D. spurca W4119/Att246-18 (FN547644)
D. celata BEG231, FACE234, W4718+19/Att1278-2 (AM713404)
0.05

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D. celata BEG231, FACE234, W4718-19/Att1278-2 (AY639235)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AM713404)
D. celata BEG230, FACE83, W4758/Att1292-2 (AY639229)
D. celata BEG230, FACE83, W4758/Att1292-2 (AY639226)
D. celata BEG230, FACE83, W4758/Att1292-2 (AY639232)
D. celata BEG232, FACE272, W4757/Att1291-2 (AY639236)
D. celata BEG232, FACE272, W4757/Att1291-2 (AY639237)
D. celata BEG230, FACE83, W4758/Att1292-2 (AY639227)
D. celata BEG232, FACE272, W4757/Att1291-2 (AY639240)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AM713417)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AM713402)
D. celata BEG231, FACE234 ,W4718-19/Att1278-2 (AM713403)
D. celata BEG232, FACE272, W4757/Att1291-2 (AY639238)
D. celata BEG230, FACE83, W4758/Att1292-2 (AY639225)
D. celata BEG233, FACE410, W5306+07/Att1500-2 (DQ350448)
D. celata BEG233, FACE410, W5306+07/Att1500-2 (DQ350452)
D. celata BEG233, FACE410, W5306+07/Att1500-2 (DQ350450)
D. celata BEG233, FACE410, W5306+07/Att1500-2 (DQ350451)
D. celata BEG232, FACE272, W4757/Att1291-2 (AY639241)
D. celata BEG230, FACE83, W4758/Att1292-2 (AY639230)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AM713418)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AM713419)
D. celata BEG233, FACE410, W5306+07/Att1500-2 (DQ350449)
D. celata BEG233, FACE410, W5306+07/Att1500-2 (DQ350453)
D. celata BEG230, FACE83, W4758/Att1292-2 (AY639231)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AY639233)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AY639234)
D. celata BEG231, FACE234, W4718-19/Att1278-2 (AY639306)
D. celata BEG232, FACE272, W4757/Att1291-2 (AY639239)
D. celata BEG230, FACE83, W4758/Att1292-2 (AY639228)
Diversispora sp. AZ237B (AF185681) 757 bp
Diversispora sp. AZ237B (AF185677) 567 bp
Diversispora sp. AZ237B (AF185679) 578 bp
Diversispora sp. AZ237B (AF185678) 332 bp
Diversispora sp. NB101 (AF185694) 517 bp
Diversispora sp. NB101 (AF185693) 577 bp
Diversispora sp. NB101 (AF185682) 688 bp
Diversispora sp. NB101 (AF185691) 528 bp
Diversispora sp. NB101 (AF185695) 567 bp
Diversispora sp. NB101 (AF185690) 516 bp
Diversispora sp. AZ237B (AF185680) 564 bp
D. eburnea AZ420A, W4729 (AM713408)
D. eburnea AZ420A, W4729 (AM713407)
D. eburnea AZ420A, W4729 (AM713410)
D. eburnea UK121 (FJ461831) 708 bp ◄
D. eburnea AZ420A, W4729 (AM713413)
D. eburnea AZ420A, W4729 (AM713416)
D. eburnea AZ420A, W4729 (AM713411)
D. eburnea AZ420A, W4729 (EF067886)
D. eburnea AZ420A, W4729 (AM713406)
D. eburnea AZ420A, W4729 (EF067888)
D. eburnea AZ420A, W4729 (AM713415)
D. eburnea AZ420A, W4729 (AM713405)
D. eburnea AZ420A, W4729 (AM713412)
D. eburnea AZ420A, W4729 (EF067887)
D. eburnea AZ420A, W4729 (AM713414)
D. eburnea AZ420A, W4729 (AM713409)
D. spurca ex-type W4119/Att246-18 (FN547647)
D. spurca ex-type W4119/Att246-18 (FN547649)
D. spurca ex-type W4119/Att246-18 (FN547648)
D. spurca ex-type W4119/Att246-18 (FN547642)
D. spurca ex-type W4119/Att246-18 (FN547646)
D. spurca ex-type W4119/Att246-18 (FN547643)
D. spurca ex-type W4119/Att246-18 (FN547644)
D. spurca ex-type W4119/Att246-18 (FN547653)
D. spurca ex-type W4119/Att246-18 (FN547639)
D. spurca ex-type W4119/Att246-18 (FN547640)
D. spurca ex-type W4119/Att246-18 (FN547654)
D. spurca ex-type W4119/Att246-18 (FN547641)
D. spurca ex-type W4119/Att246-18 (FN547650)
D. spurca ex-type W4119/Att246-18 (FN547638)
D. spurca ex-type W4119/Att246-18 (FN547645)
D. spurca WV109F (FJ461847) 713 bp ◄
D. spurca ex-type W4119/Att246-18 (FN547651)
D. spurca ex-type W4119/Att246-18 (FN547637)
D. spurca ex-type W4119/Att246-18 (FN547652)
D. spurca HA567 (FJ461848) 713 bp ◄
D. spurca SC157 (FJ461849) 710 bp ◄
D. aurantia ex-type W4728/Att1296-0 (FN547661)
D. aurantia ex-type W4728/Att1296-0 (FN547665)
D. aurantia ex-type W4728/Att1296-0 (FN547664)
D. aurantia ex-type W4728/Att1296-0 (FN547663)
D. aurantia ex-type W4728/Att1296-0 (FN547659)
D. aurantia ex-type W4728/Att1296-0 (FN547660)
D. aurantia ex-type W4728/Att1296-0 (FN547662)
D. aurantia ex-type W4728/Att1296-0 (FN547655)
D. aurantia ex-type W4728/Att1296-0 (FN547658)
D. aurantia ex-type W4728/Att1296-0 (FN547657)
D. aurantia ex-type W4728/Att1296-0 (FN547656)
D. aurantia ex-type W4728/Att1296-0 (EF581863) 760 bp
D. aurantia ex-type W4728/Att1296-0 (EF581862) 758 bp
D. aurantia ex-type W4728/Att1296-0 (EF581860) 760 bp
D. aurantia holotype (AJ849468)
D. aurantia ex-type W4728/Att1296-0 (EF581864) 761 bp
D. aurantia ex-type W4728/Att1296-0 (EF581861) 761 bp
D. trimurales FL707B (FJ461854) 709 bp ◄
D. trimurales BR608 (FJ461851) 702 bp ◄
D. epigaea BEG47, W3180/Att475-22 (FN547672)
D. epigaea BEG47, W3180/Att475-22 (FN547677)
D. epigaea BEG47, W3180/Att475-22 (FN547674)
D. epigaea BEG47, W3180/Att475-22 (FN547679)
D. epigaea BEG47, W3180/Att475-22 (FN547680)
D. epigaea BEG47, W3180/Att475-22 (FR686941)
D. epigaea BEG47, W3180/Att475-22 (FR686940)
D. epigaea BEG47, W3180/Att475-22 (FR686938)
D. epigaea BEG47, W3180/Att475-22 (FR686939)
D. epigaea BEG47, W3180/Att475-22 (FN547666)
D. epigaea BEG47, W3180/Att475-22 (FN547669)
D. epigaea BEG47, W3180/Att475-22 (FN547670)
D. epigaea BEG47, W3180/Att475-22 (FN547671)
D. epigaea BEG47, W3180/Att475-22 (FN547668)
D. epigaea BEG47, W3180/Att475-22 (FN547673)
D. epigaea BEG47, W3180/Att475-22 (FN547676)
D. epigaea BEG47, W3180/Att475-22 (FN547675)
D. epigaea BEG47, W3180/Att475-22 (FN547678)
D. epigaea BEG47, W3180/Att475-22 (FN547667)
D. epigaea BEG47, W3180/Att475-22 (FN547681)
D. epigaea BEG47 (AY842568)
D. epigaea BEG47, W5165/Att475-45 (FM876814)
D. epigaea BEG47 (AY842573)
D. epigaea BEG47, W5165/Att475-45 (FM876817)
D. epigaea BEG47 (AY842569)
D. epigaea BEG47 (AY842574)
D. epigaea BEG47, W5165/Att475-45 (FM876816)
D. epigaea BEG47, W5165/Att475-45 (FN547635)
D. epigaea BEG47 (AY842567)
D. epigaea BEG47 (FJ461852) 707 bp ◄
D. epigaea BEG47 (AM947665)
D. epigaea BEG47, W5165/Att475-45 (FM876819)
D. epigaea BEG47, W5165/Att475-45 (FM876818)
D. epigaea BEG47, W5165/Att475-45 (FM876820)
D. epigaea BEG47, W5165/Att475-45 (FN547636)
D. epigaea BEG47, W5165/Att475-45 (FM876815)
Diversispora sp. W5257 (FR686946)
Diversispora sp. W5257 (FR686948)
Diversispora sp. W5257 (FR686949)
Diversispora sp. W5257 (FR686947)
Diversispora sp. W5257 (FR686945)
Diversispora sp. W5257 (FR686958)
Diversispora sp. W5257 (FR686950)
Diversispora sp. W5257 (FR686952)
Diversispora sp. W5257 (FR686951)
Re. fulvum CL-Mart05-049 (AM418546) 767 bp
Re. fulvum CL-Mart05-111 (AM418548) 762 bp
Re. fulvum CL-Mart05-049 (AM418545) 765 bp
Re. fulvum CL-Mart05-111 (AM418547) 754 bp
Redeckera sp. *fulvum*-like AC-Pohn99-001 (consensus 2)
Re. pulvinatum CL-Mart05-035 (AM418549) 772 bp
Re. pulvinatum CL-Mart05-035 (AM418550) 767 bp
Re. megalocarpum CL-Guad05-051 (AM418552) 778 bp
Re. megalocarpum CL-Guad05-051 (AM418551) 786 bp
D. trimurales KS101 (FJ461855) 713 bp ◄
G. tortuosum JA306A (FJ461850)705 bp ◄
A. cavernata BEG33, W3293/Att209-37 (FM876791)
A. cavernata BEG33, W3293/Att209-37 (FM876790)
A. laevis W3247/Att423-4 (FN547503)
A. laevis W3247/Att423-4 (FN547502)
0.05

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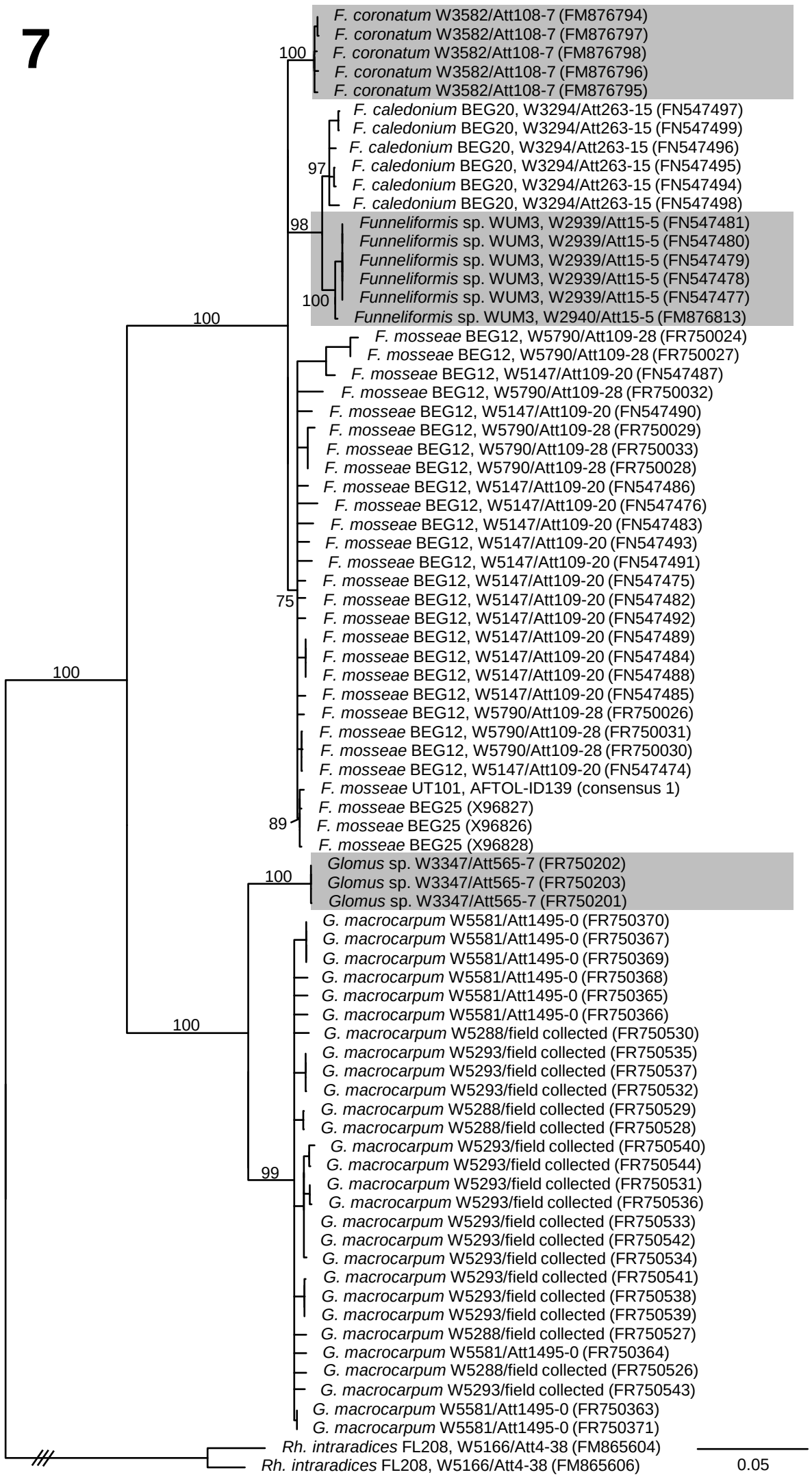
F. coronatum W3582/Att108-7 (FM876794)
F. coronatum W3582/Att108-7 (FM876797)
F. coronatum W3582/Att108-7 (FM876798)
F. coronatum W3582/Att108-7 (FM876796)
F. coronatum W3582/Att108-7 (FM876795)
F. caledonium BEG20, W3294/Att263-15 (FN547497)
F. caledonium BEG20, W3294/Att263-15 (FN547499)
F. caledonium BEG20, W3294/Att263-15 (FN547496)
F. caledonium BEG20, W3294/Att263-15 (FN547495)
F. caledonium BEG20, W3294/Att263-15 (FN547494)
F. caledonium BEG20, W3294/Att263-15 (FN547498)
Funneliformis sp. WUM3, W2939/Att15-5 (FN547481)
Funneliformis sp. WUM3, W2939/Att15-5 (FN547480)
Funneliformis sp. WUM3, W2939/Att15-5 (FN547479)
Funneliformis sp. WUM3, W2939/Att15-5 (FN547478)
Funneliformis sp. WUM3, W2939/Att15-5 (FN547477)
Funneliformis sp. WUM3, W2940/Att15-5 (FM876813)
F. mosseae BEG12, W5790/Att109-28 (FR750024)
F. mosseae BEG12, W5790/Att109-28 (FR750027)
F. mosseae BEG12, W5147/Att109-20 (FN547487)
F. mosseae BEG12, W5790/Att109-28 (FR750032)
F. mosseae BEG12, W5147/Att109-20 (FN547490)
F. mosseae BEG12, W5790/Att109-28 (FR750029)
F. mosseae BEG12, W5790/Att109-28 (FR750033)
F. mosseae BEG12, W5790/Att109-28 (FR750028)
F. mosseae BEG12, W5147/Att109-20 (FN547486)
F. mosseae BEG12, W5147/Att109-20 (FN547476)
F. mosseae BEG12, W5147/Att109-20 (FN547483)
F. mosseae BEG12, W5147/Att109-20 (FN547493)
F. mosseae BEG12, W5147/Att109-20 (FN547491)
F. mosseae BEG12, W5147/Att109-20 (FN547475)
F. mosseae BEG12, W5147/Att109-20 (FN547482)
F. mosseae BEG12, W5147/Att109-20 (FN547492)
F. mosseae BEG12, W5147/Att109-20 (FN547489)
F. mosseae BEG12, W5147/Att109-20 (FN547484)
F. mosseae BEG12, W5147/Att109-20 (FN547488)
F. mosseae BEG12, W5147/Att109-20 (FN547485)
F. mosseae BEG12, W5790/Att109-28 (FR750026)
F. mosseae BEG12, W5790/Att109-28 (FR750031)
F. mosseae BEG12, W5790/Att109-28 (FR750030)
F. mosseae BEG12, W5147/Att109-20 (FN547474)
F. mosseae UT101, AFTOL-ID139 (consensus 1)
F. mosseae BEG25 (X96827)
F. mosseae BEG25 (X96826)
F. mosseae BEG25 (X96828)
Glomus sp. W3347/Att565-7 (FR750202)
Glomus sp. W3347/Att565-7 (FR750203)
Glomus sp. W3347/Att565-7 (FR750201)
G. macrocarpum W5581/Att1495-0 (FR750370)
G. macrocarpum W5581/Att1495-0 (FR750367)
G. macrocarpum W5581/Att1495-0 (FR750369)
G. macrocarpum W5581/Att1495-0 (FR750368)
G. macrocarpum W5581/Att1495-0 (FR750365)
G. macrocarpum W5581/Att1495-0 (FR750366)
G. macrocarpum W5288/field collected (FR750530)
G. macrocarpum W5293/field collected (FR750535)
G. macrocarpum W5293/field collected (FR750537)
G. macrocarpum W5293/field collected (FR750532)
G. macrocarpum W5288/field collected (FR750529)
G. macrocarpum W5288/field collected (FR750528)
G. macrocarpum W5293/field collected (FR750540)
G. macrocarpum W5293/field collected (FR750544)
G. macrocarpum W5293/field collected (FR750531)
G. macrocarpum W5293/field collected (FR750536)
G. macrocarpum W5293/field collected (FR750533)
G. macrocarpum W5293/field collected (FR750542)
G. macrocarpum W5293/field collected (FR750534)
G. macrocarpum W5293/field collected (FR750541)
G. macrocarpum W5293/field collected (FR750538)
G. macrocarpum W5293/field collected (FR750539)
G. macrocarpum W5288/field collected (FR750527)
G. macrocarpum W5581/Att1495-0 (FR750364)
G. macrocarpum W5288/field collected (FR750526)
G. macrocarpum W5293/field collected (FR750543)
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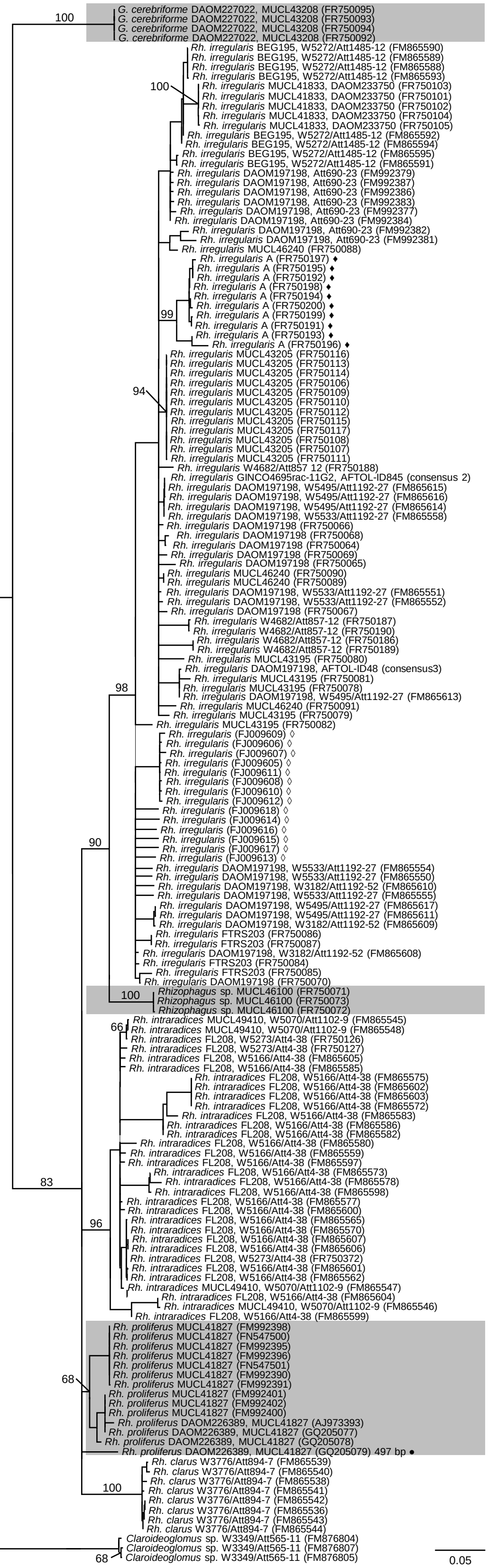
8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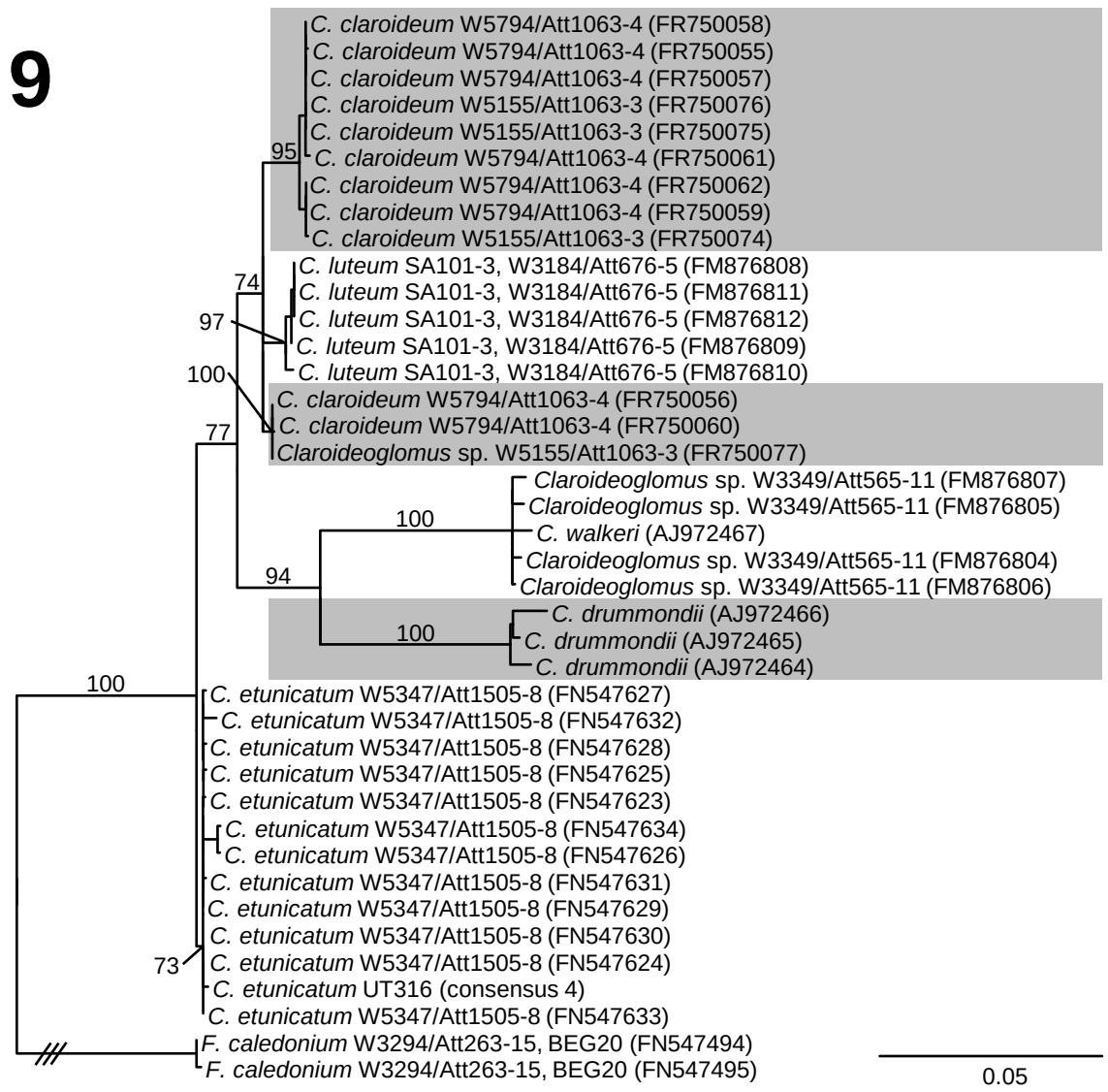
0.05

9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0.05

9. Discussion

9.1 General discussion

The aim of my thesis was to elaborate the molecular phylogeny of the ecologically and economically important AMF, for a better understanding of their evolution, diversity and applicability. These data should moreover be used to develop molecular tracing tools for AMF recognition in molecular-ecological studies. As suitable markers for phylogenetically inclusive detection of AMF were still missing, a new primer set for characterization of AMF with species-level resolution, was successfully designed and tested (chapter 4).

Using the 1.5 kb SSU-ITS-LSU fragment as baseline data, detailed DNA barcoding analyses could be conducted, including the analysis of intraspecific variability and potential DNA barcoding regions for species recognition of AMF (chapter 5). DNA barcoding could be helpful in biological research and agronomic field analyses regarding AMF, e.g. as quality control of applied inoculum or for beforehand characterization of the occurring AMF in the field. It is well known that AMF can improve plant tolerance to drought stress and pathogen resistance, but many mechanisms are not yet understood. Using a suited DNA barcoding region will allow detecting AMF-plant preferences in different environments and thus also help to uncover such yet unknown mechanisms.

Based on a ~2.7 kb (SSUfull-ITS-LSU) rDNA consensus sequence analysis, the sister-grouping of glomeromycotan fungi to *Dikarya* (Schüßler et al., 2001b; James et al., 2006), when using the SSU, ITS and/or LSU rDNA regions, was confirmed and the *Paraglomerales* were, for the first time, supported in bootstrap analyses as the most ancient lineage in the *Glomeromycota* (chapter 8, Fig. 1). Furthermore several debated revisions in the systematics of AMF could be clarified, supported or rejected based on the more comprehensive database provided, e.g. that of the *Gigasporaceae* (Oehl et al., 2008; Morton & Msiska, 2010a) and of *Entrophospora* (Sieverding & Oehl, 2006), as well as the transfer of *Kuklospora* to *Acaulospora* (Kaonongbua et al., 2010). Furthermore, the major taxonomic revision of Schüßler & Walker (2010) within the *Glomeromycota* was partially based on the SSUfull-ITS-LSU data.

This ~2.7 kb SSUfull-ITS-LSU sequence baseline was established and will become a base of a curated dataset to make the improvements in molecular detection and species recognition of AMF available for ecosystem research and AMF application.

9.2 The recent taxonomy of *Glomeromycota*

The fundamental changes in the systematics of *Glomeromycota* (Schüßler et al., 2001b), due to the large number of revised or modified revisions, indicate the needs for reliable molecular characterization and tracing tools. A revision of *Gigasporaceae* by Oehl et al. (2008) was rejected in most parts by Morton & Msiska (2010a) lacking a sufficient taxon sampling and robust phylogenetic analyses for an adequate revision. Further changes in the systematics of AMF were made, e.g. with the erection of *Intraspora* and *Kuklospora* (Sieverding & Oehl, 2006) solely based on morphology and recently revised (Kaonongbua et al., 2010) founded on molecular evidence as *Kuklospora* spp. cluster polyphyletically in *Acaulospora*. Last but not least a major revision of *Glomus*, *Diversisporaceae* and the rejection of *Intraspora* was published by Schüßler & Walker (2010), attempting to base the systematic of AMF on a natural, phylogenetic framework. The latter revision was also done to avoid the inflation of names announced at a symposium at the ICOM6 conference in Brazil, which would have led to a large number of new taxa in the *Glomeromycota*.

The results of the phylogenetic analyses of the 2.7 kb SSUfull-ITS-LSU, the SSU-ITS-LSU fragment and the SSU rRNA gene (chapter 8, Figs 1-9) are congruent with, and partly were the base for, the revision of Schüßler & Walker (2010). The data presented here also support the changes in the *Diversisporaceae*, namely the transfer of four *Glomus* species to *Diversispora* (Schüßler & Walker, 2010) based on molecular evidence (Schüßler et al., 2011 - chapter 7) and the new genus *Redeckera*, with the species *Re. fulvum*, *Re. pulvinatum* and the generic type species *Re. megalocarpum*. *Scutellospora weresubiae* was re-transferred to *Racocetra* based on the phylogenetic data shown in chapter 8, as it clusters monophyletically with this genus. The monospecific genus *Intraspora*, was rejected and *Intraspora schenckii* transferred to *Archaeospora* as it is phylogenetically placed in between *Archaeospora* cultures and thus congeneric, as demonstrated in chapter 8. The recently described *Entrophospora nevadensis* (Palenzuela et al., 2010), as well as *Otospora bareae* (Palenzuela et al., 2008) was shown to be congeneric with *Diversispora* (Schüßler et al., 2011 - chapter 7; chapter 8) and the published sequence data may be derived from contaminations. Furthermore the phylogenetic relationship of *Ambispora brasiliensis* (Goto et al., 2008), which was described based only on spore morphology, could be clarified and molecular evidence place the fungus in *Acaulospora* as *Ac. brasiliensis* (Krüger et al., 2011 - chapter 6).
It seems clear that further revisions within the *Glomeromycota* have to be done as e.g. for the genera erected by Oehl et al. (2008), representing *Scutellospora* species sensu Morton & Msiska (2010a). These genera are largely supported by the SSU and LSU analyses shown here, but the results are still based on a limited taxon sampling. A robust taxon sampling, beside the molecular tools that allow species recognition, should be the base for any major taxonomic changes.

9.3 Evolution of *Glomeromycota*

A correct natural systematics of the *Glomeromycota* should reflect the evolution of this ancient fungal phylum, which is dated back to at least 460 Mya and whose members co-evolved with land plants since their origin. Molecular clock estimates seem to be the only method to date back the origin of early fungal lineages and the *Glomeromycota*. However, due to rare fossil records and variant substitution rates in different fungal lineages this method may produce artefacts and divergence time estimates may be biased (Berbee & Taylor, 2010). There are only few fossil records for glomeromycotan fungi, such as fossil spores resembling modern glomeromycotan spores (460 Mya, Redecker et al., 2000; 400 Mya, Dotzler et al., 2006; 2009) and the well preserved arbuscules found in *Aglaophyton* (400 Mya; Remy et al., 1994). These recently discovered fossils (Dotzler et al., 2006; 2009) could be very valuable for re-calibrating molecular estimates, especially the origin of the *Gigasporaceae*.

The molecular clock estimates for the origin of the glomeromycotan lineages differ from 760 Mya to over 1000 Mya, indicating the limitations of the molecular clock methods and the lack of appropriate fossil calibration points. It is very likely that AMF arose before land plants (Brundrett, 2002) and are thus hypothesized to have played an important role in colonization of the land by plants (Pirozynski & Malloch, 1975), which is widely accepted nowadays. Functional evidence for this hypothesis was lacking so far, but Humphrey et al. (2010) recently showed support for this scenario by demonstrating that mycorrhizal *Marchantia paleaceae* (a thalloid liverwort) shows enhanced biomass production, uptake of nitrogen and phosphorus, in contrary to the non-mycorrhizal plants, when grown at CO_2 concentrations similar to them in the early Palaeozoic era. Before such mycorrhiza-like symbioses with bryophytes, AMF may have been associated with other photoautrophic organisms (Selosse & Tacon, 1999; Heckman et al., 2001) such as the unique endosymbiosis of *Geosiphon pyriformis* (Schüßler, 2002) which forms symbiosis with the cyanobacterium *Nostoc punctiforme* (Schüßler et al., 2007).

Land plants were recently dated back using an uncorrelated relaxed-clock analysis including 33 fossil calibration points to have been originated at 477 Mya (Middle Ordovician; Smith et al., 2010), but in fact Smith et al. (2010) discuss the split between bryophytes and Lycopodiophyta and not the origin of the land plant lineage. This split is consistent with the earliest known microfossil records of land plants (~470 Mya, Wellmann & Gray, 2000). Flowering plants (*Angiospermae*) were suggested to have originated 217 Mya (Late Triassic) approx. 20 My earlier than previously estimated (140 Mya, Bell et al., 2005; 190 Mya, Magallon & Sanders, 2005). It seems likely that the origin of land plants and of AMF will be dated back further in time.

9.4 Molecular phylogeny of *Glomeromycota*

The *Glomeromycota* (Schüßler et al., 2001b) and their sister-grouping to *Asco-* and *Basidiomycota* (James et al., 2006; chapter 8), was questioned by Lee & Young (2009). They demonstrated low supported sister-grouping to *Mortierella verticillata* based on the phylogenetic analyses on 14 mitochondrially encoded proteins. The relationship with the *Mortierellales* was also indicated by analyses of actin genes, RPB1 and elongation factor 1-alpha (EF-1α) (Redecker & Raab, 2006) and an analysis based on 113 nucleus-encoded proteins (Liu et al., 2009). The α- and β-tubulin gene phylogenies suggested *Chytridiomycota* as sister-group of glomeromycotan fungi (Corradi et al., 2004), while with increased taxon sampling and exclusion of the third codon position of the β-tubulin gene Msiska & Morton (2009) showed sister relationship to *Zygomycota* for *Glomeromycota*. The phylogenetic relationship of *Glomeromycota* to other fungal phyla remains unclear and varies depending on the marker used, but in contrast the monophyly of glomeromycotan fungi is supported in all analyses independent of the marker region used.

Currently only few data is available for the protein coding genes of AMF, mainly from *Rhizophagus irregularis* and closely related species, and a more comprehensive sampling of taxa is needed. Therefore only the rDNA as marker regions are discussed here, as providing the largest taxon sampling and sequence numbers have grown considerably in the last years.

Genus resolution with the SSU rDNA marker region

Despite the limited resolution power of the SSU rDNA, which was also indicated in previous studies (Bruns et al., 1991; Hofstetter et al., 2007), the SSU rDNA is still widely used for characterization of AMF in the field (Lee et al., 2008; Beck et al., 2007; Öpik et al., 2008, 2010; Turrini et al., 2008; Long et al., 2010; Ryszka et al., 2010). As the SSU rDNA provides the largest taxon sampling, Öpik et al. (2009) and Lumini et al. (2010) both conducted in-field community studies of AMF using a 454 sequencing approach based on the conserved SSU and the relatively short 200-250 bp reads. Both defined phylotypes with 97% sequence similarity, widely used for full length SSU sequences of bacteria, but clearly corresponding to above species recognition for AMF. Thus, this method may hide many AMF species, making interpretations in ecological studies difficult and error-prone. We considered the SSU rDNA region as unsuited for community analysis at species-level and DNA barcoding.

New AMF specific primers and species resolution with the ITS and LSU rDNA region

As the SSU rDNA region is unsuited for species recognition, we designed new AMF specific primers (SSUmAf-LSUmAr, SSUmCf-LSUmBr), which amplify a fragment of ~1.5-1.8 kb covering the 3' SSU,

the whole ITS and a part of the LSU rDNA region. They were tested and amplify members of all main lineages in the *Glomeromycota* (chapter 4). In a field trial using the primers for amplification of AMF DNA from plant roots we only observed two non-target sequence from over 100 processed samples, which was *Ranunculus repens* and *Rumex acetosella*. Due to the increasing number of sequence data for AMF it seems clear that the primers have to be optimized in future, as we, e.g. recently observed some mismatches for *Archaeospora schenckii* and *Archaeospora trappei*. To improve the efficiency of the primers and to prevent bias in amplification towards certain groups of AMF the concentration of the individual primers in the mixture could easily be adjusted, in future attempts, and new versions of individual primers may be designed, when necessary.

The ITS region was used as a kind of 'de facto DNA barcode' since the early 1990s for fungi (Kõljalg et al., 2005) and may provide species-level resolution. The ITS region was used for AMF to separate species e.g. in the *Ambisporaceae* (Walker et al., 2007) and in combination with the LSU rRNA gene for *Diversisporaceae* (Gamper et al., 2009), but species recognition of the ITS alone for very closely related species e.g. for *Rhizophagus intraradices* FL208 and *Rhizophagus irregularis* DAOM197198 (the latter usually wrongly named as *Glomus intraradices*, see Stockinger et al., 2009) is not always robust, due to high intraspecific variability. We considered the ITS region as useful to distinguish species, but with some limitations.

The LSU rDNA was also frequently used for identification of AMF in community analyses (van Tuinen et al., 1998; Kjøller & Rosendahl, 2000; Turnau et al., 2001; Gollotte et al., 2004; Pivato et al., 2007; Gamper et al., 2009), often covering the variable D1 or D2 region, sometimes both. The LSU-D2 region could resolve AMF species (Kjøller & Rosendahl, 2000) and most of the frequently used LSU primer pairs are designed to amplify the LSU-D2 in a nested PCR approach (Gollotte et al., 2004; Kjøller & Rosendahl, 2000). Our results now demonstrate the good resolution provided by the LSU-D2 alone, which was almost the same as for the 800 bp LSU-fragment covering both, the D1 and D2 regions. In contrast, the LSU-D1 alone could not separate over half of the analyzed AMF species (chapter 5).

Using the SSU-ITS-LSU fragment, amplified with the AMF specific primers, we could achieve species-level resolution and clarify some inconsistencies within the systematics of *Glomeromycota* (see chapter 9.1). With the SSU-ITS-LSU fragment, which covers all earlier used regions, we could analyze all available data and compare results of different studies, which was not possible before. The analysis in chapter 5 was the first using the SSU-ITS-LSU fragment to characterize AMF, therefore no other comparative data covering the complete 1.5 kb SSU-ITS-LSU fragment were available yet, but we know from personal communications that it is now used by several research groups and soon more data will be

published. Further sequence data were published and analyzed here (chapter 8), for all main phylogenetic lineages of *Glomeromycota*, which confirmed and refined the former results. This indicates that the SSU-ITS-LSU fragment carries appropriate informative regions for robust phylogenetic analyses and molecular detection of AMF at species-level. Furthermore using the SSU-ITS-LSU fragment as a phylogenetic 'backbone', species recognition was possible even with shorter fragments included (Stockinger et al., 2010 – chapter 5).

In combination with a fragment, covering almost the full length of the SSU, a robust phylogenetic analysis based on 2.7 kb SSUfull-ITS-LSU sequences was conducted, which might be used as future standard in molecular characterization of glomeromycotan fungi, also helping to fill up the gaps in the coverage provided by public sequence databases.

9.5 DNA barcoding of *Glomeromycota*

For fungi, a standardized official DNA barcode is currently lacking. Our recommendation is to use the complete 1.5 kb SSU-ITS-LSU fragment as baseline for AMF DNA barcoding, because shorter fragments failed to separate closely related species robustly (chapter 5). However, species identification is only as good as the reference sequence database (Begerow et al., 2010) and standards are needed, e.g. regarding vouchers, geographical data, correct annotation, more available sequence data, etc. Thus and because of the known problems in the international sequence databases (Bidartondo et al., 2008), curated databases such as the 'user-friendly nordic ITS ectomycorrhiza database' (UNITE, http://unite.ut.ee) and the accompanied web-based workbench PlutoF (Abarenkov et al., 2010; http://plutof.ut.ee) are needed. Presently UNITE and PlutoF only provide upload and comparison of the ITS region, but for UNITE the LSU rDNA region will also be implemented in future also for *Glomeromycota* (UNITE/NordForsk Network Meeting, Helsinki Finland, 2009). To support such databases, descriptions of new AMF species should be as accurate as possible (vouchers, geo-data, covering intraspecific sequence variability, etc.). We here publish our curated sequence database to partly overcome the current limitations of AMF species recognition in ecological studies (chapter 8).

10. Outlook

The currently 228 described AMF species are only the tip of the iceberg and it is most likely that within the next years many new species will be described. In regard to the high-throughput sequencing methods even more undescribed species will be published. Therefore it is important to have a standardized molecular characterization, e.g. the one we introduced based on a 2.7 kb SSUfull-ITS-LSU rDNA fragment (chapter 8) also covering the most likely future DNA barcode for fungi and part of the intraspecific variability. Such molecular characterization should be done for all available and morphological characterized, defined AMF cultures to improve the data-baseline for community analyses. For a reliable taxonomy and systematic of AMF, new species should be described as accurate as possible, both, morphologically and molecularly. Lacking cultures and high-quality sequence data are the biggest bottlenecks for glomeromycotan molecular-ecological research.

Further improving the dataset for protein encoding genes for AMF is also important, as comparison of phylogenetic analyses for multiple genes may solve the yet unclear relationship of the *Glomeromycota* to other fungal phyla. Partly due to such problems, the limited fossil records and variable molecular clock estimates, the understanding how AMF have evolved still is in its infancy. Molecular clock estimates are not always congruent and tend to result in earlier dating (Bromham & Penny, 2003) than estimates based on fossil records. Furthermore the fossil records are getting rare with increasing geological age (Heckmann et al., 2001) and thus the precision of molecular clock estimates cannot be proven, which was called 'the negative evidence dilemma' by Berbee & Taylor (2010). New fossil findings and refined molecular clock methods will improve the precision of the estimations about the origin of the *Glomeromycota*. It is likely that their origin will be dated back in regard to the more conserved estimation methods as it was recently done for the origin of land plants (Smith et al., 2010).

The molecular tools presented (chapter 4, 5) could be used for detection of AMF species applied from an inoculum mixture, e.g. in tree nurseries or agriculture. Knowing which AMF persists in the field the inoculum could be improved, making afforestation or agricultural application more efficient. This could be time and cost efficient with the recent GS-FLX system (~400 bp) or the upcoming upgrade of the system (~800 bp).

Third generation sequencing technologies, such as the PACBIO *RS* (Pacific Bioscience) combining the high amount of sequences generated (second generation) and the reduction of the bias introduced by PCR with projected read lengths of ≤1kb (www.pacificbioscience.com) will help to discover the biodiversity of AMF in an unknown range, further improving the knowledge about the important and potential plant preferences of these indispensable fungi.

11. References

Abarenkov K, Tedersoo L, Nilsson HR, Vellak K, Saar I, Veldre V, Parmasto E, Prous M, Aan A, Ots M, Kurina O, Ostonen I, Jõgeva J, Halapuu S, Põldmaa K, Toots M, Truu J, Larsson KH, Kõljalg U. 2010. PlutoF—a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. *Evolutionary Bioinformatics* **6**: 189-196.

Ames RN, Reid CPP, Porter LK, Cambardella C. 1983. Hyphal uptake and transport of nitrogen from two 15N-labelled sources by *Glomus mosseae*, a vesicular–arbuscular mycorrhizal fungus. *New Phytologist* **95**: 381–396.

Angelard C, Colard A, Niculita-Hirzel H, Croll D, Sanders IR, 2010. Segregation in a mycorrhizal fungus alters rice growth and symbiosis-specific gene transcription. *Current Biology*, in press

Aroca R, Porcel R, Ruiz-Lozano JM. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist* **173**: 808-816.

Auge RM, Stodola JW, Tims JE, Saxton AM. 2001. Moisture retention properties of a mycorrhizal soil. *Plant and Soil* **230**: 87-97.

Balestrini R, Magurno F, Walker C, Lumini E, Bianciotto V. 2010. Cohorts of arbuscular mycorrhizal fungi (AMF) in *Vitis vinifera*, a typical Mediterranean fruit crop. *Environmental Microbiology Reports* **2**: 594–604.

Beck A, Haug I, Oberwinkler F, Kottke I. 2007. Structural characterization and molecular identification of arbuscular mycorrhiza morphotypes of *Alzatea verticillata (Alzateaceae)*, a prominent tree in the tropical mountain rain forest of South Ecuador. *Mycorrhiza* **17**: 607-625.

Begerow D, Nilsson H, Unterseher M, Maier W. 2010. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* **87**(1): 99-108.

Bell CD, Soltis DE, Soltis PS. 2005. The age of the angiosperms: a molecular timescale without a clock. *Evolution* **59**:1245–1258.

Berbee, ML and JW Taylor. 2010. Dating the molecular clock in fungi - how close are we? *Fungal Biology Reviews* **24**: 1-16.

Birky C, Wolf C, Maughan H, Herbertson L, Henry E. 2005. Speciation and selection without sex. *Hydrobiologia* **546**: 29-45.

Bidartondo MI. 2008. Preserving accuracy in GenBank. *Science* **319**: 1616.

Błaszkowski J, Czerniawska B, Wubet T, Schäfer T, Buscot F, Renker C. 2008. *Glomus irregulare*, a new arbuscular mycorrhizal fungus in the *Glomeromycota*. *Mycotaxon* **106**: 247-267.

Börstler B, Thiéry O, Sýkorová Z, Berner A, Redecker D. 2010. Diversity of mitochondrial large subunit rDNA haplotypes of *Glomus intraradices* in two agricultural field experiments and two semi-natural grasslands. *Molecular Ecology* **19:** 1497-1511.

Börstler B, Raab PA, Thiéry O, Morton JB, Redecker D. 2008. Genetic diversity of the arbuscular mycorrhizal fungus *Glomus intraradices* as determined by mitochondrial large subunit rRNA gene sequences is considerably higher than previously expected. *New Phytologist* **180**: 452-465.

Börstler B, Renker C, Kahmen A, Buscot F. 2006. Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity. *Biology and Fertility of Soils* **42**: 286–298.

Bromham L, Penny D. 2003.The modern molecular clock. *Nature Reviews Genetics* **4**: 216-224.

Brundrett MC. 2002. Tansley Review no. 134. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* **154**: 275-304.

Bruns TD, White TJ, Taylor JW. 1991. Fungal molecular systematics. *Annual Review of Ecology and Systematics* **22**: 525-564.

Cárdenas-Flores A, Draye X, Bivort C, Cranenbrouck S, Declerck S. 2010. Impact of multispores in vitro subcultivation of *Glomus* sp. MUCL43194 (DAOM 197198) on vegetative compatibility and genetic diversity detected by AFLP. *Mycorrhiza* **20**: 415-425.

Clapp JP, Fitter AH, Young JPW. 1999. Ribosomal small subunit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp. *Molecular Ecology* **8:** 915-922.

Clapp JP, Young JPW, Merryweather JW, Fitter AH. 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist* **130:** 259-265.

Cooper KM, Tinker PB. 1978. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. II. Uptake and translocation of phosphorus, zinc and sulphur. *New Phytologist* **81**: 43-52**.**

Corradi N, Kuhn G, Sanders IR. **2004**. Monophyly of β-*tubulin* and H^+-*ATPase* gene variants in *Glomus intraradices*: consequences for molecular evolutionary studies of AM fungal genes. *Fungal Genetics and Biology* **41**: 262-273.

Croll D, Giovannetti M, Koch AM, Sbrana C, Ehinger M, Lammers PJ, Sanders IR. **2008**. Nonself vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **181**: 924-937.

Croll D, Wille L, Gamper HA, Mathimaran N, Lammers PJ, Corradi N, Sanders IR. 2008. Genetic diversity and host plant preferences revealed by simple sequence repeat and mitochondrial markers in a population of the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **178:** 672-687.

Declerck S, Séguin S, Dalpé Y. 2005. The monoxenic culture of arbuscular mycorrhizal fungi as a tool for germplasm collections. In: Declerck S, Strullu D-G, Fortin JA. eds. In vitro culture of mycorrhizas. Springer. Berlin, Heidelberg. pp. 17-30.

de la Pena E, Rodriguez Echeverria S, van der Putten WH, Freitas H, Moens M. 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. *New Phytologist* **169**: 829–840.

de la Providencia IE, De Souza FA, Fernández F, Delmas NS, Declerck S. **2005**. Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. *New Phytologist* **165**: 261–271.

Dotzler N, Krings M, Taylor TN, Agerer R. **2006**. Germination shields in *Scutellospora* (*Glomeromycota*: *Diversisporales*, *Gigasporaceae*) from the 400 million-year-old Rhynie chert. *Mycological Progress* **5**: 178-184.

Dotzler N, Walker C, Krings M, Hass H, Kerp H, Taylor TN, Agerer R. **2009**. Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. *Mycological Progress* **8**: 9-18.

Douds DD, Pfeffer PE, Shachar-Hill Y .2000. Carbon partitioning, cost and metabolism of arbuscular mycorrhizae in arbuscular mycorrhizas: physiology and function. *In* Y Kapulnick, DD Douds Jr, eds, Arbuscular Mycorrhizas: Molecular Biology and Physiology. Kluwer Academic Publishers, Dordrecht, The Netherlands (in press)

Fortin JA, Declerck S, Strullu D-G. 2005. In vitro culture of mycorrhizas. In: Declerck S, Strullu D-G, Fortin JA. eds. In vitro culture of mycorrhizas. Springer. Berlin, Heidelberg. pp. 3-14.

Frey B, Schüepp H. 1993. Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L. *New Phytologist* **124**: 221–230.

Frézal L, Leblois R. **2008**. Four years of DNA barcoding: Current advances and prospects. *Infection, Genetics and Evolution* **8**: 727-736.

Gamper HA, Walker C, Schüßler A. **2009**. *Diversispora celata* sp. nov: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. *New Phytologist* **182**: 495-506.

Gange AC. 2001. Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. *New Phytologist* **150**: 611-618.

Gerdemann JW, Trappe JM. **1974**. *Endogonaceae* in the Pacific Northwest. *Mycol. Mem.* **5**: 1-76.

Gollotte A, van Tuinen D, Atkinson D. 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. *Mycorrhiza* **14:** 111-117.

Goto BT, Maia LC, Oehl F. 2008. *Ambispora brasiliensis* a new ornamented species in the arbuscular mycorrhiza-forming Glomeromycetes. *Mycotaxon* **105:** 11-18.

Govindarajulu M, Pfeffer P, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**: 819-823.

Graham JH .2000. Assessing costs of arbuscular mycorrhizal symbiosis agroecosystems fungi. *In* GK Podila, DD Douds Jr, eds, Current Advances in Mycorrhizae Research. APS Press, St. Paul, pp 127-140

Harrison MJ, van Buuren ML. **1995**. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature* **378**: 626-629.

Haug I, Lempe J, Homeier J, Weiß M, Setaro S, Oberwinkler F, Kottke I. 2004. *Graffenrieda emarginata* (*Melastomataceae*) forms mycorrhizas with Glomeromycota and with a member of the *Hymenoscyphus ericae* aggregate in the organic soil of a neotropical mountain rain forest. *Canadian Journal of Botany* **82**: 340–356.

Hebert PD, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of birds through DNA barcodes. *PLoS Biology* **2**: e312.

Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB. **2001**. Molecular Evidence for the Early Colonization of Land by Fungi and Plants. *Science* **293**: 1129-1133.

Hedges SB, Kumar S. **2003**. Genomic clocks and evolutionary timescales. *Trends in Genetics* **19**: 200-206.

Helgason T, Fitter AH. 2009. Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). *Journal of Experimental Botany* **60:** 2465-2480.

Helgason T, Watson IJ, Young PW. **2003**. Phylogeny of the *Glomerales* and *Diversisporales* (Fungi: *Glomeromycota*) from actin and elongation factor 1-alpha sequences. *FEMS Microbiology Letters* **229**: 127-132.

Helgason T, Fitter AH, Young JPW. 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides* non-scripta (bluebell) in a seminatural woodland. *Molecular Ecology* **8:** 659-666.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. 1998. Ploughing up the wood-wide web? *Nature* **394:** 431.

Hempel S, Renker C, Buscot F. 2007. Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. *Environmental Microbiology* **9**: 1930-1938.

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf SM, James TY, Kirk PM, Lücking R, Lumbsch HT, Lutzon F, Matheny PB, Mclaughlin DJ, Powell MJ, Redhead SA, et al. **2007**. A higher-level phylogenetic classification of the Fungi. *Mycological Research* **111**: 509-547.

Hijri M, Sanders IR. **2005**. Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. *Nature* **433**: 160-163.

Hildebrandt U, Ouziad F, Marner FJ, Bothe H. 2006. The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. *FEMS Microbiol Lett* **254:** 258–267.

Hildebrandt U, Janetta K, Bothe H. **2002**. Towards growth of arbuscular mycorrhizal fungi independent of a plant host. *Applied and Environmental Microbiology* **68**: 1919–1924.

Hildebrandt U, Kaldorf M, Bothe H. 1999. The zinc violet and its colonization by arbuscular mycorrhizal fungi. *Journal of Plant Physiology* **154**: 709-717.

Hodge A, Campbell CD, Fitter AH. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413**: 297–299.

Hofstetter V, Miadlikowska J, Kauff F, Lutzoni F. 2007. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: A case study of the *Lecanoromycetes* (*Ascomycota*). *Molecular Phylogenies and Evolution* **44**: 412-426.

Humphreys CP, Franks PJ, Rees M, Bidartondo MI, Leake JR, Beerling DJ. **2010**. Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. *Nature Communications* doi:10.1038/ncomms1105.

Husband R, Herre EA, Turner SL, Gallery R, Young JPW. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology* **11**: 2669–2678.

INVAM - International Culture Collection of VA Mycorrhizal Fungi. http://invam.caf.wvu.edu/collection/collection.htm [12.12.2010]

Jakobsen I, Abbott LK, Robson AD. 1992a. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. *New Phytologist* **120**: 371-380.

Jakobsen I, Abbott LK, Robson AD. 1992b. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 2. Hyphal transport of 32P over defined distances. *New Phytologist* **120**: 509–516.

James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox C, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett D, Lutzoni F, McLaughlin D, Spatafora J, Vilgalys R. **2006**. Reconstructing the early evolution of the fungi using a six gene phylogeny. *Nature* **443**:818–822.

Jansa J, Mozafar A, Kuhn G, Anken T, Ruh R, Sanders IR, Frossard E. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications* **13:** 1164-1176.

Johansen A, Finlay RD, Olsson PA. **1996**. Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **133**: 705–-712.

Johansen A, Jakobsen I, Jensen ES. 1992. Hyphal transport of 15N-labelled nitrogen by a vesicular–arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. *New Phytologist* **122**: 281–288

Kaonongbua W, Morton JB, Bever JD. **2010**. Taxonomic revision transferring species in *Kuklospora* to *Acaulospora* (*Glomeromycota*) and a description of *Acaulospora colliculosa* sp. nov. from field collected spores. *Mycologia* **102**: 1497-1509.

Kjøller R, Rosendahl S. **2000**. Detection of arbuscular mycorrhizal fungi (*Glomales*) in roots by nested PCR and SSCP (Single Stranded Conformation Polymorphism). *Plant and Soil* **226:** 189-196.

Kõljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM. **2005**. UNITE: a database

providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist* **166**:1063–1068.

König S, Wubet T, Dormann CF, Hempel S, Renker C, Buscot F. 2010. TaqMan Real-Time PCR Assays to assess arbuscular mycorrhizal responses to field manipulation of grassland biodiversity: Effects of soil characteristics, plant species richness, and functional traits. *Applied and Environmental Microbiology* **76**: 3765–3775.

Kottke I, Haug I, Setaro S, Suárez JP, Weiß M, Preußing M, Nebel M, Oberwinkler F. 2008. Guilds of mycorrhizal fungi and their relation to trees, ericads, orchids and liverworts in a neotropical mountain rain forest. *Basic and Applied Ecology* **9:** 13-23.

Kuhn G, Hijri M, Sanders IR. **2001**. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature* **414**: 745-748.

Lanfranco L, Delpero M, Bonfante P. **1999**. Intrasporal variability of ribosomal sequences in the endomycorrhizal fungus *Gigaspora margarita*. *Molecular Ecology* **8**: 37-45.

Lee J, Young JPW. **2009**. The mitochondrial genome sequence of the arbuscular mycorrhizal fungus *Glomus intraradices* isolate 494 and implications for the phylogenetic placement of *Glomus*. *New Phytologist* **183**: 200-211.

Lee J, Lee S, Young JPW. **2008**. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology* **65:** 339-349.

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL. 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* **9**: 331-336.

Liu Y, Leigh JW, Brinkmann H, Cushion MT, Rodriguez-Ezpeleta N, Philippe H, Lang BF. **2009**. Phylogenomic analyses support the monophyly of Taphrinomycotina, including *Schizosaccharomyces* fission yeasts. *Molecular Biology and Evolution* **26:** 27-34.

Long LK, Yao Q, Guo J, Yang RH, Huang YH, Zhu HH. 2010. Molecular community analysis of arbuscular mycorrhizal fungi associated with five selected plant species from heavy metal polluted soils. *European Journal of Soil Biology*. **46**: 288-294.

Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar, Buchner A, Lai T, Steppi S, Jobb G, Förster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, König A, Liss T, Lüßmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A, Schleifer KH. 2004. ARB: a software environment for sequence data. *Nucleic Acids Research* **32:** 1363-1371.

Lumini E, Orgiazzi A, Borriello R, Bonfante P, Bianciotto V. **2010**. Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. *Environmental Microbiology* **12**: 2165-2179.

Magallón SA, Sanderson MJ. **2005**. Angiosperm divergence times: the effect of genes, codon positions, and time constraints. *Evolution* **59**:1653–1670.

Martin F, Gianinazzi-Pearson V, Hijri M, Lammers P, Requena N, Sanders IR, Shachar-Hill Y, Shapiro H, Tuskan GA, Young JPW. 2008. The long hard road to a completed *Glomus intraradices* genome. *New Phytologist* **180:** 747–750.

McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema JH, Turland NJ (eds). **2006**. International Code of Botanical Nomenclature (Vienna Code) adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005. Regnum Vegetabile 146. Ruggell: A.R.G. Ganter Verlag.

Merryweather J, Fitter A. 1998. The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. I. Diversity of fungal taxa. *New Phytologist* **138**: 117–129.

Michelson A, Rosendahl S. **1990**. The effect of VA mycorrhizal fungi, phosphorus and drought stress on the growth of *Acacia nilotica* and *Leucaena leucocephala* seedlings. *Plant and Soil* **124**: 7–13.

Miller MA; Holder MT, Vos R, Midford PE, Liebowitz T, Chan L, Hoover P, Warnow T. 2009. The CIPRES Portals. CIPRES. URL:http://www.phylo.org/sub_sections/portal. Accessed: 2009-08-04. (Archived by WebCite(r) at http://www.webcitation.org/5imQlJeQa)

Msiska Z, Morton J. **2009**. Phylogenetic analysis of the *Glomeromycota* by partial β-tubulin gene sequences. *Mycorrhiza* **19**: 1432-1890.

Morton JB. 1993. Problems and solutions for the integration of glomalean taxonomy, systematic biology, and the study of endomycorrhizal phenomena. *Mycorrhiza* **2**: 97-109.

Morton JB. 1985. Variation in mycorrhizal and spore morphology of *Glomus occultum* and *Glomus diaphanum* as influenced by plant host and soil environment. *Mycologia* **77**: 192-204.

Morton JB. **2000**. Evolution of endophytism in arbuscular mycorrhizal fungi of *Glomales*. In Microbial Endophytes (C. W. Bacon & J. H. White, eds):121±140. Marcel Dekker, New York.

Morton JB, Msiska Z. **2010a**. Phylogenies from genetic and morphological characters do not support a revision of *Gigasporaceae (Glomeromycota)* into four families and five genera. *Mycorrhiza* **20**: 483-496.

Morton JB, Msiska Z. **2010b**. Ontogeny and phylogeny of a *Scutellospora heterogama* mutant, with implications for morphological recognition of species in *Glomeromycota*. *Fungal Biology* **114**: 410-420.

Morton JB, Benny GL. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): A new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon* **37**:471-491.

Mosse B, Bowen GD. 1968. A key to the recognition of some *Endogone* spore types. *Transactions of the British Mycological Society* **51**: 469-483.

Mummey DL, Rillig MC. 2008. Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate grassland. *FEMS Microbiology Ecology* **64:** 260-270.

Oehl F, de Souza FA, Sieverding E. **2008**. Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming *Glomeromycetes*. *Mycotaxon* **106**: 311-360.

Oehl F, Sieverding E, Ineichen K, Ris EA, Boller T, Wiemken A. 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytologist* **165:** 273-283.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M. **2010**. The online database Maarj*AM* reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (*Glomeromycota*). *New Phytologist* **188**: 223-241.

Öpik M, Metsis M, Daniell TJ, Zobel M, Moora M. **2009**. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. *New Phytologist* **184**: 424-437.

Öpik M, Moora M, Zobel M, Saks Ü, Wheatley R, Wright F, Daniell T. 2008. High diversity of arbuscular mycorrhizal fungi in a boreal herb-rich coniferous forest. *New Phytologist* **179:** 867-876.

Palenzuela J, Barea JM, Ferrol N, Azcon-Aguilar C, Oehl F. **2010**. *Entrophospora nevadensis*, a new arbuscular mycorrhizal fungus from Sierra Nevada National Park (southeastern Spain). *Mycologia* **102:** 624-632.

Palenzuela J, Ferrol N, Boller T, Azcón-Aguilar C, Oehl F. **2008**. *Otospora bareai*, a new fungal species in the *Glomeromycetes* from a dolomitic shrub land in Sierra de Baza National Park (Granada, Spain). *Mycologia* **100**: 296-305.

Pawlowska TE, Taylor JW. **2004**. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature* **427**: 733-737.

Phipps CJ, Taylor TN. **1996**. Mixed arbuscular mycorrhizae from the Triassic of Antarctica. *Mycologia* **88**: 707–714.

Pirozynski KA, Malloch DW. **1975**. The origin of land plants: a matter of mycotrophism. *Biosystems* **6**:153-164.

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, van Tuinen D. 2007. *Medicago* species affect the community composition of arbuscular mycorrhizal fungi associated with roots. *New Phytologist* **176:** 197-210.

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* **35:** 7188-7196.

Raven JA, Smith SE, Smith FA. 1978. Ammonium assimilation and the role of mycorrhizas in climax communities in Scotland. *Botanical Society of Edinburgh Transactions* **43**: 27–35.

Redecker D. 2000. Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. *Mycorrhiza* **10:** 73-80.

Redecker D, Raab P. **2006**. Phylogeny of the *Glomeromycota* (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* **98**: 885-895.

Redecker D, Kodner R, Graham LE. **2000**. Glomalean fungi from the Ordovician. *Science* **289**: 1920-1921.

Remy W, Taylor TN, Hass H, Kerp H. **1994**. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences* **91**: 11841-11843.

Renker C, Weißhuhn K, Kellner H, Buscot F. 2006. Rationalizing molecular analysis of field-collected roots for assessing diversity of arbuscular mycorrhizal fungi: to pool, or not to pool, that is the question. *Mycorrhiza* **16**: 525-531.

Renker C, Blanke V, Buscot F. 2005. Diversity of arbuscular mycorrhizal fungi in grassland spontaneously developed on area polluted by a fertilizer plant. *Environmental Pollution* **135**: 255–266.

Renker C, Heinrichs J, Kaldorf M, Buscot F. 2003. Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. *Mycorrhiza* **13:** 191-198.

Requena N, Breuninger M, Franken P, Ocón A. **2003**. Symbiotic status, phosphate and sucrose regulate the expression of two plasma membrane H^+-ATPase genes from the mycorrhizal fungus *Glomus mosseae*. Plant Physiology **132**: 1540-1549.

Rippka R, Deruelles J, Waterbury J, Herdman M, Stanier R. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* **111:** 1-61.

Ryszka P, Błaszkowski J, Jurkiewicz A, Turnau K. 2010. Arbuscular mycorrhiza of *Arnica montana* under field conditions-conventional and molecular studies, *Mycorrhiza* **20***:* 551-557.

Sanders IR. 1999. No sex please, we're fungi. *Nature* **399**. 737-739.

Sanders IR. **2002**. Ecology and evolution of multigenomic arbuscular mycorrhizal fungi. *American Naturalist* **160**: S128–S141.

Sanders IR, Croll D. **2010**. Arbuscular Mycorrhiza: The challenge to understand the genetics of the fungal partner. *Annual Review of Genetics* **44**: 271-292.

Sanders FE, Tinker PB. 1971. Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas. *Nature* **233**: 278–279.

Sanders FE, Tinker PB. **1973**. Phosphate flow into mycorrhizal roots. *Pesticide Science* **4**: 385–395.

Schenck NC, Smith GS. **1982**. Additional new and unreported species of mycorrhizal fungi (*Endogonaceae*) from Florida. *Mycologia* **77**: 566-574.

Schüßler A. **2002**. Molecular phylogeny, taxonomy, and evolution of *Geosiphon pyriformis* and arbuscular mycorrhizal fungi. *Plant Soil* **244**:75-83.

Schüßler A, Walker C. **2010**. The *Glomeromycota*: a species list with new families and genera. Published by A. Schüßler & C. Walker, Gloucester UK, 2010. online available at www.amf-phylogeny.com

Schüßler A, Martin H, Cohen D, Fitz M, Wipf D. 2007. Arbuscular mycorrhiza - studies on the *Geosiphon* symbiosis lead to the characterization of the first glomeromycotan sugar transporter. (Addendum to: Schüßler A, Martin H, Cohen D, Fitz M, Wipf D (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. Nature 444: 933-936) *Plant Signaling & Behavior* 2: 431-434.

Schüßler A, Gehrig H, Schwarzott D, Walker C. **2001a**. Analysis of partial *Glomales* SSU rRNA gene sequences: implications for primer design and phylogeny. *Mycological Research* **105:** 5-15.

Schüßler A, Schwarzott D, Walker C. **2001b**. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* **105**: 1413-1421.

Schwarzott D, Schüßler A. **2001**. A simple and reliable method for SSU rRNA gene DNA extraction, amplification, and cloning from single AM fungal spores. *Mycorrhiza* **10:** 203-207.

Schwarzott D, Walker C, Schüßler A. **2001**. *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (*Glomales*), is nonmonophyletic. *Molecular Phylogenetics and Evolution* **21**: 190-197.

Seifert KA. **2009**. Progress towards DNA barcoding of fungi. *Molecular Ecology Resources* **9**: 83-89.

Selosse MA, Le Tacon F. **1998**. The land flora: A phototroph-fungus partnership? *Trends in Ecology & Evolution* **13**: 15-20.

Sieverding E, Oehl F. **2006**. Revision of *Entrophospora* and description of *Kuklospora* and *Intraspora*, two new genera in the arbuscular mycorrhizal *Glomeromycetes. Journal of Applied Botany and Food Quality* **80**: 69-81.

Simon L, Lalonde M, Bruns TD. 1992. Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied Environmental Microbiology* **58:** 291-295.

Smith SE. 1980. Mycorrhizas of autotrophic higher plants. *Biological Reviews* **55**: 475–510.

Smith SE, Read DJ. **2008**. *Mycorrhizal Symbiosis*. Amsterdam; Boston: Academic Press.

Smith SE, Read DJ. **1997**. *Mycorrhizal Symbiosis*. London; UK: Academic Press.

Smith SA, Beaulieu JM, Donoghue MJ. **2010**. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences* **107***:* 5897-5902.

Smith FA, Grace EJ, Smith SE. 2009. More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. *New Phytologist* **182:** 347-358.

Sokolski S, Dalpé Y, Séguin S, Khasa D, Lévesque CA, Piché Y. **2010**. Conspecificity of DAOM197198, the model arbuscular mycorrhizal fungus, with *Glomus irregulare*: molecular evidence with three protein-encoding genes. *Botany* **88**: 829–838.

Stamatakis A, Berger SA. **2009**. Evolutionary Placement of Short Sequence Reads, Performance & Accuracy of Evolutionary Placement Algorithms for Short Sequence Reads under Maximum Likelihood. *The Exelixis Lab, Dept. of Computer Science Technische Universität München Boltzmannstr. 3, 85748 Garching b. München, Germany*

Stamatakis A, Hoover P, Rougemont J. 2008. A Rapid Bootstrap Algorithm for the RAxML Web Servers. Systematic Biology Advance. *Systematic Biology* **57:** 758-771.

Stockinger H, Krüger M, Schüßler A. 2010. DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist* **187**: 461-474.

Stockinger H, Walker C, Schüßler A. 2009. '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. *New Phytologist* **183**: 1176-1187.

Stubblefield SP, Taylor TN, Trappe JM. **1987**. Fossil mycorrhizae: a case for symbiosis. *Science* **237**: 59-60.

Stukenbrock EH, Rosendahl S. 2005. Development and amplification of multiple co-dominant genetic markers from single spores of arbuscular mycorrhizal fungi by nested multiplex PCR. *Fungal Genetics and Biology* **42:** 73-80.

Taylor JW, Berbee ML. **2001**. Fungal molecular evolution: gene trees and geologic time. In: The Mycota, Vol VII(B), Systematics and Evolution, McLaughlin DJ, McLaughlin EG, Lemke PA (eds). *Springer Verlag, Berlin Heidelberg*, p. 229-245.

Taylor JW, Berbee ML. **2006**. Dating divergences in the Fungal Tree of Life: review and new analyses. *Mycologia* **98**: 838-849.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC. **2000**. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* **31**: 21-32.

The International Bank for the *Glomeromycota*. http://www.kent.ac.uk/bio/beg/ [12.12.2010]

Thiéry O, Börstler B, Ineichen K, Redecker D. **2010**. Evolutionary dynamics of introns and homing endonuclease ORFs in a region of the large subunit of the mitochondrial rRNA in *Glomus* species (arbuscular mycorrhizal fungi, *Glomeromycota*). *Molecular Phylogenetics and Evolution* **55**: 599-610.

Tinker PB. 1971. Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas. *Nature* **233**: 278–279.

Trappe JM. 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir GR (ed) Ecophysiology of VA mycorrhizal plants. CRC, Boca Raton, pp 5–25.

Turnau K, Ryszka P, Gianinazzi-Pearson V, van Tuinen D. 2001. Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza* **10:** 169-174.

Turrini A, Avio L, Bavila C, Giovannetti M. 2008. Characterisation of arbuscular mycorrhizal fungi in roots by means of epifluorescence microscopy and molecular methods. *Annals of Microbiology* **58**(1): 157-162.

van der Heijden MGA, Bardgett RD, van Straalen NM. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11:** 296-310.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396:** 69-72.

van de Voorde TFJ, van der Putten WH, Gamper HA, Hol WHG, Bezemer TM. 2010. Comparing arbuscular mycorrhizal communities of individual plants in a grassland biodiversity experiment. *New Phytologist* **186**: 746–754.

van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V. **1998**., Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. *Molecular Ecology* **7**: 103–111.

Vigo C, Norman JR, Hooker JE. 2000. Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. *Plant Pathology* **49**: 509–514.

Walker C, Schüßler A. 2004. Nomenclatural clarifications and new taxa in the *Glomeromycota*. *Mycological Research* **108:** 981-982.

Walker C, Vestberg M. 1994. A Simple and Inexpensive Method for Producing and Maintaining Closed Pot Cultures of Arbuscular Mycorrhizal Fungi. *Agricultural Science in Finland* **3:**233-240

Walker C, Vestberg M, Demircik F, Stockinger H, Saito M, Sawaki H, Nishmura I, Schüßler A. **2007**. Molecular phylogeny and new taxa in the *Archaeosporales* (*Glomeromycota*): *Ambispora fennica* gen. sp nov., *Ambisporaceae* fam. nov., and emendation of *Archaeospora* and *Archaeosporaceae*. *Mycological Research* **111**: 137-153.

Wang B, Qiu Y-L. **2006.** Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**: 299-363.

Wang YY, Vestberg M, Walker C, Hurme T, Zhang XP, Lindström K. 2008. Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China. *Mycorrhiza* **18:** 59-68.

Wellman CH, Gray J.**2000**. The microfossil record of early land plants. *Philosophical Transactions of the Royal Society B: Biological Science* **355**:717–732.

Wildeman AG, Nazar RN. 1981. Studies on the secondary structure of 5.8S rRNA from a Thermophile, *Thermomyces lanuginosus*. *The Journal of Biological Chemistry* **256:** 5675-5682.

Wubet T, Weiß M, Kottke I, Teketay D, Oberwinkler F. 2006. Phylogenetic analysis of nuclear small subunit rDNA sequences suggests that the endangered African Pencil Cedar, *Juniperus procera*, is associated with distinct members of *Glomeraceae*. *Mycological Research* **110:** 1059-1069.

Wubet T, Weiß M, Kottke I, Oberwinkler F. 2004. Morphology and molecular diversity of arbuscular mycorrhizal fungi in wild and cultivated yew (*Taxus baccata*). *The Canadian Journal of Botany* **81:** 255-266.

Wubet T, Kottke I, Teketay D, Oberwinkler F. 2003. Mycorrhizal status of indigenous trees in dry afromontane forests of Ethiopia. *Forest Ecology and Management* **179**:387–399.

12. Acknowledgment

First I want to thank my supervisor PD Dr. Arthur Schüßler for his support, expertise and the opportunity to work in his lab.
Furthermore Herbert Stockinger, Claudia Krüger and Macarena Marin are acknowledged for the kind and productive atmosphere in the office and laboratory, fruitful discussions and help of any kind.
Many Thanks to Christopher Walker for his expertise and his tireless advice about AMF cultures and all morphological issues.
I would like to thank the members of AG Parniske, AG Brachmann, AG Ott and AG Lahaye for the nice atmosphere and help when needed.

Last but not least I want to thank my family for their love, reassurement and motivation during my PhD thesis.

13. Appendix

13.1 Supplementary data – chapter 5

The following data are supplementary material for the publication ‘DNA barcoding of arbuscular mycorrhizal fungi’.

Figure S1

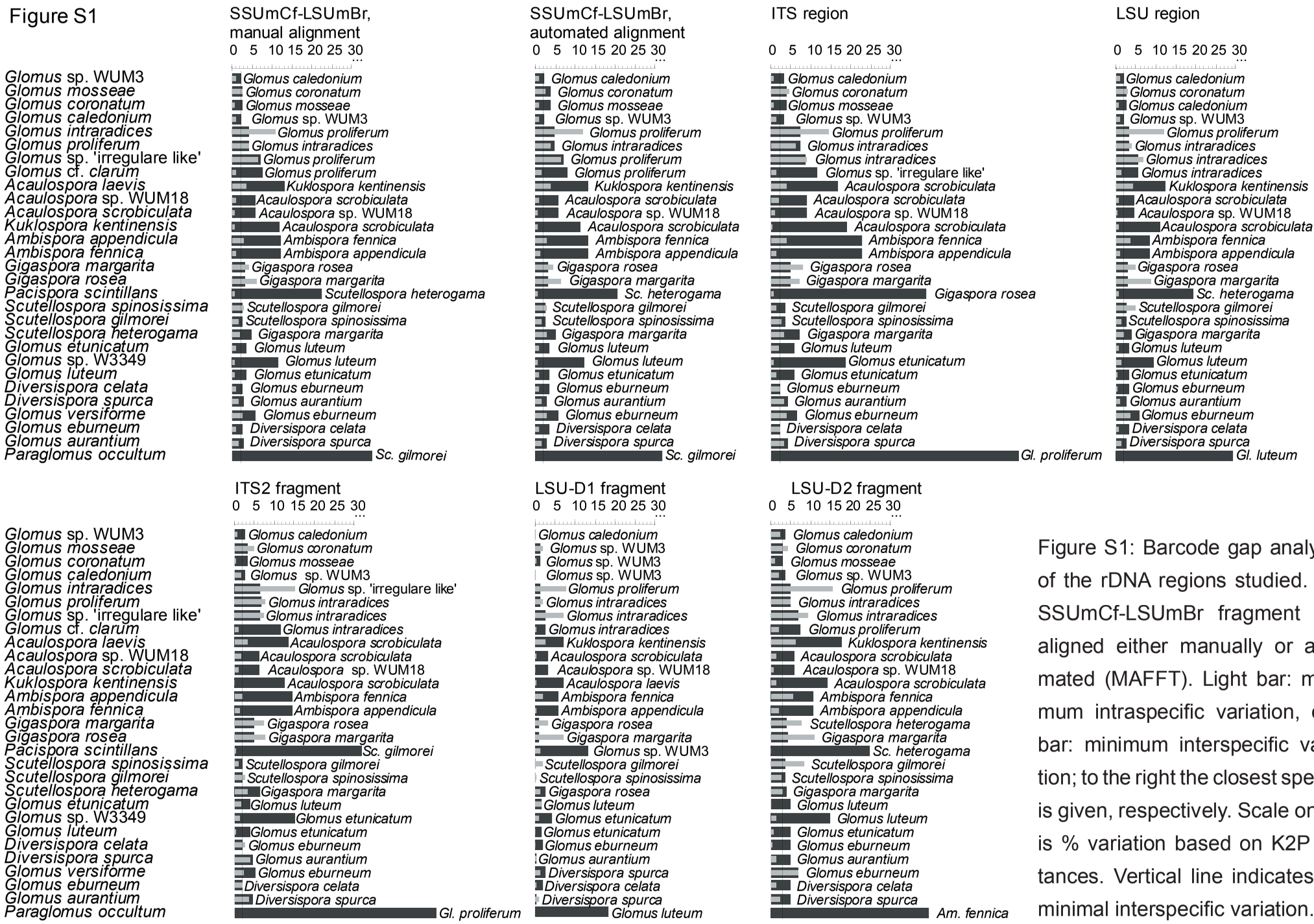


Figure S1: Barcode gap analyses of the rDNA regions studied. The SSUmCf-LSUmBr fragment was aligned either manually or automated (MAFFT). Light bar: maximum intraspecific variation, dark bar: minimum interspecific variation; to the right the closest species is given, respectively. Scale on top is % variation based on K2P distances. Vertical line indicates the minimal interspecific variation.

Figure S2: A: Barcode gap analyses of the *Ambisporaceae* including database sequences of the complete ITS region and the ITS2 fragment. For the environmental sequences, the distance to the closest related species (or clade) is higher than the maximal intraspecific distance of *Am. appendicula* (11 sequences), *Am. leptoticha* (26), and *Am. callosa* (34). The intraspecific variation in the ITS region of the *Ambispora* species ranged from 2.3 to 7.3 %. *Am. leptoticha* lacked a barcode gap to *Am. appendicula*. B: Barcode gap analyses of the *Diversisporaceae*, including database sequences. All species showed an intraspecific variation within the ITS of below 7 %, except *Gl. fulvum* (5 sequences). The high variation of approx. 15 % in *Gl. fulvum* is reduced to <10 % if sequence AM818544 is left out, which originated from a field collected specimen different from those giving rise to the other sequences. The analysis of the LSU region with additional database sequences showed the intraspecific variation: *Di. celata* 2.6 % (26 sequences), *Gl. versiforme* 4.1 % (29 sequences), *Gl. aurantium* 1.9 % (14 sequences). When including the 'outliers' (lower right graphs) that likley do not correspond to the respective species, for *Gl. versiforme* (without EU346868, isolate HDAM-4) the variation was 9.1 % and for *Gl. aurantium* (without EF581861,64) 8.1 %, whereby both species lost the barcode gap to their neighbors.

For explanation of the graphs, see Figure S1.

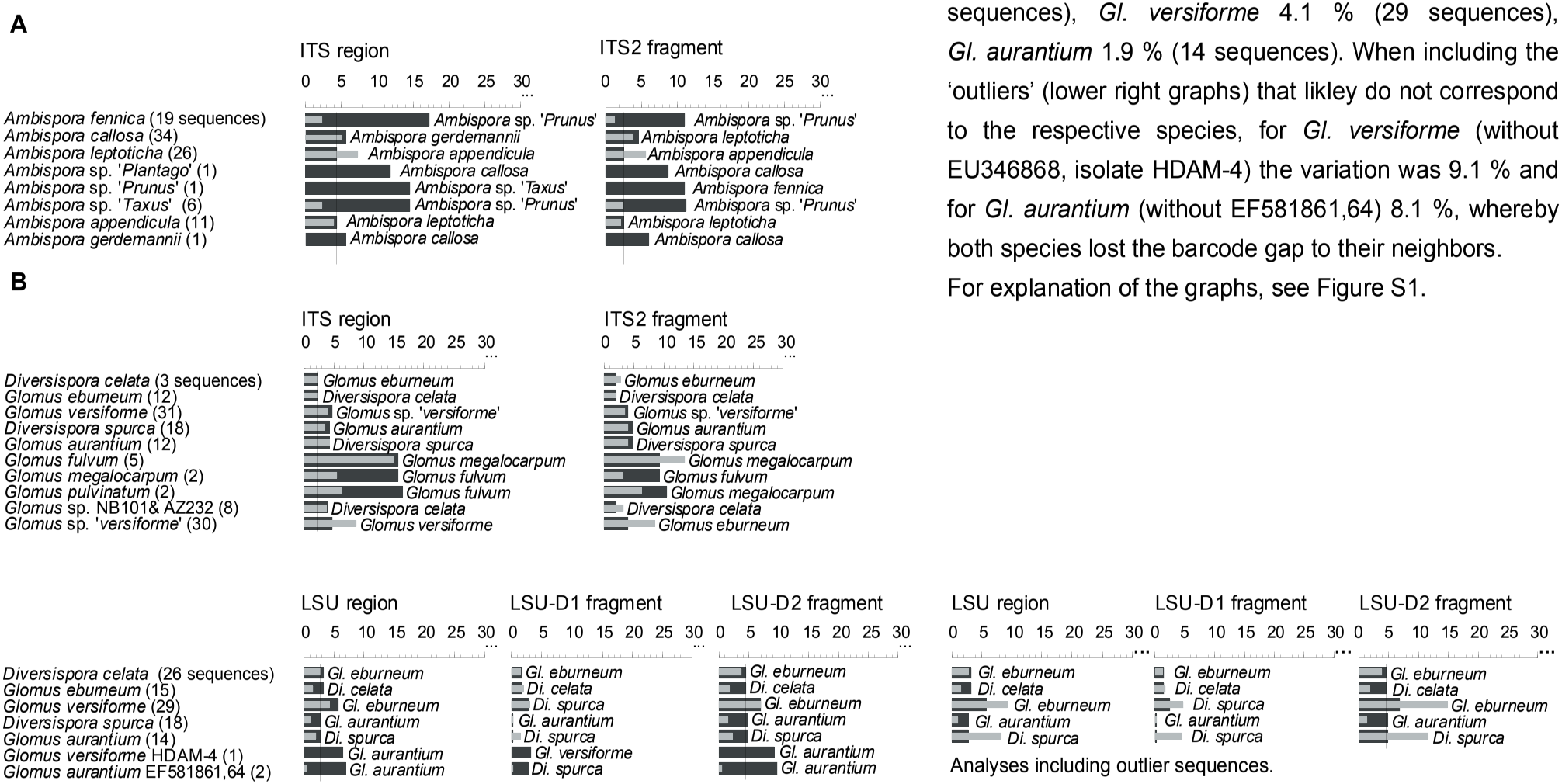


Figure S3: For legend see next page.

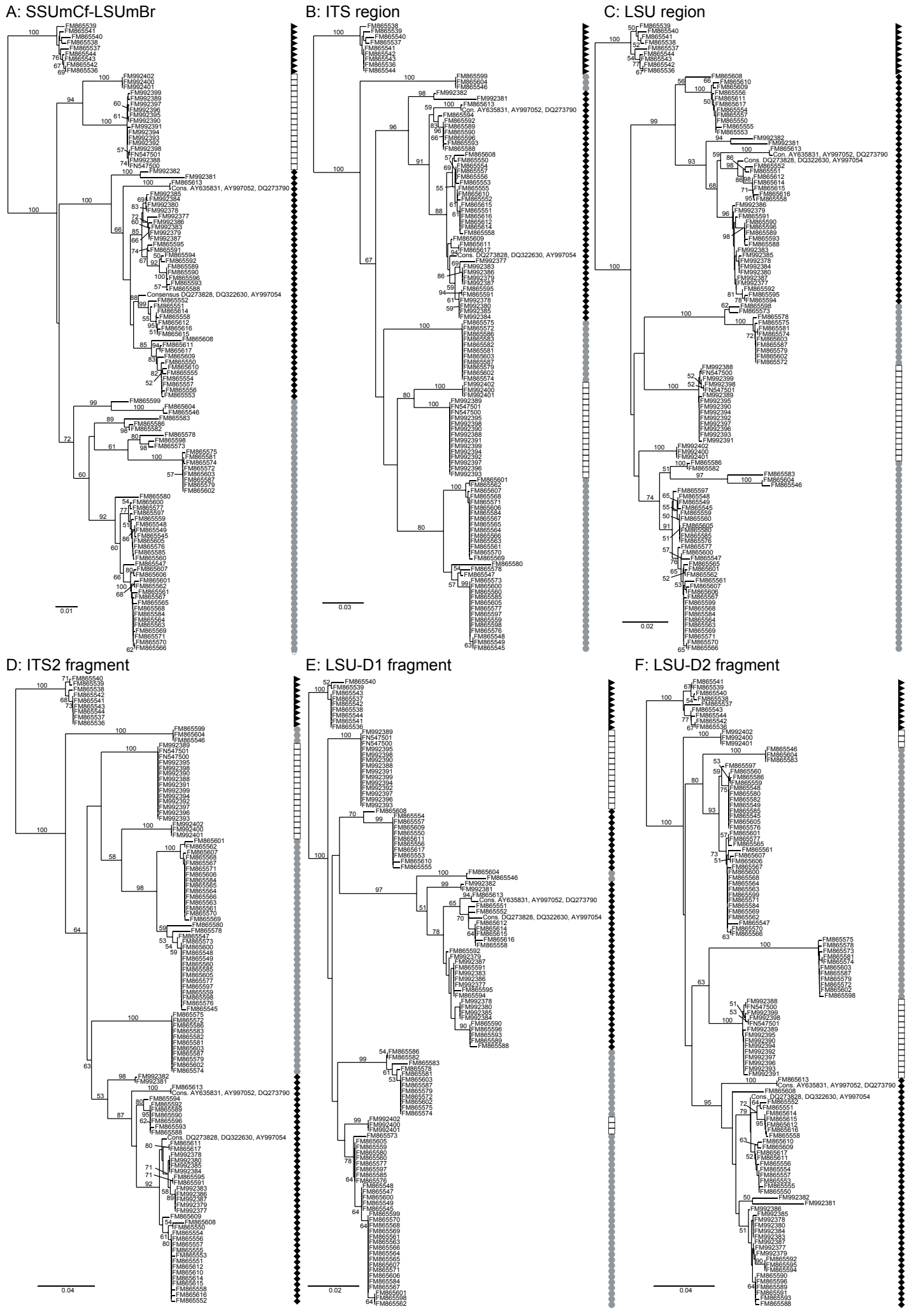


Figure S3: SSUmCf-LSUmBr (A), ITS region (B), LSU region (C), ITS2 fragment (D), LSU-D1 fragment (E), or LSU-D2 fragment (F) neighbour joining (NJ) analyses, 1000 bootstraps (BS), of *Glomus* Group Ab from the core dataset. *Glomus* cf. *clarum* (►), *Gl. intraradices* (●), *Gl. proliferum* (□), *Glomus* sp. 'irregulare-like' (◆).

Figure S4: SSUmCf-LSUmBr (A), ITS region (B), LSU region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) NJ analyses (1000 BS) of *Glomus* Group Aa from the core dataset. *Glomus mosseae* (■), *Glomus* sp. WUM3 (●), *Gl. coronatum* (▲), *Gl. caledonium* (►).

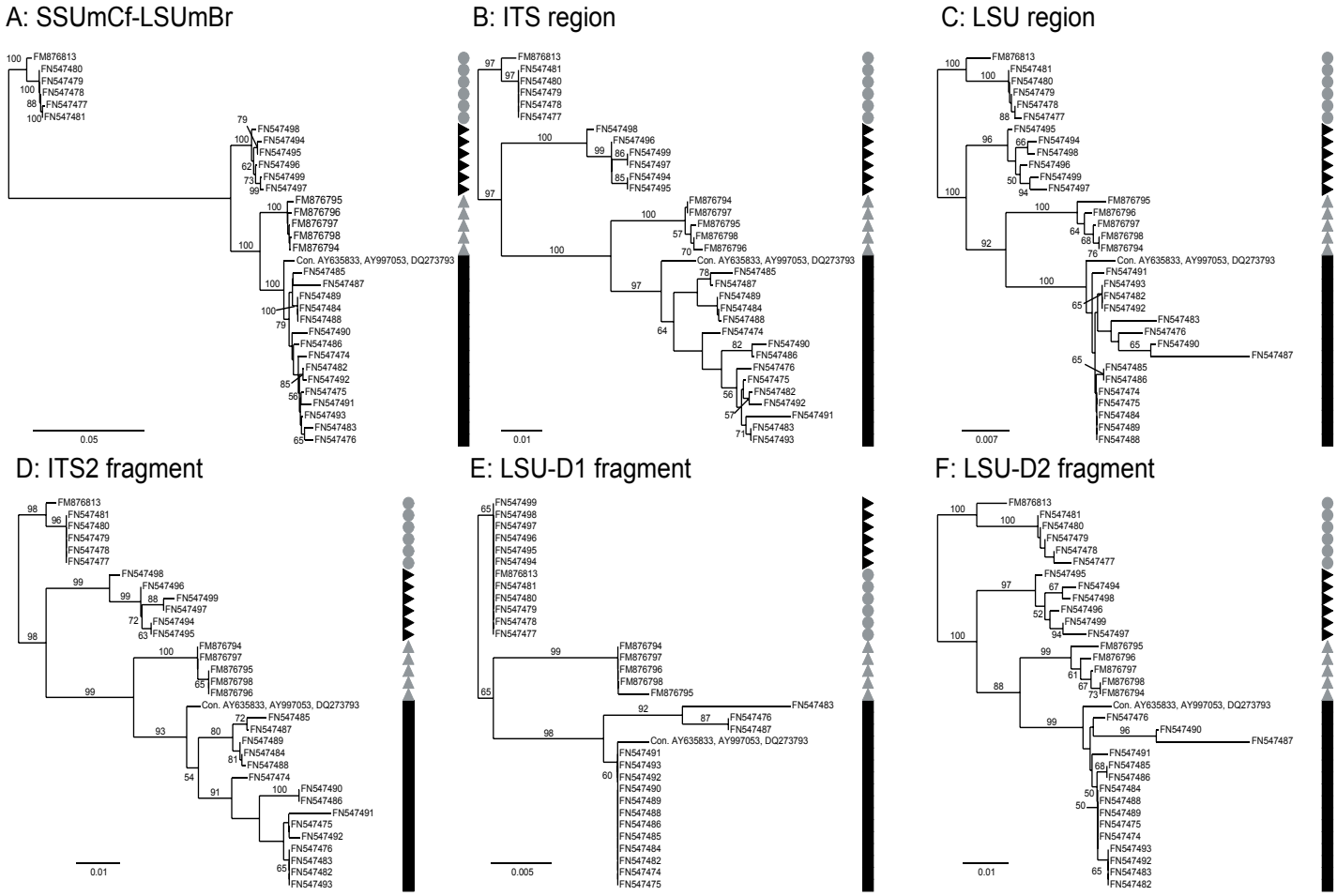
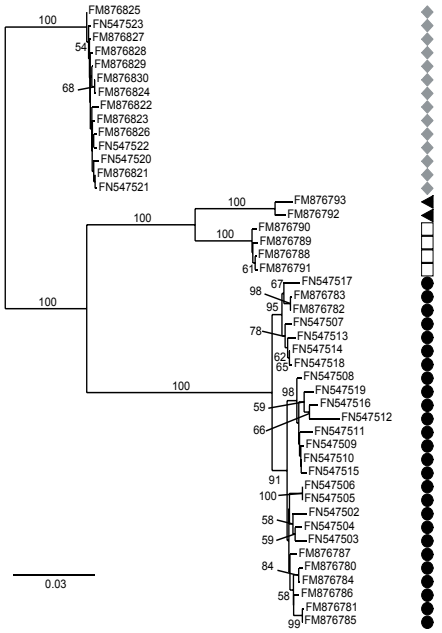
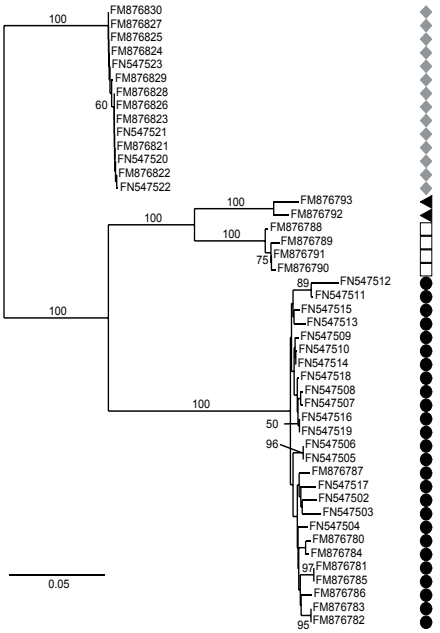


Figure S5: SSUmCf-LSUmBr (A), ITS region (B), LSU region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) NJ analyses (1000 BS) of *Acaulosporaceae* from the core dataset. *Kuklospora kentinensis* (◆), *Acaulospora* sp. WUM18 (◄), *Ac. scrobiculata* (□), *Ac. laevis* (●).

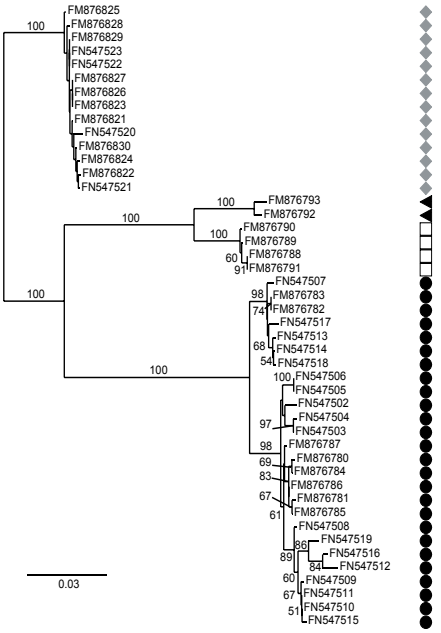
A: SSUmCf-LSUmBr



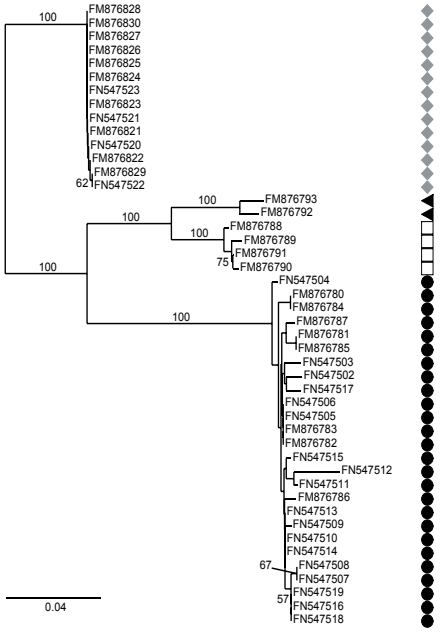
B: ITS region



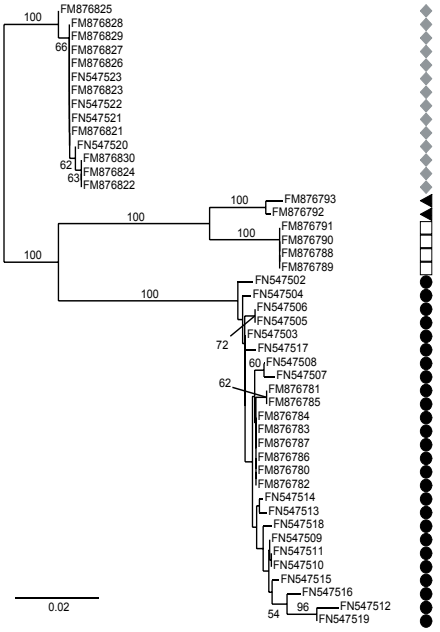
C: LSU region



D: ITS2 fragment



E: LSU-D1 fragment



F: LSU-D2 fragment

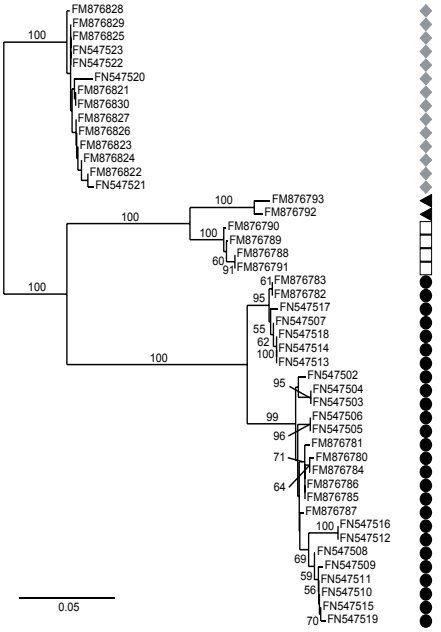


Figure S6: SSUmCf-LSUmBr (A), ITS region (B), LSU region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) NJ analyses (1000 BS) of *Glomus* Group B from the core dataset. *Glomus* sp. W3349 (◆), *Gl. luteum* (□), *Gl. etunicatum* (●).

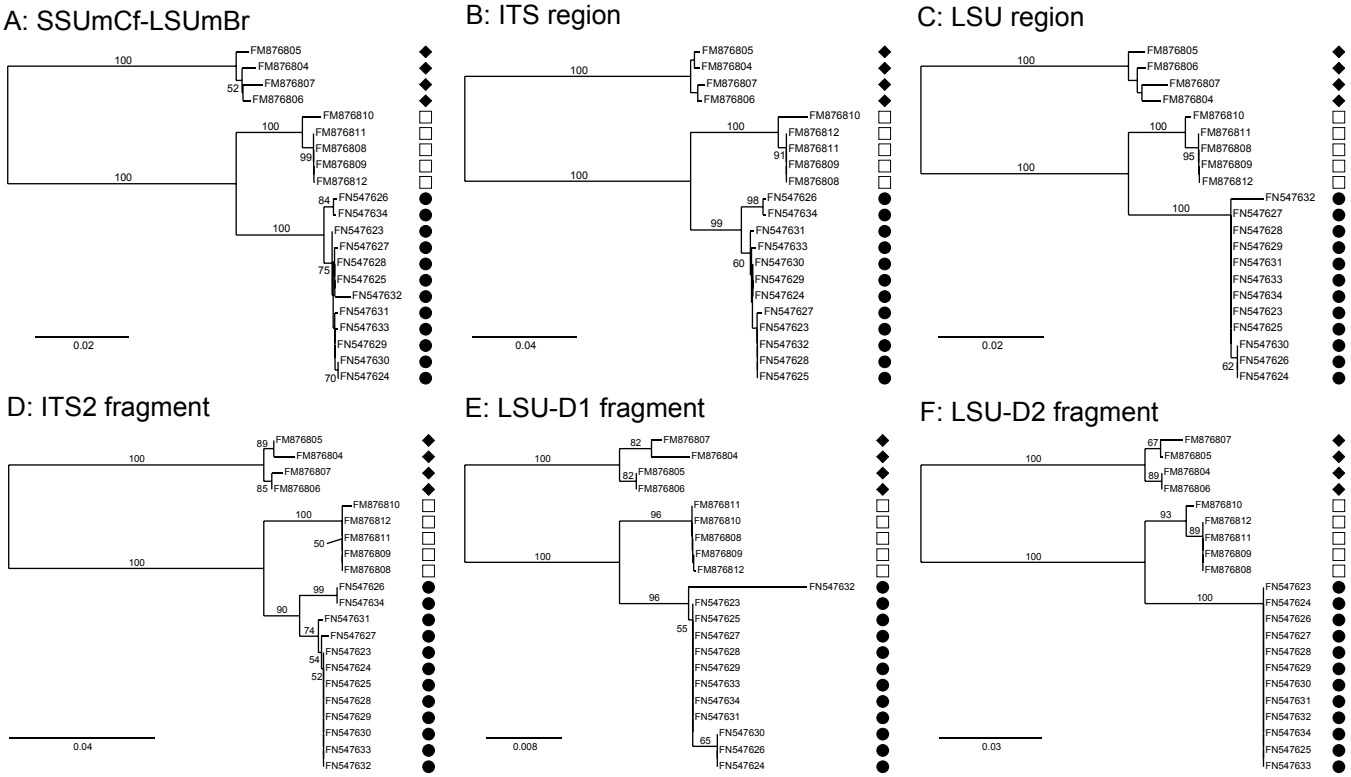
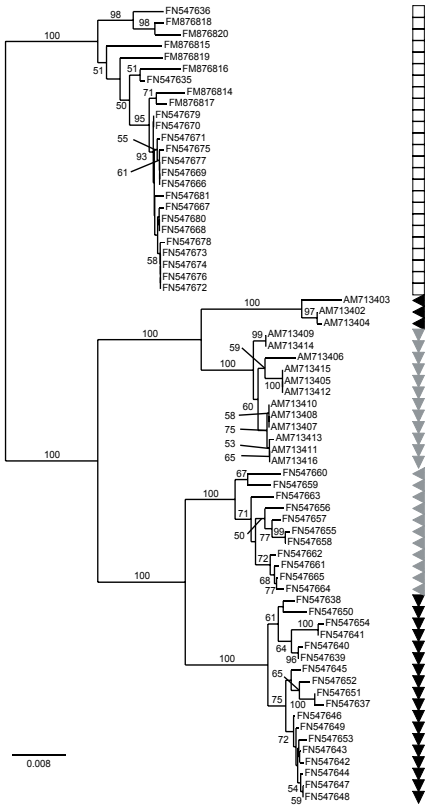
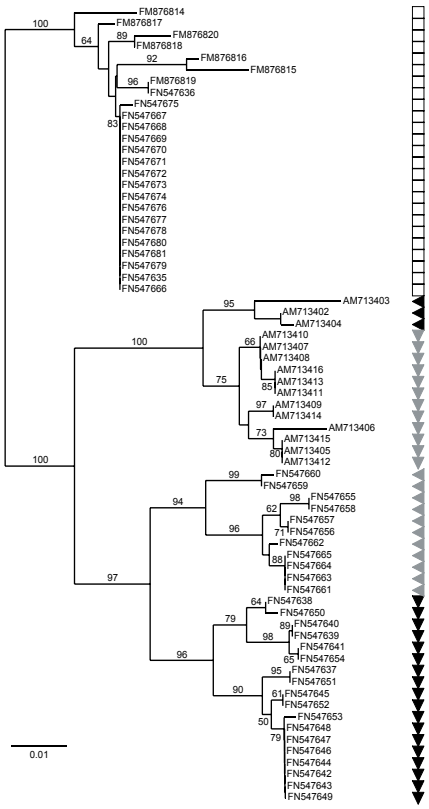


Figure S7: SSUmCf-LSUmBr (A), ITS region (B), LSU region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) NJ analyses (1000 BS) of *Diversisporaceae* from the core dataset. *Glomus eburneum* (▼), *Gl. aurantium* (◄), *Gl. versiforme* (□), *Diversispora celata* (◄), *Di. spurca* (▼).

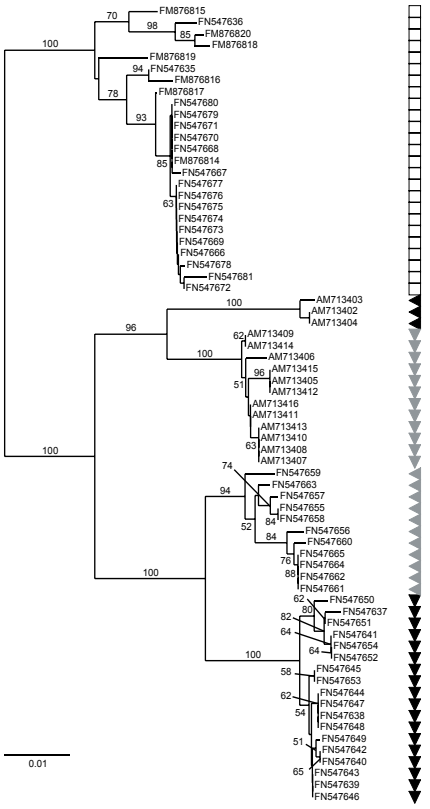
A: SSUmCf-LSUmBr



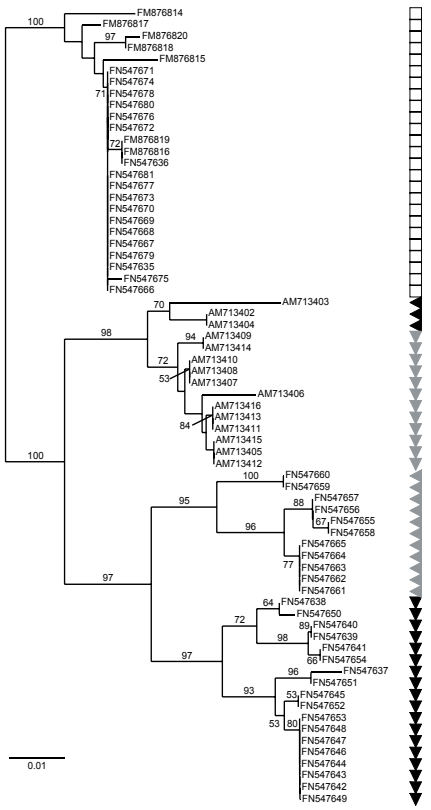
B: ITS region



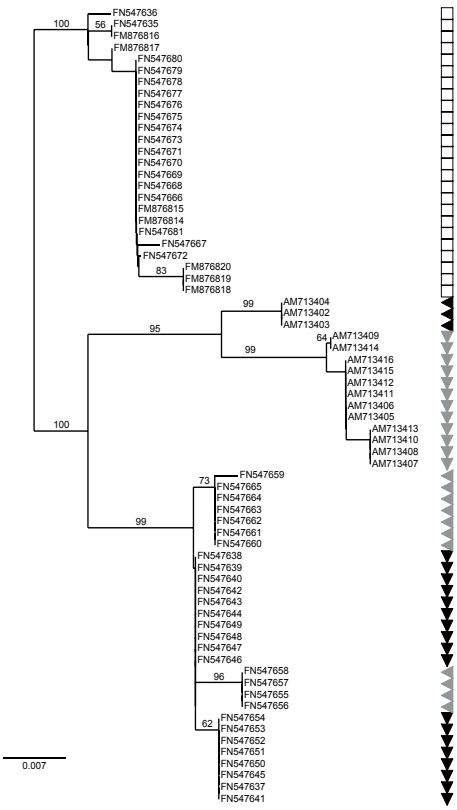
C: LSU region



D: ITS2 fragment



E: LSU-D1 fragment



F: LSU-D2 fragment

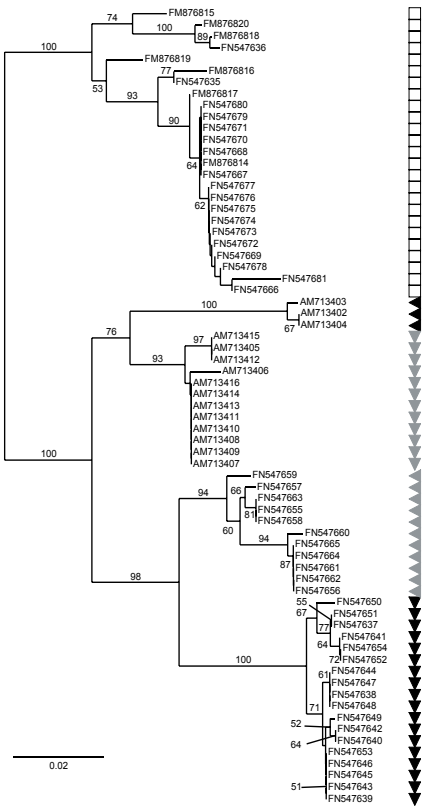
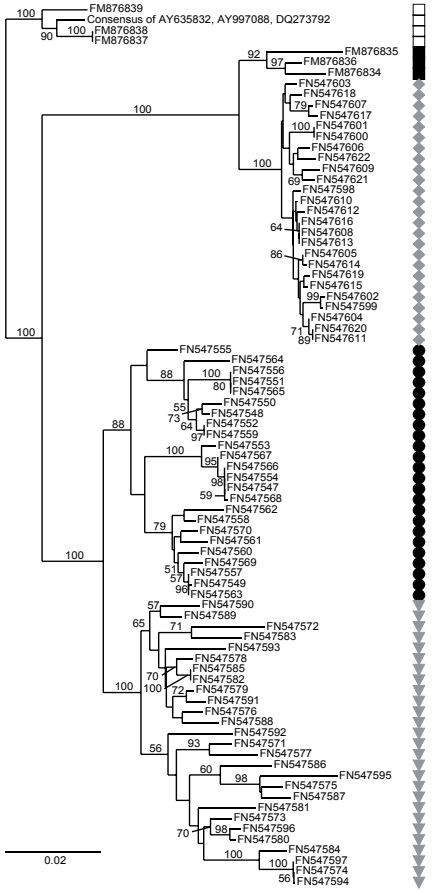


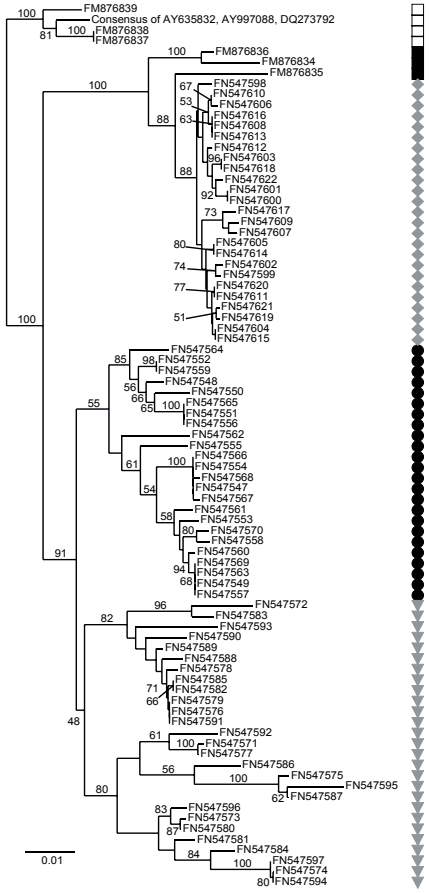
Figure S8: SSUmCf-LSUmBr (A), ITS region (B), LSU region (C), ITS2 fragment (D), LSU-D1 fragment (E), LSU-D2 fragment (F) NJ analyses (1000 BS) of *Gigasporaceae* from core dataset. *Scutellospora spinosissima* (■), *Sc. heterogama* (□), *Gigaspora rosea* (▼), *Sc. gilmorei* (◆), *Gi. margarita* (●).

A: SSUmCf-LSUmBr

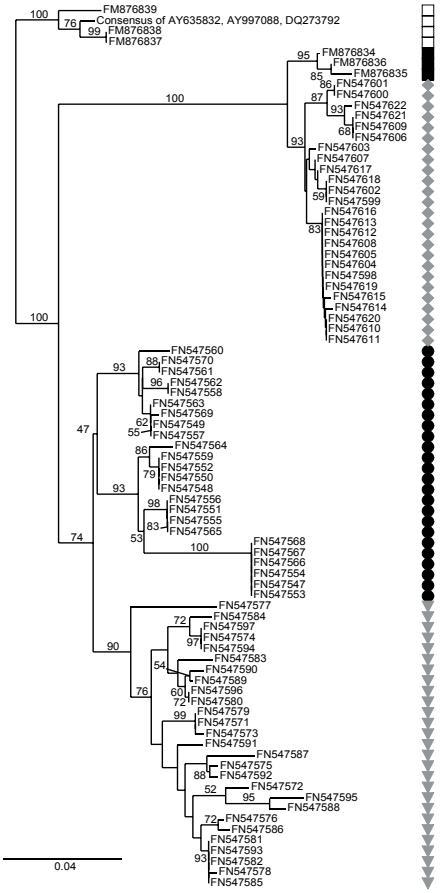


B: ITS region

C: LSU region



D: ITS2 fragment



E: LSU-D1 fragment

F: LSU-D2 fragment

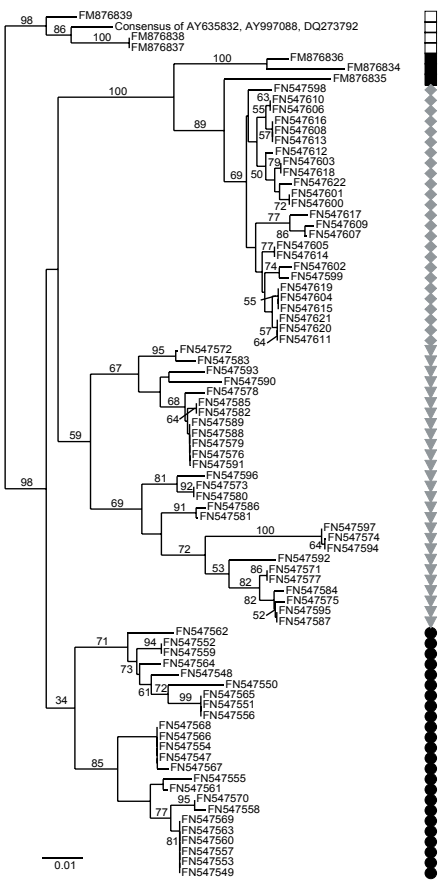


Figure S9: ITS region (A) and ITS2 fragment (B) NJ analyses (1000 BS) of the *Ambisporaceae*. *Ambispora gerdemannii* (▼), *Am. leptoticha* (⊠), *Am. callosa* (◄), *Am. fennica* (▲), *Am. appendicula* (◆), *Ambispora* sp. from *Plantago* (■), from *Prunus* (□), from *Taxus* (►).

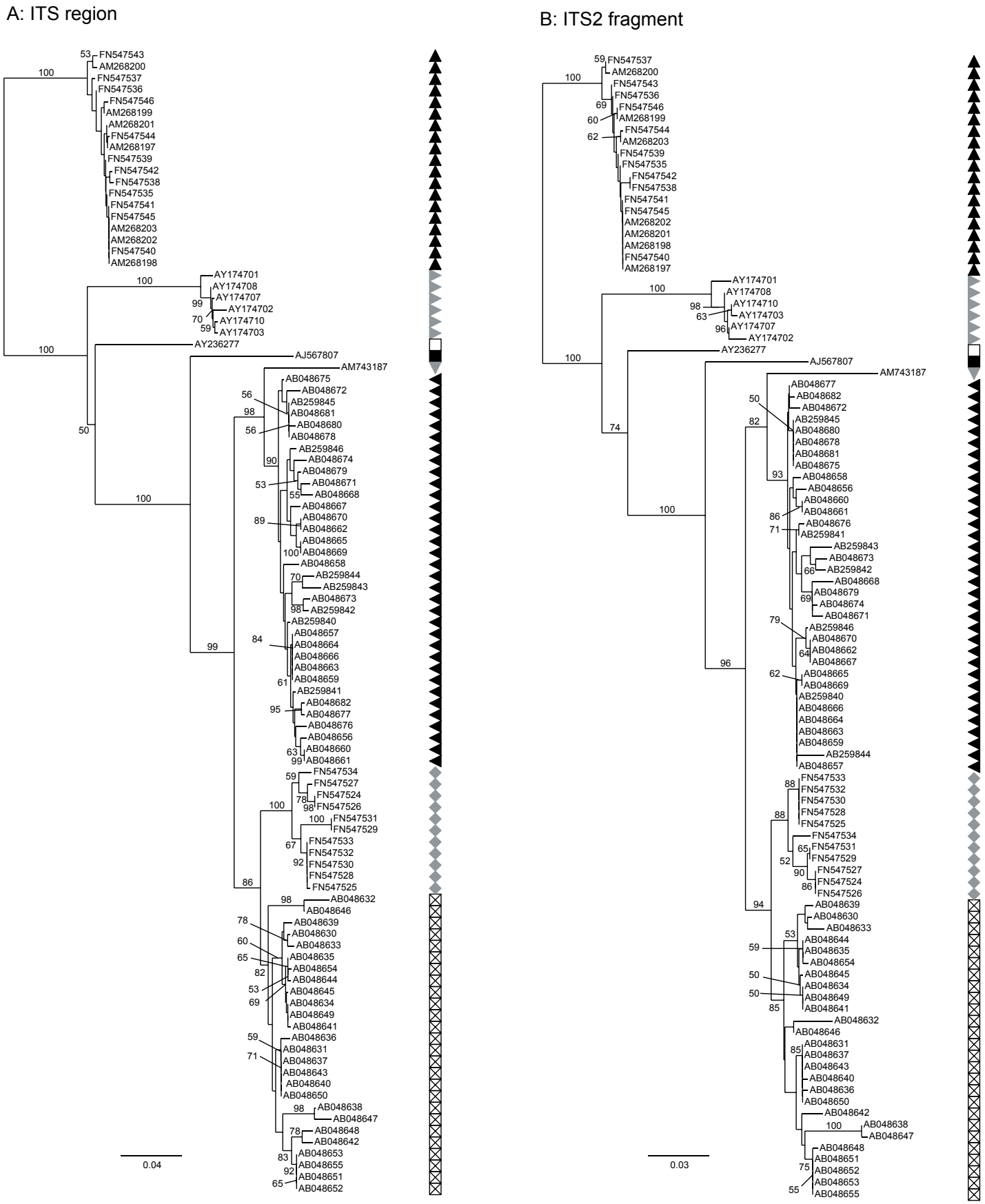


Figure S10: ITS region (A) and ITS2 fragment (B) NJ analyses (1000 BS) of the *Diversisporaceae*. *Glomus eburneum* (▼), *Gl. aurantium* (◄), *Gl. versiforme* (□), *Diversispora celata* (◄), *Di. spurca* (▼), *Gl. megalocarpum* (●), *Gl. fulvum* (●), *Gl. pulvinatum* (►), *Gl.* sp. NB101 (▲), *Gl.* sp. AZ37B (▲), *Gl.* sp. '*versiforme*' environmental (⊠).

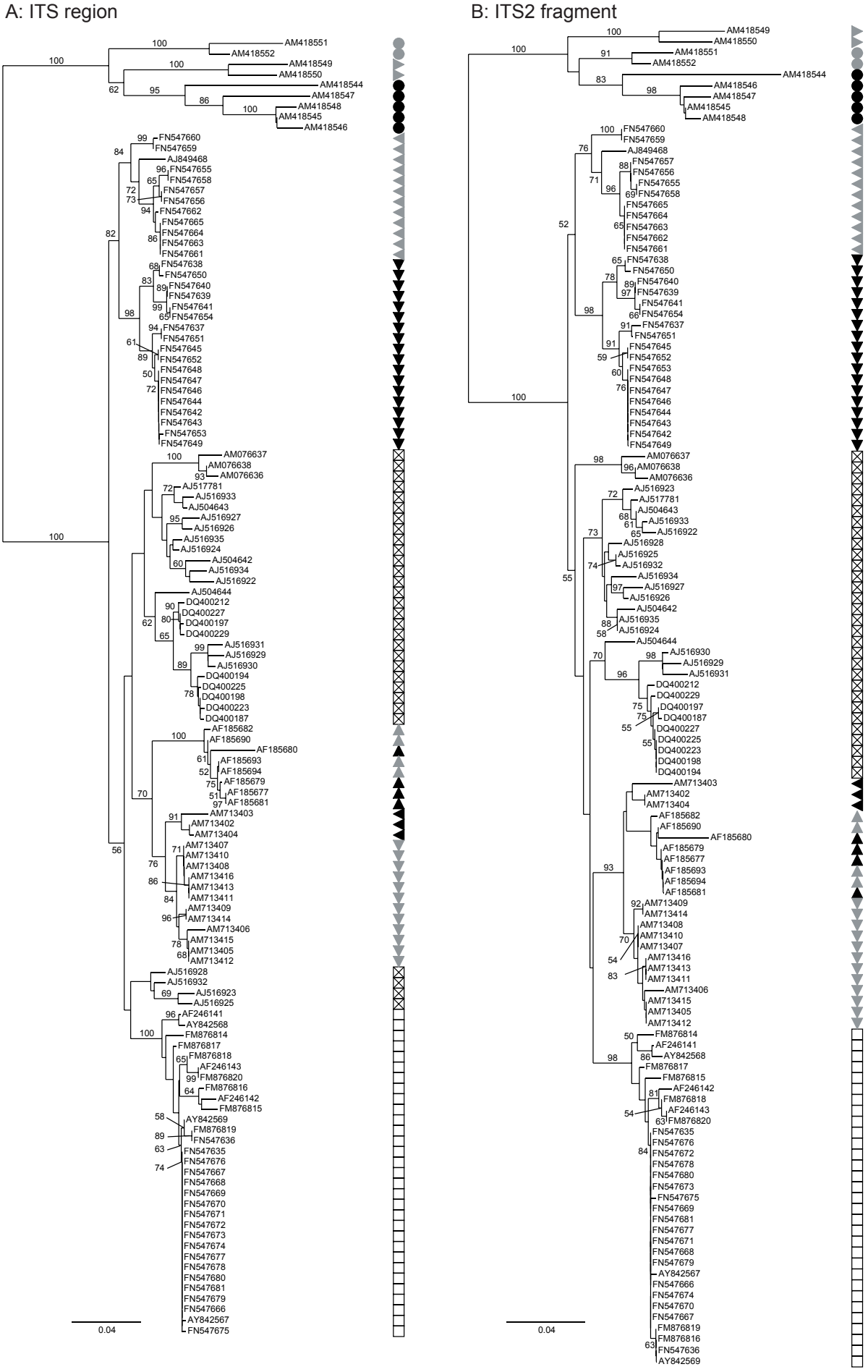
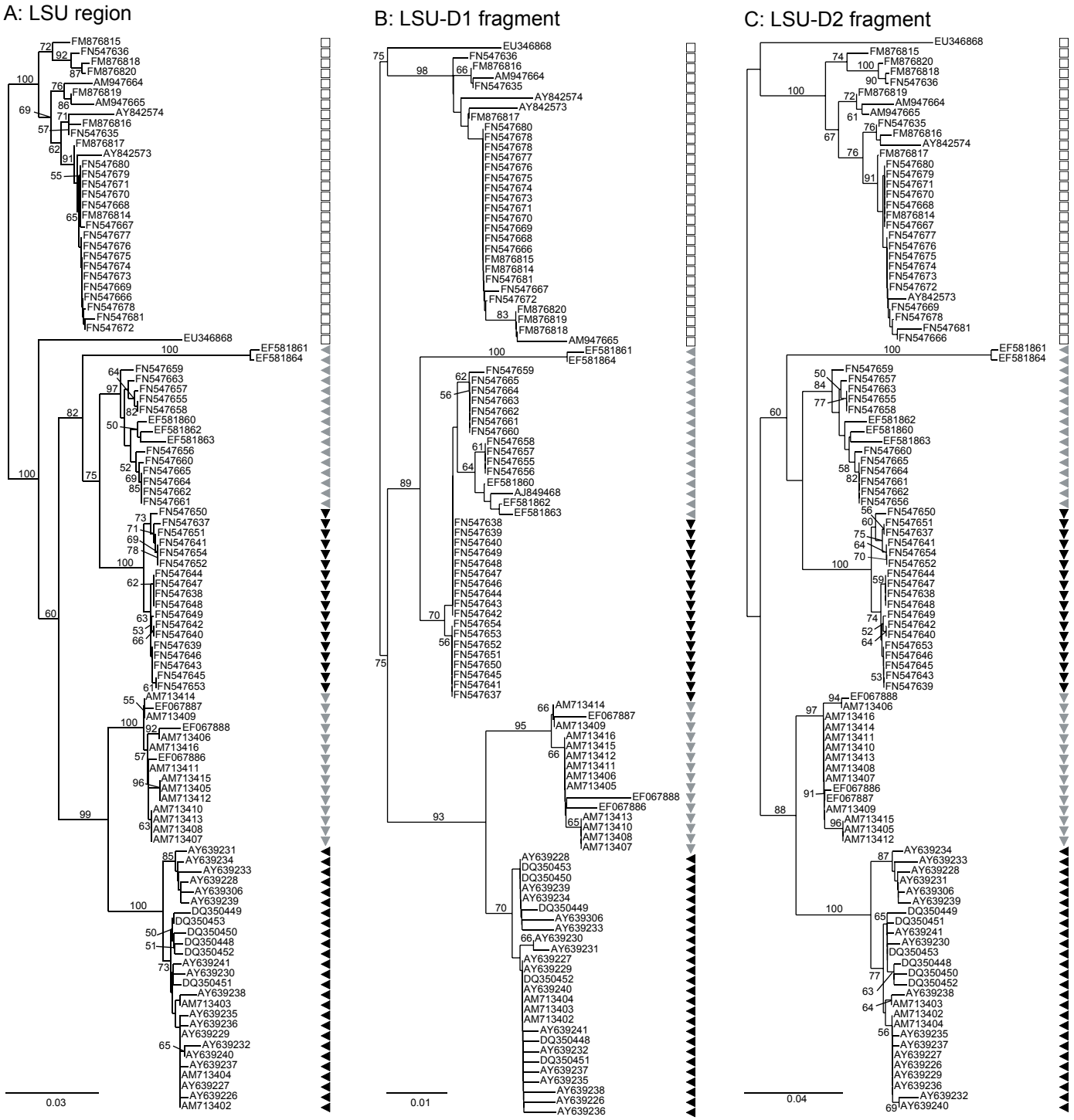


Figure S11: LSU region (A), LSU-D1 fragment (B) and LSU-D2 fragment (C) NJ analyses (1000 BS) of the *Diversisporaceae*. *Glomus eburneum* (▼), *Gl. aurantium* (◄), *Gl. versiforme* (□), *Di. celata* (◄), *Di. spurca* (▼).



Supplementary Tables S1 – S7

Table S1: Sequences used to assemble the core dataset. Number of spores used for DNA extraction is shown, if known (ss, single spore; ms, multi-spore), as well as cloning numbers (in parentheses, following the number of sequences) and the primers used for the sequences published here (in parentheses, following the accession numbers; [n], amplified by nested PCR).

Identifier, culture (Att)/voucher (W) used	Species name	No. of sequences	DNA extraction	Acc Nos.
BEG12, Att109-20/W5147	*Glomus mosseae*	7 (pHS110), 8 (pHS101)	1 x ss	FN547474-6,82-93 (SSUmCf-LSUmBr [n])
WUM3, Att15-5/W2939	*Glomus* sp. WUM3	5 (pMK23)	ss	FN547477-81 (SSUGlom1-NDL22 [n])
MUCL41827, -/-	*Glomus proliferum*	2 (pHS113)	ss	FN547500-1(SSUmCf-LSUmBr [n])
BEG13, -/W5258	*Acaulospora laevis*	7 (pHS054)	ss	FN547507-12, 16 (SSUmAf-LR4+2)
none, Att423-4/W3077	*Acaulospora* cf. *laevis*	6 (pHS032)	ss	FN547502-6,17 (SSUmAf-LR4+2)
BEG26, -/-	*Acaulospora* cf. *laevis*	5 (pHS030)	ss	FN547513-5,18,19 (SSUGlom1-NDL22[n])
INVAM TW111, Att1499-9/W5346	*Kuklospora kentinensis*	4 (pHS098)	ss	FN547520-3 (SSUmCf-LSUmBr [n])
none, Att1235-2/W5156	*Ambispora appendicula*	11 (pMK096)	ms (3 spores)	FN547524-34 (SSUmAf-LSUmAr)
none, Att200-23/W4752	*Ambispora fennica*	12 (pMK094)	ss	FN547535-46 (SSUmCf-LSUmBr [n])
BEG34, -/-	*Gigaspora margarita*	24 (pHS108)	ss	FN547547-70 (SSUmAf-LSUmAr)
DAOM194757, Att1509-20/W5384	*Gigaspora rosea*	6 (pHS106), 18 (pHS105), 3 (pHS104)	1 x ss	FN547571-97 (SSUmCf-LSUmBr [n])
FCPC1145, Att590-16/W5342	*Scutellospora gilmorei*	21 (pHS107), 5 (pHS103)	1 x ss	FN547598-622 (SSUmCf-LSUmBr [n])
none, Att1505-8/W5347	*Glomus etunicatum*	12 (pHS112)	ss	FN547623-34 (SSUGlom1-NDL22 [n])
BEG20, Att263-15/W3294	*Glomus caledonium*	6 (pHS031)	ss	FN547494-9 (SSUGlom1-NDL22 [n])
BEG47, Att475-45/W5165	*Glomus versiforme*	2 (pHS034)	ss	FN547635-6 (SSUGlom1-NDL22 [n])
BEG47, Att475-22/W3180	*Glomus versiforme*	10 (pMK73), 6 (pMK72)	2 x ss	FN547666-81 (SSUmAf-LR4+2)
none, Att1296-0/W4728	*Glomus aurantium*	11 (pHS109)	ss	FN547655-65 (SSUmCf-LSUmBr [n])
none, Att246-18/W4119	*Diversispora spurca*	18 (pHS100)	ss	FN547637-54 (SSUmCf-LSUmBr [n])
WUM18, Att869-3/-	*Acaulospora* sp. WUM18	2 [1]	ss	FM876792-3
BEG33, Att209-37/-	*Acaulospora scrobiculata*	4 [1]	ss	FM876788-91
BEG231, FACE#234	*Diversispora celata*	3 [2]	ms	AM713402-4
INVAM AZ420A, Att1290-5/W4729	*Glomus eburneum*	12 [2]	ms	AM713405-16
BEG28, Att108-7/-	*Glomus coronatum*	5 [1]	ss	FM876794-8
WUM3, Att15-5/W2940	*Glomus* sp. WUM3	1 [1]	ss	FM876813

INVAM SA101, Att676-5/-	*Glomus luteum*	5 [1]	ss	FM876808-12
none, Att565-11/W3349	*Glomus* sp. W3349	4 [1]	ss	FM876804-7
WUM11, Att862-7/W2928	*Acaulospora laevis*	8 [1]	ss	FM876780-7
none, Att894-7/-	*Glomus* cf. *clarum*	9 [3]	ss	FM865536-44
DAOM197198 related, -/W5533, W5495, W3182, W5499; BEG195, -/W5272	*Glomus* sp. 'irregulare-like'	39 [3]	4 x ss, 1 x ms (3 spores)	FM865550-8, FM865588-96, FM865608-17, FM992377-87
INVAM FL208, -/W5413, W5166, W5507; MUCL49410, -/W5070	*Glomus intraradices*	45 [3]	4 x ss	FM865545-49, FM865559-87, FM865597-607
none, -/W4545	*Pacispora scintillans*	2 [1]	ss	FM876831-2
INVAM TW111, Att1499-9/W5346	*Kuklospora kentinensis*	10 [1]	ss	FM876821-30
MUCL41827, -/-	*Glomus proliferum*	15 [3]	1 x ss, 1 x ms	FM992388-402
none, -/W3009	*Scutellospora spinosissima*	3 [1]	ss	FM876834-6
BEG35, Att334-16/-	*Scutellospora heterogama*	3 [1]	ss	FM876837-9
BEG47, Att475-45/W5165	*Glomus versiforme*	7 [1]	ss	FM876814-20
AFTOL-139, INVAM UT101/ BL022	*Glomus mosseae*	1 [4]	unknown	Consensus AY635833 + AY997053 + DQ273793
AFTOL-845, 4695rac-11G2/ BL095	*Glomus* sp. 'irregulare-like'	1 [4]	unknown	Consensus DQ273828 + DQ322630 + AY997054
AFTOL-48, MUCL 43194/DAOM181602	*Glomus* sp. 'irregulare-like'	1 [4]	ms	Consensus AY635831 + AY997052 + DQ273790
AFTOL-138, INVAM FL225/ BL021	*Scutellospora heterogama*	1 [4]	unknown	Consensus AY635832 + AY997088 + DQ273792
AFTOL-844, INVAM IA702/ BL093	*Paraglomus occultum*	1 [4]	unknown	Consensus DQ322629 + DQ273827 + AY997069

[1] Krüger *et al.* 2009, [2] Gamper *et al.* 2009, [3] Stockinger *et al.* 2009, [4] James *et al.* 2006

Table S2: Sequences used for analysis of the *Ambisporaceae* ITS region (see Figure S9).

Accession	Species	Culture/voucher
FN547524	*Ambispora appendicula*	Att1235-2/W5156
FN547525	*Ambispora appendicula*	Att1235-2/W5156
FN547526	*Ambispora appendicula*	Att1235-2/W5156
FN547527	*Ambispora appendicula*	Att1235-2/W5156
FN547528	*Ambispora appendicula*	Att1235-2/W5156
FN547529	*Ambispora appendicula*	Att1235-2/W5156
FN547530	*Ambispora appendicula*	Att1235-2/W5156
FN547531	*Ambispora appendicula*	Att1235-2/W5156
FN547532	*Ambispora appendicula*	Att1235-2/W5156
FN547533	*Ambispora appendicula*	Att1235-2/W5156
FN547534	*Ambispora appendicula*	Att1235-2/W5156
AB048656	*Ambispora callosa*	MAFF520057/W4769
AB048657	*Ambispora callosa*	MAFF520057/W4769
AB048658	*Ambispora callosa*	MAFF520057/W4769
AB048659	*Ambispora callosa*	MAFF520057/W4769
AB048660	*Ambispora callosa*	MAFF520057/W4769
AB048661	*Ambispora callosa*	MAFF520057/W4769
AB048662	*Ambispora callosa*	MAFF520057/W4769
AB048663	*Ambispora callosa*	MAFF520057/W4769
AB048664	*Ambispora callosa*	MAFF520057/W4769
AB048665	*Ambispora callosa*	MAFF520057/W4769
AB048666	*Ambispora callosa*	MAFF520057/W4769
AB048667	*Ambispora callosa*	MAFF520057/W4769
AB048668	*Ambispora callosa*	MAFF520057/W4769
AB048669	*Ambispora callosa*	MAFF520057/W4769
AB048670	*Ambispora callosa*	MAFF520057/W4769
AB048671	*Ambispora callosa*	MAFF520058/W4771
AB048672	*Ambispora callosa*	MAFF520058/W4771
AB048673	*Ambispora callosa*	MAFF520058/W4771
AB048674	*Ambispora callosa*	MAFF520058/W4771
AB048675	*Ambispora callosa*	MAFF520058/W4771
AB048676	*Ambispora callosa*	MAFF520058/W4771
AB048677	*Ambispora callosa*	MAFF520058/W4771
AB048678	*Ambispora callosa*	MAFF520058/W4771
AB048679	*Ambispora callosa*	MAFF520058/W4771
AB048680	*Ambispora callosa*	MAFF520058/W4771
AB048681	*Ambispora callosa*	MAFF520058/W4771
AB048682	*Ambispora callosa*	MAFF520058/W4771
AB259840	*Ambispora callosa*	OK-m1/W4768
AB259841	*Ambispora callosa*	OK-m1/W4768
AB259842	*Ambispora callosa*	OK-m1/W4768
AB259843	*Ambispora callosa*	OK-m1/W4768
AB259844	*Ambispora callosa*	MAFF520073/W4752
AB259845	*Ambispora callosa*	MAFF520073/W4752
AB259846	*Ambispora callosa*	MAFF520073/W4752
AM268197	*Ambispora fennica*	Att200-11/W3569
AM268198	*Ambispora fennica*	Att200-23/W4752
AM268199	*Ambispora fennica*	Att200-11/W3569

AM268200	*Ambispora fennica*	Att200-11/W3569
AM268201	*Ambispora fennica*	Att200-11/W3569
AM268202	*Ambispora fennica*	Att200-11/W3569
AM268203	*Ambispora fennica*	Att200-23/W4752
FN547535	*Ambispora fennica*	Att200-23/W4752
FN547536	*Ambispora fennica*	Att200-23/W4752
FN547537	*Ambispora fennica*	Att200-23/W4752
FN547538	*Ambispora fennica*	Att200-23/W4752
FN547539	*Ambispora fennica*	Att200-23/W4752
FN547540	*Ambispora fennica*	Att200-23/W4752
FN547541	*Ambispora fennica*	Att200-23/W4752
FN547542	*Ambispora fennica*	Att200-23/W4752
FN547543	*Ambispora fennica*	Att200-23/W4752
FN547544	*Ambispora fennica*	Att200-23/W4752
FN547545	*Ambispora fennica*	Att200-23/W4752
FN547546	*Ambispora fennica*	Att200-23/W4752
AM743187	*Ambispora gerdemannii*	INVAM AU215
AB048630	*Ambispora leptoticha*	MAFF520055/W4770
AB048631	*Ambispora leptoticha*	MAFF520055/W4770
AB048632	*Ambispora leptoticha*	MAFF520055/W4770
AB048633	*Ambispora leptoticha*	MAFF520055/W4770
AB048634	*Ambispora leptoticha*	MAFF520055/W4770
AB048635	*Ambispora leptoticha*	MAFF520055/W4770
AB048636	*Ambispora leptoticha*	MAFF520055/W4770
AB048637	*Ambispora leptoticha*	MAFF520055/W4770
AB048638	*Ambispora leptoticha*	MAFF520055/W4770
AB048639	*Ambispora leptoticha*	MAFF520055/W4770
AB048640	*Ambispora leptoticha*	MAFF520055/W4770
AB048641	*Ambispora leptoticha*	MAFF520055/W4770
AB048642	*Ambispora leptoticha*	MAFF520055/W4770
AB048643	*Ambispora leptoticha*	MAFF520055/W4770
AB048644	*Ambispora leptoticha*	MAFF520055/W4770
AB048645	*Ambispora leptoticha*	MAFF520055/W4770
AB048646	*Ambispora leptoticha*	MAFF520055/W4770
AB048647	*Ambispora leptoticha*	MAFF520055/W4770
AB048648	*Ambispora leptoticha*	MAFF520055/W4770
AB048649	*Ambispora leptoticha*	MAFF520055/W4770
AB048650	*Ambispora leptoticha*	MAFF520055/W4770
AB048651	*Ambispora leptoticha*	MAFF520055/W4770
AB048652	*Ambispora leptoticha*	MAFF520055/W4770
AB048653	*Ambispora leptoticha*	MAFF520055/W4770
AB048654	*Ambispora leptoticha*	MAFF520055/W4770
AB048655	*Ambispora leptoticha*	MAFF520055/W4770
AJ567807	*Am*. sp. from *Plantago lanceolata*	environmental
AY236277	*Am*. sp. from *Prunus africana*	environmental
AY174701	*Am.* sp. from *Taxus baccata*	environmental
AY174702	*Am.* sp. from *Taxus baccata*	environmental
AY174703	*Am.* sp. from *Taxus baccata*	environmental
AY174707	*Am.* sp. from *Taxus baccata*	environmental
AY174708	*Am.* sp. from *Taxus baccata*	environmental
AY174710	*Am.* sp. from *Taxus baccata*	environmental

Table S3: Sequences used for analyses of the *Diversisporaceae* ITS region (see Figure S10).

Accession	Species	Culture/voucher
AM713402	*Diversispora celata*	FACE234; BEG231
AM713403	*Diversispora celata*	FACE234; BEG231
AM713404	*Diversispora celata*	FACE234; BEG231
FN547637	*Diversispora spurca*	Att246-18/W4119
FN547638	*Diversispora spurca*	Att246-18/W4119
FN547639	*Diversispora spurca*	Att246-18/W4119
FN547640	*Diversispora spurca*	Att246-18/W4119
FN547641	*Diversispora spurca*	Att246-18/W4119
FN547642	*Diversispora spurca*	Att246-18/W4119
FN547643	*Diversispora spurca*	Att246-18/W4119
FN547644	*Diversispora spurca*	Att246-18/W4119
FN547645	*Diversispora spurca*	Att246-18/W4119
FN547646	*Diversispora spurca*	Att246-18/W4119
FN547647	*Diversispora spurca*	Att246-18/W4119
FN547648	*Diversispora spurca*	Att246-18/W4119
FN547649	*Diversispora spurca*	Att246-18/W4119
FN547650	*Diversispora spurca*	Att246-18/W4119
FN547651	*Diversispora spurca*	Att246-18/W4119
FN547652	*Diversispora spurca*	Att246-18/W4119
FN547653	*Diversispora spurca*	Att246-18/W4119
FN547654	*Diversispora spurca*	Att246-18/W4119
AM418549	*G. pulvinatum*	environmental
AM418550	*G. pulvinatum*	environmental
AJ849468	*Glomus aurantium*	Holotype. Błaszkowski J., 2444 (DPP)
FN547655	*Glomus aurantium*	Att1296-0/W4728
FN547656	*Glomus aurantium*	Att1296-0/W4728
FN547657	*Glomus aurantium*	Att1296-0/W4728
FN547658	*Glomus aurantium*	Att1296-0/W4728
FN547659	*Glomus aurantium*	Att1296-0/W4728
FN547660	*Glomus aurantium*	Att1296-0/W4728
FN547661	*Glomus aurantium*	Att1296-0/W4728
FN547662	*Glomus aurantium*	Att1296-0/W4728
FN547663	*Glomus aurantium*	Att1296-0/W4728
FN547664	*Glomus aurantium*	Att1296-0/W4728
FN547665	*Glomus aurantium*	Att1296-0/W4728
AM713405	*Glomus eburneum*	AZ420A/W4729
AM713406	*Glomus eburneum*	AZ420A/W4729
AM713407	*Glomus eburneum*	AZ420A/W4729
AM713408	*Glomus eburneum*	AZ420A/W4729
AM713409	*Glomus eburneum*	AZ420A/W4729
AM713410	*Glomus eburneum*	AZ420A/W4729
AM713411	*Glomus eburneum*	AZ420A/W4729
AM713412	*Glomus eburneum*	AZ420A/W4729
AM713413	*Glomus eburneum*	AZ420A/W4729
AM713414	*Glomus eburneum*	AZ420A/W4729
AM713415	*Glomus eburneum*	AZ420A/W4729
AM713416	*Glomus eburneum*	AZ420A/W4729
AM418544	*Glomus fulvum*	environmental

AM418545	*Glomus fulvum*	environmental
AM418546	*Glomus fulvum*	environmental
AM418547	*Glomus fulvum*	environmental
AM418548	*Glomus fulvum*	environmental
AM418551	*Glomus megalocarpum*	environmental
AM418552	*Glomus megalocarpum*	environmental
AF185677	*Glomus* sp.	INVAM AZ237B
AF185679	*Glomus* sp.	INVAM AZ237B
AF185680	*Glomus* sp.	INVAM AZ237B
AF185681	*Glomus* sp.	INVAM AZ237B
AF185682	*Glomus* sp.	INVAM NB101
AF185690	*Glomus* sp.	INVAM NB101
AF185693	*Glomus* sp.	INVAM NB101
AF185694	*Glomus* sp.	INVAM NB101
AJ504642	*Glomus* sp. 'versiforme'	environmental
AJ504643	*Glomus* sp. 'versiforme'	environmental
AJ504644	*Glomus* sp. 'versiforme'	environmental
AJ516922	*Glomus* sp. 'versiforme'	environmental
AJ516923	*Glomus* sp. 'versiforme'	environmental
AJ516924	*Glomus* sp. 'versiforme'	environmental
AJ516925	*Glomus* sp. 'versiforme'	environmental
AJ516926	*Glomus* sp. 'versiforme'	environmental
AJ516927	*Glomus* sp. 'versiforme'	environmental
AJ516928	*Glomus* sp. 'versiforme'	environmental
AJ516929	*Glomus* sp. 'versiforme'	environmental
AJ516930	*Glomus* sp. 'versiforme'	environmental
AJ516931	*Glomus* sp. 'versiforme'	environmental
AJ516932	*Glomus* sp. 'versiforme'	environmental
AJ516933	*Glomus* sp. 'versiforme'	environmental
AJ516934	*Glomus* sp. 'versiforme'	environmental
AJ516935	*Glomus* sp. 'versiforme'	environmental
AJ517781	*Glomus* sp. 'versiforme'	environmental
AM076636	*Glomus* sp. 'versiforme'	environmental
AM076637	*Glomus* sp. 'versiforme'	environmental
AM076638	*Glomus* sp. 'versiforme'	environmental
DQ400187	*Glomus* sp. 'versiforme'	environmental
DQ400194	*Glomus* sp. 'versiforme'	environmental
DQ400197	*Glomus* sp. 'versiforme'	environmental
DQ400198	*Glomus* sp. 'versiforme'	environmental
DQ400212	*Glomus* sp. 'versiforme'	environmental
DQ400223	*Glomus* sp. 'versiforme'	environmental
DQ400225	*Glomus* sp. 'versiforme'	environmental
DQ400227	*Glomus* sp. 'versiforme'	environmental
DQ400229	*Glomus* sp. 'versiforme'	environmental
AF246141	*Glomus versiforme*	BEG47
AF246142	*Glomus versiforme*	BEG47
AF246143	*Glomus versiforme*	BEG47
AY842567	*Glomus versiforme*	BEG47
AY842568	*Glomus versiforme*	BEG47
AY842569	*Glomus versiforme*	BEG47
FM876814	*Glomus versiforme*	BEG47/W5165

FM876815	*Glomus versiforme*	BEG47/W5165
FM876816	*Glomus versiforme*	BEG47/W5165
FM876817	*Glomus versiforme*	BEG47/W5165
FM876818	*Glomus versiforme*	BEG47/W5165
FM876819	*Glomus versiforme*	BEG47/W5165
FM876820	*Glomus versiforme*	BEG47/W5165
FN547635	*Glomus versiforme*	BEG47/W5165
FN547636	*Glomus versiforme*	BEG47/W5165
FN547666	*Glomus versiforme*	BEG47/W3180
FN547667	*Glomus versiforme*	BEG47/W3180
FN547668	*Glomus versiforme*	BEG47/W3180
FN547669	*Glomus versiforme*	BEG47/W3180
FN547670	*Glomus versiforme*	BEG47/W3180
FN547671	*Glomus versiforme*	BEG47/W3180
FN547672	*Glomus versiforme*	BEG47/W3180
FN547673	*Glomus versiforme*	BEG47/W3180
FN547674	*Glomus versiforme*	BEG47/W3180
FN547675	*Glomus versiforme*	BEG47/W3180
FN547676	*Glomus versiforme*	BEG47/W3180
FN547677	*Glomus versiforme*	BEG47/W3180
FN547678	*Glomus versiforme*	BEG47/W3180
FN547679	*Glomus versiforme*	BEG47/W3180
FN547680	*Glomus versiforme*	BEG47/W3180
FN547681	*Glomus versiforme*	BEG47/W3180

Table S4: Sequences used for analyses of the *Diversisporaceae* LSU region (see Figure S11).

Accession	Species	Culture/voucher
AM713402	*Diversispora celata*	BEG231 (FACE234)
AM713403	*Diversispora celata*	BEG231 (FACE234)
AM713404	*Diversispora celata*	BEG231 (FACE234)
AM713405	*Glomus eburneum*	AZ420A/W4729
AM713406	*Glomus eburneum*	AZ420A/W4729
AM713407	*Glomus eburneum*	AZ420A/W4729
AM713408	*Glomus eburneum*	AZ420A/W4729
AM713409	*Glomus eburneum*	AZ420A/W4729
AM713410	*Glomus eburneum*	AZ420A/W4729
AM713411	*Glomus eburneum*	AZ420A/W4729
AM713412	*Glomus eburneum*	AZ420A/W4729
AM713413	*Glomus eburneum*	AZ420A/W4729
AM713414	*Glomus eburneum*	AZ420A/W4729
AM713415	*Glomus eburneum*	AZ420A/W4729
AM713416	*Glomus eburneum*	AZ420A/W4729
FN547635	*Glomus versiforme*	BEG47/W5165
FN547636	*Glomus versiforme*	BEG47/W5165
FM876814	*Glomus versiforme*	BEG47/W5165
FM876815	*Glomus versiforme*	BEG47/W5165
FM876816	*Glomus versiforme*	BEG47/W5165
FM876817	*Glomus versiforme*	BEG47/W5165
FM876818	*Glomus versiforme*	BEG47/W5165
FM876819	*Glomus versiforme*	BEG47/W5165
FM876820	*Glomus versiforme*	BEG47/W5165
FN547637	*Diversispora spurca*	Att246-18/W4119
FN547638	*Diversispora spurca*	Att246-18/W4119
FN547639	*Diversispora spurca*	Att246-18/W4119
FN547640	*Diversispora spurca*	Att246-18/W4119
FN547641	*Diversispora spurca*	Att246-18/W4119
FN547642	*Diversispora spurca*	Att246-18/W4119
FN547643	*Diversispora spurca*	Att246-18/W4119
FN547644	*Diversispora spurca*	Att246-18/W4119
FN547645	*Diversispora spurca*	Att246-18/W4119
FN547646	*Diversispora spurca*	Att246-18/W4119
FN547647	*Diversispora spurca*	Att246-18/W4119
FN547648	*Diversispora spurca*	Att246-18/W4119
FN547649	*Diversispora spurca*	Att246-18/W4119
FN547650	*Diversispora spurca*	Att246-18/W4119
FN547651	*Diversispora spurca*	Att246-18/W4119
FN547652	*Diversispora spurca*	Att246-18/W4119
FN547653	*Diversispora spurca*	Att246-18/W4119
FN547654	*Diversispora spurca*	Att246-18/W4119
FN547655	*Glomus aurantium*	Att1296-0/W4728
FN547656	*Glomus aurantium*	Att1296-0/W4728
FN547657	*Glomus aurantium*	Att1296-0/W4728
FN547658	*Glomus aurantium*	Att1296-0/W4728
FN547659	*Glomus aurantium*	Att1296-0/W4728

FN547660	*Glomus aurantium*	Att1296-0/W4728
FN547661	*Glomus aurantium*	Att1296-0/W4728
FN547662	*Glomus aurantium*	Att1296-0/W4728
FN547663	*Glomus aurantium*	Att1296-0/W4728
FN547664	*Glomus aurantium*	Att1296-0/W4728
FN547665	*Glomus aurantium*	Att1296-0/W4728
FN547666	*Glomus versiforme*	BEG47/W3180
FN547667	*Glomus versiforme*	BEG47/W3180
FN547668	*Glomus versiforme*	BEG47/W3180
FN547669	*Glomus versiforme*	BEG47/W3180
FN547670	*Glomus versiforme*	BEG47/W3180
FN547671	*Glomus versiforme*	BEG47/W3180
FN547672	*Glomus versiforme*	BEG47/W3180
FN547673	*Glomus versiforme*	BEG47/W3180
FN547674	*Glomus versiforme*	BEG47/W3180
FN547675	*Glomus versiforme*	BEG47/W3180
FN547676	*Glomus versiforme*	BEG47/W3180
FN547677	*Glomus versiforme*	BEG47/W3180
FN547678	*Glomus versiforme*	BEG47/W3180
FN547679	*Glomus versiforme*	BEG47/W3180
FN547680	*Glomus versiforme*	BEG47/W3180
FN547681	*Glomus versiforme*	BEG47/W3180
AY842574	*Glomus versiforme*	BEG47/W3180
AY842573	*Glomus versiforme*	BEG47/W3180
EF067888	*Glomus eburneum*	INVAM AZ420A
EF067887	*Glomus eburneum*	INVAM AZ420A
EF067886	*Glomus eburneum*	INVAM AZ420A
AM947665	*Glomus versiforme*	BEG47
AM947664	*Glomus versiforme*	BEG47
EU346868	*Glomus versiforme*	HDAM-4
AY639306	*Diversispora celata*	BEG231 (FACE234)
AY639235	*Diversispora celata*	BEG231 (FACE234)
AY639234	*Diversispora celata*	BEG231 (FACE234)
AY639233	*Diversispora celata*	BEG231 (FACE234)
AY639241	*Diversispora celata*	BEG232 (FACE272)
AY639240	*Diversispora celata*	BEG232 (FACE272)
AY639239	*Diversispora celata*	BEG232 (FACE272)
AY639238	*Diversispora celata*	BEG232 (FACE272)
AY639237	*Diversispora celata*	BEG232 (FACE272)
AY639236	*Diversispora celata*	BEG232 (FACE272)
DQ350448	*Diversispora celata*	BEG233 (FACE410)
DQ350449	*Diversispora celata*	BEG233 (FACE410)
DQ350450	*Diversispora celata*	BEG233 (FACE410)
DQ350451	*Diversispora celata*	BEG233 (FACE410)
DQ350452	*Diversispora celata*	BEG233 (FACE410)
DQ350453	*Diversispora celata*	BEG233 (FACE410)
AY639232	*Diversispora celata*	BEG230 (FACE83)
AY639231	*Diversispora celata*	BEG230 (FACE83)
AY639230	*Diversispora celata*	BEG230 (FACE83)
AY639229	*Diversispora celata*	BEG230 (FACE83)
AY639228	*Diversispora celata*	BEG230 (FACE83)

AY639227	*Diversispora celata*	BEG230 (FACE83)
AY639226	*Diversispora celata*	BEG230 (FACE83)
EF581864	*Glomus aurantium*	Att1296-0/W4728
EF581863	*Glomus aurantium*	Att1296-0/W4728
EF581862	*Glomus aurantium*	Att1296-0/W4728
EF581861	*Glomus aurantium*	Att1296-0/W4728
EF581860	*Glomus aurantium*	Att1296-0/W4728

Table S5: Sequences used for analysis of the *Glomus* Group Aa ITS region (see Figure 3).

Accession	Species	Culture/voucher
X96842	*Glomus* cf. *fasciculatum*	BEG58
X96843	*Glomus* cf. *fasciculatum*	BEG58
AY035642	*Glomus caledonium*	JJ36
AY035646	*Glomus caledonium*	JJ40
AY035647	*Glomus caledonium*	JJ41
AY035651	*Glomus caledonium*	BEG161
FN547494	*Glomus caledonium*	BEG20/W3294
FN547495	*Glomus caledonium*	BEG20/W3294
FN547496	*Glomus caledonium*	BEG20/W3294
FN547497	*Glomus caledonium*	BEG20/W3294
FN547498	*Glomus caledonium*	BEG20/W3294
FN547499	*Glomus caledonium*	BEG20/W3294
AJ890365	*Glomus coronatum*	IMA3
AJ890366	*Glomus coronatum*	IMA3
FM213083	*Glomus coronatum*	environmental
FM213084	*Glomus coronatum*	environmental
FM213085	*Glomus coronatum*	environmental
FM213086	*Glomus coronatum*	environmental
FM213087	*Glomus coronatum*	environmental
FM213088	*Glomus coronatum*	environmental
FM876794	*Glomus coronatum*	BEG28 (Att108-7)
FM876795	*Glomus coronatum*	BEG28 (Att108-7)
FM876796	*Glomus coronatum*	BEG28 (Att108-7)
FM876797	*Glomus coronatum*	BEG28 (Att108-7)
FM876798	*Glomus coronatum*	BEG28 (Att108-7)
X96844	*Glomus coronatum*	BEG28
X96845	*Glomus coronatum*	BEG28
X96846	*Glomus coronatum*	BEG28
X96838	*Glomus dimorphicum*	BEG59
X96839	*Glomus dimorphicum*	BEG59
X96840	*Glomus dimorphicum*	BEG59
X96841	*Glomus dimorphicum*	BEG59
AF231469	*Glomus geosporum*	unknown
AJ319778	*Glomus geosporum*	unknown
AJ319779	*Glomus geosporum*	unknown
AJ319780	*Glomus geosporum*	unknown
AJ319781	*Glomus geosporum*	unknown
AJ319782	*Glomus geosporum*	unknown
AJ319783	*Glomus geosporum*	unknown
AJ319784	*Glomus geosporum*	unknown
AJ319785	*Glomus geosporum*	unknown
AJ319786	*Glomus geosporum*	unknown
AJ319787	*Glomus geosporum*	unknown
AJ319788	*Glomus geosporum*	unknown
AJ319789	*Glomus geosporum*	unknown
AJ319790	*Glomus geosporum*	unknown
AJ319791	*Glomus geosporum*	unknown

AJ319792	*Glomus geosporum*	unknown
AJ319793	*Glomus geosporum*	unknown
AJ319794	*Glomus geosporum*	unknown
AJ319795	*Glomus geosporum*	unknown
AJ319796	*Glomus geosporum*	unknown
AJ319797	*Glomus geosporum*	unknown
AJ319798	*Glomus geosporum*	unknown
AJ319799	*Glomus geosporum*	unknown
AJ319800	*Glomus geosporum*	unknown
AJ319801	*Glomus geosporum*	unknown
AJ319802	*Glomus geosporum*	unknown
AJ319803	*Glomus geosporum*	unknown
FJ009619	*Glomus geosporum*	unknown
FJ009620	*Glomus geosporum*	unknown
FJ009621	*Glomus geosporum*	unknown
FJ009622	*Glomus geosporum*	unknown
AF004689	*Glomus monosporum*	INVAM IT102
AF004690	*Glomus monosporum*	INVAM FR115
AF125195	*Glomus monosporum*	INVAM FR115
AF161043	*Glomus mosseae*	environmental (GMO1a)
AF161044	*Glomus mosseae*	environmental (GMO1b)
AF161045	*Glomus mosseae*	environmental (GM01c)
AF161046	*Glomus mosseae*	environmental (GMO1d)
AF161047	*Glomus mosseae*	environmental (GMO1e)
AF161048	*Glomus mosseae*	environmental (GMO1f)
AF161049	*Glomus mosseae*	environmental (GMO1g)
AF161050	*Glomus mosseae*	environmental (GMO1h)
AF161051	*Glomus mosseae*	environmental (GMO1i)
AF161052	*Glomus mosseae*	environmental (GMO1j)
AF161053	*Glomus mosseae*	environmental (GMO1l)
AF161054	*Glomus mosseae*	environmental (GMO1)
AF161055	*Glomus mosseae*	environmental (GMO2a)
AF161056	*Glomus mosseae*	environmental (GMO2b)
AF161057	*Glomus mosseae*	environmental (GMO2c)
AF161058	*Glomus mosseae*	environmental (GMO2e)
AF161059	*Glomus mosseae*	environmental (GMO3a)
AF161060	*Glomus mosseae*	environmental (GMO3b)
AF161061	*Glomus mosseae*	environmental (GMO3c)
AF161062	*Glomus mosseae*	environmental (GM03d)
AF161063	*Glomus mosseae*	environmental (GM03e)
AF161064	*Glomus mosseae*	environmental (GM03f)
AF166276	*Glomus mosseae*	environmental (GMO2d)
AJ849469	*Glomus mosseae*	unknown
AJ919273	*Glomus mosseae*	INVAM AZ225C
AJ919274	*Glomus mosseae*	INVAM AZ225C
AJ919275	*Glomus mosseae*	INVAM NB114
AJ919276	*Glomus mosseae*	INVAM IN101C
AJ919277	*Glomus mosseae*	INVAM FL156
AJ919278	*Glomus mosseae*	INVAM FL156
AM076635	*Glomus mosseae*	environmental
AM157131	*Glomus mosseae*	ISCB13

AM157132	*Glomus mosseae*	ISCB17
AM157133	*Glomus mosseae*	ISCB22
AM157134	*Glomus mosseae*	ISCB19
AM157135	*Glomus mosseae*	ISCB20
AM423114	*Glomus mosseae*	IMA1
AM423115	*Glomus mosseae*	IMA1
AM423116	*Glomus mosseae*	BEG25
AM423117	*Glomus mosseae*	BEG25
AM423118	*Glomus mosseae*	BEG25
AM423119	*Glomus mosseae*	BEG25
AY035650	*Glomus mosseae*	BEG160
AY035652	*Glomus mosseae*	BEG161
AY236331	*Glomus mosseae*	SP301
AY236332	*Glomus mosseae*	SP302
AY236333	*Glomus mosseae*	SP303
AY236334	*Glomus mosseae*	SP304
AY236335	*Glomus mosseae*	SP305
AY236336	*Glomus mosseae*	SP306
AY997053	*Glomus mosseae*	INVAM UT101 (AFTOL-ID 139)
DQ400127	*Glomus mosseae*	environmental
DQ400128	*Glomus mosseae*	environmental
DQ400129	*Glomus mosseae*	environmental
DQ400130	*Glomus mosseae*	environmental
DQ400131	*Glomus mosseae*	environmental
DQ400132	*Glomus mosseae*	environmental
DQ400134	*Glomus mosseae*	environmental
DQ400136	*Glomus mosseae*	environmental
DQ400137	*Glomus mosseae*	environmental
DQ400138	*Glomus mosseae*	environmental
DQ400139	*Glomus mosseae*	environmental
DQ400141	*Glomus mosseae*	environmental
DQ400142	*Glomus mosseae*	environmental
DQ400144	*Glomus mosseae*	environmental
DQ400146	*Glomus mosseae*	environmental
DQ400149	*Glomus mosseae*	environmental
DQ400151	*Glomus mosseae*	environmental
DQ400158	*Glomus mosseae*	environmental
DQ400160	*Glomus mosseae*	environmental
EF989113	*Glomus mosseae*	environmental
EF989114	*Glomus mosseae*	environmental
EF989115	*Glomus mosseae*	environmental
EF989116	*Glomus mosseae*	environmental
EF989117	*Glomus mosseae*	environmental
FN547474	*Glomus mosseae*	BEG12
FN547475	*Glomus mosseae*	BEG12
FN547476	*Glomus mosseae*	BEG12
FN547482	*Glomus mosseae*	BEG12
FN547483	*Glomus mosseae*	BEG12
FN547484	*Glomus mosseae*	BEG12
FN547485	*Glomus mosseae*	BEG12
FN547486	*Glomus mosseae*	BEG12

FN547487	*Glomus mosseae*	BEG12
FN547488	*Glomus mosseae*	BEG12
FN547489	*Glomus mosseae*	BEG12
FN547490	*Glomus mosseae*	BEG12
FN547491	*Glomus mosseae*	BEG12
FN547492	*Glomus mosseae*	BEG12
FN547493	*Glomus mosseae*	BEG12
U31996	*Glomus mosseae*	BEG 12
U49264	*Glomus mosseae*	UKJII8
U49265	*Glomus mosseae*	INVAM FL156
X84232	*Glomus mosseae*	BEG12
X84233	*Glomus mosseae*	BEG12
X96826	*Glomus mosseae*	BEG25
X96827	*Glomus mosseae*	BEG25
X96828	*Glomus mosseae*	BEG25
X96829	*Glomus mosseae*	BEG55
X96830	*Glomus mosseae*	BEG54
X96831	*Glomus mosseae*	BEG54
X96832	*Glomus mosseae*	BEG54
X96833	*Glomus mosseae*	BEG57
X96834	*Glomus mosseae*	BEG57
X96835	*Glomus mosseae*	BEG57
X96836	*Glomus mosseae*	BEG61
X96837	*Glomus mosseae*	BEG61
FM876813	*Glomus* sp. WUM3	WUM3/W2940
FN547477	*Glomus* sp. WUM3	WUM3/W2939
FN547478	*Glomus* sp. WUM3	WUM3/W2939
FN547479	*Glomus* sp. WUM3	WUM3/W2939
FN547480	*Glomus* sp. WUM3	WUM3/W2939
FN547481	*Glomus* sp. WUM3	WUM3/W2939

Table S6: Sequences used for analysis of the *Glomus* Group Aa LSU-D2 fragment (see Figure 3).

Accession	Species	Culture/voucher
FN547474	*Glomus mosseae*	BEG12/W5147
FN547475	*Glomus mosseae*	BEG12/W5147
FN547476	*Glomus mosseae*	BEG12/W5147
FN547477	*Glomus* sp.	WUM3/W2939
FN547478	*Glomus* sp.	WUM3/W2939
FN547479	*Glomus* sp.	WUM3/W2939
FN547480	*Glomus* sp.	WUM3/W2939
FN547481	*Glomus* sp.	WUM3/W2939
FN547482	*Glomus mosseae*	BEG12/W5147
FN547483	*Glomus mosseae*	BEG12/W5147
FN547484	*Glomus mosseae*	BEG12/W5147
FN547485	*Glomus mosseae*	BEG12/W5147
FN547486	*Glomus mosseae*	BEG12/W5147
FN547487	*Glomus mosseae*	BEG12/W5147
FN547488	*Glomus mosseae*	BEG12/W5147
FN547489	*Glomus mosseae*	BEG12/W5147
FN547490	*Glomus mosseae*	BEG12/W5147
FN547491	*Glomus mosseae*	BEG12/W5147
FM876813	*Glomus* sp.	WUM3/W2940
FN547492	*Glomus mosseae*	BEG12/W5147
FN547493	*Glomus mosseae*	BEG12/W5147
FM876798	*Glomus coronatum*	BEG28 (Att108-7)
FM876796	*Glomus coronatum*	BEG28 (Att108-7)
FM876797	*Glomus coronatum*	BEG28 (Att108-7)
FM876794	*Glomus coronatum*	BEG28 (Att108-7)
FM876795	*Glomus coronatum*	BEG28 (Att108-7)
FN547494	*Glomus caledonium*	BEG20/W3294
FN547495	*Glomus caledonium*	BEG20/W3294
FN547496	*Glomus caledonium*	BEG20/W3294
FN547497	*Glomus caledonium*	BEG20/W3294
FN547498	*Glomus caledonium*	BEG20/W3294
FN547499	*Glomus caledonium*	BEG20/W3294
AF145741	*Glomus constrictum*	BEG130
AF145747	*Glomus fragilistratum*	BEG05
AF145735	*Glomus mosseae*	BEG25
AF145745	*Glomus caledonium*	BEG20
AF145740	*Glomus coronatum*	BEG49
AF145742	*Glomus geosporum*	BEG90
AF396789	*Glomus caledonium*	RMC658
AF396794	*Glomus caledonium*	RWC658
AF145736	*Glomus mosseae*	BEG85
AF396799	*Glomus caledonium*	SC_658
AJ510239	*Glomus caledonium*	BEG86
AF396788	*Glomus mosseae*	243
AF396793	*Glomus mosseae*	243
AF396798	*Glomus mosseae*	243
AY639156	*Glomus mosseae*	8

AY639157	*Glomus mosseae*	8
AY639158	*Glomus mosseae*	8
AY639160	*Glomus mosseae*	environmental
AY639162	*Glomus mosseae*	environmental
AY639163	*Glomus mosseae*	environmental
AY639164	*Glomus mosseae*	101
AY639270	*Glomus mosseae*	environmental
AY639159	*Glomus mosseae*	environmental
AY639274	*Glomus mosseae*	environmental
AY639281	*Glomus mosseae*	209
AY639271	*Glomus mosseae*	environmental
AY639278	*Glomus mosseae*	102
AY639280	*Glomus mosseae*	BEG224 (FACE 130)
AY639161	*Glomus mosseae*	environmental
AY639165	*Glomus mosseae*	BEG224 (FACE 130)
AY639166	*Glomus mosseae*	BEG224 (FACE 130)
AY639167	*Glomus mosseae*	BEG224 (FACE 130)
AY639168	*Glomus mosseae*	BEG224 (FACE 130)
AY639169	*Glomus mosseae*	BEG224 (FACE 130)
AY639170	*Glomus mosseae*	BEG224 (FACE 130)
AY639171	*Glomus mosseae*	209
AY639172	*Glomus mosseae*	209
AY639173	*Glomus mosseae*	209
AY639174	*Glomus mosseae*	209
AY639266	*Glomus mosseae*	8
AY639267	*Glomus mosseae*	8
AY639268	*Glomus mosseae*	8
AY639269	*Glomus mosseae*	8
AY639272	*Glomus mosseae*	environmental
AY639273	*Glomus mosseae*	environmental
AY639276	*Glomus mosseae*	environmental
AY639277	*Glomus mosseae*	101
DQ469128	*Glomus mosseae*	505
AJ628059	*Glomus caledonium*	BEG86
AJ510241	*Glomus geosporum*	BEG11
DQ273793	*Glomus mosseae*	INVAM UT101 (AFTOL-ID 139)
AY639279	*Glomus mosseae*	BEG224 (FACE 130)
FJ790678	*Glomus mosseae*	DDAM
EU931286	*Glomus geosporum*	BEG199
EU931285	*Glomus geosporum*	BEG199
EU931284	*Glomus geosporum*	BEG199
EU931283	*Glomus geosporum*	BEG199
EU931282	*Glomus geosporum*	BEG199
EU931281	*Glomus geosporum*	BEG199
EU931280	*Glomus geosporum*	BEG199
EU931279	*Glomus geosporum*	BEG199
EU931278	*Glomus geosporum*	BEG199
EU931277	*Glomus geosporum*	BEG199
EU931276	*Glomus geosporum*	BEG199
EU931275	*Glomus geosporum*	BEG199
EU931274	*Glomus geosporum*	BEG199

EU931273	*Glomus geosporum*	BEG211
EU931272	*Glomus geosporum*	BEG211
EU931271	*Glomus geosporum*	BEG211
EU931270	*Glomus geosporum*	BEG211
EU931269	*Glomus geosporum*	BEG211
EU931267	*Glomus geosporum*	BEG211
EU931266	*Glomus geosporum*	BEG211
EU931265	*Glomus geosporum*	BEG211
EU931264	*Glomus geosporum*	BEG211
EU931263	*Glomus geosporum*	BEG211
EU931262	*Glomus geosporum*	BEG211
EU931261	*Glomus geosporum*	BEG211
EU346866	*Glomus mosseae*	HDAM-2
EU234489	*Glomus mosseae*	BEG116
AM158954	*Glomus mosseae*	BEG167
AM158953	*Glomus mosseae*	BEG167
DQ469131	*Glomus mosseae*	505
DQ469130	*Glomus mosseae*	505
DQ469129	*Glomus mosseae*	505
DQ469127	*Glomus mosseae*	505
DQ469126	*Glomus mosseae*	505
DQ469125	*Glomus mosseae*	505
AJ459412	*Glomus mosseae*	environmental
AJ628057	*Glomus mosseae*	BEG29
AJ628056	*Glomus mosseae*	BOL3
AJ628055	*Glomus mosseae*	BOL1
AJ628054	*Glomus mosseae*	V150
AJ628053	*Glomus mosseae*	V249
AJ628052	*Glomus mosseae*	V293
AJ628051	*Glomus mosseae*	V91
AJ628050	*Glomus mosseae*	V296
AJ628049	*Glomus mosseae*	V296
AF145746	*Glomus caledonium*	BEG86
AF145743	*Glomus geosporum*	BEG106
AF145738	*Glomus mosseae*	BEG84
AF145737	*Glomus mosseae*	BEG83
AJ271924	*Glomus mosseae*	HM-CL1
AJ510240	*Glomus caledonium*	BEG20
AF389014	*Glomus mosseae*	BEG68
AF389013	*Glomus mosseae*	BEG68
AF389012	*Glomus mosseae*	BEG68
AF389011	*Glomus mosseae*	BEG68
AF389010	*Glomus mosseae*	BEG68
AF389009	*Glomus mosseae*	BEG68
AF389008	*Glomus mosseae*	BEG68
GQ330818	*Glomus mosseae*	AU34
GQ330817	*Glomus mosseae*	AU33
GQ330815	*Glomus mosseae*	AU8
GQ330814	*Glomus mosseae*	AU2
GQ330813	*Glomus mosseae*	WUM16
GQ330811	*Glomus mosseae*	Narrabii

GQ330807	*Glomus mosseae*	Bur11
GQ330806	*Glomus mosseae*	INVAM JA205c
GQ330805	*Glomus mosseae*	BEG229
GQ330800	*Glomus mosseae*	BEG55
GQ330797	*Glomus mosseae*	INVAM NB103c
GQ330793	*Glomus mosseae*	INVM SF1171
GQ330791	*Glomus mosseae*	INVAM CU134a
GQ330789	*Glomus mosseae*	DKB01D4
GQ330788	*Glomus mosseae*	DKK04D22
GQ330787	*Glomus mosseae*	DKGm1
GQ330785	*Glomus mosseae*	Sp813
GQ330784	*Glomus mosseae*	Sp6314
GQ330783	*Glomus mosseae*	Sp4318
GQ330781	*Glomus mosseae*	Sp2735
GQ330780	*Glomus mosseae*	Sp1841
GQ330779	*Glomus mosseae*	BEG128
GQ330778	*Glomus mosseae*	BEG124
GQ330777	*Glomus mosseae*	BEG85
GQ330774	*Glomus mosseae*	Dk11107
GQ330773	*Glomus mosseae*	Dk21107
GQ330772	*Glomus mosseae*	Dk17107
GQ330771	*Glomus mosseae*	BEG230
GQ330768	*Glomus mosseae*	Dk23135
GQ330760	*Glomus mosseae*	INVAM WY111
GQ330757	*Glomus mosseae*	INVAM MT107
GQ330756	*Glomus mosseae*	INVAM OR229
GQ330754	*Glomus mosseae*	INVAM SC226
GQ330749	*Glomus mosseae*	INVAM MN101
GQ330748	*Glomus mosseae*	INVAM MI210
GQ330747	*Glomus mosseae*	INVAM ON201
GQ330744	*Glomus mosseae*	INVAM WI101
GQ330743	*Glomus mosseae*	INVAM NV106
GQ330742	*Glomus mosseae*	INVAM IN101

Table S7: Barcode gap analyses with TaxonGap 2.3 based on pairwise comparison of K2P distances based on a manual or automated alignment (MAFFT) of the large SSUmCf-LSUmBr fragment. Variation is given in % K2P distances. The closest species and presence or absences of a barcode gap were identical for the manual and MAFFT alignments, respectively. Seq, number of sequences; CS, closest species; BG, barcode gap; Max. ISV, maximum intraspecific variation; Min. ISV, minimum intraspecific variation; ?, unknown.

					manual alignment		**MAFFT alignment**	
Family	**Species**	**Seq**	**CS**	**BG**	**Max. ISV**	**Min. ISV**	**Max. ISV**	**Min. ISV**
	Glomus mosseae	16	*Gl. coronatum*	Yes	2.52	2.66	2.58	3.78
Glomeraceae	*Gl.* sp. WUM3	6	*Gl. caledonium*	Yes	0.85	2.1	0.85	2.09
(*Glomus* Group	*Gl. coronatum*	5	*Gl. mosseae*	Yes	0.5	2.66	1.01	3.78
Aa)	*Gl. caledonium*	3	*Gl.* sp. WUM3	Yes	0.8	2.1	0.8	2.09
	Gl. intraradices	47	*Gl. proliferum*	No	10.77	4.29	11.75	4.7
Glomeraceae	*Gl. proliferum*	15	*Gl. intraradices*	Yes	4.02	4.29	3.89	4.7
(*Glomus* Group	*Gl.* sp. 'irregulare-like'	39	*Gl. proliferum*	Yes	6.43	7	6.29	6.94
Ab)	*Gl. clarum*	9	*Gl. proliferum*	Yes	1.09	7.58	1.59	7.96
	Acaulospora laevis	26	*Ku. kentinensis & Ac. scrobiculata*	Yes	3.42	13.07	3.99	13.16
	Acaulospora sp. WUM18	2	*Ac. scrobiculata*	Yes	1.02	5.66	1.02	5.66
	Ac. scrobiculata	4	*Ac.* sp. WUM18	Yes	0.47	5.66	0.47	5.66
Acaulosporaceae	*Kuklospora kentinensis*	14	*Ac. scrobiculata*	Yes	0.54	11.98	0.54	11.38
	Ambispora appendicula	11	*Am. fennica*	Yes	2.87	12.11	2.87	13.26
Ambisporaceae	*Am. fennica*	12	*Am. appendicula*	Yes	1	12.11	1.14	13.26
	Gigaspora margarita	24	*Gi. rosea*	No	4.15	3.26	4.42	3.34
	Gi. rosea	27	*Gi. margarita*	No	6.17	3.26	6.53	3.34
	Sc. gilmorei	25	*Sc. spinosissima*	Yes	1.55	2.64	1.62	2.5
	Sc. spinosissima	3	*Sc. gilmorei*	No	2.84	2.64	2.84	2.5
Gigasporaceae	*Sc. heterogama*	4	*Gi. margarita*	Yes	1.95	4.69	2.74	5.07
Glomeraceae	*Glomus* sp. W3349	4	*Gl. luteum*	Yes	0.77	11.54	0.71	12.27
(*Glomus* Group	*Gl. etunicatum*	12	*Gl. luteum*	Yes	0.93	3.64	0.94	3.63
B)	*Gl. luteum*	5	*Gl. etunicatum*	Yes	0.64	3.64	0.96	3.63
	Diversispora celata	3	*Gl. eburneum*	Yes	0.9	2.61	0.83	3.39
	Gl. eburneum	12	*Di. celata*	Yes	0.92	2.61	0.92	3.39
	Gl. versiforme	25	*Gl. eburneum*	Yes	2.52	5.81	2.79	5.64
	Diversispora spurca	18	*Gl. aurantium*	Yes	1.59	2.73	1.66	2.87
Diversisporaceae	*Gl. aurantium*	11	*Di. spurca*	Yes	1.71	2.73	1.71	2.87
Paraglomeraceae	*Paraglomus occultum*	1	*Sc. gilmorei*	?	-	34.93	-	31.7
Pacisporaceae	*Pacispora scintillans*	2	*Sc. heterogama*	Yes	0.62	22.59	0.55	20.55

13.2 Supplementary data – chapter 6

The following table is supplementary material for the publication '*Acaulospora brasiliensis* comb. nov. and *Acaulospora alpina* (*Glomeromycota*) from upland Scotland: morphology, molecular phylogeny and DNA based detection in roots'.

Table S1. Colour of spores observed in water with reflected light at 3100 K. Colours are either unmatched to a chart, or are matched with the Royal Botanic Garden Edinburgh colour chart or are given in Munsell notation (Anon 1969; Anon 1990).

Voucher	Observed colour of spores
W4514	non matched: orange brown
W4699	ochraceous to ochre (9-11 RBG)
W4786	reddish yellow to yellowish red (5YR 7.8-6.8 Munsell)
W4796	non matched: pale yellow brown
W4833	pale sienna (pale 11 RBG)
W5125	sienna (11 RBG)
W5473	non matched: yellow brown
W5516	pale ochraceous to sienna (6-11 RBG)
W5748	non matched: pale yellow
W5751	very pale brown to yellow to brownish yellow to yellowish brown (10YR 8/3-5/8 Munsell)
W5755	non matched: yellow to brownish yellow
W5759	non matched: pale yellow brown
W5762	non matched: yellow to yellow brown
W5765	non matched: pale yellow to yellow brown

13.3 Supplementary data – chapter 7

The following data are supplementary material of the publication 'Arbuscular mycorrhizal fungi: biogeography and molecular systematics of the *Diversisporaceae*, with special reference to *Diversispora epigaea* (formerly known as '*Glomus versiforme* BEG47')'

Supplementary Figure 1.

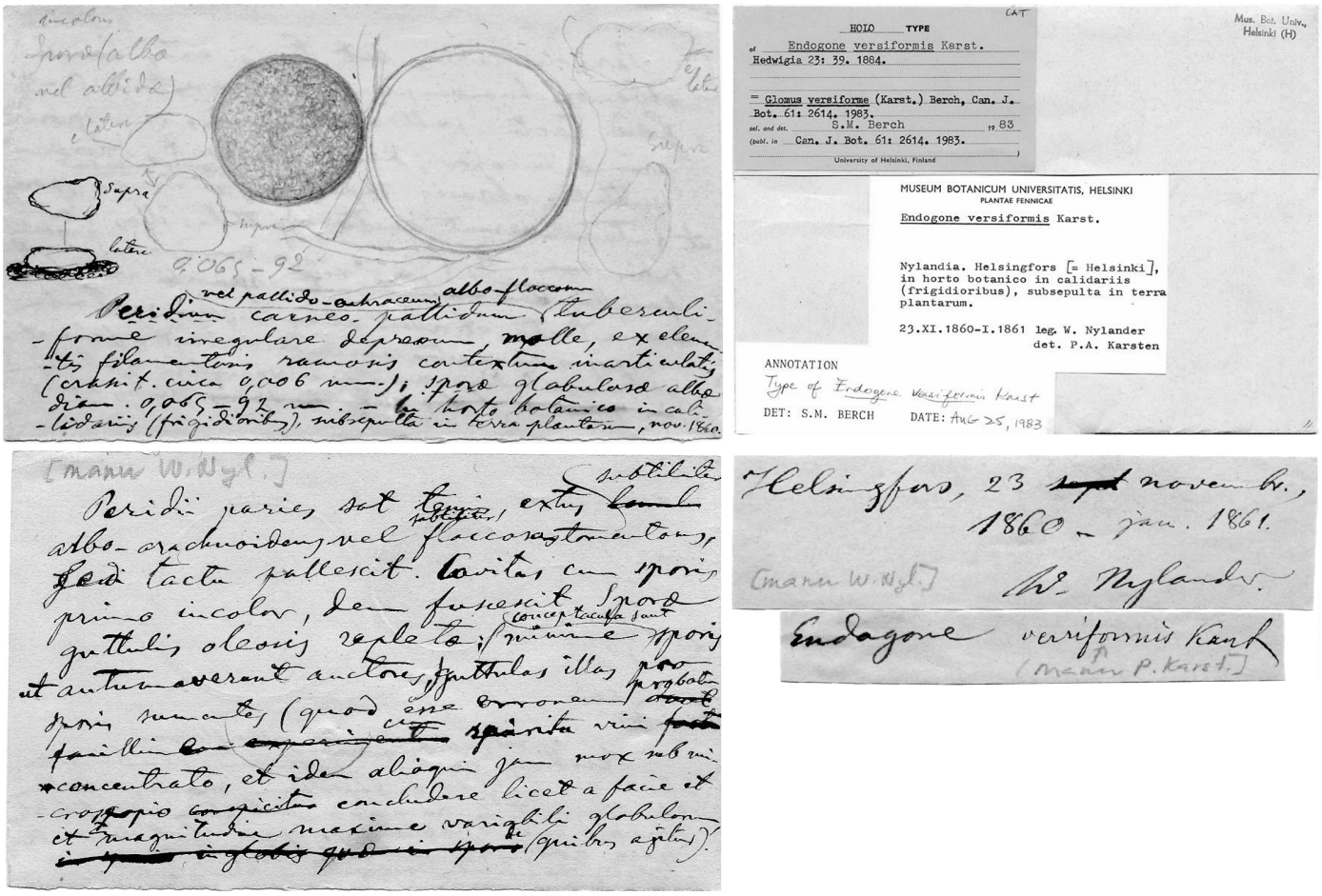
Phylogenetic tree derived from the extended dataset analysis of members of the *Diversisporaceae*, including all environmental *Diversisporaceae* sequences available from the public databases. RAxML maximum likelihood tree with bootstrap support shown at the branches. Branchings with bootstrap support below 50% are shown as polytomies. The sequences not included in the analysis in Fig. 2 all cluster in the *Diversispora* clade, except one (DQ357079 from Portugal).

Supplementary Figure 2.

The handwritten labels and notes of Nylund (23 Nov 1860 - Jan 1861), and annotations included in the herbarium packet containing the holotype of *Glomus versiforme* (basionym *Endogone versiformis*), its translation, and the protologue and its translation of *Endogone versiformis* (Karsten 1884).

Supplementary Figure 1

Supplementary Figure 2.



The notes of Nylund (23 Nov 1860 - Jan 1861) are transcribed as "*Peridium carneo, pallidum vel pallido-ochraceum, albo-floccosum, tuberculiforme irregulare depressum, molle, ex elementis filamentaris ramosis contextum inarticulatis (crassit. circa 0.006 mm), sporae globulosae albae diam. 0.065–92 mm. In horto botanico in calidariis* [as '*calilidariis*'] *(frigidioribus), subsepulta in terra plantarum, nov. 1860*". This is translated as "Peridium carnose, pale to pale-ochraceous, whitish floccose, tubercular irregularly flattened, soft, composed of filamentous branching aseptate elements (about 0.006 mm [6 µm] thick), white, globular spores with a diameter of 0.065-92 mm [65-92 µm]. In botanical garden in greenhouses (temperate), buried in the soil of plants, Nov. 1860."

Continued on the reverse with notes that are very difficult to transcribe. We transcribe the Latin as: "*Peridii paries sat tenuis, extus subtiliter albo-arachnoideus vel floccoso-tomentosus, sed tactu pallescit. Cavitas cum sporis primo incolor, dein fuscescit. Sporae guttulis oleosis repletae; conceptacula sunt (minime sporis ut autunaverant auctores,) guttulas illas pro sporis sumentes (quod esse erroneum probatum facillime cum spiritu vini concentrato, et idem alioquin iam mox sub microscopico concludere licet a facie et* [as 'et et'] *magnitudine maxime variabili globulorum (quibus agitur)!*" The following translation is based on this transcript, whereas it should be kept in mind that many words were difficult to read or misspelled: "Wall of the peridium moderately thin, outer layer finer, white-interwoven or floccose-tomentose, but when touched becoming yellowish. Interior containing the spores initially colorless, then darkening. Spores filled with oily guttules; being within a receptacle these guttules (small spores by earlier authors) appearing to be spores (which is an artefact easily tested with concentrated ethanol, and the same in general immediately then can be seen under the microscope concluding from appearance and greatly variable size of the globules (which lead to that interpretation).

The protologue of *Endogone versiformis* (Karsten 1884) "*Peridia tuberculiformia, irregularia, mollia, ex hyphis ramosis inarticulatis, circa 6 mmm crassis contexta, carneo-pallida vel pallido-ochracea, albofloccosa, sicca subochraceae, usque ad 1 cm lata. Sporangia subsphaeroidea, albida, diam. 65–95 mmm. Sporae sporoidae. In horto botanico Helsingforsiensi in calidariis (frigidioribus), subsepulta in terra plantarum m., Nov.-Jan.*" is translated as "Peridium tubercular, irregular, soft, composed of aseptate racemose hyphae, about 6 mmm [6 µm] thick, pale flesh coloured or pale ochraceous, white-woolly, dry pale yellowish, up to 1 cm wide. Spores [as 'sporangia'] sub-globose, white, diameter 65-95 mmm [65-95 µm]. Spores spore-like. In the botanical garden Helsinki in glasshouses (temperate), buried in the soil of plants, Nov. - Jan."

Supplementary Table 1.
Strict consensus sequences used in the phylogenetic analyses.

Diversispora aurantia	AJ849468 (type culture); FN547655-65, AM713432, EF581860,62,63,80-83 (W4728/Att1296-0 (ex-type soil trap culture)
Diversispora celata	AM713423-25, AY639225-32, EF581865-68 (W4758/Att1292-2, BEG230 = FACE83); AM713426-28, AY639236-41, EF581873-76 (W4757/Att1291-2, BEG232 = FACE272); DQ350448-53 (W5306-07/Att1500-2 = BEG233 = FACE410); AM713417-22, AM713402-04, AY639233-35, AY639306, EF581869-72 (ex-type single spore culture W4718-19/Att1278-2, BEG231 = FACE234)
Diversispora eburnea	AM713405-16,29-31, EF067886-88, EF581877-79 (AZ420A W4729/Att1290-5, ex-type culture)
Diversispora epigaea (all ex-type cultures, originally from Oregon State University, USA, all in the same culture-lineage as BEG47):	AJ132666 (BEG47 from BEG at INRA Dijon, France); AJ276088 (W3221/Att475-21 from C. Walker, Hampshire, England); AM947665, AY842567-69,73,74, FJ461852, FM876814-20, FN547635,36, (W5165/Att475-45 from P. Bonfante, Torino via C. Walker, UK via B. Blal, Dijon, France via INRA, Dijon, France via Rothamsted Experimental Station, UK); FN547666-81 (W3180/Att475-22); X86687, Y17651, FR686938-42 (HC/F-E01 from P. Bonfante, Torino, Italy via J. Trappe, Corvallis, USA)
***Diversispora* sp. W2423**	AJ301863, AJ276076, Y17644, AJ301860, FR686943-44 (W2423/Att382-16)
***Diversispora* sp. 'NamAri'**	AF185677-81 (in sequence database as *Glomus* sp. AZ237B; in INVAM culture collection as *Glomus intraradices* AZ237B); AF185682,90,91, AF185693-95 (in sequence database as *Glomus* sp. NB101; in INVAM culture collection as *Glomus intraradices* NB101)
***Diversispora* sp. W5257**	FR686945-52, FR686958 (W5257)
Diversispora spurca (all ex-type cultures)	AJ276077 (W3239/Att246-4); AJ276078, Y17649,50, FR686953 (W2396/Att246-4); FN547637-54, FR686954 (W4119/Att246-18)
Diversispora trimurales	FR686956-57 (W4124/Att1152-1)
Redeckera fulvum	AM418543-44 (AC/Pohn99-001)
Redeckera megalocarpum (from type material)	AM418551,52 (CL/Guad05-051)
Redeckera pulvinatum	AM418549,50 (CL/Mart05-035)
Otospora bareai (thought to be from the type material)	AM400229, AM905318 (assembly of non-overlapping 5' and 3' regions of SSU rRNA gene)
***Acaulospora lacunosa* BEG78**	FR719957, AJ891110-13, AJ510230
***Acaulospora laevis* AU211**	AJ250847, AJ242499, FJ461802
***Acaulospora cavernata* BEG33** (given as *A. scrobiculata* at BEG)	AJ306442, FM876788-91
***Acaulospora* sp. WUM18** (equivalent to INVAM AU103A)	AJ306441, FM876792,93
***Claroideoglomus claroideum* BEG14** (=*Glomus claroideum*)	AJ301851,52, AJ276075, Y17636, AF235007
Rhizophagus irregularis GINCO4695rac-11G2 (AFTOL-ID845) (=*Glomus irregulare*)	DQ322630, AY997054, DQ273828
***Funneliformis mosseae* UT101** (AFTOL-ID139) (=*Glomus mosseae*)	AY635833, AY997053, DQ273793
***Gigaspora rosea* DAOM194757**	X58726, AJ410746,47, FN547571-97
***Pacispora scintillans* W4545** (vouchD1, sample3)	FM876831,32, AJ619952-55
***Racocetra castanea* BEG1** (ex-type culture)	AF038590, AJ002874, AJ313169-75, FJ461867, FN423706,07, U31997,98, Y12076
***Scutellospora heterogama* BEG35**	AJ306434, FM876837-39
***Scutellospora nodosa* BEG4**	FM876833-36, AJ306436

Supplementary Table 2.
Diversispora epigaea (=*Glomus versiforme* BEG47, =*Glomus epigaeum*) samples studied.

Voucher (W)	Culture Attempt (Att)	Collection Date	Collector	Locality
90	475-1. Pot culture with *Asparagus officinalis* established from a single 'sporocarp'	13 December 1977	B. Daniels	USA, Oregon, Benton Co., USDA- ARS, Horticultural Crops Research Unit, Tropical Greenhouse. HOLOTYPE. Trappe 5174. OSC39475
100	475-1. As above	12 April 1979	B. Daniels	As above
407	475-3. No culture or collection data	July 1978. Date known only to month	B. Daniels	As above
1518	475-7. From pot culture with *Sorghum bicolor*	24 January 1981	D. Egel	USA, Ames, Iowa, Iowa State Univeristy Forestry Greenhouse
526	475-6. No culture or collection data except 'Menge 0-1'	13 January 1982	J. Menge	USA, California, University of California, Riverside
1640	475-14. Pot culture with *Allium porrum*	2 December1991	C. Grace	UK, England, Hertfordshire, Harpenden, Rothamsted Experiment Station
1641	475-17. No culture or collection data	1990. Date known only to year	F. Sanders	UK, England, Yorkshire, Leeds, University of Leeds, Dept. of Plant Sciences
1728	475-12. No culture or collection data	19 March 1993	B. Breuinett	Italy, Torino, University of Torino, Departimento di Biologia Vegetale
2336	475-18. Pot culture with *Plantago. lanceolata*	11 October 1995	A. Broome	UK, Scotland, Midlothian, Roslin, Forest Research, Northern Research Station
2842	475-20. No culture or collection data	1 October 1996	P. Bonfante	Italy, Torino, University of Torino, Departimento di Biologia Vegetale
3180	475-22. No culture or collection data	15 October 1996	P. Bonfante	Italy, Torino, University of Torino, Departimento di Biologia Vegetale
3206	475-21. Pot culture with *P. lanceolata*	13 January 1999	C. Walker	UK, England, Hampshire, Efford, Horticultural Research International
3221	475-21. Pot culture with *P. lanceolata*	24 February 1999	C. Walker	UK, England, Hampshire, Efford, Horticultural Research International
3537	475-30. Pot culture with *P. lanceolata*	3 November 2000	M. Vestberg	Finland, Vihtavuori, Laukaa Research & Elite Plant Laboratory
3581	475-21. Pot culture with *P. lanceolata*	5 February 2001	C. Walker	UK, England, Hampshire, Efford, Horticultural Research International
3864	475-21. Pot culture with *P. lanceolata*	15 March2002	C. Walker	UK, England, Hampshire, Efford, Horticultural Research International
4475	475-38. No culture or collection data	12 June 2003	B. Blal	France, Dijon, Biorize
4560	475-39. Pot culture with *P. lanceolata*	9 December 2003	C. Walker	Belgium, Louvain-la-Neuve, Catholic University of Louvain
4565	475-40. Pot culture with *P. lanceolata*	15 December 2003	C. Walker	UK, Scotland, Royal Botanic Garden Edinburgh
5164	475-40. Pot culture with *P. lanceolata*	15 April 2006	C. Walker	UK, England, Gloucester (moved from Edinburgh)
5065	475-44. Pot culture with *P. lanceolata*	30 January 2007	G. Bending	UK, England, Wellesbourne, University of Warwick
5066	475-44. Pot culture with *P. lanceolata*	30 January 2007	G. Bending	UK, England, Wellesbourne, University of Warwick
5117	475-45. Pot culture with	08 February 2007	M. Naumann	Italy, Torino, University of Torino,

	Trifolium repens			Departimento di Biologia Vegetale
5165	475-45. Pot culture with *T. repens*	28 April 2007	M. Naumann	Italy, Torino, University of Torino, Departimento di Biologia Vegetale
5167	475-45. Pot culture with *T. repens*	28 April 2007	M. Naumann	Italy, Torino, University of Torino, Departimento di Biologia Vegetale
5170	475-46. Pot culture with *P. lanceolata*	16 May 2007	M. Naumann	Italy, Torino, University of Torino, Departimento di Biologia Vegetale
5260	475-45. Pot culture with *T. repens*	1 June 2007	M. Naumann	Italy, Torino, University of Torino, Departimento di Biologia Vegetale
5358	475-45. Pot culture with *T. repens*	25 July 2007	M. Naumann	Italy, Torino, University of Torino, Departimento di Biologia Vegetale
5606	475-55. No culture or collection data	1 February 2009	M. Harrison	USA, New York, Ithaca, Boyce Thompson Institute for Plant Research
5707	475-59. Pot culture with *P. lanceolata*	25 January 2010	C. Walker	UK, England, Wellesbourne, University of Warwick
5708	475-60. Pot culture with *P. lanceolata*	25 January 2010	C. Walker	UK, England, Wellesbourne, University of Warwick
5728	475-56. Pot culture with *P. lanceolata*, *Festuca ovina* agg. *Lotus japonicus* var. gifu	3 March 2010	A. Schüßler	Germany, Martinsried, Ludwig-Maximilians-University Munich
5724	475-48. Pot culture with *P. lanceolata*	9 March 2010	M. Krüger	Germany, Martinsried, Ludwig-Maximilians-University Munich
5725	475-49. Pot culture with *P. lanceolata*	9 March 2010	M. Krüger	Germany, Martinsried, Ludwig-Maximilians-University Munich
5726	475-57. Pot culture with *P. lanceolata*	9 March 2010	M. Krüger	Germany, Martinsried, Ludwig-Maximilians-University Munich
5727	475-47. Pot culture with *P. lanceolata*	9 March 2010	A. Schüßler	Germany, Martinsried, Ludwig-Maximilians-University Munich
5786	475-61. Pot culture with *P. lanceolata*	24 June 2010	C. Walker	UK, England, Gloucester
5835	475-61. Pot culture with *P. lanceolata*	23 September 2010	C. Walker	UK, England, Gloucester
5848	475-66. Pot culture with *P. lanceolata*	26 October 2010	C. Krüger	Germany, Martinsried, Ludwig-Maximilians-University Munich
5849	475-71. Pot culture with *P. lanceolata*	26 October 2010	C. Krüger	Germany, Martinsried, Ludwig-Maximilians-University Munich

13.4 Supplementary data – chapter 8

The following data are supplementary material of the publication 'A 3 kb, three-rDNA-loci phylogenetic framework for arbuscular mycorrhizal fungi - from phylum to species resolution'.

Supplementary Figure S1

Maximum likelihood phylogenetic tree based on the nuclear SSU-ITS-LSU rDNA of *Glomeraceae*, except *Rhizophagus* and *Sclerocystis*, including public database sequences of >500 bp. *Rhizophagus* species were used as outgroup. Branches receiving less than 60% bootstrap support (1000 bootstraps) were collapsed to polytomies, long branches were shortened by 50% as indicated with two diagonal slashes or by 75% indicated with three slashes. Bootstrap values are given for branches among but not within different cultures. Scale bar, number of substitutions per site. The annotation marked with (consensus) was computed from a strict consensus sequence of the accession numbers given in the tree.

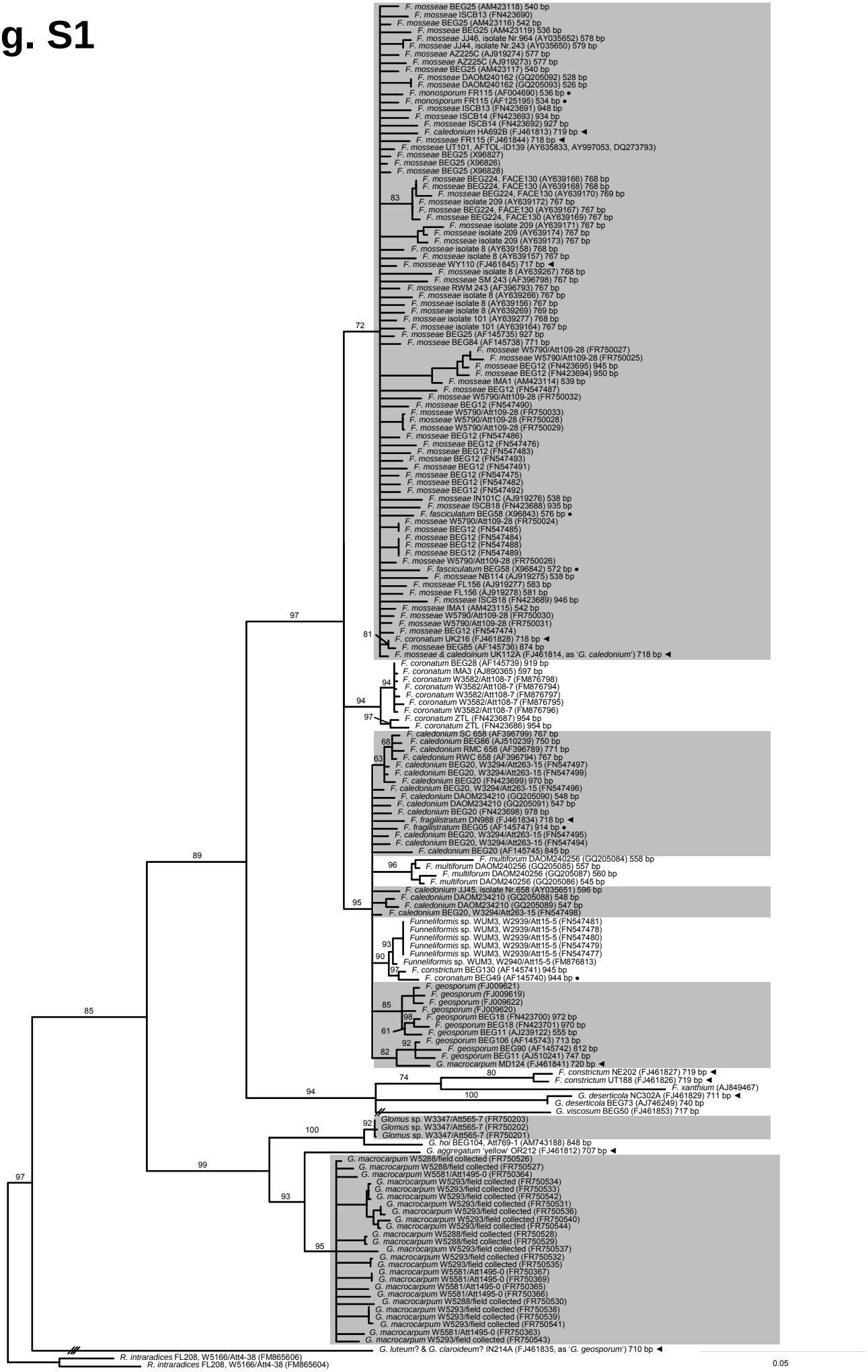
Supplementary Figure S2

Maximum likelihood phylogenetic tree based on nuclear SSU-ITS-LSU rDNA of the *Claroideoglomeraceae*, including public database sequences of ≥450 bp, *Funneliformis* was used as outgroup. Branches receiving less than 60% bootstrap support (1000 bootstraps) were collapsed to polytomies, long branches were shortened by 50% as indicated with two diagonal slashes or by 75% indicated with three slashes. Bootstrap values are given for branches among but not within different cultures. Scale bar, number of substitutions per site.

Supplementary Table S1

List of sequence identifiers derived from this and related studies published by the authors, with their current species affiliations and, for recently changed names, synonyms, their source publication, culture identifier, clone number type of culture, sample used for DNA extraction, and geographic origin when known. (#), if more than one number is shown, respective clones had identical sequences; (*) all cultures are pot cultures if not otherwise stated; ROC, root organ culture (monoxenic).

Fig. S1


F. mosseae BEG25 (AM423118) 540 bp
F. mosseae ISCB13 (FN423690)
F. mosseae BEG25 (AM423116) 542 bp
F. mosseae BEG25 (AM423119) 536 bp
F. mosseae JJ46, isolate Nr.964 (AY035652) 578 bp
F. mosseae JJ44, isolate Nr.243 (AY035650) 579 bp
F. mosseae AZ225C (AJ919274) 577 bp
F. mosseae AZ225C (AJ919273) 577 bp
F. mosseae BEG25 (AM423117) 540 bp
F. mosseae DAOM240162 (GQ205092) 528 bp
F. mosseae DAOM240162 (GQ205093) 526 bp
F. monosporum FR115 (AF004690) 536 bp ●
F. monosporum FR115 (AF125195) 534 bp ●
F. mosseae ISCB13 (FN423691) 948 bp
F. mosseae ISCB14 (FN423693) 934 bp
F. mosseae ISCB14 (FN423692) 927 bp
F. caledonium HA692B (FJ461813) 719 bp ◄
F. mosseae FR115 (FJ461844) 718 bp ◄
F. mosseae UT101, AFTOL-ID139 (AY635833, AY997053, DQ273793)
F. mosseae BEG25 (X96827)
F. mosseae BEG25 (X96826)
F. mosseae BEG25 (X96828)
F. mosseae BEG224, FACE130 (AY639166) 768 bp
F. mosseae BEG224, FACE130 (AY639168) 768 bp
F. mosseae BEG224, FACE130 (AY639170) 769 bp
F. mosseae isolate 209 (AY639172) 767 bp
F. mosseae BEG224, FACE130 (AY639167) 767 bp
F. mosseae BEG224, FACE130 (AY639169) 767 bp
F. mosseae isolate 209 (AY639171) 767 bp
F. mosseae isolate 209 (AY639174) 767 bp
F. mosseae isolate 209 (AY639173) 767 bp
F. mosseae isolate 8 (AY639158) 768 bp
F. mosseae isolate 8 (AY639157) 767 bp
F. mosseae WY110 (FJ461845) 717 bp ◄
F. mosseae isolate 8 (AY639267) 768 bp
F. mosseae SM 243 (AF396798) 767 bp
F. mosseae RWM 243 (AF396793) 767 bp
F. mosseae isolate 8 (AY639266) 767 bp
F. mosseae isolate 8 (AY639156) 767 bp
F. mosseae isolate 8 (AY639269) 769 bp
F. mosseae isolate 101 (AY639277) 768 bp
F. mosseae isolate 101 (AY639164) 767 bp
F. mosseae BEG25 (AF145735) 927 bp
F. mosseae BEG84 (AF145738) 771 bp
F. mosseae W5790/Att109-28 (FR750027)
F. mosseae W5790/Att109-28 (FR750025)
F. mosseae BEG12 (FN423695) 945 bp
F. mosseae BEG12 (FN423694) 950 bp
F. mosseae IMA1 (AM423114) 539 bp
F. mosseae BEG12 (FN547487)
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F. mosseae W5790/Att109-28 (FR750028)
F. mosseae W5790/Att109-28 (FR750029)
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F. mosseae BEG12 (FN547476)
F. mosseae BEG12 (FN547483)
F. mosseae BEG12 (FN547493)
F. mosseae BEG12 (FN547491)
F. mosseae BEG12 (FN547475)
F. mosseae BEG12 (FN547482)
F. mosseae BEG12 (FN547492)
F. mosseae IN101C (AJ919276) 538 bp
F. mosseae ISCB18 (FN423688) 935 bp
F. fasciculatum BEG58 (X96843) 576 bp ●
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F. mosseae FL156 (AJ919277) 583 bp
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F. mosseae IMA1 (AM423115) 542 bp
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F. mosseae W5790/Att109-28 (FR750031)
F. mosseae BEG12 (FN547474)
F. coronatum UK216 (FJ461828) 718 bp ◄
F. mosseae BEG85 (AF145736) 874 bp
F. mosseae & caledoinum UK112A (FJ461814, as 'G. caledonium') 718 bp ◄
F. coronatum BEG28 (AF145739) 919 bp
F. coronatum IMA3 (AJ890365) 597 bp
F. coronatum W3582/Att108-7 (FM876798)
F. coronatum W3582/Att108-7 (FM876794)
F. coronatum W3582/Att108-7 (FM876797)
F. coronatum W3582/Att108-7 (FM876795)
F. coronatum W3582/Att108-7 (FM876796)
F. coronatum ZTL (FN423687) 954 bp
F. coronatum ZTL (FN423686) 954 bp
F. caledonium SC 658 (AF396799) 767 bp
F. caledonium BEG86 (AJ510239) 750 bp
F. caledonium RMC 658 (AF396789) 771 bp
F. caledonium RWC 658 (AF396794) 767 bp
F. caledonium BEG20, W3294/Att263-15 (FN547497)
F. caledonium BEG20, W3294/Att263-15 (FN547499)
F. caledonium BEG20 (FN423699) 970 bp
F. caledonium BEG20, W3294/Att263-15 (FN547496)
F. caledonium DAOM234210 (GQ205090) 548 bp
F. caledonium DAOM234210 (GQ205091) 547 bp
F. caledonium BEG20 (FN423698) 978 bp
F. fragilistratum DN988 (FJ461834) 718 bp ◄
F. fragilistratum BEG05 (AF145747) 914 bp ●
F. caledonium BEG20, W3294/Att263-15 (FN547495)
F. caledonium BEG20, W3294/Att263-15 (FN547494)
F. caledonium BEG20 (AF145745) 845 bp
F. multiforum DAOM240256 (GQ205084) 558 bp
F. multiforum DAOM240256 (GQ205085) 557 bp
F. multiforum DAOM240256 (GQ205087) 560 bp
F. multiforum DAOM240256 (GQ205086) 545 bp
F. caledonium JJ45, isolate Nr.658 (AY035651) 596 bp
F. caledonium DAOM234210 (GQ205088) 548 bp
F. caledonium DAOM234210 (GQ205089) 547 bp
F. caledonium BEG20, W3294/Att263-15 (FN547498)
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Funneliformis sp. WUM3, W2939/Att15-5 (FN547478)
Funneliformis sp. WUM3, W2939/Att15-5 (FN547480)
Funneliformis sp. WUM3, W2939/Att15-5 (FN547479)
Funneliformis sp. WUM3, W2939/Att15-5 (FN547477)
Funneliformis sp. WUM3, W2940/Att15-5 (FM876813)
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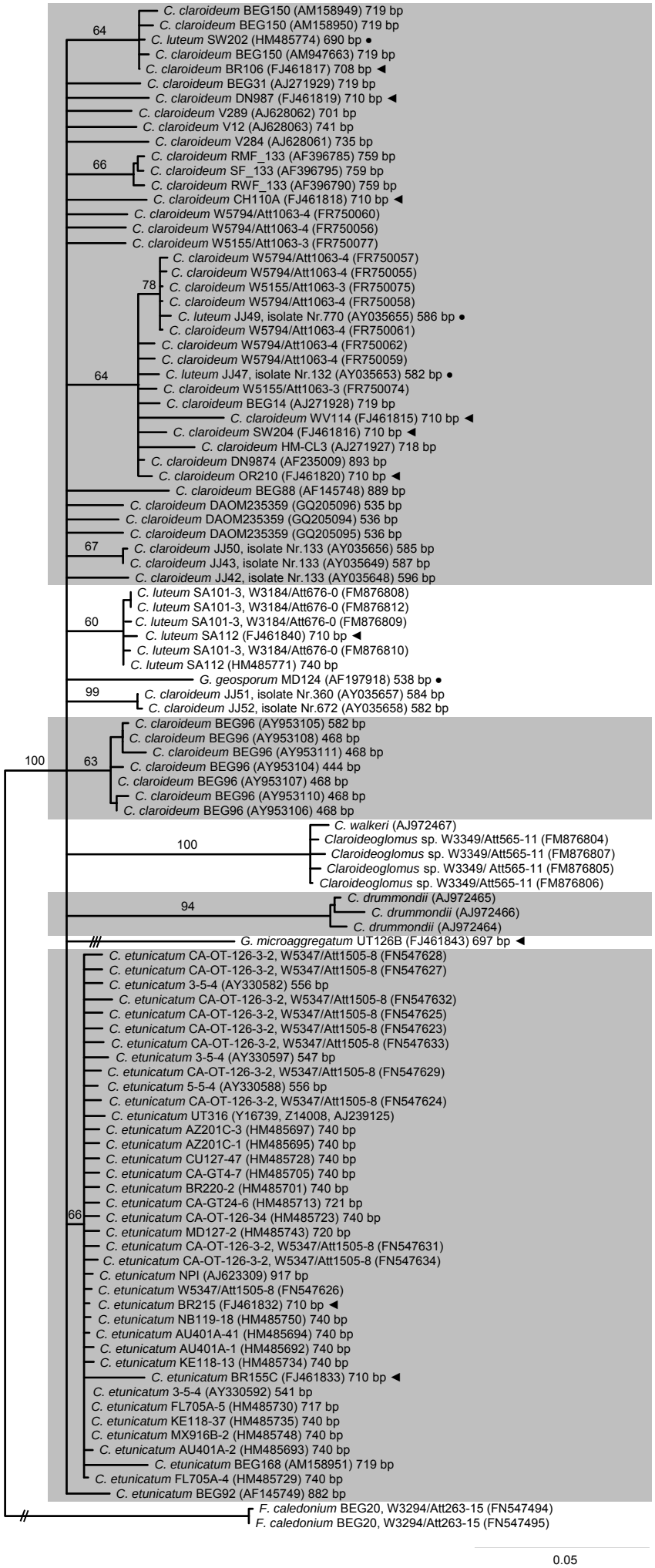
F. coronatum BEG49 (AF145740) 944 bp ●
F. geosporum (FJ009621)
F. geosporum (FJ009619)
F. geosporum (FJ009622)
F. geosporum (FJ009620)
F. geosporum BEG18 (FN423700) 972 bp
F. geosporum BEG18 (FN423701) 970 bp
F. geosporum BEG11 (AJ239122) 555 bp
F. geosporum BEG106 (AF145743) 713 bp
F. geosporum BEG90 (AF145742) 812 bp
F. geosporum BEG11 (AJ510241) 747 bp
G. macrocarpum MD124 (FJ461841) 720 bp ◄
F. constrictum NE202 (FJ461827) 719 bp ◄
F. constrictum UT188 (FJ461826) 719 bp ◄
F. xanthium (AJ849467)
G. deserticola NC302A (FJ461829) 711 bp ◄
G. deserticola BEG73 (AJ746249) 740 bp
G. viscosum BEG50 (FJ461853) 717 bp
Glomus sp. W3347/Att565-7 (FR750203)
Glomus sp. W3347/Att565-7 (FR750202)
Glomus sp. W3347/Att565-7 (FR750201)
G. hoi BEG104, Att769-1 (AM743188) 848 bp
G. aggregatum 'yellow' OR212 (FJ461812) 707 bp ◄
G. macrocarpum W5288/field collected (FR750526)
G. macrocarpum W5288/field collected (FR750527)
G. macrocarpum W5581/Att1495-0 (FR750364)
G. macrocarpum W5293/field collected (FR750534)
G. macrocarpum W5293/field collected (FR750533)
G. macrocarpum W5293/field collected (FR750542)
G. macrocarpum W5293/field collected (FR750531)
G. macrocarpum W5293/field collected (FR750536)
G. macrocarpum W5293/field collected (FR750540)
G. macrocarpum W5293/field collected (FR750544)
G. macrocarpum W5288/field collected (FR750528)
G. macrocarpum W5288/field collected (FR750529)
G. macrocarpum W5293/field collected (FR750537)
G. macrocarpum W5293/field collected (FR750532)
G. macrocarpum W5293/field collected (FR750535)
G. macrocarpum W5581/Att1495-0 (FR750367)
G. macrocarpum W5581/Att1495-0 (FR750369)
G. macrocarpum W5581/Att1495-0 (FR750365)
G. macrocarpum W5581/Att1495-0 (FR750366)
G. macrocarpum W5288/field collected (FR750530)
G. macrocarpum W5293/field collected (FR750538)
G. macrocarpum W5293/field collected (FR750539)
G. macrocarpum W5293/field collected (FR750541)
G. macrocarpum W5581/Att1495-0 (FR750363)
G. macrocarpum W5293/field collected (FR750543)
G. luteum? & G. claroideum? IN214A (FJ461835, as 'G. geosporum') 710 bp ◄
R. intraradices FL208, W5166/Att4-38 (FM865606)
R. intraradices FL208, W5166/Att4-38 (FM865604)
0.05

Fig. S2


C. claroideum BEG150 (AM158949) 719 bp
C. claroideum BEG150 (AM158950) 719 bp
64
C. luteum SW202 (HM485774) 690 bp ●
C. claroideum BEG150 (AM947663) 719 bp
C. claroideum BR106 (FJ461817) 708 bp ◄
C. claroideum BEG31 (AJ271929) 719 bp
C. claroideum DN987 (FJ461819) 710 bp ◄
C. claroideum V289 (AJ628062) 701 bp
C. claroideum V12 (AJ628063) 741 bp
C. claroideum V284 (AJ628061) 735 bp
C. claroideum RMF_133 (AF396785) 759 bp
66
C. claroideum SF_133 (AF396795) 759 bp
C. claroideum RWF_133 (AF396790) 759 bp
C. claroideum CH110A (FJ461818) 710 bp ◄
C. claroideum W5794/Att1063-4 (FR750060)
C. claroideum W5794/Att1063-4 (FR750056)
C. claroideum W5155/Att1063-3 (FR750077)
C. claroideum W5794/Att1063-4 (FR750057)
C. claroideum W5794/Att1063-4 (FR750055)
78
C. claroideum W5155/Att1063-3 (FR750075)
C. claroideum W5794/Att1063-4 (FR750058)
C. luteum JJ49, isolate Nr.770 (AY035655) 586 bp ●
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C. claroideum W5794/Att1063-4 (FR750059)
64
C. luteum JJ47, isolate Nr.132 (AY035653) 582 bp ●
C. claroideum W5155/Att1063-3 (FR750074)
C. claroideum BEG14 (AJ271928) 719 bp
C. claroideum WV114 (FJ461815) 710 bp ◄
C. claroideum SW204 (FJ461816) 710 bp ◄
C. claroideum HM-CL3 (AJ271927) 718 bp
C. claroideum DN9874 (AF235009) 893 bp
C. claroideum OR210 (FJ461820) 710 bp ◄
C. claroideum BEG88 (AF145748) 889 bp
C. claroideum DAOM235359 (GQ205096) 535 bp
C. claroideum DAOM235359 (GQ205094) 536 bp
C. claroideum DAOM235359 (GQ205095) 536 bp
67
C. claroideum JJ50, isolate Nr.133 (AY035656) 585 bp
C. claroideum JJ43, isolate Nr.133 (AY035649) 587 bp
C. claroideum JJ42, isolate Nr.133 (AY035648) 596 bp
C. luteum SA101-3, W3184/Att676-0 (FM876808)
C. luteum SA101-3, W3184/Att676-0 (FM876812)
60
C. luteum SA101-3, W3184/Att676-0 (FM876809)
C. luteum SA112 (FJ461840) 710 bp ◄
C. luteum SA101-3, W3184/Att676-0 (FM876810)
C. luteum SA112 (HM485771) 740 bp
G. geosporum MD124 (AF197918) 538 bp ●
99
C. claroideum JJ51, isolate Nr.360 (AY035657) 584 bp
C. claroideum JJ52, isolate Nr.672 (AY035658) 582 bp
C. claroideum BEG96 (AY953105) 582 bp
C. claroideum BEG96 (AY953108) 468 bp
100
C. claroideum BEG96 (AY953111) 468 bp
63
C. claroideum BEG96 (AY953104) 444 bp
C. claroideum BEG96 (AY953107) 468 bp
C. claroideum BEG96 (AY953110) 468 bp
C. claroideum BEG96 (AY953106) 468 bp
C. walkeri (AJ972467)
Claroideoglomus sp. W3349/Att565-11 (FM876804)
100
Claroideoglomus sp. W3349/Att565-11 (FM876807)
Claroideoglomus sp. W3349/ Att565-11 (FM876805)
Claroideoglomus sp. W3349/Att565-11 (FM876806)
C. drummondii (AJ972465)
94
C. drummondii (AJ972466)
C. drummondii (AJ972464)
G. microaggregatum UT126B (FJ461843) 697 bp ◄
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547628)
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547627)
C. etunicatum 3-5-4 (AY330582) 556 bp
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547632)
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547625)
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547623)
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547633)
C. etunicatum 3-5-4 (AY330597) 547 bp
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547629)
C. etunicatum 5-5-4 (AY330588) 556 bp
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547624)
C. etunicatum UT316 (Y16739, Z14008, AJ239125)
C. etunicatum AZ201C-3 (HM485697) 740 bp
C. etunicatum AZ201C-1 (HM485695) 740 bp
C. etunicatum CU127-47 (HM485728) 740 bp
C. etunicatum CA-GT4-7 (HM485705) 740 bp
C. etunicatum BR220-2 (HM485701) 740 bp
C. etunicatum CA-GT24-6 (HM485713) 721 bp
66
C. etunicatum CA-OT-126-34 (HM485723) 740 bp
C. etunicatum MD127-2 (HM485743) 720 bp
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547631)
C. etunicatum CA-OT-126-3-2, W5347/Att1505-8 (FN547634)
C. etunicatum NPI (AJ623309) 917 bp
C. etunicatum W5347/Att1505-8 (FN547626)
C. etunicatum BR215 (FJ461832) 710 bp ◄
C. etunicatum NB119-18 (HM485750) 740 bp
C. etunicatum AU401A-41 (HM485694) 740 bp
C. etunicatum AU401A-1 (HM485692) 740 bp
C. etunicatum KE118-13 (HM485734) 740 bp
C. etunicatum BR155C (FJ461833) 710 bp ◄
C. etunicatum 3-5-4 (AY330592) 541 bp
C. etunicatum FL705A-5 (HM485730) 717 bp
C. etunicatum KE118-37 (HM485735) 740 bp
C. etunicatum MX916B-2 (HM485748) 740 bp
C. etunicatum AU401A-2 (HM485693) 740 bp
C. etunicatum BEG168 (AM158951) 719 bp
C. etunicatum FL705A-4 (HM485729) 740 bp
C. etunicatum BEG92 (AF145749) 882 bp
F. caledonium BEG20, W3294/Att263-15 (FN547494)
F. caledonium BEG20, W3294/Att263-15 (FN547495)
0.05

Supporting Table 1. Annotation of sequences from our laboratory, which were used in the analyses.

rDNA region	accession no.	clone no.	species name (synonym, if recently changed)	type-information	attempt no.	voucher	other identifier(s)	culture type or sample origin	DNA extraction from	reference	geographical origin	collector or originator
SSU	FN825898	FD22-1	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU	FN825899	FD22-6	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU	FN825900	MK113-1	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825901	MK109-1	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825902	MK109-2	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825903	MK065-7	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825904	MK065-6	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825905	MK065-5	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825906	MK065-4	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825907	MK064-6	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825908	MK064-4	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825909	MK062-3	Acaulospora brasiliensis (Ambispora brasiliensis		Att1211-0	W4699		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825910	CK032-4	Acaulospora brasiliensis (Ambispora brasiliensis		Att1210-5	W5473		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825911	CK032-2	Acaulospora brasiliensis (Ambispora brasiliensis		Att1210-5	W5473		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU-ITS-LSU	FN825912	CK032-1	Acaulospora brasiliensis (Ambispora brasiliensis		Att1210-5	W5473		soil trap	single spore	Krüger et al. 2011	UK, Perthshire, Meall nan Tarmachar	C Walker
SSU	FR750213	FD002-1 (+3)	Acaulospora capsicula		Att1186-5	W4681		single spore (= isolate	single spore	Krüger et al. 2011	Australia, New South Wales, Sydney	P McGee
SSU	AJ306442	WD159-1-1	Acaulospora cavernata	epitype predecessor	Att209-37	W3293	BEG33	multispore	single spore	Schüßler et al. 2001	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FM876788	MK006-1	Acaulospora cavernata	epitype predecessor	Att209-37	W3293	BEG33	multispore	single spore	Krüger et al. 2009	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FM876789	MK006-2	Acaulospora cavernata	epitype predecessor	Att209-37	W3293	BEG33	multispore	single spore	Krüger et al. 2009	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FM876790	MK006-3	Acaulospora cavernata	epitype predecessor	Att209-37	W3293	BEG33	multispore	single spore	Krüger et al. 2009	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FM876791	MK006-4	Acaulospora cavernata	epitype predecessor	Att209-37	W3293	BEG33	multispore	single spore	Krüger et al. 2009	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FR750063	CK086-4	Acaulospora colombiana	ex-type	Att1476-8	W5795	CIAT C-10; INVAM CL356	pot culture (details unknown)	single spore	this study	Colombia, Meta, Carimagua	J Spain
SSU-ITS-LSU	FR750168	MK095-1	Acaulospora entreriana	ex-type	Att1541-1	W5476		multispore	single spore	this study	Argentina, Colón County, Ubajay	S Velazquez
SSU-ITS-LSU	FR750169	MK095-2	Acaulospora entreriana	ex-type	Att1541-1	W5476		multispore	single spore	this study	Argentina, Colón County, Ubajay	S Velazquez
SSU-ITS-LSU	FR750170	MK095-3	Acaulospora entreriana	ex-type	Att1541-1	W5476		multispore	single spore	this study	Argentina, Colón County, Ubajay	S Velazquez
SSU-ITS-LSU	FR750171	MK095-4	Acaulospora entreriana	ex-type	Att1541-1	W5476		multispore	single spore	this study	Argentina, Colón County, Ubajay	S Velazquez
SSU-ITS-LSU	FR750172	MK095-5	Acaulospora entreriana	ex-type	Att1541-1	W5476		multispore	single spore	this study	Argentina, Colón County, Ubajay	S Velazquez
SSU-ITS-LSU	FR750173	MK095-6	Acaulospora entreriana	ex-type	Att1541-1	W5476		multispore	single spore	this study	Argentina, Colón County, Ubajay	S Velazquez
SSU-ITS-LSU	FM876821	HS098-1	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876822	HS098-2	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876823	HS098-5	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876824	HS098-16	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876825	HS098-20	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876826	HS098-23	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876827	HS098-24	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876828	HS098-29	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876829	HS098-56	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FM876830	HS098-57	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Krüger et al. 2009	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FN547520	HS098-38	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Stockinger et al. 2010	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FN547521	HS098-43	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Stockinger et al. 2010	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FN547522	HS098-21	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Stockinger et al. 2010	Taiwan, Tainam	C-G Wu
SSU-ITS-LSU	FN547523	HS098-35	Acaulospora kentinensis (Kuklospora kentinensis	authenticated	Att1499-9	W5346	TW111A	pot culture (details unknown)	single spore	Stockinger et al. 2010	Taiwan, Tainam	C-G Wu
SSU	FR719957	WD195-1-2	Acaulospora lacunosa		Att626-8	W3289	BEG78	multispore	single spore	this study	USA, New Hampshire	D Watson
SSU	Y17633	WD95–1-4	Acaulospora laevis		Att896–8	W3107	WUM46	multispore	single spore	Schüßler et al. 2001	Australia, Western Australia, Jarrahdale	D Jasper
SSU	FR750214	FD061	Acaulospora laevis		Att423-4	W3247		multispore	single spore	this study	UK, Sutherland, Elphin	C Walker
SSU-ITS-LSU	FN547502	HS032-80	Acaulospora laevis		Att423-4	W3247		multispore	single spore	Stockinger et al. 2010	UK, Sutherland, Elphin	C Walker
SSU-ITS-LSU	FN547503	HS032-82	Acaulospora laevis		Att423-4	W3247		multispore	single spore	Stockinger et al. 2010	UK, Sutherland, Elphin	C Walker
SSU-ITS-LSU	FN547504	HS032-88	Acaulospora laevis		Att423-4	W3247		multispore	single spore	Stockinger et al. 2010	UK, Sutherland, Elphin	C Walker
SSU-ITS-LSU	FN547505	HS032-81	Acaulospora laevis		Att423-4	W3247		multispore	single spore	Stockinger et al. 2010	UK, Sutherland, Elphin	C Walker
SSU-ITS-LSU	FN547506	HS032-89	Acaulospora laevis		Att423-4	W3247		multispore	single spore	Stockinger et al. 2010	UK, Sutherland, Elphin	C Walker
SSU-ITS-LSU	FN547517	HS032-69	Acaulospora laevis		Att423-4	W3247		multispore	single spore	Stockinger et al. 2010	UK, Sutherland, Elphin	C Walker
SSU-ITS-LSU	FN547513	HS030-1	Acaulospora laevis		none (material from BEG	none	BEG26	multispore	single spore	Stockinger et al. 2010	China	V. Gianinazzi-Pearsor
SSU-ITS-LSU	FN547514	HS030-22	Acaulospora laevis		none (material from BEG	none	BEG26	multispore	single spore	Stockinger et al. 2010	China	V. Gianinazzi-Pearsor
SSU-ITS-LSU	FN547515	HS030-2	Acaulospora laevis		none (material from BEG	none	BEG26	multispore	single spore	Stockinger et al. 2010	China	V. Gianinazzi-Pearsor
SSU-ITS-LSU	FN547518	HS030-9	Acaulospora laevis		none (material from BEG	none	BEG26	multispore	single spore	Stockinger et al. 2010	China	V. Gianinazzi-Pearsor
SSU-ITS-LSU	FN547519	HS030-26	Acaulospora laevis		none (material from BEG	none	BEG26	multispore	single spore	Stockinger et al. 2010	China	V. Gianinazzi-Pearsor
SSU-ITS-LSU	FM876780	HS028-4	Acaulospora laevis		Att862-7	W2928	WUM11, AU221?	multispore	single spore	Krüger et al. 2009	Australia, Shire of Dandaragan, Badgingarra	L Abbott
SSU-ITS-LSU	FM876781	HS028-10	Acaulospora laevis		Att862-7	W2928	WUM11, AU221?	multispore	single spore	Krüger et al. 2009	Australia, Shire of Dandaragan, Badgingarra	L Abbott
SSU-ITS-LSU	FM876782	HS028-12	Acaulospora laevis		Att862-7	W2928	WUM11, AU221?	multispore	single spore	Krüger et al. 2009	Australia, Shire of Dandaragan, Badgingarra	L Abbott
SSU-ITS-LSU	FM876783	HS028-17	Acaulospora laevis		Att862-7	W2928	WUM11, AU221?	multispore	single spore	Krüger et al. 2009	Australia, Shire of Dandaragan, Badgingarra	L Abbott
SSU-ITS-LSU	FM876784	HS028-22	Acaulospora laevis		Att862-7	W2928	WUM11, AU221?	multispore	single spore	Krüger et al. 2009	Australia, Shire of Dandaragan, Badgingarra	L Abbott
SSU-ITS-LSU	FM876785	HS028-25	Acaulospora laevis		Att862-7	W2928	WUM11, AU221?	multispore	single spore	Krüger et al. 2009	Australia, Shire of Dandaragan, Badgingarra	L Abbott
SSU-ITS-LSU	FM876786	HS028-6	Acaulospora laevis		Att862-7	W2928	WUM11, AU221?	multispore	single spore	Krüger et al. 2009	Australia, Shire of Dandaragan, Badgingarra	L Abbott
SSU-ITS-LSU	FM876787	HS028-1	Acaulospora laevis		Att862-7	W2928	WUM11, AU221?	multispore	single spore	Krüger et al. 2009	Australia, Shire of Dandaragan, Badgingarra	L Abbott
SSU-ITS-LSU	FN547507	HS054-36	Acaulospora laevis	ex-epitype	Att192-10	W5258	BEG13	multispore	single spore	Stockinger et al. 2010	New Zealand, unknown locatior	B Mosse
SSU-ITS-LSU	FN547508	HS054-1	Acaulospora laevis	ex-epitype	Att192-10	W5258	BEG13	multispore	single spore	Stockinger et al. 2010	New Zealand, unknown locatior	B Mosse
SSU-ITS-LSU	FN547509	HS054-2	Acaulospora laevis	ex-epitype	Att192-10	W5258	BEG13	multispore	single spore	Stockinger et al. 2010	New Zealand, unknown locatior	B Mosse
SSU-ITS-LSU	FN547510	HS054-42	Acaulospora laevis	ex-epitype	Att192-10	W5258	BEG13	multispore	single spore	Stockinger et al. 2010	New Zealand, unknown locatior	B Mosse
SSU-ITS-LSU	FN547511	HS054-24	Acaulospora laevis	ex-epitype	Att192-10	W5258	BEG13	multispore	single spore	Stockinger et al. 2010	New Zealand, unknown locatior	B Mosse
SSU-ITS-LSU	FN547512	HS054-35	Acaulospora laevis	ex-epitype	Att192-10	W5258	BEG13	multispore	single spore	Stockinger et al. 2010	New Zealand, unknown locatior	B Mosse
SSU-ITS-LSU	FN547516	HS054-7	Acaulospora laevis	ex-epitype	Att192-10	W5258	BEG13	multispore	single spore	Stockinger et al. 2010	New Zealand, unknown locatior	B Mosse
SSU	AJ306441	WD157-2-3	Acaulospora sp.		Att869-3	W2941	WUM18	multispore	single spore	Krüger et al. 2008	Australia, Nedlands, Univ. of Western Australia	L Abbott
SSU-ITS-LSU	FM876792	MK005-1	Acaulospora sp.		Att869-3	W2941	WUM18	multispore	single spore	Krüger et al. 2009	Australia, Nedlands, Univ. of Western Australia	L Abbott
SSU-ITS-LSU	FM876793	MK005-2	Acaulospora sp.		Att869-3	W2941	WUM18	multispore	single spore	Krüger et al. 2009	Australia, Nedlands, Univ. of Western Australia	L Abbott
SSU	FR750204	WD210-1-1	Acaulospora spinosa	ex-type	Att165-9	W3574		multispore	single spore	this study	USA, Iowa, Boone	C Walker
SSU-ITS-LSU	FR750151	MK038-11	Acaulospora spinosa	ex-type	Att165-9	W3574		multispore	single spore	this study	USA, Iowa, Boone	C Walker
SSU-ITS-LSU	FR750152	MK038-15	Acaulospora spinosa	ex-type	Att165-9	W3574		multispore	single spore	this study	USA, Iowa, Boone	C Walker
SSU-ITS-LSU	FR750153	MK038-32	Acaulospora spinosa	ex-type	Att165-9	W3574		multispore	single spore	this study	USA, Iowa, Boone	C Walker
SSU-ITS-LSU	FR750154	MK038-41	Acaulospora spinosa	ex-type	Att165-9	W3574		multispore	single spore	this study	USA, Iowa, Boone	C Walker
SSU-ITS-LSU	FR750155	MK038-42	Acaulospora spinosa	ex-type	Att165-9	W3574		multispore	single spore	this study	USA, Iowa, Boone	C Walker
SSU-ITS-LSU	FR750156	MK038-44	Acaulospora spinosa	ex-type	Att165-9	W3574		multispore	single spore	this study	USA, Iowa, Boone	C Walker
SSU-ITS-LSU	FN547524	MK096-1	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547525	MK096-10	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547526	MK096-11	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547527	MK096-12	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547528	MK096-2	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547529	MK096-3	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547530	MK096-4	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547531	MK096-5	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547532	MK096-6	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547533	MK096-7	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU-ITS-LSU	FN547534	MK096-8	Ambispora appendicula	authenticated	Att1235-1	W5156		multispore	multi spore (3)	Stockinger et al. 2010	Brazil, Mato Grosso, between Barra do Gracas & Poxore	J Spain
SSU	AM268192	FD36-3-3	Ambispora fennica	type-culture predecesso	Att200-11	W3569		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
SSU	AM268193	FD98-1	Ambispora fennica	type-culture predecesso	Att200-11	W3569		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
SSU	AM268195	FD35-8	Ambispora fennica	type-culture predecesso	Att200-11	W3570		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
SSU	AM268194	WD265-1-1	Ambispora fennica	type-culture predecesso	Att200-21	W3847		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
SSU	AM268196	FD120-2	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547535	MK094-1	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547536	MK094-10	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547537	MK094-11	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547538	MK094-12	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547539	MK094-2	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547540	MK094-3	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547541	MK094-4	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547542	MK094-5	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547543	MK094-6	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547544	MK094-7	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547545	MK094-8	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FN547546	MK094-9	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Stockinger et al. 2010	Finland, Kurikka, Jyllintaival	M Vestberg
SSU-ITS-LSU	FR750157	MK074-1	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	this study	Finland, Kurikka, Jyllintaival	M Vestberg
ITS	AM268198	FD120-13	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
ITS	AM268203	FD120-6	Ambispora fennica	ex-type	Att200-23	W4752		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
ITS	AM268197	FD120-10	Ambispora fennica	type-culture predecesso	Att200-11	W3569		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
ITS	AM268199	FD120-7	Ambispora fennica	type-culture predecesso	Att200-11	W3569		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
ITS	AM268200	FD114-12	Ambispora fennica	type-culture predecesso	Att200-11	W3569		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
ITS	AM268201	FD114-5	Ambispora fennica	type-culture predecesso	Att200-11	W3569		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
ITS	AM268202	FD114-6	Ambispora fennica	type-culture predecesso	Att200-11	W3569		single spore (= isolate	single spore	Walker et al. 2007	Finland, Kurikka, Jyllintaival	M Vestberg
SSU	AJ301861	WD147–1-1	Ambispora leptoticha		Att733-0	W3442	NC176	single spore (= isolate	single spore (acaulos	Schüßler et al. 2001	USA, North Carolina, Durham	J Bever

SSU	FR773150	WD198_1	Archaeospora schenkii (Intraspora schenckii		Att58-6	W3571	CIAT -C133-8	multispore	single spore	this study	Colombia, Cundinamarca, between Cajic & Tabic	E Sieverding
SSU-ITS-LSU	FR750020	CK077-1 (2+4+5)	Archaeospora schenkii (Intraspora schenckii		Att212-4	W5673		multispore	single spore	this study	Argentina, Puna region	A Menendez
SSU-ITS-LSU	FR750021	CK077-3 (+6)	Archaeospora schenkii (Intraspora schenckii		Att212-4	W5673		multispore	single spore	this study	Argentina, Puna region	A Menendez
SSU-ITS-LSU	FR750022	CK077-6	Archaeospora schenkii (Intraspora schenckii		Att212-4	W5673		multispore	single spore	this study	Argentina, Puna region	A Menendez
SSU-ITS-LSU	FR750023	CK077-7	Archaeospora schenkii (Intraspora schenckii		Att212-4	W5673		multispore	single spore	this study	Argentina, Puna region	A Menendez
SSU	AM114274	WD103-3-10	Archaeospora trappe		Att186-1	W3179		soil trap	single spore	this study	Austria, Tyrol, Schulterberg	P Schweiger
SSU	Y17634	WD103–3-8	Archaeospora trappe		Att186–1	W3179		soil trap	single spore	Schüßler et al. 2001	Austria, Tyrol, Schulterberg	P Schweiger
SSU-ITS-LSU	FR750034	CK082-10	Archaeospora trappe		Att178-3	W5791		multispore	single spore	this study	UK, Midlothian, Dolphinton	C Walker
SSU-ITS-LSU	FR750035	CK082-3	Archaeospora trappe		Att178-3	W5791		multispore	single spore	this study	UK, Midlothian, Dolphinton	C Walker
SSU-ITS-LSU	FR750036	CK082-4	Archaeospora trappe		Att178-3	W5791		multispore	single spore	this study	UK, Midlothian, Dolphinton	C Walker
SSU-ITS-LSU	FR750037	CK082-5 (+8+9)	Archaeospora trappe		Att178-3	W5791		multispore	single spore	this study	UK, Midlothian, Dolphinton	C Walker
SSU-ITS-LSU	FR750038	CK082-7	Archaeospora trappe		Att178-3	W5791		multispore	single spore	this study	UK, Midlothian, Dolphinton	C Walker
SSU	AJ276080	KL14–4a	Claroideoglomus claroideum (Glomus claroideum		none (material from BEG	none	BEG23	multispore	single spore	Schüßler et al. 2001	Czech Republic, Nový Bydzov, Knezic	M Gryndler
SSU	Y17642	TR5-5	Claroideoglomus claroideum (Glomus claroideum		none (material from BEG	none	BEG23	multispore	single spore	Schüßler et al. 2001	Czech Republic, Nový Bydzov, Knezic	M Gryndler
SSU	AJ276079	KL4–2	Claroideoglomus claroideum (Glomus claroideum		Att79–3	W1843	BEG31	multispore	single spore	Schüßler et al. 2001	Finland, Laukaa, Hohc	M Vestberg
SSU	Y17641	KL4-1	Claroideoglomus claroideum (Glomus claroideum		Att79-3	W1843	BEG31	multispore	single spore	Schüßler et al. 2001	Finland, Laukaa, Hohc	M Vestberg
SSU	AJ276075	KL2–9a	Claroideoglomus claroideum (Glomus claroideum		none (material from BEG	none	BEG14	pot culture (details unknown	single spore	Schüßler et al. 2001	Denmark, Zealanc	S Rosendahl
SSU	Y17636	GCL-1	Claroideoglomus claroideum (Glomus claroideum		none (material from BEG	none	BEG14	pot culture (details unknown	single spore	Schüßler et al. 2001	Denmark, Zealanc	S Rosendahl
SSU	AJ301851	KL2-7	Claroideoglomus claroideum (Glomus claroideum		none (material from BEG	none	BEG14	pot culture (details unknown	single spore	Schüßler et al. 2001	Denmark, Zealanc	S Rosendahl
SSU	AJ301852	KL2-10a	Claroideoglomus claroideum (Glomus claroideum		none (material from BEG	none	BEG14	pot culture (details unknown	single spore	Schüßler et al. 2001	Denmark, Zealanc	S Rosendahl
SSU-ITS-LSU	FR750055	CK085-1	Claroideoglomus claroideum (Glomus claroideum		Att1063-4	W5794	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750056	CK085-2	Claroideoglomus claroideum (Glomus claroideum		Att1063-4	W5794	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750057	CK085-3	Claroideoglomus claroideum (Glomus claroideum		Att1063-4	W5794	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750058	CK085-4	Claroideoglomus claroideum (Glomus claroideum		Att1063-4	W5794	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750059	CK085-5	Claroideoglomus claroideum (Glomus claroideum		Att1063-4	W5794	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750060	CK085-6	Claroideoglomus claroideum (Glomus claroideum		Att1063-4	W5794	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750061	CK085-8	Claroideoglomus claroideum (Glomus claroideum		Att1063-4	W5794	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750062	CK085-9	Claroideoglomus claroideum (Glomus claroideum		Att1063-4	W5794	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750074	HS035-33	Claroideoglomus claroideum (Glomus claroideum		Att1063-3	W5155	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750075	HS035-44	Claroideoglomus claroideum (Glomus claroideum		Att1063-3	W5155	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750076	HS035-52	Claroideoglomus claroideum (Glomus claroideum		Att1063-3	W5155	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU-ITS-LSU	FR750077	HS035-61	Claroideoglomus claroideum (Glomus claroideum		Att1063-3	W5155	SW210	multispore	single spore (= isolate	this study	Switzerland, Canton Thurgau, Tänikor	J Jansa
SSU	FR750216	WD249-1-1	Claroideoglomus etunicatum (Glomus etunicatum		Att367-3	W3808		multispore	single spore (= isolate	this study	UK, Strathclyde Region, Cambuslang	C Walker
SSU	FR750217	WD255-2-1	Claroideoglomus etunicatum (Glomus etunicatum		Att843-1	W3815		multispore	single spore (= isolate	this study	Mexico, Yucatan	R Rodriguez
SSU	Y17639	WD106–2-2	Claroideoglomus etunicatum (Glomus etunicatum		Att678–4	W3093	UT316–8	pot culture (details unknown	single spore	Schüßler et al. 2001	USA, no location data	Unknown
SSU-ITS-LSU	FN547623	HS112-36	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547624	HS112-24	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547625	HS112-40	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547626	HS112-15	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547627	HS112-13	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547628	HS112-17	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547629	HS112-39	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547630	HS112-23	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547631	HS112-5	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547632	HS112-18	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547633	HS112-6	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU-ITS-LSU	FN547634	HS112-28	Claroideoglomus etunicatum (Glomus etunicatum		Att1505-8	W5347	CA-OT-126-3-2	single spore (= isolate) ROC	single spore	Stockinger et al. 2010	USA, California, Berkeley	T Pawlowska
SSU	FR773151	WD99_5_1	Claroideoglomus lamellosum (Glomus lamellosum)	ex-'isotype'	Att244-7	W3158	DAOM212349 (note: a later ROC subculture carries same no., but contains Rh. irregularis)	multispore (approx. 25 spores)	single spore	this study	Canada, Ontario, Wasaga Beach Provincial Park	Y Dalpé
SSU	FR773152	WD99_5_2	Claroideoglomus lamellosum (Glomus lamellosum	ex-'isotype	Att244-7	W3158	DAOM212349	multispore (approx. 25 spores	single spore	this study	Canada, Ontario, Wasaga Beach Provincial Park	Y Dalpé
SSU	AJ276087	WD100–2-6	Claroideoglomus lamellosum (Glomus lamellosum	ex-'isotype	Att244–13	W3160	DAOM212349	single spore (= isolate	single spore	Schüßler et al. 2001	Canada, Ontario, Wasaga Beach Provincial Park	Y Dalpé
SSU	AJ276083	WD116–1-2	Claroideoglomus lamellosum (Glomus lamellosum		Att872–13	W3161		single spore (= isolate	single spore	Schüßler et al. 2001	Iceland, Vadlasandur,	S Greipsson
SSU	AJ276089	WD141–1	Claroideoglomus luteum (Glomus luteum)	authenticated	Att676-5 (formerly 676-0)	W3090	SA101-3	pot culture (details unknown	single spore	Schüßler et al. 2001	Canada, Saskatchewar	N Talukdar
SSU	Y17645	KL12-1	Claroideoglomus luteum (Glomus luteum)	authenticated	Att676-4 (formerly 676-0)	W3184	SA101-1	pot culture (details unknown	single spore	Schüßler et al. 2001	Canada, Saskatchewar	N Talukdar
SSU-ITS-LSU	FM876808	MK002-1	Claroideoglomus luteum (Glomus luteum)	authenticated	Att676-5 (formerly 676-0)	W3090	SA101-3	pot culture (details unknown	single spore	Krüger et al. 2009	Canada, Saskatchewar	N Talukdar
SSU-ITS-LSU	FM876809	MK020-1	Claroideoglomus luteum (Glomus luteum)	authenticated	Att676-5 (formerly 676-0)	W3090	SA101-3	pot culture (details unknown	single spore	Krüger et al. 2009	Canada, Saskatchewar	N Talukdar
SSU-ITS-LSU	FM876810	MK020-2	Claroideoglomus luteum (Glomus luteum)	authenticated	Att676-5 (formerly 676-0)	W3090	SA101-3	pot culture (details unknown	single spore	Krüger et al. 2009	Canada, Saskatchewar	N Talukdar
SSU-ITS-LSU	FM876811	MK020-3	Claroideoglomus luteum (Glomus luteum)	authenticated	Att676-5 (formerly 676-0)	W3090	SA101-3	pot culture (details unknown	single spore	Krüger et al. 2009	Canada, Saskatchewar	N Talukdar
SSU-ITS-LSU	FM876812	MK020-6	Claroideoglomus luteum (Glomus luteum)	authenticated	Att676-5 (formerly 676-0)	W3090	SA101-3	pot culture (details unknown	single spore	Krüger et al. 2009	Canada, Saskatchewar	N Talukdar
SSU	AJ301856	WD175-1-5	Claroideoglomus sp		Att565-11	W3349		individual spore cluste	single spore	Schwarzott et al. 2001	UK, Yorkshire, York	J Merryweather
SSU-ITS-LSU	FM876804	MK007-1	Claroideoglomus sp		Att565-11	W3349		individual spore cluste	single spore	Krüger et al. 2009	UK, Yorkshire, York	J Merryweather
SSU-ITS-LSU	FM876805	MK007-2	Claroideoglomus sp		Att565-11	W3349		individual spore cluste	single spore	Krüger et al. 2009	UK, Yorkshire, York	J Merryweather
SSU-ITS-LSU	FM876806	MK007-3	Claroideoglomus sp		Att565-11	W3349		individual spore cluste	single spore	Krüger et al. 2009	UK, Yorkshire, York	J Merryweather
SSU-ITS-LSU	FM876807	MK007-4	Claroideoglomus sp		Att565-11	W3349		individual spore cluste	single spore	Krüger et al. 2009	UK, Yorkshire, York	J Merryweather
SSU	FR750220	WD252_1_6	Claroideoglomus sp.		Att757-1	W3814		single spore (= isolate	single spore	this study	Germany, Darmstadt, Truppenübungsplatz	C Walker & A. Schüßler
SSU	FR750221	WD279_1_3	Claroideoglomus sp.		Att844-2	W3816		single spore (= isolate	single spore	this study	Mexico, Veracruz, Antigua	C Walker
SSU	AM713432	FD102-5	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Gamper et al. 2009	W'tzerland, Eschikon-Lindau	J Blaszkowsky
SSU-ITS-LSU	FN547655	HS109-27	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547656	HS109-7	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547657	HS109-22	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547658	HS109-29	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547659	HS109-2	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547660	HS109-4	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547661	HS109-5	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547662	HS109-6	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547663	HS109-15	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547664	HS109-25	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU-ITS-LSU	FN547665	HS109-43	Diversispora aurantia (Glomus aurantium	ex-type	Att1296-0	W4728	Blaskowsky 1219-T1	pot culture (details unknown	single spore	Stockinger et al. 2010	Israel, Tel-Aviv	J Blaszkowsky
SSU	AM713423	FD155-14	Diversispora celata		Att1292-2	W4758	BEG230, FACE 83	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713424	FD155-01	Diversispora celata		Att1292-2	W4758	BEG230, FACE 83	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713425	FD155-13	Diversispora celata		Att1292-2	W4758	BEG230, FACE 83	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713426	FD154-16	Diversispora celata		Att1291-2	W4757	BEG232, FACE272	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713427	FD154-14	Diversispora celata		Att1291-2	W4757	BEG232, FACE272	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713428	FD154-15	Diversispora celata		Att1291-2	W4757	BEG232, FACE272	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713420	HS004-10	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713421	HS004-9	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713422	HS004-1	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
LSU	AM713417	HS006-1	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
LSU	AM713418	HS006-6	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
LSU	AM713419	HS006-5	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU-ITS-LSU	AM713402	HS005-1	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU-ITS-LSU	AM713403	HS005-2	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU-ITS-LSU	AM713404	HS005-4	Diversispora celata	ex-type	Att1278-2	W4718/W4719	BEG231, FACE234	single spore (= isolate	multi spores (250)	Gamper et al. 2009	Switzerland, Eschikon-Lindau	H Gamper
SSU	AM713429	HS003-11	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU	AM713430	HS003-12	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU	AM713431	HS003-10	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713405	CK235-39	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713406	CK235-31	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713407	CK235-35	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713408	CK235-22	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713409	CK235-41	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713410	CK235-36	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713411	CK235-38	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713412	CK235-42	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713413	CK235-43	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713414	CK235-44	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713415	CK235-46	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz
SSU-ITS-LSU	AM713416	CK235-47	Diversispora eburnea (Glomus eburneum	ex-type	Att1290-5	W4729	AZ420A	multispore	multi spores (120)	Gamper et al. 2009	USA, Arizona, Lewis Springs	J Stutz

SSU	AJ132666	TR29-9	Diversispora epigaea (Glomus epigaeum	ex-type	none (material from BEG	none	BEG47	individual spore cluste	single spore	Schüßler et al. 2001	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU	AJ276088	WD111–2-1	Diversispora epigaea (Glomus epigaeum	ex-type	Att475–21	W3221	BEG47	individual spore cluste	single spore	Schüßler et al. 2001	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU	X86687	WD191-3-5	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Gehrig et al. 1996	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU	Y17651	TR15-B6a	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Schüßler et al. 2001	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FM876814	HS036-4	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Krüger et al. 2009	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FM876815	HS036-6	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Krüger et al. 2009	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FM876816	HS036-7	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Krüger et al. 2009	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FM876817	HS036-10	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Krüger et al. 2009	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FM876818	HS036-12	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Krüger et al. 2009	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FM876819	HS036-13	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Krüger et al. 2009	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FM876820	HS036-23	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Krüger et al. 2009	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547635	HS034-1	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547636	HS034-2	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-45	W5165	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547666	MK073-02	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547667	MK072-02	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547668	MK072-03	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547669	MK072-06	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547670	MK073-01	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547671	MK073-05	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547672	MK073-07	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547673	MK073-09	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547674	MK073-10	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547675	MK073-11	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547676	MK073-14	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547677	MK072-01	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547678	MK073-03	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547679	MK072-04	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547680	MK073-04	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FN547681	MK072-08	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Stockinger et al. 2010	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FR686938	MK072-5	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Schüßler et al. 2011	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU-ITS-LSU	FR686942	MK073-12	Diversispora epigaea (Glomus epigaeum	ex-type	Att475-22	W3180	BEG47	individual spore cluste	single spore	Schüßler et al. 2011	USA, Oregon, Corvallis, Oregon State Universit	B Hetrick nee Daniels
SSU	AJ276076	KL6	Diversispora sp		Att382-16	W2423		multispore	single spore	Schüßler et al. 2001	UK, Strathclyde Region, Cambuslang	C Walker
SSU	AJ301860	KL6-9b1	Diversispora sp		Att382-16	W2423		multispore	single spore	Schwarzott et al. 2001	UK, Strathclyde Region, Cambuslang	C Walker
SSU	AJ301863	KL6-6	Diversispora sp		Att382-16	W2423		multispore	single spore	Schwarzott et al. 2001	UK, Strathclyde Region, Cambuslang	C Walker
SSU	Y17644	ASGE-10	Diversispora sp		Att382-16	W2423		multispore	single spore	Schüßler et al. 2001	UK, Strathclyde Region, Cambuslang	C Walker
SSU	AJ276077	WD115–1-9	Diversispora spurca	ex-type	Att246–4	W3239	FCPC1000	multispore	single spore	Schüßler et al. 2001	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU	AJ276078	KL1-3a	Diversispora spurca	ex-type	Att246–4	W2396	FCPC1000	multispore	single spore	Schüßler et al. 2001	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU	FR686953	KL1-2	Diversispora spurca	ex-type	Att246-4	W2396	FCPC1000	multispore	single spore	Schüßler et al. 2011	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU	Y17649	KL1-1	Diversispora spurca	ex-type	Att246-4	W2396	FCPC1000	multispore	single spore	Schüßler et al. 2001	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU	Y17650	HG-17	Diversispora spurca	ex-type	Att246-4	W2396	FCPC1000	multispore	single spore	Schüßler et al. 2001	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU	FR686954	WD296-1-6	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Schüßler et al. 2011	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547637	HS100-25	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547638	HS100-26	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547639	HS100-21	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547640	HS100-31	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547641	HS100-2	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547642	HS100-34	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547643	HS100-5	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547644	HS100-40	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547645	HS100-28	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547646	HS100-3	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547647	HS100-4	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547648	HS100-18	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547649	HS100-19	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547650	HS100-33	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547651	HS100-24	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547652	HS100-36	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547653	HS100-38	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU-ITS-LSU	FN547654	HS100-39	Diversispora spurca	ex-type	Att246-18	W4119		single spore (= isolate	single spore	Stockinger et al. 2010	USA, Arizona, Tucson, Univ. of Arizona	M Pfeiffer
SSU	FR686956	WD294B-1-3	Diversispora trimurales		Att1152-1	W4124		multispore	single spore	Schüßler et al. 2011	Poland, Szczecin	J Blaszkowsky
SSU	FR686957	WD294-1-1	Diversispora trimurales		Att1152-1	W4124		multispore	single spore	Schüßler et al. 2011	Poland, Szczecin	J Blaszkowsky
SSU	AJ301854	TR9-11	Funneliformis caledonium (Glomus caledonium		none (material from BEG	none	BEG15	pot culture (details unknown	single spore	Schüßler et al. 2001	Denmark, Skjern	I Jakobsen
SSU	Y17653	KL10–2	Funneliformis caledonium (Glomus caledonium		none (material from BEG	none	BEG15	pot culture (details unknown	single spore	Schüßler et al. 2001	Denmark, Skjern	I Jakobsen
SSU	AJ301853	KL9-1	Funneliformis caledonium (Glomus caledonium		Att263-15	W3294	BEG20	multispore	single spore	Schwarzott et al. 2001	UK, Bedfordshire, Woburn	D Hayman
SSU	Y17635	WD115–1	Funneliformis caledonium (Glomus caledonium		Att263–15	W3294	BEG20	multispore	single spore	Schüßler et al. 2001	UK, Bedfordshire, Woburn	D Hayman
SSU-ITS-LSU	FN547494	HS031-34	Funneliformis caledonium (Glomus caledonium		Att263-15	W3294	BEG20	multispore	single spore	Stockinger et al. 2010	UK, Bedfordshire, Woburn	D Hayman
SSU-ITS-LSU	FN547495	HS031-33	Funneliformis caledonium (Glomus caledonium		Att263-15	W3294	BEG20	multispore	single spore	Stockinger et al. 2010	UK, Bedfordshire, Woburn	D Hayman
SSU-ITS-LSU	FN547496	HS031-38	Funneliformis caledonium (Glomus caledonium		Att263-15	W3294	BEG20	multispore	single spore	Stockinger et al. 2010	UK, Bedfordshire, Woburn	D Hayman
SSU-ITS-LSU	FN547497	HS031-41	Funneliformis caledonium (Glomus caledonium		Att263-15	W3294	BEG20	multispore	single spore	Stockinger et al. 2010	UK, Bedfordshire, Woburn	D Hayman
SSU-ITS-LSU	FN547498	HS031-45	Funneliformis caledonium (Glomus caledonium		Att263-15	W3294	BEG20	multispore	single spore	Stockinger et al. 2010	UK, Bedfordshire, Woburn	D Hayman
SSU-ITS-LSU	FN547499	HS031-57	Funneliformis caledonium (Glomus caledonium		Att263-15	W3294	BEG20	multispore	single spore	Stockinger et al. 2010	UK, Bedfordshire, Woburn	D Hayman
SSU	FR750212	FD001-1 (+2)	Funneliformis constrictum (Glomus constrictum)		Att756-1	W3809	Darmstad Att6-7	single spore (= isolate	single spore	this study	Germany, Darmstadt, Truppenübungsplatz	C Walker & A. Schüßler
SSU	Y17637	TR27B-10	Funneliformis coronatum (Glomus coronatum		none (material from BEG	none	BEG22	multispore	single spore	Schüßler et al. 2001	Australia, South Australia, Loxton	V Gianinazzi-Pearson
SSU	AJ276086	WD93–2-1	Funneliformis coronatum (Glomus coronatum		Att1143–5	W3153	COG1	single spore (= isolate	single spore	Schüßler et al. 2001	Australia, New South Wales, Sydney	P McGee
SSU	FR773144	WD203_1_1	Funneliformis coronatum (Glomus coronatum	ex-type	Att108-7	W3582	BEG28, UY285	multispore	single spore	this study	Italy, Tuscany, Folonica	M Giovannetti
SSU	FR773145	WD203_1_4	Funneliformis coronatum (Glomus coronatum	ex-type	Att108-7	W3582	BEG28, UY285	multispore	single spore	this study	Italy, Tuscany, Folonica	M Giovannetti
SSU-ITS-LSU	FM876794	MK028-8	Funneliformis coronatum (Glomus coronatum	ex-type	Att108-7	W3582	BEG28, UY285	multispore	single spore	Krüger et al. 2009	Italy, Tuscany, Follonica	M Giovannetti
SSU-ITS-LSU	FM876795	MK028-9	Funneliformis coronatum (Glomus coronatum	ex-type	Att108-7	W3582	BEG28, UY285	multispore	single spore	Krüger et al. 2009	Italy, Tuscany, Follonica	M Giovannetti
SSU-ITS-LSU	FM876796	MK028-10	Funneliformis coronatum (Glomus coronatum	ex-type	Att108-7	W3582	BEG28, UY285	multispore	single spore	Krüger et al. 2009	Italy, Tuscany, Follonica	M Giovannetti
SSU-ITS-LSU	FM876797	MK028-11	Funneliformis coronatum (Glomus coronatum	ex-type	Att108-7	W3582	BEG28, UY285	multispore	single spore	Krüger et al. 2009	Italy, Tuscany, Follonica	M Giovannetti
SSU-ITS-LSU	FM876798	MK028-12	Funneliformis coronatum (Glomus coronatum	ex-type	Att108-7	W3582	BEG28, UY285	multispore	single spore	Krüger et al. 2009	Italy, Tuscany, Follonica	M Giovannetti
SSU	AJ276085	WD114–3-3	Funneliformis fragilistratum (Glomus fragilistratum	ex-type	Att112–6	W3238		multispore	single spore	Schüßler et al. 2001	Denmark, Skjern/Ringkøbing, Hanning	I Jakobsen
SSU	AJ132664	TR12B-6a	Funneliformis geosporum (Glomus geosporum		none (material from BEG	none	BEG11	single spore (= isolate	single spore	Schüßler et al. 2001	UK, Kent, Ramsgate	J Dodd
SSU	AJ245637	KL11-1a	Funneliformis geosporum (Glomus geosporum		none (material from BEG	none	BEG11	single spore (= isolate	single spore	Schüßler et al. 2001	UK, Kent, Ramsgate	J Dodd
SSU	Y17643	TR12C-8	Funneliformis geosporum (Glomus geosporum		none (material from BEG	none	BEG11	single spore (= isolate	single spore	Schüßler et al. 2001	UK, Kent, Ramsgate	J Dodd
SSU	FR750227	WD319_2_2	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-25	W4540	BEG12	single spore (= isolate	single spore	this study	England, Kent, East Malling	B Mosse (isolated by L Whitfield
SSU-ITS-LSU	FN547474	HS101-C1	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547475	HS101-A1	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547476	HS110-38	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547482	HS101-F1	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547483	HS110-22	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547484	HS101-C2	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547485	HS110-39	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547486	HS110-37	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547487	HS110-9	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547488	HS101-B2	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547489	HS101-D2	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547490	HS110-4	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547491	HS101-B1	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547492	HS101-D1	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FN547493	HS110-15	Funneliformis mosseae (Glomus mosseae	epitype predecessor	Att109-20	W5147	BEG12	single spore (= isolate	single spore	Stockinger et al. 2010	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750024	CK081-1	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750025	CK081-10	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750026	CK081-11	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750027	CK081-2	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750028	CK081-3	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750029	CK081-4	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750030	CK081-6	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750031	CK081-7	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750032	CK081-8	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU-ITS-LSU	FR750033	CK081-9	Funneliformis mosseae (Glomus mosseae	epitype	Att109-28	W5790	BEG12	single spore (= isolate	single spore	this study	UK, Kent, East Malling	B Mosse
SSU	AJ301864	WD160-1-1	Funneliformis sp.		Att15-5	W2940	WUM3	multispore	single spore (dark m	Schwarzott et al. 2001	Australia, Merredin	W Porter
SSU	AJ301865	WD169-1-4	Funneliformis sp.		Att15-5	W2939	WUM3	multispore	single spore (pale m	Schwarzott et al. 2001	Australia, Merredin	W Porter
SSU-ITS-LSU	FM876813	MK010-01	Funneliformis sp.		Att15-5	W2940	WUM3	multispore	single spore	Krüger et al. 2009	Australia, Merredin	W Porter
SSU-ITS-LSU	FN547477	MK023-4	Funneliformis sp.		Att15-5	W2939	WUM3	multispore	single spore	Stockinger et al. 2010	Australia, Merredin	W Porter
SSU-ITS-LSU	FN547478	MK023-7	Funneliformis sp.		Att15-5	W2939	WUM3	multispore	single spore	Stockinger et al. 2010	Australia, Merredin	W Porter
SSU-ITS-LSU	FN547479	MK023-2	Funneliformis sp.		Att15-5	W2939	WUM3	multispore	single spore	Stockinger et al. 2010	Australia, Merredin	W Porter
SSU-ITS-LSU	FN547480	MK023-10	Funneliformis sp.		Att15-5	W2939	WUM3	multispore	single spore	Stockinger et al. 2010	Australia, Merredin	W Porter
SSU-ITS-LSU	FN547481	MK023-8	Funneliformis sp.		Att15-5	W2939	WUM3	multispore	single spore	Stockinger et al. 2010	Australia, Merredin	W Porter

SSU	Y15905	HGGeo1-Ba	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Schüßler et al. 2001	Germany, Bieber, Biebergemünc	A Schüßler
SSU	Y17831	TR17-2	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Schüßler et al. 2001	Germany, Bieber, Biebergemünc	A Schüßler
SSU	AJ276074	HGGeo1-Ca	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Schüßler et al. 2001	Germany, Bieber, Biebergemünc	A Schüßler
SSU	AM183923	WD205-1-1	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	James et al. 2006	Germany, Bieber, Biebergemünc	A Schüßler
SSU	X86686	GEO2 (=GEOB2)	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	multi spore	Gehrig et al. 1996	Germany, Bieber, Biebergemünc	A Schüßler
SSU	Y15904	HGGeo1-Aa	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Schüßler et al. 2001	Germany, Bieber, Biebergemünc	A Schüßler
SSU-5.8S-LSU	FM876840	MK044-1	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Krüger et al. 2009	Germany, Bieber, Biebergemünc	A Schüßler
SSU-5.8S-LSU	FM876841	MK044-23	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Krüger et al. 2009	Germany, Bieber, Biebergemünc	A Schüßler
SSU-5.8S-LSU	FM876842	MK044-36	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Krüger et al. 2009	Germany, Bieber, Biebergemünc	A Schüßler
SSU-5.8S-LSU	FM876843	MK044-35	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Krüger et al. 2009	Germany, Bieber, Biebergemünc	A Schüßler
SSU-5.8S-LSU	FM876844	MK044-9	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	Krüger et al. 2009	Germany, Bieber, Biebergemünc	A Schüßler
5.8S	AM268204	FD112-2	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	James et al. 2006	Germany, Bieber, Biebergemünc	A Schüßler
LSU	AM183920	pAS36A+C.2, pAS3	Geosiphon pyriformis		none (cyanobacteria symbiosis		GEO1	multi-bladders	single spore	James et al. 2006	Germany, Bieber, Biebergemünc	A Schüßler
SSU	AJ276091	WD131–7	Gigaspora candida		Att26–19	W3292	BEG17	single spore (= isolate	single spore	Schüßler et al. 2001	Republic of China, Taiwar	W Chou
SSU	Y17646	TR28–1a	Gigaspora margarita		none (material from BEG	none	BEG34	multispore	single spore	Schüßler et al. 2001	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FR750039	CK083-1	Gigaspora margarita		Att256-18	W5792	BEG34	multispore	single spore	this study	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FR750040	CK083-2	Gigaspora margarita		Att256-18	W5792	BEG34	multispore	single spore	this study	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FR750041	CK083-3	Gigaspora margarita		Att256-18	W5792	BEG34	multispore	single spore	this study	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FR750042	CK083-4	Gigaspora margarita		Att256-18	W5792	BEG34	multispore	single spore	this study	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FR750043	CK083-6	Gigaspora margarita		Att256-18	W5792	BEG34	multispore	single spore	this study	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FR750044	CK083-7	Gigaspora margarita		Att256-18	W5792	BEG34	multispore	single spore	this study	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FR750045	CK083-8	Gigaspora margarita		Att256-18	W5792	BEG34	multispore	single spore	this study	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547547	HS108-10	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547548	HS108-11	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547549	HS108-12	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547550	HS108-13	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547551	HS108-14	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547552	HS108-16	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547553	HS108-18	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547554	HS108-19	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547555	HS108-21	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547556	HS108-23	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547557	HS108-24	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547558	HS108-27	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547559	HS108-28	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547560	HS108-31	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547561	HS108-32	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547562	HS108-36	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547563	HS108-4	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547564	HS108-41	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547565	HS108-43	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547566	HS108-45	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547567	HS108-47	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547568	HS108-5	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547569	HS108-6	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU-ITS-LSU	FN547570	HS108-7	Gigaspora margarita		none (material from Univ. Torino	none	BEG34	multispore ROC	single spore	Stockinger et al. 2010	Unknown, possibly New Zealanc	Uncertain, possibly J Crush
SSU	Y17647	TR26-6	Gigaspora rosea		none (material from BEG	none	BEG9	multispore	single spore	Schüßler et al. 2001	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547571	HS105-E10	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547572	HS105-E7	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547573	HS105-E8	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547574	HS105-E9	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547575	HS105-F10	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547576	HS105-F7	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547577	HS105-F8	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547578	HS105-F9	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547579	HS105-G10	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547580	HS105-H10	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547581	HS105-H7	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547582	HS106-B11	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547583	HS106-C11	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547584	HS106-D11	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547585	HS106-E12	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547586	HS106-F11	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547587	HS106-H11	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547588	HS104-C4	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547589	HS104-F3	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547590	HS104-G3	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547591	HS105-B7	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547592	HS105-C7	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547593	HS105-C8	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547594	HS105-C9	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547595	HS105-D5	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547596	HS105-D8	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FN547597	HS105-D9	Gigaspora rosea		Att1509-19	W5384	DAOM194757	multispore	single spore	Stockinger et al. 2010	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750174	MK097-1	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750175	MK097-10	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750176	MK097-11	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750177	MK097-12	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750178	MK097-13	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750179	MK097-2	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750180	MK097-3	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750181	MK097-4	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750182	MK097-5	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750183	MK097-6	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750184	MK097-7	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU-ITS-LSU	FR750185	MK097-8	Gigaspora rosea		Att1509-13	W2856	DAOM194757	multispore (2 spores) ROC	single spore	this study	Unknown, thought to be from USA	Unknown collector
SSU	AJ276090	WD143–12	Gigaspora sp.		none	W2992		field collected spores (sand dunes	single spore	Schüßler et al. 2001	Argentina, Buenos Aires, Tres Arroya	M Cabellc
SSU-ITS-LSU	FM876800	MK021-6	Gigaspora sp.		none	W2992		field collected spores (sand dunes	single spore	Krüger et al. 2009	Argentina, Buenos Aires, Tres Arroya	M Cabellc
SSU-ITS-LSU	FM876801	MK021-2	Gigaspora sp.		none	W2992		field collected spores (sand dunes	single spore	Krüger et al. 2009	Argentina, Buenos Aires, Tres Arroya	M Cabellc
SSU-ITS-LSU	FM876802	MK021-1	Gigaspora sp.		none	W2992		field collected spores (sand dunes	single spore	Krüger et al. 2009	Argentina, Buenos Aires, Tres Arroya	M Cabellc
SSU-ITS-LSU	FM876803	MK003-1	Gigaspora sp.		none	W2992		field collected spores (sand dunes	single spore	Krüger et al. 2009	Argentina, Buenos Aires, Tres Arroya	M Cabellc
SSU-ITS-LSU	FM876799	MK021-10	Gigaspora sp.		none	W2992		field collected spores (sand dunes	single spore	Krüger et al. 2009	Argentina, Buenos Aires, Tres Arroya	M Cabellc
SSU	FR750376	MK111-4	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750526	HS093-32	Glomus macrocarpum		none	W5288		field collected spores (sporocarp	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750527	HS093-34	Glomus macrocarpum		none	W5288		field collected spores (sporocarp	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750528	HS093-45	Glomus macrocarpum		none	W5288		field collected spores (sporocarp	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750529	HS093-48	Glomus macrocarpum		none	W5288		field collected spores (sporocarp	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750530	HS093-49	Glomus macrocarpum		none	W5288		field collected spores (sporocarp	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750531	HS094-1	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750532	HS094-10	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750533	HS094-11	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750534	HS094-12	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750535	HS094-14	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750536	HS094-15	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750537	HS094-16	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750538	HS094-17 (+19+22)	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750539	HS094-18	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750540	HS094-20 (+21)	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750541	HS094-30	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750542	HS094-4	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750544	HS094-6	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU-ITS-LSU	FR750543	HS094-5	Glomus macrocarpum		none	W5293		field collected spores (sporocarp	single spore	this study	UK, Gloucestershire, Painswick	C Walker
SSU	FR772325	MK112-3	Glomus macrocarpum	ex-epitype	Att1495-0	W5605		multispore (sporocarp fragment	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750363	CK076-1	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750364	CK076-10	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750365	CK076-11	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750366	CK076-13	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750367	CK076-2 (+3)	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750368	CK076-4	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750369	CK076-5	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750370	CK076-6 (+7+8+14)	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker
SSU-ITS-LSU	FR750371	CK076-9	Glomus macrocarpum	epitype	Att1495-0	W5581		multispore (from sporocarp W5288	single spore	this study	UK, Cardiff, Morganstowr	C Walker

SSU	AJ301857	WD145-6-4	Glomus sp.		Att565-7	W3347		individual spore cluste	single spore	Schwarzott et al. 2001	UK, Yorkshire, Pretty Wood	J Merryweather
SSU-ITS-LSU	FR750201	MK110-1 (+6)	Glomus sp.		Att565-7	W3347		individual spore cluste	single spore	this study	UK, Yorkshire, Pretty Wood	J Merryweather
SSU-ITS-LSU	FR750202	MK110-2 (+3+5)	Glomus sp.		Att565-7	W3347		individual spore cluste	single spore	this study	UK, Yorkshire, Pretty Wood	J Merryweather
SSU-ITS-LSU	FR750203	MK110-4	Glomus sp.		Att565-7	W3347		individual spore cluste	single spore	this study	UK, Yorkshire, Pretty Wood	J Merryweather
SSU	Y17652	WD107–1-2	Glomus viscosum (contaminant?)		Att179–8	W3207	BEG27	multispore	single spore	Schüßler et al. 2001	Europe, from compost of unknown origir	M Giovannetti
SSU	FR750224	WD315_2_1	Pacispora franciscana		Att961-1	W3850		multispore	single spore	this study	Poland, Pomerani, Lipki	J Błaszkowsky
SSU	FR750225	WD315_2_4	Pacispora franciscana		Att961-1	W3850		multispore	single spore	this study	Poland, Pomerani, Lipki	J Błaszkowsky
SSU	FR750226	WD315_2_5	Pacispora franciscana		Att961-1	W3850		multispore	single spore	this study	Poland, Pomerani, Lipki	J Błaszkowsky
SSU	FR750219	WD121_3	Pacispora franciscana		Att599-7	W3251		soil trap	single spore	this study	Germany, Niedersachsen, Braunschweic	P Vandenkoornhuyse
SSU	FR750375	WD121-3	Pacispora franciscana		Att599-7	W3251		soil trap	single spore	this study	Germany, Niedersachsen, Braunschweic	P Vandenkoornhuyse
SSU	AJ619944	WD273-3-2	Pacispora scintillans		Att961-1	W3849		multispore	single spore	Walker et al. 2004	Poland, Pomerani, Lipki	J Błaszkowsky
SSU	AJ619945	WD273-3-3	Pacispora scintillans		Att961-1	W3849		multispore	single spore	Walker et al. 2004	Poland, Pomerani, Lipki	J Błaszkowsky
SSU	AJ619946	WD273-3-5	Pacispora scintillans		Att961-1	W3849		multispore	single spore	Walker et al. 2004	Poland, Pomerani, Lipki	J Błaszkowsky
SSU	AJ619947	WD273-3-1	Pacispora scintillans		Att961-1	W3849		multispore	single spore	Walker et al. 2004	Poland, Pomerani, Lipki	J Błaszkowsky
SSU	AJ619940	WD245-1-1	Pacispora scintillans		none	W3793		field collected spores	single spore	Walker et al. 2004	Griesheim, Germany	C Walker & A. Schüßler
SSU	AJ619941	WD245-1-2	Pacispora scintillans		none	W3793		field collected spores	single spore	Walker et al. 2004	Griesheim, Germany	C Walker & A. Schüßler
SSU	AJ619942	WD245-1-5	Pacispora scintillans		none	W3793		field collected spores	single spore	Walker et al. 2004	Griesheim, Germany	C Walker & A. Schüßler
SSU	AJ619943	WD245-1-6	Pacispora scintillans		none	W3793		field collected spores	single spore	Walker et al. 2004	Griesheim, Germany	C Walker & A. Schüßler
SSU	AJ619948	WD274-3-1	Pacispora scintillans		none	W3862		field collected spores	single spore	Walker et al. 2004	UK, Dorset, East Lulworth	C Walker
SSU	AJ619949	WD274-3-2	Pacispora scintillans		none	W3862		field collected spores	single spore	Walker et al. 2004	UK, Dorset, East Lulworth	C Walker
SSU	AJ619950	WD274-3-3	Pacispora scintillans		none	W3862		field collected spores	single spore	Walker et al. 2004	UK, Dorset, East Lulworth	C Walker
SSU	AJ619951	WD274-3-4	Pacispora scintillans		none	W3862		field collected spores	single spore	Walker et al. 2004	UK, Dorset, East Lulworth	C Walker
SSU	AJ619952	WD200-2-3	Pacispora scintillans		none	W4545		field collected spores	single spore	Walker et al. 2004	Griesheim, Germany	C Walker & A. Schüßler
SSU	AJ619953	WD200-2-4	Pacispora scintillans		none	W4545		field collected spores	single spore	Walker et al. 2004	Griesheim, Germany	C Walker & A. Schüßler
SSU	AJ619954	WD200-2-5	Pacispora scintillans		none	W4545		field collected spores	single spore	Walker et al. 2004	Griesheim, Germany	C Walker & A. Schüßler
SSU	AJ619955	WD200-2-6	Pacispora scintillans		none	W4545		field collected spores	single spore	Walker et al. 2004	Griesheim, Germany	C Walker & A. Schüßler
SSU-ITS-LSU	FM876831	MK027-1	Pacispora scintillans		none	W4545		field collected spores	single spore	Krüger et al. 2009	Griesheim, Germany	C Walker & A. Schüßler
SSU-ITS-LSU	FM876832	MK027-2	Pacispora scintillans		none	W4545		field collected spores	single spore	Krüger et al. 2009	Griesheim, Germany	C Walker & A. Schüßler
SSU-ITS-LSU	FR750046	CK084-1	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750047	CK084-10	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750048	CK084-12	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750049	CK084-13	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750050	CK084-4	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750051	CK084-5 (+11+14)	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750052	CK084-6	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750053	CK084-7	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750054	CK084-8	Paraglomus brasilianum		Att260-8	W5793		single spore (= isolate	single spore	this study	Brazil, Minus, Januaria	J Spain
SSU-ITS-LSU	FR750083	HS038-1	Paraglomus laccatum		Att960-3	W5141		multispore	single spore	this study	UK, from compost of unknown origin	C Walker
SSU	AJ276081	WD108–2-1	Paraglomus occultum		Att677–4	W3166	IA702–3	pot culture (details unknown)	single spore	Schüßler et al. 2001	USA, Iowa, Boone	N Klopfenstein for C Walker
SSU	AJ276082	WD117–1-1	Paraglomus occultum		Att677–3	W3091	IA702–3	pot culture (details unknown)	single spore	Schüßler et al. 2001	USA, Iowa, Boone	N Klopfenstein for C Walker
SSU	AJ306435	WD167-1-6	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	Schüßler et al. 2001	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750136	MK022-05	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750137	MK022-06	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750138	MK022-07	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750139	MK022-10	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750140	MK022-11	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750141	MK022-13	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750142	MK022-17	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750143	MK022-19	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750144	MK022-20	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750145	MK022-22	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750146	MK022-23	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750147	MK022-24	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750148	MK022-25	Racocetra fulgida (Scutellospora fulgida		none	W2993		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU	AJ306444	WD170-1-4	Racocetra weresubiae (Scutellospora weresubiae		none	W2988		field collected spores	single spore	Schüßler et al. 2001	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750134	MK011-1	Racocetra weresubiae (Scutellospora weresubiae		none	W2988		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU-ITS-LSU	FR750135	MK011-2	Racocetra weresubiae (Scutellospora weresubiae		none	W2988		field collected spores	single spore	this study	Argentina, Buenos Aires, Tres Arroya	M Cabello
SSU	AJ276084	WD125–1	Rhizophagus clarus (Glomus clarum)		Att72–1	W3163	BR147B-8	pot culture (details unknown)	single spore	Schüßler et al. 2001	Brazil (proprietary; no data	M Lin
SSU	FR773148	WD236_1_4	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	this study	Iceland, Sanda	S Greipsson
SSU	FR773149	WD236_1_3	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	this study	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865536	HS029-10	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865537	HS029-17	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865538	HS029-2	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865539	HS029-22	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865540	HS029-24	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865541	HS029-26	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865542	HS029-28	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865543	HS029-29	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU-ITS-LSU	FM865544	HS029-6	Rhizophagus clarus (Glomus clarum)		Att894-7	W3776		single spore (= isolate	single spore	Stockinger et al. 2009	Iceland, Sanda	S Greipsson
SSU	Y17640	KL5–3	Rhizophagus fasciculatus (Glomus fasciculatum		none (material from BEG	none	BEG53	single spore (= isolate	single spore	Schüßler et al. 2001	Canada, Quebec, La Pocatière	V Furlan
SSU	FR750205	CK087-1	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-70	W5570	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU	FR750206	CK087-2	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-70	W5570	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU	FR750207	CK087-3	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-70	W5570	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU	FR750208	CK087-4	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-70	W5570	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU	FR750209	CK087-5	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-70	W5570	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU	FR750210	CK087-6	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-70	W5570	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU	FR750211	CK087-7	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-70	W5570	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865597	HS099-11	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865598	HS099-14	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865599	HS099-16	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865600	HS099-3	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865601	HS099-32	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865602	HS099-36	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865603	HS099-40	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865604	HS099-41	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865605	HS099-47	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865606	HS099-6	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865607	HS099-8	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-57	W5507	FL208 (MUCL49413)	one spore cluster from ROC (= isolate	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865545	HS051-14	Rhizophagus intraradices (Glomus intraradices	re-isolate from type locality	Att1102-12	W5070	MUCL49410	ROC	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865546	HS051-20	Rhizophagus intraradices (Glomus intraradices	re-isolate from type locality	Att1102-12	W5070	MUCL49410	ROC	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865547	HS051-24	Rhizophagus intraradices (Glomus intraradices	re-isolate from type locality	Att1102-12	W5070	MUCL49410	ROC	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865548	HS051-39	Rhizophagus intraradices (Glomus intraradices	re-isolate from type locality	Att1102-12	W5070	MUCL49410	ROC	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865549	HS051-49	Rhizophagus intraradices (Glomus intraradices	re-isolate from type locality	Att1102-12	W5070	MUCL49410	ROC	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865559	HS080-12	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865560	HS080-16	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865561	HS086-2	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865562	HS086-4	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865563	HS086-5	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865564	HS089-11	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865565	HS089-14	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865566	HS089-15	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865567	HS089-16	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865568	HS089-17	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865569	HS089-19	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865570	HS089-6	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865571	HS089-7	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865572	HS096-10	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-41	W5413	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865573	HS096-11	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-41	W5413	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865574	HS096-12	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-41	W5413	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865575	HS096-16	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-41	W5413	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865576	HS096-19	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-41	W5413	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865577	HS096-22	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-41	W5413	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865578	HS096-27	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-41	W5413	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FM865579	HS096-28	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-41	W5413	FL208	root fragment	single spore	Stockinger et al. 2009	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FR750372	HS089-18	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5273	FL208	root fragment	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FR750126	HS080-32	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragments	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec
SSU-ITS-LSU	FR750127	HS080-7	Rhizophagus intraradices (Glomus intraradices	ex-type	Att4-38	W5166	FL208	root fragments	single spore	this study	USA, Orlando, Clermont-Minneola	S. Nemec

SSU	FR750223	WD313_1_1	Rhizophagus irregularis (Glomus irregulare		Att1225-1	W4533	LW139	single spore (= isolate	single spore	this study	UK, Berkshire, Maidenheac	L Whitfield
SSU	FR750222	WD303_6_3	Rhizophagus irregularis (Glomus irregulare)		none (material from GINCO)	none	DAOM212349 (=MUCL43195) (note: DAOM212349 is used in the literature for two different cultures of different species; see Cl. lamellosum entries)	ROC	spores from ROC	this study	Canada, Ontario, Wasaga Beach Provincial Park	GINCO (G Mitrow, Y Dalpé)
SSU	FR750228	WDG_lam_3	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM212349 (=MUCL43195)	ROC	spores from ROC	this study	Canada, Ontario, Wasaga Beach Provincial Park	GINCO (G Mitrow, Y Dalpé)
SSU-ITS-LSU	FR750078	HS037-10	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM212349 (=MUCL43195)	ROC	single spore	this study	Canada, Ontario, Wasaga Beach Provincial Park	GINCO (G Mitrow, Y Dalpé)
SSU-ITS-LSU	FR750079	HS037-23	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM212349 (=MUCL43195)	ROC	single spore	this study	Canada, Ontario, Wasaga Beach Provincial Park	GINCO (G Mitrow, Y Dalpé)
SSU-ITS-LSU	FR750080	HS037-4	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM212349 (=MUCL43195)	ROC	single spore	this study	Canada, Ontario, Wasaga Beach Provincial Park	GINCO (G Mitrow, Y Dalpé)
SSU-ITS-LSU	FR750081	HS037-5	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM212349 (=MUCL43195)	ROC	single spore	this study	Canada, Ontario, Wasaga Beach Provincial Park	GINCO (G Mitrow, Y Dalpé)
SSU-ITS-LSU	FR750082	HS037-8	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM212349 (=MUCL43195)	ROC	single spore	this study	Canada, Ontario, Wasaga Beach Provincial Park	GINCO (G Mitrow, Y Dalpé)
SSU-ITS-LSU	FR750084	HS043-17	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	FTRS203	ROC	single spore	this study	Unknown	sent from LLN by S Cranenbrouck
SSU-ITS-LSU	FR750085	HS043-4	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	FTRS203	ROC	single spore	this study	Unknown	sent from LLN by S Cranenbrouck
SSU-ITS-LSU	FR750087	HS043-7	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	FTRS203	ROC	single spore	this study	Unknown	sent from LLN by S Cranenbrouck
SSU-ITS-LSU	FR750086	HS043-6	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	FTRS203	ROC	single spore	this study	Unknown	sent from LLN by S Cranenbrouck
SSU-ITS-LSU	FR750106	HS058-1 (+23+44)	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750107	HS058-10 (+40)	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750108	HS058-11	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750109	HS058-12	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750110	HS058-13	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750111	HS058-15	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750112	HS058-2	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750113	HS058-27	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750114	HS058-30	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750115	HS058-38	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750116	HS058-6 (+29)	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750117	HS058-7	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL43205	ROC	single spore	this study	Canada, Québec, Terrebonne	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750089	HS050-25	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL46240	ROC	single spore	this study	Canada, Québec, Buckingham	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750088	HS050-1	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL46240	ROC	single spore	this study	Canada, Québec, Buckingham	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750090	HS050-30	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL46240	ROC	single spore	this study	Canada, Québec, Buckingham	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750091	HS050-44	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL46240	ROC	single spore	this study	Canada, Québec, Buckingham	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750101	HS057-14 (+19)	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL41833	single vesicle (spore?) from roo	single spore	this study	Spain, Canary Islands	GINCO
SSU-ITS-LSU	FR750102	HS057-17	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL41833	single vesicle (spore?) from roo	single spore	this study	Spain, Canary Islands	GINCO
SSU-ITS-LSU	FR750103	HS057-23	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL41833	single vesicle (spore?) from roo	single spore	this study	Spain, Canary Islands	GINCO
SSU-ITS-LSU	FR750104	HS057-24	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL41833	single vesicle (spore?) from roo	single spore	this study	Spain, Canary Islands	GINCO
SSU-ITS-LSU	FR750105	HS057-39	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	MUCL41833	single vesicle (spore?) from roo	single spore	this study	Spain, Canary Islands	GINCO
SSU-ITS-LSU	FR750191	MK108-1	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750192	MK108-10	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750193	MK108-2	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750194	MK108-3	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750195	MK108-4	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750196	MK108-5	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750197	MK108-6	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750198	MK108-7	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750199	MK108-8	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750200	MK108-9	Rhizophagus irregularis (Glomus irregulare		none	none		soil trap	single spore	this study	Poland, location unknown	J Blaszkowsky
SSU-ITS-LSU	FR750186	MK100-1	Rhizophagus irregularis (Glomus irregulare		Att857-12	W4682	WUM38	single spore (= isolate	single spore	this study	Australia, Argyle	K. Clarke
SSU-ITS-LSU	FR750187	MK100-2 (+3+6)	Rhizophagus irregularis (Glomus irregulare		Att857-12	W4682	WUM38	single spore (= isolate	single spore	this study	Australia, Argyle	K. Clarke
SSU-ITS-LSU	FR750188	MK100-4	Rhizophagus irregularis (Glomus irregulare		Att857-12	W4682	WUM38	single spore (= isolate	single spore	this study	Australia, Argyle	K. Clarke
SSU-ITS-LSU	FR750189	MK100-5	Rhizophagus irregularis (Glomus irregulare		Att857-12	W4682	WUM38	single spore (= isolate	single spore	this study	Australia, Argyle	K. Clarke
SSU-ITS-LSU	FR750190	MK100-7	Rhizophagus irregularis (Glomus irregulare		Att857-12	W4682	WUM38	single spore (= isolate	single spore	this study	Australia, Argyle	K. Clarke
SSU-ITS-LSU	FR750064	HS027-1	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM197198 (=DAOM181602, =MUCL43194)	multispore ROC	single spore	this study	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FR750065	HS027-18	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM197198 (=DAOM181602, =MUCL43194)	multispore ROC	single spore	this study	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FR750066	HS027-2	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM197198 (=DAOM181602, =MUCL43194)	multispore ROC	single spore	this study	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FR750067	HS027-21	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM197198 (=DAOM181602, =MUCL43194)	multispore ROC	single spore	this study	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FR750068	HS027-24	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM197198 (=DAOM181602, =MUCL43194)	multispore ROC	single spore	this study	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FR750069	HS027-27	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM197198 (=DAOM181602, =MUCL43194)	multispore ROC	single spore	this study	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FR750070	HS027-6	Rhizophagus irregularis (Glomus irregulare		none (material from GINCO)	none	DAOM197198 (=DAOM181602, =MUCL43194)	multispore ROC	single spore	this study	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865550	HS059-1	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865551	HS059-12	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865552	HS059-20	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865553	HS059-23	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865554	HS059-2	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865555	HS059-38	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865556	HS059-47	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865557	HS059-7	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865558	HS059-9	Rhizophagus irregularis (Glomus irregulare		Att1192-44	W5533	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865608	MK009-1	Rhizophagus irregularis (Glomus irregulare		Att1192-53	W3182	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865609	MK009-3	Rhizophagus irregularis (Glomus irregulare		Att1192-53	W3182	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865610	MK009-4	Rhizophagus irregularis (Glomus irregulare		Att1192-53	W3182	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865611	MK041-10	Rhizophagus irregularis (Glomus irregulare		Att1192-27	W5495	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	multi spores (3)	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865612	MK041-20	Rhizophagus irregularis (Glomus irregulare		Att1192-27	W5495	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	multi spores (3)	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865613	MK041-23	Rhizophagus irregularis (Glomus irregulare		Att1192-27	W5495	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	multi spores (3)	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865614	MK041-24	Rhizophagus irregularis (Glomus irregulare		Att1192-27	W5495	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	multi spores (3)	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865615	MK041-7	Rhizophagus irregularis (Glomus irregulare		Att1192-27	W5495	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	multi spores (3)	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865616	MK041-9	Rhizophagus irregularis (Glomus irregulare		Att1192-27	W5495	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	multi spores (3)	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM865617	MK041-21	Rhizophagus irregularis (Glomus irregulare		Att1192-27	W5495	DAOM197198 (=DAOM181602, =MUCL43194)	ROC	multi spores (3)	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992377	HS111-8	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992378	HS111-20	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992379	HS111-22	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992380	HS111-44	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992381	HS111-21	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992382	HS111-6	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992383	HS111-48	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992384	HS111-16	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992385	HS111-7	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992386	HS111-43	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU-ITS-LSU	FM992387	HS111-41	Rhizophagus irregularis (Glomus irregulare		Att690-23	W5499	DAOM197198 (=DAOM181602, =MUCL43194)	root fragment	single spore	Stockinger et al. 2009	Canada, Québec, Pont Rouge	GINCO (C Plenchette, V Furlan)
SSU	Y17648	WD113-4-1	Rhizophagus manihotis (Glomus manihotis	ex-type	Att575–9	W3224	CIAT C-1-1	multispore	single spore	Schüßler et al. 2001	Colombia, Cauca, Santander de Quilacha	R Howeler
SSU	FR773146	WD82_2_1	Rhizophagus manihotis (Glomus manihotis		Att1597-10	W3095	FL879-6	pot culture (details unknown)	single spore	this study	USA, Florida, White Springs	D Sylvia
SSU	FR773147	WD82_2_2	Rhizophagus manihotis (Glomus manihotis		Att1597-10	W3095	FL879-6	pot culture (details unknown)	single spore	this study	USA, Florida, White Springs	D Sylvia
SSU	Y17638	TR31-11	Rhizophagus manihotis (Glomus manihotis		none (material from INVAM)	none	FL879-3	pot culture (details unknown)	single spore	Schüßler et al. 2001	USA, Florida, White Springs	D Sylvia
SSU-ITS-LSU	FN547500	HS113-5	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2010	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FN547501	HS113-36	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2010	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992388	HS113-1	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992389	HS113-14	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992390	HS113-17	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992391	HS113-2	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992392	HS113-20	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992393	HS113-21	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992394	HS113-25	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992395	HS113-26	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992396	HS113-27	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992397	HS113-29	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992398	HS113-33	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992399	HS113-8	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	single spore	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992400	HS116-IIC4	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	multi spores (10)	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992401	HS116-IIC6	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	multi spores (10)	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU-ITS-LSU	FM992402	HS117-IID	Rhizophagus proliferus (Glomus proliferum	ex-type	none (material from GINCO)	none	DAOM226389 (=MUCL41827)	root fragment ROC	multi spores (10)	Stockinger et al. 2009	Guadeloupe, Capastere-Bel-Eau, Neufchatea	J Riseé
SSU	FR750373	WD194-1-1	Rhizophagus sp.		none	W3563		field collected spores	single spore	this study	Uganda, Kigeza, Kisor	T Pettitt
SSU-ITS-LSU	FR750092	HS055-34	Rhizophagus sp. (Glomus cerebriforme, but questionable determinatior		none (material from GINCO)	none	DAOM227022 (=MUCL43208)	ROC	single spore	this study	Canada, Québec, Lotbinière	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750093	HS055-39	Rhizophagus sp. (Glomus cerebriforme, but questionable determinatior		none (material from GINCO)	none	DAOM227022 (=MUCL43208)	ROC	single spore	this study	Canada, Québec, Lotbinière	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750094	HS055-43	Rhizophagus sp. (Glomus cerebriforme, but questionable determinatior		none (material from GINCO)	none	DAOM227022 (=MUCL43208)	ROC	single spore	this study	Canada, Québec, Lotbinière	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750095	HS055-47	Rhizophagus sp. (Glomus cerebriforme, but questionable determinatior		none (material from GINCO)	none	DAOM227022 (=MUCL43208)	ROC	single spore	this study	Canada, Québec, Lotbinière	GINCO (Y Dalpé, S Seguin)
SSU-ITS-LSU	FR750071	HS033-11	Rhizophagus sp. (Glomus sp.		none (material from GINCO)	none	MUCL46100	ROC (unknown if isolate	single spore	this study	Unknown	GINCO (Strullu)
SSU-ITS-LSU	FR750072	HS033-2	Rhizophagus sp. (Glomus sp.		none (material from GINCO)	none	MUCL46100	ROC (unknown if isolate	single spore	this study	Unknown	GINCO (Strullu)
SSU-ITS-LSU	FR750073	HS033-9	Rhizophagus sp. (Glomus sp.		none (material from GINCO)	none	MUCL46100	ROC (unknown if isolate	single spore	this study	Unknown	GINCO (Strullu)
SSU	FR750374	WD193-2-3	Rhizophagus vesiculiferus (Glomus vesiculiferum		Att14-8	W2857		plant trap	single spore	this study	Canada, Quebec	V Furlan
SSU	AJ276092	WD66–5	Scutellospora aurigloba		Att860-10	W3121	WUM53	multispore	single spore	Schüßler et al. 2001	Australia, Western Australia, Cape	Jayasundra F
SSU	AJ276093	WD66–26	Scutellospora aurigloba		Att860-10	W3121	WUM53	multispore	single spore	Schüßler et al. 2001	Australia, Western Australia, Cape	Jayasundra F

SSU	AJ306443	WD153-1-1	Scutellospora calospora	epitype predecessor	Att333-17	W3290	BEG32	multispore	single spore	Schüßler et al. 2001	UK, Scotland, Midlothian	C Walker
SSU	AJ306445	WD162-1-5	Scutellospora calospora	epitype predecessor	Att333-17	W3290	BEG32	multispore	single spore	Schüßler et al. 2001	UK, Scotland, Midlothian	C Walker
SSU	AJ306446	WD162-1-6	Scutellospora calospora	epitype predecessor	Att333-17	W3290	BEG32	multispore	single spore	Schüßler et al. 2001	UK, Scotland, Midlothian	C Walker
SSU	AJ276094	WD140–3	Scutellospora gilmore		Att590–1	W3085	from FCPC1145	single spore (= isolate	single spore	Schüßler et al. 2001	USA, Oregon, Newport	I Ho
SSU	FR773142	FD055	Scutellospora gilmore		Att590-7	W3557	from FCPC1145	single spore (= isolate	single spore	this study	USA, Oregon, Newport	I Ho
SSU	FR773143	FD088_2	Scutellospora gilmore		Att590-7	W3557	from FCPC1145	single spore (= isolate	single spore	this study	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547598	HS103-A3	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547599	HS103-B3	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547600	HS103-C3	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547601	HS103-D3	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547602	HS103-G2	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547603	HS107-2	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547604	HS107-9	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547605	HS107-4	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547606	HS107-32	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547607	HS107-1	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547608	HS107-10	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547609	HS107-3	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547610	HS107-30	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547611	HS107-8	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547612	HS107-22	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547613	HS107-26	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547614	HS107-27	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547615	HS107-28	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547616	HS107-11	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547617	HS107-13	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547618	HS107-14	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547619	HS107-17	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547620	HS107-29	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547621	HS107-20	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU-ITS-LSU	FN547622	HS107-21	Scutellospora gilmore		Att590-16	W5342	from FCPC1145	single spore (= isolate	single spore	Stockinger et al. 2010	USA, Oregon, Newport	I Ho
SSU	AJ306434	WD163-2-6	Scutellospora heterogama		Att334-16	W3214	BEG35	pot culture (details unknown)	single spore	Schüßler et al. 2001	Unknown, probably USA	Unknown collector
SSU-ITS-LSU	FM876837	MK029-3	Scutellospora heterogama		Att334-16	W3214	BEG35	pot culture (details unknown)	single spore	Krüger et al. 2009	Unknown, probably USA	Unknown collector
SSU-ITS-LSU	FM876838	MK029-5	Scutellospora heterogama		Att334-16	W3214	BEG35	pot culture (details unknown)	single spore	Krüger et al. 2009	Unknown, probably USA	Unknown collector
SSU-ITS-LSU	FM876839	MK029-4	Scutellospora heterogama		Att334-16	W3214	BEG35	pot culture (details unknown)	single spore	Krüger et al. 2009	Unknown, probably USA	Unknown collector
SSU	FR774917	FD121-5	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750158	MK075-1	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750159	MK075-2	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750160	MK075-5	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750161	MK076-1	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750162	MK076-2	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750163	MK076-4	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750164	MK076-5	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750165	MK076-6	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750166	MK076-7	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750167	MK076-8	Scutellospora heterogama		Att1283-1	W4733	Goldsboro 2003-38	multispore	single spore	this study	USA, North Carolina, Goldsboro	D Watson
SSU-ITS-LSU	FR750012	CK066-1(+7+13)	Scutellospora heterogama		Att1577-4	W5611	FL654	pot culture (details unknown)	single spore	this study	USA, Florida	A Adholeya
SSU-ITS-LSU	FR750013	CK066-2(+9)	Scutellospora heterogama		Att1577-4	W5611	FL654	pot culture (details unknown)	single spore	this study	USA, Florida	A Adholeya
SSU-ITS-LSU	FR750014	CK066-3(+4)	Scutellospora heterogama		Att1577-4	W5611	FL654	pot culture (details unknown)	single spore	this study	USA, Florida	A Adholeya
SSU-ITS-LSU	FR750015	CK066-5	Scutellospora heterogama		Att1577-4	W5611	FL654	pot culture (details unknown)	single spore	this study	USA, Florida	A Adholeya
SSU-ITS-LSU	FR750016	CK066-6(+8+10+11)	Scutellospora heterogama		Att1577-4	W5611	FL654	pot culture (details unknown)	single spore	this study	USA, Florida	A Adholeya
SSU-ITS-LSU	FR750017	CK067-1(+2)	Scutellospora heterogama		Att1577-4	W5611	FL654	pot culture (details unknown)	single spore	this study	USA, Florida	A Adholeya
SSU-ITS-LSU	FR750018	CK067-3	Scutellospora heterogama		Att1577-4	W5611	FL654	pot culture (details unknown)	single spore	this study	USA, Florida	A Adholeya
SSU-ITS-LSU	FR750019	CK067-4	Scutellospora heterogama		Att1577-4	W5611	FL654	pot culture (details unknown)	single spore	this study	USA, Florida	A Adholeya
SSU	AJ306436	WD188-2-3	Scutellospora nodosa	ex-epitype	Att209-44	W3485	BEG4	single spore (= isolate	single spore	Schüßler et al. 2001	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FM876833	MK008-1	Scutellospora nodosa	ex-epitype	Att209-44	W3485	BEG4	single spore (= isolate	single spore	Krüger et al. 2009	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FM876834	MK008-2	Scutellospora nodosa	ex-epitype	Att209-44	W3485	BEG4	single spore (= isolate	single spore	Krüger et al. 2009	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FM876835	MK008-4	Scutellospora nodosa	ex-epitype	Att209-44	W3485	BEG4	single spore (= isolate	single spore	Krüger et al. 2009	UK, Northumberland, Kielder	C Walker
SSU-ITS-LSU	FM876836	MK008-5	Scutellospora nodosa	ex-epitype	Att209-44	W3485	BEG4	single spore (= isolate	single spore	Krüger et al. 2009	UK, Northumberland, Kielder	C Walker
SSU	FR750215	FD142-4	Scutellospora pellucida		Att1295-9	W4761	CL750A (from CIAT, Colombia)	pot culture (details unknown)	single spore	this study	Colombia, Timbio, Cauca	E Sieverding
SSU	AJ306437	WD186-1-2	Scutellospora spinosissima	isotype	Att664-1	W3009		soil trap	single spore	Schüßler et al. 2001	Venezuela, Bolivar, Iboribo	G Cuenca
SSU-ITS-LSU	FR750149	MK024-1(+2+4+5)	Scutellospora spinosissima	isotype	Att664-1	W3009		soil trap	single spore	this study	Venezuela, Bolivar, Iboribo	G Cuenca
SSU-ITS-LSU	FR750150	MK024-3(+6+8)	Scutellospora spinosissima	isotype	Att664-1	W3009		soil trap	single spore	this study	Venezuela, Bolivar, Iboribo	G Cuenca

Supporting Information S3 - Consensus sequences used for Fig. 1.

1: AJ306442, FM876788-91; **2**: AJ306441, FM876792-93; **3**: FR750204, FR750151-56; **4**: AJ250847, AJ242499, FJ461802; **5**: FN825898-912; **6**: FR719957, AJ891110-13, AJ510230; **7**: AJ276077-78, FN547637-54, Y17649-50, FR686953,54; **8**: AJ849468, AM713432, EF581860,62,63,80-83, FN547655-65; **9**: AM713417-22, AM713402-04, AY639233-35, AY639306, EF581869-72; **10**: AM713405-16,29-31, EF067886-88, EF581877-79; **11**: AJ132666, AJ276088, AM947665, AY842567-69,73-74,FJ461852,FM876814-20,FN547635-36,66-81, FR686938, FR686942, X86687, Y17651; **12**: AJ306434, FM876837-39; **13**: AY635832, AY997088, DQ273792; **14**: AJ276090, FM876799-803; **15**: X58726, AJ410746-47, FN547571-97, FR750174-85; **16**: FM876833-36, AJ306436; **17**: AF038590, AJ002874, AJ313169-75, FJ461867, FN423706-07, U31997-98, Y12076; **18**: AJ306437, FR750149-50; **19**: FM876831-32, AJ619952-55; **20**: AJ301857, FR750201-03; **21**: FR750531-44, FR750376; **22**: FR750526-30, FR750363-71, FR772325; **23**: AY635833, AY997053, DQ273793; **24**: FN423694,95, FN547474-76, FN547482-93, U31995,96, U96139, X84232,33; **25**: FR773144+45, FM876794-98; **26**: AJ301864-65, FM876813, FN547477-81; **27**: AF145745, AJ301853, FN423698-99, FN547494-99, Y17635; **28**: FR750222, FR750228, FR750078-82; **29**: AY635831, AY997052, DQ273790; **30**: DQ322630, AY997054, DQ273828; **31**: AF213462, AJ973393, FM992388-402, FN547500-01, GQ205077-79; **32**: AF185661-68, AM980860-63, FM865559-79, FM865597-607, FR750205-11; **33**: AJ437105-06, AJ133706, FJ461846; **34**: AJ301856, FM876804-07; **35**: AJ276089, U36591, Y17645, FM876808-12; **36**: FN547535-46, FR750157, AM268192-203; **37**: AM183923, AM183920, X86686, Y17831, Y15904-05, AJ276074, FM876840-44; **38**: FR773150, FR750020-23; **39**: DQ322629, AY997069, DQ273827.

Supporting Information S4 - Consensus sequences used for Fig. 2.

1: AJ306434, FM876837-39; **2**: FR774917, FR750158-67; **3**: AY635832, AY997088, DQ273792; **4**: AB041344-45, AB048683-90; **5**: AJ871270-73; **6**: AJ132662-63, AJ504639, Y12075, Y17647, AJ410748-50; **7**: AJ276091, AJ539263; **8**: X58726, AJ410746-47, FN547571-97, FR750174-85; **9**: AJ276090, FM876799-803; **10**: Y17646, AF001053, AJ852011, FN547547-70; **11**: FM876833-36, AJ306436; **12**: AJ276094, FR773142-43, FN547598-622; **13**: AJ871274-75; **14**: AJ306444, FR750134-35; **15**: AF038590, AJ002874, AJ313169-75, FJ461867, FN423706-07, U31997-98, Y12076; **16**: AJ306435, FR750136-48; **17**: AJ306437, FR750149-50; **18**: AJ306443, AJ306445-46, AJ510231; **19**: AJ276092, AJ276093; **20**: AJ619940-43; **21**: AJ619944-47; **22**: AJ619948-51; **23**: FM876831-32, AJ619952-55; **24**: AM713423-25, AY639225-32, EF581865-68; **25**: AM713417-22, AM713402-04, AY639233-35, AY639306, EF581869-72; **26**: AM713426-28, AY639236-41, EF581873-76; **27**: AM713405-16, AM713429-31, EF067886-88, EF581877-79; **28**: AJ301863, AJ276076, Y17644, AJ301860; **29**: AM400229, AM905318; **30**: AJ849468, AM713432, EF581860,62-63,80-83, FN547655-65; **31**: AJ276077-78, FN547637-54, Y17649-50, FR686953, FR686954; **32**: AJ132666, AJ276088, AM947665, AY842567-69,73-74, FJ461852, FM876814-20, FN547635-36,66-81, FR686938, FR686942, X86687, Y17651; **33**: FR686956, FR686957; **34**: AM418543-44; **35**: AJ306442, FM876788-91; **36**: AJ306441, FM876792-93; **37**: FR750204, FR750151-56; **38**: FR719957, AJ891110-13, AJ510230; **39**: AJ250847, AJ242499, FJ461802; **40**: FR750214, FN547502-06, FN547517; **41**: FN825898-912; **42**: U96140, X96826-28, AM423116-19, AF145735; **43**: AY635833, AY997053, DQ273793; **44**: FN423694-95, FN547474-76, FN547482-93, FR750227, U31995-96, U96139, X84232-33; **45**: AJ919277-78, Z14007; **46**: AJ132664, Y17643, AJ245637; **47**: AJ301864-65, FM876813, FN547477-81; **48**: AF145745, AJ301853, FN423698-99, FN547494-99, Y17635; **49**: Y17653, AJ301854; **50**: FR773144-45, FM876794-98; **51**: HM153415-19; **52**: FR750531-44, FR750376; **53**: FR750526-30, FR750363-71, FR772325; **54**: AJ301857, FR750201-03; **55**: AY635831, AY997052, DQ273790; **56**: FR750222, FR750228, FR750078-82; **57**: FJ009605-10, FJ009612-18; **58**: DQ322630, AY997054, DQ273828; **59**: AF185661-68, AM980860-63, FM865559-87, FM865597-607, FR750205-11; **60**: AF213462, AJ973393, FM992388-402, FN547500-01, GQ205077-79; **61**: FR773148-49; **62**: FR773146-47, U36590, Y17638, FJ461842; **63**: AJ437105-06, AJ133706, FJ461846; **64**: GU059534-43; **65**: HM153420-24; **66**: Y17639, Z14008, AJ239125; **67**: FR773151, 52, AJ276087; **68**: AJ276079, Y17641; **69**: AJ301851-52, AJ276075,

Y17636, AF235007; **70**: Y17642, AJ276080; **71**: AJ276089, U36591, Y17645, FM876808-12; **72**: AJ301856, FM876804-07; **73**: AJ006793, AJ012201; **74**: AJ301861, AJ006466, AJ006794-97, AJ012109-10; **75**: AB047302-04, AB015052, AB048630-55; **76**: AB047305-07, AB048656-70; **77**: AB047308-09, AB048671-82; **78**: FN547535-46, AM268192-93, AM268195-203, FR750157; **79**: FN820272-74, FN820272-75; **80**: AM183923, AM183920, AM268204, X86686, Y17831, Y15904-05, AJ276074, FM876840-44; **81**: AJ006801, AJ243419; **82**: AJ006800, AJ243420; **83**: FR773150, FR750020-23; **84**: Y17634, AM114274; **85**: AJ012203, AJ012112; **86**: DQ322629, AY997069, DQ273827.

Supporting Information S5 - Consensus sequences used for Fig. 3.

1: AJ006793, AJ012201; **2**: AJ301861, AJ006466, AJ006794-97, AJ012109-10; **3**: AJ012111, AM743187, AJ510233; **4**: AM183923, AM183920, AM268204; **5**: AJ006800, AJ243420; **6**: AJ006801, AJ243419; **7**: AJ012203, AJ012112; **8**: DQ322629, AY997069, DQ273827; **9**: AJ006799, AJ012113.

14. Contribution of the author

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species level detection of *Glomeromycota*: one PCR primer set for all arbuscular mycorrhizal fungi. *New Phytologist* **183**: 212-223.

Manuela Krüger did all the testings, on cloned genes and field material, of the new primers developed together with A. Schüßler and H. Stockinger, and most of the newly published sequences were generated by her. Together with A. Schüßler she wrote the main parts of the manuscript.

Stockinger H, Krüger M, Schüßler A. 2010. DNA barcoding for arbuscular mycorrhiza fungi. *New Phytologist* **187**: 461-474.

Manuela Krüger generated a relevant part of the new sequences published and proofread the manuscript.

Krüger M, Walker C, Schüßler A. 2011. *Acaulospora brasiliensis* comb. nov. and *Acaulospora alpina* (*Glomeromycota*) from upland Scotland: morphology, molecular phylogeny and DNA-based detection in roots. *Mycorrhiza* **21**: 577–587.

Manuela Krüger generated most of the new sequences published (from vouchers and from field material), conducted the phylogenetic analyses, and wrote the manuscript together with C. Walker and A. Schüßler.

Schüßler A, Krüger M, Walker C. 2011. Revealing natural relationships among arbuscular mycorrhizal fungi: culture line BEG47 represents *Diversispora epigaea*, not *Glomus versiforme*. PLoS ONE **6**: e23333.

Manuela Krüger generated part of the new sequences published and proofread the manuscript.

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. 2011. A phylogenetic framework for the natural systematics of arbuscular mycorrhizal fungi: from phylum to species-level resolution and environmental deep sequencing. resubmitted to *New Phytologist* 19. August 2011.

Significant parts of this publication were worked out by Manuela Krüger. She generated approximately one third of the new sequences published, performed the phylogenetic analyses and wrote parts of the manuscript.

I hereby confirm the above statements:

Manuela Krüger PD Dr. Arthur Schüßler

15. Curriculum vitae

Personal Details	Date of Birth: 15.07.1980 (Zwickau)
	Nationality: German
	Marital Status: unmarried
Education:	
Study	
06.05.2011	Rigorosum (oral examination), PhD thesis mark: 1
Oct. 2006 - Feb. 2011	LMU Munich, Department Biology I, PhD student, Topic: 'Molecular phylogeny, taxonomy and evolution of arbuscular mycorrhizal fungi - DNA-based characterization and identification of *Glomeromycota'*
Oct. 1999 - Nov. 2005	TU Bergakademie Freiberg (University of Freiberg), course of study: Applied Natural Science
27.10. 2005	Degree examination, mark: 1.9
Mar. 2005 - Sep. 2005	Degree dissertation within the Environmental Microbiology group of the TU-Bergakademie Freiberg, Topic: 'Diversity of the *Archaea* in the water of the Wettinquelle in Bad Brambach'
Oct. 1999 - Nov. 2005	Study period: consolidation Biotechnology
Work Experience	
Mar. 2006 - Sep. 2006	UFZ - Department of the Helmholtz-Centre for Environmental Research Halle/Saale, group of François Buscot, Project: 'Design of oligonucleotide probes based on the CLONDIAG chip technlogy for in-field detection of glomeromycotan fungi' - Development of glomeromycota sequence database in ARB - Design and test of the designed probes - First application of the pilot-phylochip
Training courses	
Dec. 2006	Attendance at the ARB course from Ribocon, Bremen
Nov. 2004 - Mar. 2005	Attendance at the course 'Communication Skills Intermediate' (English)
Apr. 2003 - Jul. 2003	Attendance at the course 'Databases for Natural Scientists'

Apr. 2002 - Sep. 2002	Certificate of the 'Umfassende Sachkunde' § 5 of the Chemikalienverbotsordnung (German Chemistry Law)

Publication List

Peer-reviewed Journals:

Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A. 2011. A phylogenetic framework for the natural systematics of arbuscular mycorrhizal fungi: from phylum to species-level resolution and environmental deep sequencing. resubmitted to *New Phytologist* 19. August 2011.

Schüßler A, **Krüger M**, Walker C. 2011. Revealing natural relationships among arbuscular mycorrhizal fungi: culture line BEG47 represents *Diversispora epigaea*, not *Glomus versiforme*. PLoS ONE **6**: e23333.

Krüger M, Walker C, Schüßler A. 2011. *Acaulospora brasiliensis* comb. nov. and *Acaulospora alpina* (*Glomeromycota*) from upland Scotland: morphology, molecular phylogeny and DNA-based detection in roots. *Mycorrhiza* **21**: 577–587.

Remén C, **Krüger M**, Cassel-Lundhagen A. 2010. Successful analysis of gut contents in fungal-feeding oribatid mites by combining body-surface washing and PCR. *Soil Biology and Biochemistry* 42: 1952-1957.

Stockinger H, **Krüger M**, Schüßler A. 2010. DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist* 187: 461-474.

(This paper was discussed by Ursula Eberhardt. 2010. A constructive step towards selecting a DNA barcode for fungi. *New Phytologist* 187: 265-268.)

Krüger M, Stockinger H, Krüger C, Schüßler A. 2009. DNA-based species-level detection of arbuscular mycorrhizal fungi: one PCR primer set for all AMF. *New Phytologist* 183: 212-223.

Symposia and Conferences

Talks

UNITE/NordForsk Network Meeting, ‘Molecular characterisation, DNA barcoding and 454 sequencing of AM fungi’, Helsinki (Finland), 28.-29. October 2009

6th International Conference on Mycorrhizas (ICOM6), 'Phylogenetic analyses of the *Glomeromycota* with species level resolution, based on a 3.3 kb fragment in the rDNA region', Belo Horizonte (Brazil), 9.-14. August 2009

3rd TRACEAM international Summer School, 'New primers for characterisation and community-analyses of arbuscular mycorrhizal fungi (AMF)', Munich (Germany), 7.-9. April 2009

Poster presentations

Workshop 'Symbiotic interactions', 'DNA Barcoding and sequence based in-field species detection of arbuscular mycorrhizal fungi', Munich (Germany), 19.-20. November 2009

Workshop 'Mycorrhizas in Tropical Forests', 'Molecular phylogeny and evolution of arbuscular mycorrhizal fungi - an update', Loja (Ecuador), 22.-25. September 2008

Bi-national Symposium of the DFG Research Unit 816 (FOR816), 'ITS-region DNA barcoding for arbuscular mycorrhizal fungi', Loja (Ecuador), 11.-12. September 2008

Eidesstattliche Erklärung:

Hiermit erkläre ich, dass ich die vorliegende Arbeit, abgesehen von den in ihr ausdrücklich genannten Hilfen, selbständig verfasst habe.

München, den 1. Februar 2011

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Unterschrift