

ORIGINAL ARTICLE

A population genomics approach shows widespread geographical distribution of cryptic genomic forms of the symbiotic fungus *Rhizophagus irregularis*

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Arbuscular mycorrhizal fungi (AMF; phylum Glomeromycota) associate with plants forming one of the most successful microbe–plant associations. The fungi promote plant diversity and have a potentially important role in global agriculture. Plant growth depends on both inter- and intra-specific variation in AMF. It was recently reported that an unusually large number of AMF taxa have an intercontinental distribution, suggesting long-distance gene flow for many AMF species, facilitated by either long-distance natural dispersal mechanisms or human-assisted dispersal. However, the intercontinental distribution of AMF species has been questioned because the use of very low-resolution markers may be unsuitable to detect genetic differences among geographically separated AMF, as seen with some other fungi. This has been untestable because of the lack of population genomic data, with high resolution, for any AMF taxa. Here we use phylogenetics and population genomics to test for intra-specific variation in *Rhizophagus irregularis*, an AMF species for which genome sequence information already exists. We used ddRAD sequencing to obtain thousands of markers distributed across the genomes of 81 *R. irregularis* isolates and related species. Based on 6 888 variable positions, we observed significant genetic divergence into four main genetic groups within *R. irregularis*, highlighting that previous studies have not captured underlying genetic variation. Despite considerable genetic divergence, surprisingly, the variation could not be explained by geographical origin, thus also supporting the hypothesis for at least one AMF species of widely dispersed AMF genotypes at an intercontinental scale. Such information is crucial for understanding AMF ecology, and how these fungi can be used in an environmentally safe way in distant locations.
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Introduction

In 1934, Baas-Becking (1934) was the first to propose a worldwide distribution of microorganisms, saying that ‘Everything is everywhere, the environment selects’. A recent study of the global distribution of arbuscular mycorrhizal fungi (AMF), comprising the phylum Glomeromycota, suggests that the statement by Baas-Becking might be true for this phylum. Using virtual taxa (VT; Opik *et al.*, 2010), discriminated by a 520 bp region of the 18 S (small sub-unit or SSU) rRNA gene, 93% of all AMF taxa were found to be present on more than one continent and 34% of species occurred on all continents except Antarctica

(Davison *et al.*, 2015). Global distribution of one-third of a phylum in eukaryotes is particularly unusual, especially in organisms with a low dispersal rates (Tedersoo *et al.*, 2014). Records of cosmopolitan species, excluding marine (Finlay, 2002) and invasive or pest species (Margaritopoulos *et al.*, 2009), are rare. Birds have great dispersal capabilities, but even so, only six out of 10 000 species have a cosmopolitan distribution. Even those six species exhibit distinct genetic substructure (Monti *et al.*, 2015).

Several findings in fungal population genetics suggest that, for some species, a cosmopolitan distribution reflects hidden endemism (Taylor *et al.*, 2006). Some species first thought to be cosmopolitan are actually comprised of cryptic species with distinct geographical ranges such as the Basidiomycete *Schizophyllum commune* (James *et al.*, 1999), *Aspergillus fumigatus* and six other cited Ascomycete species (Pringle *et al.*, 2005).

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Morton (1990) hypothesized that AMF speciation occurred prior to the breakup of Pangea and that fungal lineages co-evolved with the emergence of plant species thereafter, thus, resulting in global distribution. Alternatively, Davison *et al.* (2015) favoured a hypothesis based on a more recent VT-molecular clock phylogeny that such widespread distribution was the result of a recent dispersal of spores, mediated by strong storms, as well as previously underestimated human activities. In either case, interpretations may be misleading if there is an underestimation of true species and an overestimation of species with an intercontinental distribution. The use of such short SSU sequences and a 97% similarity threshold to construct VT taxa (Davison *et al.*, 2015) might not be informative enough to resolve significant AMF clades (Bruns and Taylor, 2016; Schlaeppli *et al.*, 2016). Their argument is based on the premise that two taxa that share a 97% similarity in their SSUs may have diverged genetically many millions of years ago into different species. Davison *et al.* (2015) justified the use of VT because they are 'phylogenetically defined sequence groups that exhibit a taxonomic resolution similar to that of morphological species'. However, the absence of clear morphological distinctions and indistinguishable life history traits between *Rhizophagus intraradices* and *Rhizophagus irregularis*, together with highly supported divergence based on rDNA sequences (Stockinger *et al.*, 2009), provides compelling evidence of cryptic speciation in the *Rhizophagus* clade. Similarly, other species may be genetically divergent but are morphologically similar (Rosendahl *et al.*, 2009). This is more likely to occur in small eukaryotes with limited phenotypic space for evolution of new traits but with a less constrained capacity for genotypic evolution (Taylor *et al.*, 2000). Discovery of these cryptic taxa requires the sampling of globally distributed AMF taxa and the application of population genomics tools because a large number of markers distributed across the genome would give a finer resolution suitable to detect genetic differences among populations (Bruns and Taylor, 2016; Opik *et al.*, 2016).

A high throughput sequencing technique, double digested restriction-site associated DNA sequencing (ddRAD-seq) is a reliable way of obtaining a large number of genome-wide markers (Parchman *et al.*, 2012; Peterson *et al.*, 2012). This overcomes bias created by use of single gene sequences and low similarity thresholds (for example, the SSU rRNA gene). This approach was previously applied to *R. irregularis*, an AMF species that has a putative global distribution and can be cultured under *in vitro* conditions. *In vitro* culturing allows the extraction of DNA that is free of contaminant microorganisms or plant DNA. This technique was previously shown to be reliable for *Fusarium* (Talas and McDonald, 2015) and in a study measuring inter- and intra-isolate variation in a population of *R. irregularis* (Wyss *et al.*, 2016). This multi-locus approach can

provide a much finer resolution of the divergence between different *R. irregularis* isolates, which in turn can provide data to resolve whether this species has evolved under strong or relaxed geographical constraints.

Describing the genetic diversity of AMF species, and especially the genetic diversity of *R. irregularis* at a wide geographical scale, is fundamental to understanding biogeographic patterns. However, it is not only important for resolving biogeographic questions but also has strong implications for interpretation of ecological, agronomic, and environmental studies. First, AMF are important symbiotic partners that interact with many plant species. Approximately 300–1600 predicted AMF taxa form symbioses with over 200 000 plant species (van der Heijden *et al.*, 2015). The fungi have the capacity to efficiently absorb nutrients, particularly phosphate, from the soil and give them to the plant (Smith and Read, 2008) and richness of AMF taxa promotes plant diversity (van der Heijden *et al.*, 1998). Most ecological studies have not considered the role of intra-specific AMF variation even though intra-specific differences in AMF can have larger effects on P uptake and variation in plant growth than inter-specific differences (Munkvold *et al.*, 2004; Mensah *et al.*, 2015; Rodriguez and Sanders, 2015). Thus, measurements of genetic variation within AMF species could be highly ecologically relevant. Second, genetic variability in *R. irregularis* population causes significant variation in plant growth (Koch *et al.*, 2006). The potential of using naturally existing genetic variation in this species to develop more efficient strains to increase plant growth has been demonstrated by Angelard *et al.* (2010) where five-fold differences in rice growth could be achieved due to genetic variation in *R. irregularis* strains. Defining the total genetic variation in this AMF species is important for future programmes using the genetic variation in this fungus for more efficient inoculants (Sanders, 2010). Third, *in vitro* grown *R. irregularis* significantly increases yields of the globally important crop cassava (Ceballos *et al.*, 2013). *R. irregularis* is potentially a good candidate species for large-scale inoculation of tropical crops because it can be mass-produced in contaminant-free *in vitro* conditions and because it appears to have a global distribution. However, before introducing high growth-promoting *R. irregularis* isolates from one location to another, it is important to establish with population genomics techniques the risk of introducing genetically novel isolates into a new environment with the potential to become invasive. If *R. irregularis* has a very wide geographical distribution of very genetically similar isolates then the risk of introducing exotic genetic material is low. If there is strong geographically determined genetic structure among *R. irregularis* populations then such a risk is much higher (Rodriguez and Sanders, 2015).

In this study, 61 isolates identified morphologically and genetically as *R. irregularis* were obtained

from various locations across Europe, North Africa, Middle East and North America and were propagated *in vitro* and sequenced with ddRAD-seq. To the 61 isolates, data from 20 other isolates, that had been sequenced previously with the same ddRAD-seq protocol by Wyss *et al.* (2016), were added. We addressed three central questions: (i) Is the low level of endemism reported by Davison *et al.* (2015) applicable to *R. irregularis* and related species when using high resolution genome-wide markers that are capable of showing within population genetic variation? (ii) Is there significant genetic diversification among continents? (iii) Is there evidence that some quantitative traits vary in accordance with the genetic variation observed in this fungal species?

Materials and methods

Fungal isolates and culturing

R. irregularis (known previously as *Glomus intraradices*, *Glomus irregulare* or more recently as *Rhizoglyphus irregulare*) were obtained from collections and biobanks such as BEG (<http://www.i-beg.eu>), GINCO (<http://www.mycorrhiza.be/ginco-bel/>) and INVAM (<https://invam.wvu.edu/>), from small enterprises: Symbiom (Czech Republic; <https://www.symbiom.cz/en/>) and INOQ (Germany), and from research groups (IRTA: <http://www.irta.cat>, TERI: <http://www.teriin.org>, or were present in our group at the University of Lausanne. For more details on nomenclature, see Supplementary Note 1. Sixty-one isolates were collected (Supplementary Table 1). The collection included the *R. irregularis* holotype, DAOM197198, cultured *in vitro* in two laboratories (Switzerland: DAOM197198-CH and Czech Republic: DAOM197198-CZ), the holotype of *R. intraradices*, FL208 grown *in vitro* from pot culture material provided by INVAM and *R. proliferus* that was used as an outgroup for some analyses. All isolates in this study were cultured *in vitro* with Ri T-DNA transformed carrot roots in a two-compartment culture system (St-Arnaud *et al.*, 1996) to obtain fungal spores free from host plant DNA or other microorganisms. Some isolates were received as soil samples and were transferred to *in vitro* single spore cultures (Supporting Information, Supplementary Note 2).

DNA extraction, amplification and sequencing of the phosphate transporter and the SSU

Three-month-old sporulating *in vitro* cultures were used for DNA extraction. The medium in compartments containing only spores and hyphae was dissolved in 500 ml of citrate buffer (0.0062 M of citric acid anhydrous and 0.0028 M of sodium citrate tribasic dihydrate) for 1 h with a magnetic stirrer. Spores and hyphae were collected by filtering in citrate buffer through a sieve with 32 µm openings and rinsed with ddH₂O. Spores and hyphae were frozen in liquid nitrogen and ground with a sterile

pestle. DNA was extracted with the DNeasy Plant Mini Kit (QIAGEN Inc., Venlo, Netherlands) following the manufacturer's instructions. Species identity of each isolate was verified by sequencing the Phosphate Transporter Gene (PTG), which is considered a phylogenetically informative marker by Sokolski *et al.* (2011).

The primers AML2 and NS31, previously used by Davison *et al.* (2015), were used to amplify the 18 S (SSU) rRNA gene of all isolates. This was done to observe whether this marker could reveal genetic differences among the isolates used in this study and to compare those differences with the genetic variation revealed with ddRAD-seq data. The amplified PCR product was cloned with a StrataClone PCR Cloning Kit (Agilent Technologies, Santa Clara, CA, USA) following manufacturer protocols and one to two clones were sequenced. For more information of PTG and SSU amplification, sequencing and sequence cleaning see Supplementary Note 3.

ddRAD sequencing

Double-digest restriction-site associated DNA sequencing was carried out on DNA of the 61 isolates with three to five independent biological replicates per isolate (Wyss *et al.*, 2016; Supplementary Note 4). The sampling design is given in Supplementary Note 5. The ddRAD-seq protocol was performed on 233 DNA samples. Between 1 and 25 ng µl⁻¹ of genomic DNA was digested with the *EcoRI* and *MseI* enzymes (NEB). Between 36 and 90 samples were pooled into each of four libraries with independent barcode for each sample. Library quality was verified on a Fragment Analyser (Advanced Analytical Technologies, Inc., Ankeny, IA, USA). Each library was then sequenced at the Lausanne Genomics Technologies Facility (GTF) on an Illumina HiSeq sequencer in one lane to give 100 bp paired-end reads.

In silico workflow

An additional ddRAD-seq data set containing 20 isolates of *R. irregularis*, with 3 biological replicates of each isolate, was included in the analyses (Wyss *et al.*, 2016), thus increasing the total number of isolates analysed to 81 (and the number of samples analysed to 299). Read quality was controlled with FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The computations were performed at the Vital-IT (<http://www.vital-it.ch>) centre for high-performance computing of the Swiss Institute of Bioinformatics (SIB). After Illumina adaptor and low quality read trimming and demultiplexing (Supplementary Note 6), the reads were mapped to the DAOM197198 *R. irregularis* genome assembly N6 with the Novoalign software (Novocraft-Technologies, 2014). We chose this assembly, because it was the most complete single nucleus genome assembly of this fungus to date (Lin *et al.*, 2014).

FreeBayes v1.0.2 (Garrison and Marth, 2012) software was used to call single-nucleotide polymorphisms (SNPs), insertions and deletions (indels) and multiple nucleotide polymorphisms (MNPs). The $-p$ option was set up to 10, assuming a potential number of alleles up to 10 and a minimum frequency of each allele of 10%. VCFfilter from the VCFLib library (Garrison and Marth, 2012) was used to call positions with a phred quality >30 . Markers falling in coding and non-coding regions were defined based on a prediction of coding regions with GeneMark-ES (Terhovichnissyan *et al.*, 2008; Wyss *et al.*, 2016). Positions identified as repeated elements in the genome, using RepeatModeler Open-1-0 (Smit and Hubley, 2008) and RepeatMasker Open-3.0 (Smit *et al.*, 1996), were removed from subsequent analyses (Wyss *et al.*, 2016). An *in silico* double digestion *EcoRI-MseI* in the original genome allowed identification of predicted fragments and all fragments that potentially mapped two or more times to the DAOM197198 genome. We did not consider samples with less than 450 000 reads after demultiplexing, and samples with less than 300 000 reads uniquely mapping to the N6 assembly for further analyses. This decreased the number of samples to 262 (Supplementary Table 2). The four libraries, RAD7, RAD8, RAD10B and RAD12 are available at NCBI (bioproject accession number PRJNA357003).

SNP data sets

Three data sets were generated and analysed. All the positions included in these data sets were shared among all isolates, so that no missing data was present for any isolate or replicate. These positions were located in coding and non-coding regions and contained at least 10 reads of coverage. The first data set included all 262 samples and included 498 mono-allelic and poly-allelic positions in total. This data set allowed us to conduct analyses to study genetic variation among all isolates and all species included in this study. A second data set included only one replicate of each isolate, where the replicate chosen to represent each isolate was the one with the highest number of uniquely mapped reads and the highest number of loci covered. This allowed us to conduct more precise analysis on the genetic differences among all isolates using a larger number of polymorphic sites than that available in data set 1. Using this selection, a total of 2491 mono-allelic positions were concatenated for 68 isolates. In this data set, one isolate out of the 14 identified using the

PTG sequences as *R. proliferus* was used as an outgroup. A third data set comprised a single replicate of each isolate identified in the PTG tree as *R. irregularis*. By considering polymorphic positions that were only shared among all isolates identified as *R. irregularis*, a database with a larger number of polymorphic positions could be generated, allowing us to conduct analyses with a high resolution to identify and quantify genetic differences within the species *R. irregularis*. In this data set, 6888 mono-allelic positions were concatenated. A summary of the three data sets is found in Table 1 and information about the samples used in each of the three data sets is found in Supplementary Table 2.

Phylogenetic analyses and 18 S rRNA clustering

Phylogenetic analysis of the PTG is described in Supplementary Note 3. After sequence cleaning, SSU cloned sequences of each isolate were clustered at 97, 98 and 99% similarity thresholds with the script *pick_otus* and the *uclust* clustering algorithm in the QIIME environment (Caporaso *et al.*, 2010; Edgar, 2010). Each clone was also blasted against the MaarjAM database (Opik *et al.*, 2010) and the best VT hit was retained. The sequences used for the PTG phylogeny comprised 71 from this study and 25 from Sokolski *et al.* (2011). All PTG sequences used to reconstruct this phylogeny have been deposited in NCBI GenBank as accession numbers KY348541—KY348610 and KY436236. The SSU sequences were deposited in the NCBI GenBank with accession numbers KY436237–KY436352.

Scalar distances were calculated according to the equation 2 of Wyss *et al.* (2016). These distances were computed on the first ddRAD-seq dataset and were analysed with the package *ape*, version 3.5 (Paradis *et al.*, 2004) in the R software 3.3.2. A dendrogram was built using the function *hclust* and *plot.phylo* (Wyss *et al.*, 2016). A bootstrapping method described by Wyss *et al.* (2016), was applied to calculate support values. The second and third data sets were analysed with Mr Bayes (Huelsenbeck and Ronquist, 2001), implementing Markov chain Monte Carlo (MCMC) probability analysis. A mixed model was set up with the command `lset nst = mixed rates = gamma`. The MCMC chains were run for 1 000 000 generations with the reversible jump MCMC (RJ-MCMC) procedure, avoiding the selection of only one model of substitution rate. The chains were run until the standard deviation (s.d.) of split

Table 1 The three data sets used for the phylogenetic analysis and population structure analysis

	No. of samples	No. of isolates	No. of sites	Taxa included in data set
Data set 1	265	81	489	<i>R. intraradices</i> , <i>R. irregularis</i> , <i>Rhizophagus</i> sp. LPA8-CH3, <i>R. proliferus</i>
Data set 2	68	68	2491	<i>R. intraradices</i> , <i>R. irregularis</i> , <i>Rhizophagus</i> sp. LPA8-CH3, <i>R. proliferus</i> (as outgroup)
Data set 3	59	59	6888	<i>R. irregularis</i>

frequencies reached 0.01. All PSRF+ values reached a value of 1.

Population structure, species and clade delimitation

A constrained correspondence analysis (CCA) built with the function *cca* from the package *vegan*, version 2.4-1 (Dixon, 2003) was applied to the first data set and based on a matrix of scalar distances among the 262 samples and the 498 positions. We did this to examine how all the different isolates used in this study would cluster.

BP&P (Yang and Rannala, 2010, version 3.3) and STRUCTURE (Pritchard *et al.*, 2000, version), two programmes based on the Bayesian algorithm, were used in order to define species and genotype boundaries. First, the 2491 positions of the second data set were concatenated and tested under the multispecies coalescent (MSC) model (Yang, 2002; Rannala and Yang, 2003). Using this model, species were delimited using a user-specified guide tree (Yang and Rannala, 2010; Rannala and Yang, 2013). The BPP software only evaluates the models that can be generated by collapsing nodes on the guide tree using the reversible jumps algorithm. We have added this text into the manuscript. The specific guide tree used is based on the phylogeny generated in Mr Bayes, as follows:

(*R. proliferus*, (*R. intraradices*, (*Rhizophagus* sp. LPA8-CH3, ((*R. irregularis* Gp3, *R. irregularis* Gp4), ((*R. irregularis* Gp1A, *R. irregularis* Gp1B), *R. irregularis* Gp2))))),

where *Rhizophagus* sp. LPA8-CH3 formed a distinct sister clade to *R. irregularis* comprising two isolates, *R. intraradices* is a known species, and *R. irregularis* groups Gp1A, Gp1B, Gp2, Gp3, Gp4 are distinct genetic groups within the phylogenetic analysis. Three models were run with varying ancestral population size (θ) and root age (τ_0) following the protocol of Leaché and Fujita (2010) (Supplementary Note 7).

Population structure in the third data set was analysed using an admixture ancestry model with the programme STRUCTURE, without prior knowledge of location and with correlated allele frequencies between populations. STRUCTURE (Pritchard *et al.*, 2000) is a programme suitable for defining species groups (K) even when the Hardy-Weinberg equilibrium is not respected and is also robust for analysis of population structure with data originating from organisms with low recombination rates (Falush *et al.*, 2003, 2007). The MCMC chains were run for 100 000 generations, with 10 000 generations of burn-in. Each run from K1 to K7 was replicated 10 times. The delta K (Evanno *et al.*, 2005) was computed using STRUCTURE HARVESTER (Earl and vonHoldt, 2012) in order to define the most likely number of K present in the sample. In order to concatenate all the replicates for each K , CLUMPP was used (Jakobsson and Rosenberg, 2007). Graphical output of cluster assignment as barplots and pie

charts for mapping each isolate to their geographical origin was performed in R.

Finally, an analysis of molecular variance (AMOVA) was performed on the data using Continent as a factor with the highest number of isolates (that is, Europe and North America), with 1000 permutations, based on scalar genetic distances (*pegas* package version 0.9, Paradis, 2010). A Mantel test with the package *ape* (Paradis *et al.*, 2004, version 3.5) and with 1000 permutation was also performed to test for isolation by distance, by correlating a geographical distance matrix and the matrix of phylogenetic scalar distance based on data set 3 derived from ddRAD-seq.

A previous study (Croll *et al.*, 2008) analysed the genetic structure among 29 *R. irregularis* isolates with a reduced set of markers comprising ten microsatellites, two mitochondrial LSU gene introns and one nuclear gene intron. Those 29 isolates were also present in this study, allowing a comparison of the results. We produced a matrix of phylogenetic scalar distance, based on the ddRAD-seq data set, but this time with the 29 isolates. This matrix was then compared with a Mantel test to a matrix of phylogenetic Jaccard distance based on 13 markers (Croll *et al.*, 2008). This analysis combined with the other analyses indicated that the ddRAD-seq data were robust (Supplementary Note 8).

Measurement of hyphal density

Previous studies have shown differences in hyphal density among some of the isolates used in this study. We wanted to see whether hyphal density patterns of *R. irregularis* varied in accordance with the genetic differences observed among *R. irregularis* isolates. After sequencing and analyzing all ddRAD-seq of each isolate, nine isolates spread across the major branches of the phylogeny were cultured for 3 months *in vitro* with five replicates. There were only three replicates of one of the isolates, DAOM197198-CZ. Following 3 months of growth, each plate was photographed using a camera attached to a stereomicroscope in order estimate hyphal density (Supplementary Note 9).

Results

Phylogeny based on phosphate transporter gene and SSU gene clustering

Sequences of the PTG ranged between 800 and 1191 bp in length, with 282 SNPs. Previously published sequences (Sokolski *et al.*, 2011) and sequences of the reference isolates FL208 and DAOM197198, combined with the other PTG sequences from this study, resolved three *Rhizophagus* species; *R. proliferus*, *R. intraradices* and *R. irregularis* (Figure 1a). Twenty-two different haplotypes were identified with a nucleotide diversity (PiT) of 0.0806. In the *R. irregularis* clade, 13 haplotypes were detected with a nucleotide

diversity of 0.0158 and 49 segregating sites. As expected, sequences of the reference strain DAOM197198 clustered within the *R. irregularis* clade. A highly supported sister clade to *R. irregularis* was designated as a distinct putative species *Rhizophagus* sp. LPA8-CH3 based on the two isolates comprising this group. The isolate FL208, considered as the holotype of *R. intraradices* (Stockinger *et al.*, 2009), clustered with five other isolates and with a previously published sequence of *R. intraradices*.

Sequences of the same SSU region studied by Davison *et al.* (2015) of 554 bp, revealed that at the 97% similarity threshold the different *Rhizophagus* species used in this study could not be discriminated from each other (Figure 1b; Supplementary Table 3). Only at the 99% similarity threshold, could the 3 species of *Rhizophagus* plus the putative species be distinguished from each other (Figure 1b). At the 99% similarity threshold, *R. proliferus*, *R. intraradices* and the putative *Rhizophagus* sp. LPA8-CH3, each occurred in one separate VT (as seen in the

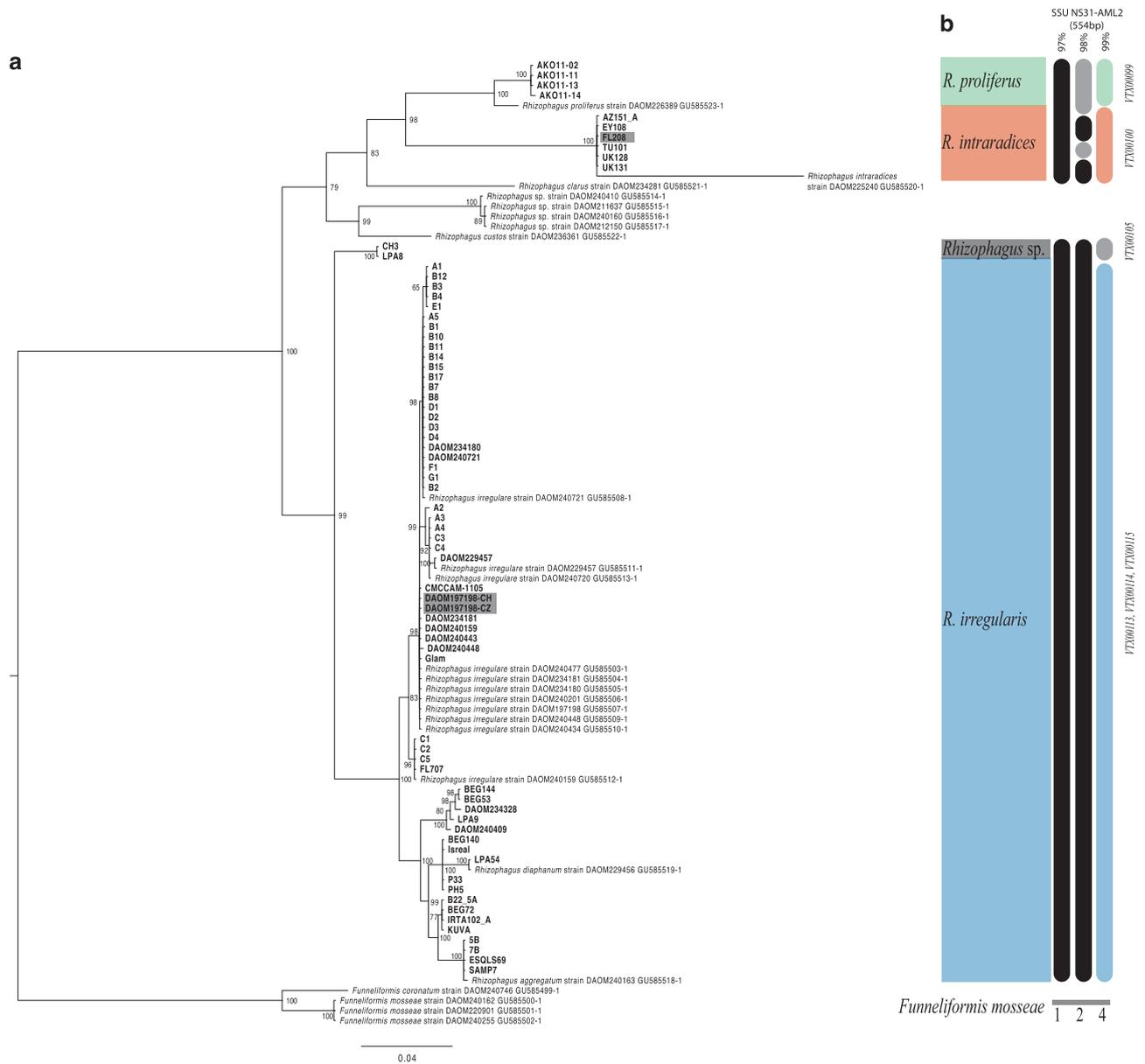


Figure 1 Phylogeny constructed using phosphate transporter gene (PTG) sequences (a) and schematic clustering of the SSU (b). Additional sequences from Sokolski *et al.* (2011). Sequences of *Funneliformis mosseae* were used to root the tree. Holotypes of *R. intraradices* (FL208) and *R. irregularis* (DAOM197198) shown in dark grey. *R. proliferus* (green), *R. intraradices* (red), *R. irregularis* (blue) and *Rhizophagus* sp. LPA8-CH3 (dark grey). Numbers at nodes represent bootstrap support for consensus tree bipartitions. SSU sequences for all isolates clustered at 97, 98 and 99% and corresponding virtual taxa number (VT) of the first hit after blast on MaarjAM database.

MaarjAM database). *R. irregularis* isolates occurred in three different VT.

Analysis of ddRAD-seq data

Each of the four ddRAD-seq libraries generated more than 300 million reads (Supplementary Table 4). Barcodes to de-multiplex the samples in each library are listed in Supplementary Table 2.

Sequencing details for each replicate of each isolate are reported in Supplementary Table 2. The ddRAD sequencing on DNA from 81 isolates resulted in 509 563 positions covered with a mean of 12 282 SNPs and 1936 indels per isolate when compared with the reference genome assembly. Not all of these positions had sufficient depth of coverage across all isolates in all samples. Among *R. intraradices* isolates, the number of positions in non-repeated coding and non-coding regions, covered by at least 10 reads, ranged from 23 668 in UK131-1 to 80 460 in UK128-1. Among *R. proliferus* isolates, the number of positions ranged from 60 795 in AKO11-11-2 to 77 186 in AKO11-13-1. Among *R. irregularis* isolates,

positions ranged from 73 030 in ESQSL69-1 to 361 950 in DAOM240448-5.

Based on the phylogenies of both ddRAD-seq data sets 1 and 2, and a CCA built with the first data set (Supplementary Figure 1; Figures 2 and 3), similar clade structure to that found with the PTG phylogeny was obtained: *R. proliferus* ($n=14$), *R. intraradices* ($n=6$) and *R. irregularis* ($n=59$). These data provided additional support for recognising *Rhizophagus* sp. LPA8-CH3 ($n=2$) as being genetically distinct from *R. irregularis* and *R. intraradices*. Four main branches within the *R. irregularis* tree as well as four main groups of isolates within *R. irregularis* were clearly recognised in the CCA.

Phylogeography and population genetic structure

Rhizophagus irregularis and *R. intraradices* isolates co-occurred in Europe, the Middle East, North Africa and Northern America (Supplementary Figure 2). We analysed the speciation probability among *Rhizophagus* species as well as within *R. irregularis*. The MSC model implemented with data set 2 using BP&P showed that in three models,

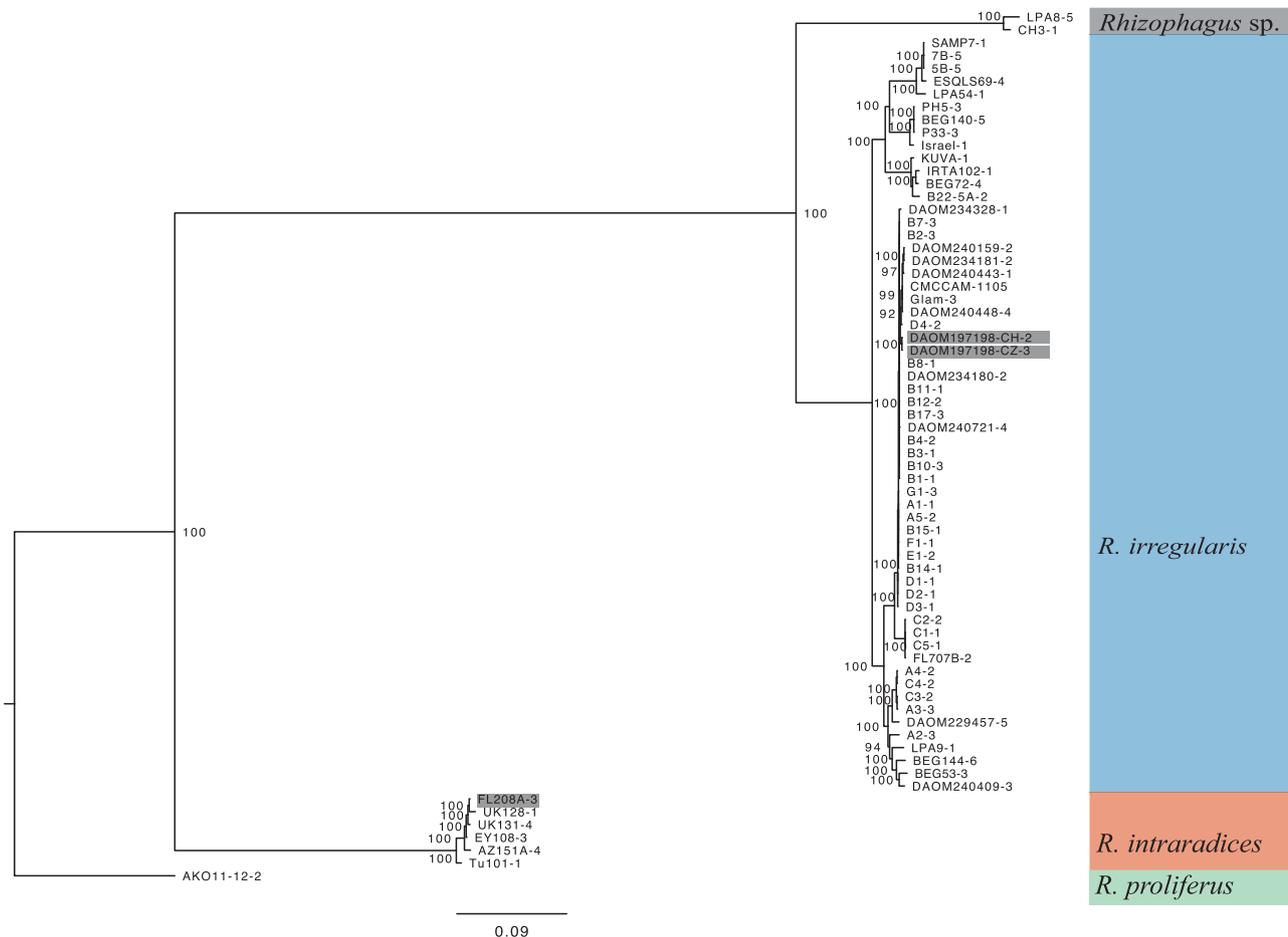


Figure 2 Phylogenetic tree based on a concatenation of 2491 SNPs across the genome (data set 2) of three species of *Rhizophagus* and *Rhizophagus* sp. LPA8-CH3 and 68 isolates. Only one replicate per isolate was used. Names are composed of the isolate name followed by the replicate number (short name in Supplementary Table 2). Numbers at nodes represent bootstrap support for consensus tree bipartitions. Colour coding follows that of Figure 1.

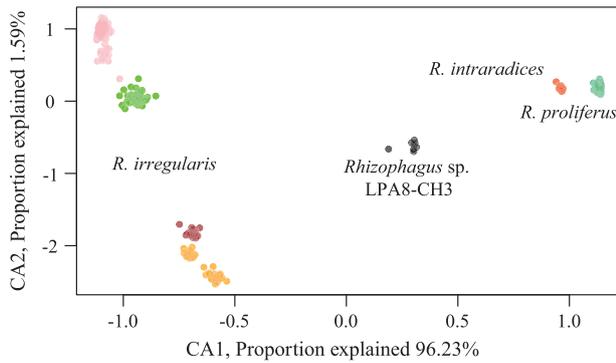


Figure 3 Constrained correspondence analysis (CCA) built on a scalar distance matrix computed among all samples and replicates ($n=262$) and based on 489 shared mono-allelic or poly-allelic positions. The four AMF species are well separated along the X-axis. Colour coding follows that of previous figures. In this figure the colour for *R. irregularis* is split into four main groups (pink, green, brown, orange).

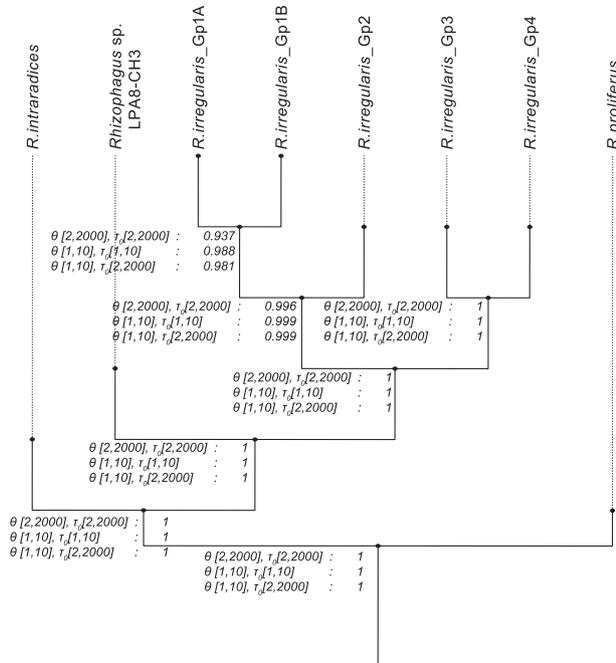


Figure 4 Bayesian species delimitation tree (based on data set 2). Marginal probabilities of speciation for three models with variable population size (θ) and divergence time (τ_0) are presented at each node.

the three distinct species were highly supported: *R. irregularis*, *R. intraradices* and *R. proliferus* and one putative species: *Rhizophagus* sp. LPA8-CH3 (Figure 4). A probability of >0.95 would normally be considered likely to represent a speciation event. In this analysis we also found significant divergence between groups Gp3 and Gp4 and between groups Gp1 and Gp2 within *R. irregularis*. Two of the three tests exceeded the >0.95 probability threshold for genetic differentiation between Gp1A and Gp1B.

The analysis using STRUCTURE and the Delta K on data set 3 (59 *R. irregularis* isolates) suggested the possibility of either two or four divergent clades

within the sampled *R. irregularis* isolates (Figure 5a), with Delta K values supporting a slightly higher probability of four groups than two (Supplementary Figure 3). BP&P and STRUCTURE analyses collectively suggested that the *R. irregularis* isolates were divided into at least four well-defined genetic groups.

The distribution of the divergent genetic groups of *R. irregularis* did not follow a geographical pattern (Figure 5b). Indeed, the Mantel test used to test for isolation by distance and an AMOVA using genetic distance and Continents as factor (America=9, Europe=37) did not reveal significant genetic differences (AMOVA: $df=1$, $P=0.395$) between the populations of the two different continents or any significant isolation by distance (z-statistic: 112835, P-value: 0.518). Moreover, isolates of each of the four genetic groups could be found in highly distant locations. For example, *R. irregularis* group Gp4 occurred in several geographically distant places in north America (Florida and Canada), in Europe from Switzerland to Finland and in North Africa (Tunisia). Similar patterns were evident in the other genetic groups. Two strains (DAOM234181 and DAOM240159) appeared to be the same clonal haplotype and originated from two locations separated by almost 4000 km in Canada. Isolates belonging to the two close genetic groups Gp3 and Gp4 coexisted in the same soil in Switzerland and both groups also co-occurred in geographically close sampling sites in Canada.

The population structure of 29 *R. irregularis* isolates measured using 13 markers (Croll *et al.*, 2008) reflected that observed with the large number of SNPs identified in this study. The Jaccard distances among isolates measured with microsatellite data were significantly correlated with the scalar distance calculated on 2100 positions from ddRAD-seq data (Supplementary Figure 4, Mantel statistic $r: 0.9624$, $P\text{-value}<0.001$).

Extraradical hyphal density

Data set 3 provided the finest resolution to discriminate between *R. irregularis* isolates and showed, based on 6888 SNPs, that genetic variation within each of the four main genetic groups also existed (Figure 6a). Significant differences in extraradical hyphal density among the nine chosen *R. irregularis* isolates, and among three genetic groups (Gp1, Gp3 and Gp4), was found (Figure 6b; lmer; Genetic groups: $dF=2$, F value=5.94, $P=0.035^*$). Isolates in the genetic group Gp3 produced a significantly higher density of extraradical hyphae than those in Gp4. Other comparisons between genetic groups were non-significant (Gp3–4, $P=0.01^*$; Gp1–4, $P=0.09$; Gp1–3, $P=0.18$).

Discussion

Describing the genetic diversity of AMF species, and especially that of the broadly distributed *R.*

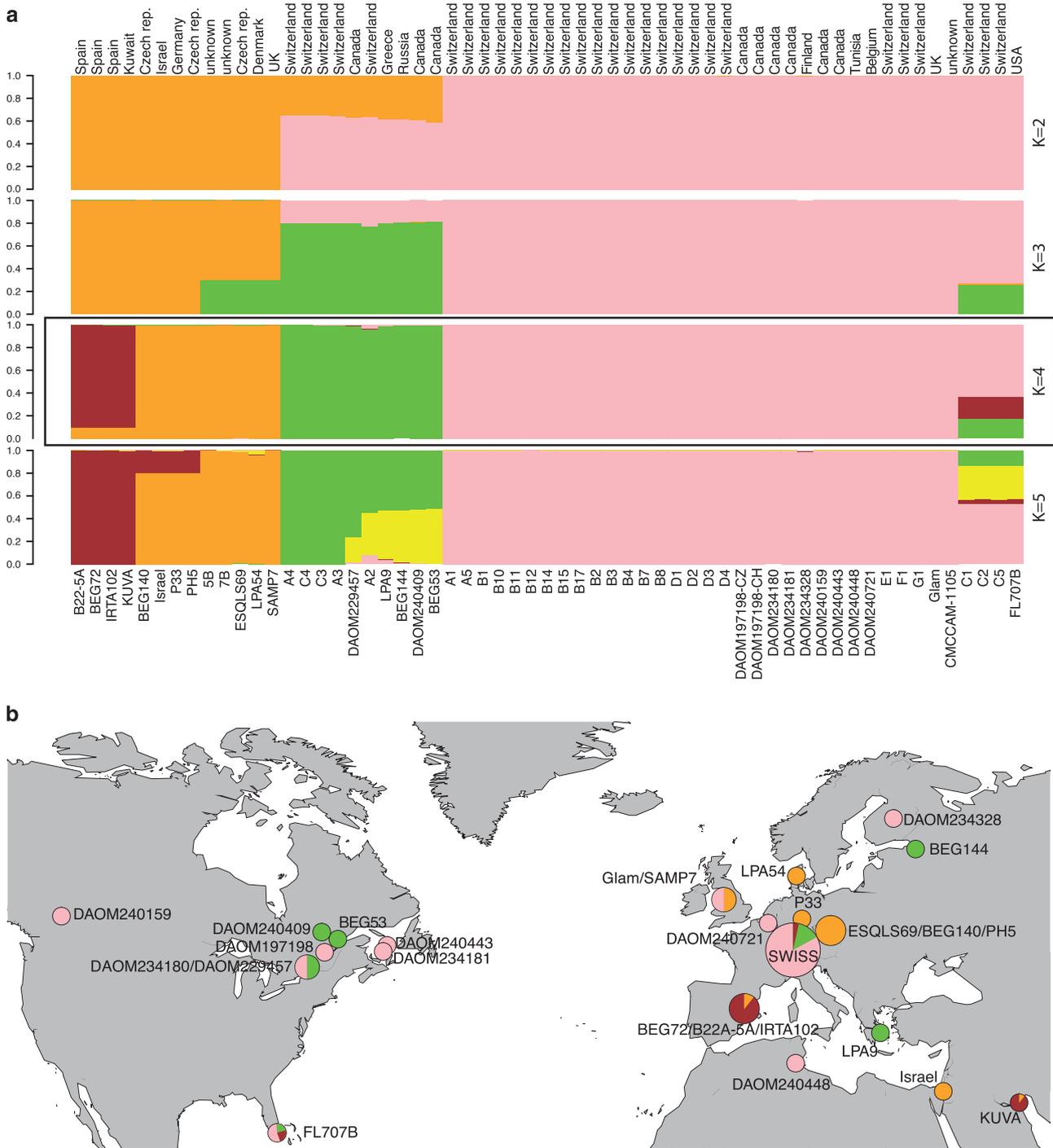


Figure 5 Assignment to 4 genetic groups with STRUCTURE and distribution map of genetic groups. (a) Vertical bars represent one *R. irregularis* isolate of and colours represent the assignment to different genetic groups (K). The delta K value for $K = 4$ was slightly higher than for $K = 2$. (b) Colour coding corresponds to results obtained with STRUCTURE, based on data set 3. Several isolates are of unknown geographical origin and are not presented here. The name of each isolate is written near its respective pie chart, except for the 29 Swiss isolates that are represented as the Swiss population.

irregularis group, is fundamental to understanding their biogeographic patterns. Data confirmed that both *R. irregularis* and *R. intraradices* are widely distributed geographically across at least two continents (North America and Europe). Moreover, the

four cryptic genomic forms within the species *R. irregularis*, identified by thousands of SNP markers obtained in this study, also have a broad geographic distribution. These data collectively support the hypothesis of Davison *et al.* (2015) that endemism

is low in some Glomeromycota, not only at the 97% level of clustering resolution using VT and at the species level, but also amongst significantly diverged genomic forms within a species.

We confirmed with both the PTG and ddRAD-seq loci that the two reference isolates FL208 and DAOM197198 (holotypes of *R. intraradices* and *R. irregularis*, respectively) indeed belonged to two distinct clades and that a number of the other isolates in this study also clustered into these two clades. The four distinct genetic groups identified by variation in thousands of genome-wide SNPs among *R. irregularis* isolates were not fully resolved by the PTG and SSU phylogenies. This result stresses the need for higher resolution and larger number of markers for understanding AMF biogeography.

While the four genetically defined *R. irregularis* groups cannot be fully ranked at the species level in this study, the data indicate that, at most, a negligible amount of gene flow occurs among the groups, even though they coexist in the same soil. Thus, rather than referring to these as different cryptic species, we define the genetically different groups as cryptic genomics forms of the fungus, meaning that they are genetically distinct, but would likely not be distinguishable by morphological studies or using single gene markers. Anastomosis has been observed in the laboratory at very low frequency between isolates in groups Gp3 and Gp4 originating from the same location (Croll et al., 2009). However, if this were common in nature, then such defined genetic groups would not be expected in the data sets generated in this study because intermediates should occur.

The four genetic groups were found across large geographical distances. The most striking examples were genetic groups Gp3 and Gp4, which occur at several different localities in Europe, Northern Africa, Canada and the USA. Some isolates from the same genetic group occurred more than 8000 km apart. The four genetic groups described here were detected because of the high resolution obtained by the large number of ddRAD-seq markers. Thus, Bruns and Taylor (2016) were correct that low-resolution markers such as the one used by Davison et al. (2015) would have failed to detect such genetic groups and would have assigned them to only one VT. However, regardless of the level of resolution informed by the SNPs or the 18 S (SSU) rRNA, the same pattern of low endemism observed by Davison et al. (2015) was evident for *R. irregularis*. This suggests, as proposed by another study (Rosendahl et al., 2009), that at least the AMF species studied in detail so far, from the species level down to the intra-specific level, have a wide distribution attributable either to high dispersal via anthropogenic activities (Davison et al., 2015) or by slower dispersal over millions of years (Morton, 1990). Since many of the fungi in this study were isolated from agricultural fields, human

agriculture may well be responsible for the dispersal of this species. We do not, however, consider it likely that the wide distribution of some of the groups is due to the application of exotic inoculum containing *Rhizophagus irregularis* because the more widespread use of such inocula is actually very recent and many of the donated cultures were isolated from the soil long before the use of commercial inocula.

The considerable genomic variability described here for a large set of isolates has not previously been reported for *R. irregularis*. Given that genetically different AMF isolates can cause large differences in plant growth (Munkvold et al., 2004; Koch et al., 2006), it would now be important to identify if variation in plant growth during the symbiosis with these different forms is greater among or within *R. irregularis* genetic groups. In this present study, we found evidence that the extraradical hyphal density, a phenotypic trait that is known to impact the phosphate acquisition capacity of the fungus and benefit to the plant (Jakobsen et al., 1992), differed significantly among genetic groups. Identifying whether the within species genetic and phenotypic variability in these fungi has consequences for plant ecology would be an important step to understand the link between fungal communities and plant communities as well as an important step for using AMF in agriculture for increasing crop yields.

The focus on the biogeography of *R. irregularis* in this study has broad agronomic implications. Genetically different *R. irregularis* applied to rice (Angelard et al., 2010) resulted in differential rice growth. Significant yield increases in cassava in the field have been achieved by applying *in vitro* produced *R. irregularis* (Ceballos et al., 2013). *R. irregularis* is a potentially strong candidate species for large-scale inoculation of tropical crops because it can be mass produced in contaminant-free *in vitro* conditions and because of its global distribution. The occurrence of *R. irregularis* genotypes distributed across diverse environments suggests broad adaptability to different soil types.

The introduction of non-native exotic strains with a strong geographically determined genetic structure among populations could be perceived as a risk for potential invasiveness (Rodriguez and Sanders, 2015; Schlaeppi et al., 2016). However, that concern is alleviated for *R. irregularis*, given that distribution of genetically similar isolates is widespread. For example, if isolates Gp3 and Gp4 from Switzerland were introduced in a field sites in Canada, the presence of these genotypes there negate any classification as exotic. We propose that a population genomic study of *R. irregularis* from tropical soils should be undertaken to verify if similar patterns occur.

Attention then would focus on the design of multi-isolate commercial inocula, where relatedness of isolates growing on the same plant strongly impacts

plant biomass (Roger *et al.*, 2013). The variation found in the 81 isolates of this study provides a bank of genetic diversity that can be utilised to test for crop compatibility and symbiotic efficiency and effectiveness. The variability described in this study has the advantage that each isolate is referenced and stored as *in vitro* pure culture and could be at any time mass-produced and used for breeding programmes (Rodriguez and Sanders, 2015), ecological experiments (Koch *et al.*, 2006) and agronomic trials (Ceballos *et al.*, 2013).

This study is, to our knowledge, the first to show that almost-clonal isolates of *R. irregularis* occurred in highly distant localities up to 4000 km apart. It also confirms the findings of Davison *et al.* (2015) of low endemism and similar genomic forms on multiple continents. At the same time, our results also show that, as Bruns and Taylor (2016) suggested, considerable genetic divergence can indeed be hidden by the use of low-resolution markers. The presence of different genetic groups of *R. irregularis* across the globe should be now investigated by isolating and genotyping isolates from agronomic and natural ecosystems from other continents such as Australia, Africa, Asia and South America.

Conflict of Interest

The authors declare no conflict of interest.

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References

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. *Curr Biol* **20**: 1216–1221.

Baas Becking LGM. (1934). *Geobiologie of Inleiding Tot de Milieukunde*. W.P. Van Stockum & Zoon (in Dutch): The Hague, The Netherlands.

Bruns TD, Taylor JW. (2016). Comment on 'Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism'. *Science* **351**: 826–b.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK *et al.* (2010). QIIME allows

analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–336.

Ceballos I, Ruiz M, Fernandez C, Pena R, Rodriguez A, Sanders IR. (2013). The *in vitro* mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the globally important food security crop cassava. *Plos One* **8**: e70633.

Croll D, Wille L, Gamper HA, Mathimaran N, Lammers PJ, Corradi N *et al.* (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 Phytol* **178**: 672–687.

Croll D, Giovannetti M, Koch AM, Sbrana C, Ehinger M, Lammers PJ *et al.* (2009). Nonselving vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytol* **181**: 924–937.

Davison J, Moora M, Ouml, pik M, Adholeya A, Ainsaar L *et al.* (2015). Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**: 970–973.

Dixon P. (2003). VEGAN, a package of R functions for community ecology. *J Veg Sci* **14**: 927–930.

Earl DA, Vonholdt BM. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* **4**: 359–361.

Edgar RC. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.

Evanno G, Regnaut S, Goudet J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* **14**: 2611–2620.

Falush D, Stephens M, Pritchard JK. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* **164**: 1567–1587.

Falush D, Stephens M, Pritchard JK. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol Ecol Notes* **7**: 574–578.

Finlay BJ. (2002). Global dispersal of free-living microbial eukaryote species. *Science* **296**: 1061–1063.

Garrison E, Marth G. (2012). Haplotype-based variant detection from short-read sequencing. arXiv preprint arXiv:1207.3907 [q-bio.GN].

Huelsenbeck JP, Ronquist F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

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

Jakobsson M, Rosenberg NA. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801–1806.

James TY, Porter D, Hamrick JL, Vilgalys R. (1999). Evidence for limited intercontinental gene flow in the cosmopolitan mushroom, *Schizophyllum commune*. *Evolution* **53**: 1665–1677.

Koch AM, Croll D, Sanders IR. (2006). Genetic variability in a population of arbuscular mycorrhizal fungi

- causes variation in plant growth. *Ecol Lett* **9**: 103–110.
- Leaché AD, Fujita MK. (2010). Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proc R Soc B Biol Sci* **277**: 3071–3077.
- Lin K, Limpens E, Zhang ZH, Ivanov S, Saunders DGO, Mu DS *et al.* (2014). Single nucleus genome sequencing reveals high similarity among nuclei of an endomycorrhizal fungus. *Plos Genet* **10**: e1004078.
- Margaritopoulos JT, Kasprócz L, Malloch GL, Fenton B. (2009). Tracking the global dispersal of a cosmopolitan insect pest, the peach potato aphid. *BMC Ecol* **9**: 13.
- Mensah JA, Koch AM, Antunes PM, Kiers ET, Hart M, Bucking H. (2015). High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. *Mycorrhiza* **25**: 533–546.
- Monti F, Duriez O, Arnal V, Dominici JM, Sforzi A, Fusani L *et al.* (2015). Being cosmopolitan: evolutionary history and phylogeography of a specialized raptor, the Osprey *Pandion haliaetus*. *BMC Evolution Biol* **15**: 255.
- Morton JB. (1990). Species and clones of arbuscular mycorrhizal fungi (Glomales, zygomycetes) - their role in macroevolutionary and microevolutionary processes. *Mycotaxon* **37**: 493–515.
- Munkvold L, Kjoller R, Vestberg M, Rosendahl S, Jakobsen I. (2004). High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytol* **164**: 357–364.
- Novocraft-Technologies. (2014). Novoalign. Available from <http://www.novocraft.com>.
- Opik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM *et al.* (2010). The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol* **188**: 223–241.
- Opik M, Davison J, Moora M, Partel M, Zobel M. (2016). Response to comment on 'Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism'. *Science* **351**: 826–c.
- Paradis E, Claude J, Strimmer K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.
- Paradis E. (2010). pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics* **26**: 419–420.
- Parchman TL, Gompert Z, Mudge J, Schilkey FD, Benkman CW, Buerkle CA. (2012). Genome-wide association genetics of an adaptive trait in lodgepole pine. *Mol Ecol* **21**: 2991–3005.
- Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. (2012). Double Digest RADseq: an inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. *Plos One* **7**: e37135.
- Pringle A, Baker DM, Platt JL, Wares JP, Latge JP, Taylor JW. (2005). Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus *Aspergillus fumigatus*. *Evolution* **59**: 1886–1899.
- Pritchard JK, Stephens M, Donnelly P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.
- Rannala B, Yang ZH. (2003). Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* **164**: 1645–1656.
- Rannala B, Yang ZH. (2013). Improved reversible jump algorithms for bayesian species delimitation. *Genetics* **194**: 245–253.
- Rodriguez A, Sanders IR. (2015). The role of community and population ecology in applying mycorrhizal fungi for improved food security. *ISME J* **9**: 1053–1061.
- Roger A, Colard A, Angelard C, Sanders IR. (2013). Relatedness among arbuscular mycorrhizal fungi drives plant growth and intraspecific fungal coexistence. *ISME J* **7**: 2137–2146.
- 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.
- Sanders IR. (2010). 'Designer' mycorrhizas? Using natural genetic variation in AM fungi to increase plant growth. *ISME J* **4**: 1081–1083.
- Schlaeppli K, Bender SF, Mascher F, Russo G, Patrignani A, Camenzind T *et al.* (2016). High-resolution community profiling of arbuscular mycorrhizal fungi. *New Phytol* **212**: 780–791.
- Smit AFA, Hubble R. (2008). RepeatModeler Open-1.0 <http://www.repeatmasker.org>.
- Smit AFA, Hubble R, Green P. (1996). RepeatMasker Open-3.0 <http://www.repeatmasker.org>.
- Smith SE, Read DJ. (2008). *Mycorrhizal Symbiosis* 3rd ed. Elsevier Ltd.: London, UK.
- Sokolski S, Dalpe Y, Piche Y. (2011). Phosphate transporter genes as reliable gene markers for the identification and discrimination of arbuscular mycorrhizal fungi in the genus *Glomus*. *Appl Environ Microbiol* **77**: 1888–1891.
- St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA. (1996). Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in the absence of host roots. *Mycol Res* **100**: 328–332.
- Stockinger H, Walker C, Schussler A. (2009). 'Glomus intraradices DAOM197198', a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. *New Phytol* **183**: 1176–1187.
- Talas F, McDonald BA. (2015). Genome-wide analysis of *Fusarium graminearum* field populations reveals hotspots of recombination. *BMC Genomics* **16**: 966.
- Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS *et al.* (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal Genet Biol* **31**: 21–32.
- Taylor JW, Turner E, Townsend JP, Dettman JR, Jacobson D. (2006). Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. *Philos Trans R Soc B Biol Sci* **361**: 1947–1963.
- Tedersoo L, Bahram M, Polme S, Koljalg U, Yorou NS, Wijesundera R *et al.* (2014). Global diversity and geography of soil fungi. *Science* **346**: 1078.
- Ter-Hovhannisyann V, Lomsadze A, Chernoff YO, Borodovsky M. (2008). Gene prediction in novel fungal genomes using an *ab initio* algorithm with unsupervised training. *Genome Res* **18**: 1979–1990.
- van der Heijden MGA, Klironomos JN, Ursic M, Moutoglou P, Streitwolf-Engel R, Boller T *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.

- van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytol* **205**: 1406–1423.
- Wyss T, Masclaux FG, Rosikiewicz P, Pagni M, Sanders IR. (2016). Population genomics reveals that within-fungus polymorphism is common and maintained in populations of the mycorrhizal fungus *Rhizophagus irregularis*. *ISME J* **10**: 2514–2526.
- Yang ZH. (2002). Likelihood and Bayes estimation of ancestral population sizes in hominoids using data from multiple loci. *Genetics* **162**: 1811–1823.
- Yang ZH, Rannala B. (2010). Bayesian species delimitation using multilocus sequence data. *Proc Natl Acad Sci USA* **107**: 9264–9269.



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