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Research

Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest

Matthew E. Smith[1], Terry W. Henkel[2], M. Catherine Aime[3], Alex K. Fremier[4] and Rytas Vilgalys[1]

[1]Department of Biology, Duke University, Durham, NC 27708, USA; [2]Department of Biological Sciences, Humboldt State University, Arcata, CA 95521, USA; [3]Department of Plant Pathology & Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA; [4]Department of Fish and Wildlife Resources, University of Idaho, Moscow, ID 83844, USA

Author for correspondence:
Matthew E. Smith
Tel: +1 919 660 7369
Email: trufflesmith@gmail.com

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Summary

• The ectomycorrhizal (ECM) symbiosis was historically considered restricted to the temperate zones, but recent studies have shown the importance of this symbiosis across the tropics. We examined ECM fungal diversity, host plant phylogeny and ECM host preferences in a rainforest dominated by the leguminous host plants *Dicymbe corymbosa*, *Dicymbe altsonii* and *Aldina insignis*.

• Ectomycorrhizal fungi were identified by internal transcribed spacer rDNA sequencing and host species were verified with chloroplast *trnL* sequencing. To test whether *Dicymbe* and *Aldina* represent independent gains of the ECM symbiosis, we constructed a Fabaceae phylogeny using *MatK* and *trnL*. We identified four independent ECM lineages within the Fabaceae.

• We detected a diverse community of 118 ECM species dominated by the /clavulina, /russula-lactarius, /boletus, and /tomentella-thelephora lineages. Ectomycorrhizal species in Agaricales, Atheliales and Polyporales may represent previously unrecognized tropical-endemic ECM lineages. Previous studies suggested that ECM fungi did not diversify in the tropics, but the /clavulina lineage appears to have a center of diversity in tropical South America.

• *Dicymbe* and *Aldina* represent independent gains of the ECM symbiosis in Fabaceae but their fungal symbionts showed no host preferences. Spatial factors are more important than hosts in structuring the ECM fungal community in this ecosystem.

Introduction

Ectomycorrhizal (ECM) fungi are a diverse group of mutualistic root symbionts that receive carbon from their host plants and in return provide enhanced nutrient uptake and resistance to stress and disease (Smith & Read, 2008). Although the ECM symbiosis has been known for > 100 yr (Frank, 1885), most studies on ECM ecology and biodiversity have focused on northern temperate forests and a narrow range of host plant families (e.g. Pinaceae, Fagaceae) (Alexander, 2006; Dickie & Moyersoen, 2008). Because of this 'higher latitude bias' in ECM studies, it was widely believed that ECM symbioses occurred predominantly in temperate and boreal biomes (Molina *et al.*, 1992; Alexander, 2006). Conversely, the tropics were thought to be dominated by arbuscular mycorrhizal (AM) plants, a view reinforced by mycorrhizal surveys of tropical sites where this was indeed the case (Redhead, 1968; Thomazini, 1974; Bereau *et al.*, 1997). However, the assumption that ECM fungi are absent or 'ecologically insignificant' in the tropics has eroded as the symbiosis has been documented in tropical habitats worldwide (Fassi & Fontana, 1962; Alexander & Högberg, 1986; Newbery *et al.*, 2000; Henkel *et al.*, 2002; Alexander & Lee, 2005; Moyersoen, 2006; Riviere *et al.*, 2007; Tedersoo *et al.*, 2007, 2010b; Morris *et al.*, 2009; Diédhiou *et al.*, 2010; Peay *et al.*, 2010b; Tedersoo & Nara, 2010). Ectomycorrhizal fungi are ecologically important in some tropical systems because they mitigate plant stress (Bandou *et al.*, 2006) and enhance seedling establishment and growth (Newbery *et al.*, 2002;

Henkel *et al.*, 2005a; McGuire, 2007). The facilitative effects of the symbiosis have been suggested to promote local dominance by tropical ECM trees (Newbery *et al.*, 1997; Torti *et al.*, 2001; Henkel, 2003) and decrease negative-feedback effects caused by pathogens in tropical forests (Alexander & Lee, 2005; Mangan *et al.*, 2010).

Despite the ecological importance of tropical ECM symbioses, the fungal symbionts remain poorly studied and relatively little is known about their biodiversity. The general latitudinal gradient of diversity (LGD) posits that biodiversity should increase with decreasing latitude and thus reach peak values in the tropics (Hillebrand, 2004). This pattern has been well documented in many groups of organisms, such as amphibians (Wiens *et al.*, 2006), mollusks (Jablonski *et al.*, 2006), angiosperms (Wright, 2002) and endophytic fungi (Arnold & Lutzoni, 2007). However, latitudinal diversity patterns for most fungal groups remain unresolved (Amend *et al.*, 2010; Tedersoo & Nara, 2010). Recent work from Amazonia suggests that lowland Neotropical ECM fungi exhibit low diversity and high host preference for dispersed, low-density hosts (Tedersoo *et al.*, 2010c). By contrast, other studies have documented high ECM fungal diversity in tropical cloud forests of Mexico (Morris *et al.*, 2009), mixed dipterocarp forests of Borneo (Peay *et al.*, 2010b) and West African forests dominated by leguminous trees (Diédhiou *et al.*, 2010; Tedersoo *et al.*, 2010b). These contrasting patterns indicate that ECM fungal diversity relationships are complex in the tropics and unlikely to be governed purely by latitudinal gradients (Tedersoo & Nara, 2010).

The factors that influence ECM fungal diversity patterns in different plant communities are not fully understood. Phylogenetically distant host plants occurring in sympatry usually host distinct ECM fungal communities (Ishida *et al.*, 2007; Tedersoo *et al.*, 2008; Smith *et al.*, 2009). As many ECM fungi show some degree of host preference, host plant diversity could be a key driver of both local and global ECM fungal diversity (Dickie, 2007). Host preferences appear to be mediated by host plant identity (Tedersoo *et al.*, 2008) but environmental influences such as litter quality and timing of litter deposition are also important (Aponte *et al.*, 2010). Host phylogeny and physiognomy also influence ECM fungal preferences (Ishida *et al.*, 2007; Morris *et al.*, 2008).

Diversity and community structure of ECM fungi may also be influenced by the size of host plants and their distribution on the landscape (Morris *et al.*, 2008; Tedersoo *et al.*, 2010c). Small, widely dispersed sympatric host plants should host fewer ECM species because of limited root resources, reduced inoculum potential, and stochastic limits on fungal colonization (Peay *et al.*, 2007, 2010a). These factors may interact to influence ECM fungal preferences and community composition among co-occurring host plants, but such interactions have not been fully explored.

Leguminous ECM trees are locally and regionally abundant in northeastern South America (Henkel, 2003; ter Steege *et al.*, 2006). Sporocarp surveys indicate high ECM fungal diversity at some sites (e.g. Singer & Aguiar, 1986; Henkel *et al.*, 2002), but there have been no comprehensive molecular studies of ECM mycobionts on leguminous hosts in this region. In this study we examined ECM fungal diversity and host preferences on the roots of three ECM tree species that occur sympatrically at high densities in the central Guiana Shield region of South America: *Dicymbe corymbosa*, *Dicymbe altsonii* and *Aldina insignis*. These are large, late-successional trees that reach the canopy in primary rainforests. The congeneric *D. corymbosa* and *D. altsonii* are presumably close relatives whereas *Aldina* is phylogenetically distant from *Dicymbe* (Doyle *et al.*, 1997; Pennington *et al.*, 2001). These two genera likely represent independent gains of the ECM habit from AM-forming ancestors in *Fabaceae*, but this hypothesis has not been explicitly tested (Alexander, 1989).

The high density of ECM trees and diversity of putative ECM fungi in combination with the sympatric occurrence of two putatively phylogenetically distant host genera prompted us to address the following questions: What is the ECM fungal diversity on the roots of the three host tree species?; What are the phylogenetic relationships among species of ECM Fabaceae and do *Dicymbe* and *Aldina* represent independent gains of the ECM habit?; Do the ECM fungal symbionts show preferences among the three host species?; and Do ECM fungi in this ecosystem show evidence of tropical origin or diversification?

Materials and Methods

Study site

The study site is located in primary rainforest in the Upper Potaro River Basin in the Pakaraima Mountains of Guyana and is part of one of the world's largest intact forest ecosystems (Fig. 1; Potapov *et al.*, 2008). The area receives *c.* 3500–4000 mm rainfall per year with peaks during May–July and December–January. Mean daily maximum temperatures range from 25 to 29°C and minima from 19 to 21°C. A base camp was established in an upland forest identified by Degagne *et al.* (2008) as co-dominated by the ECM canopy trees *D. corymbosa* Spruce ex Benth. and *D. altsonii* Sandw. (Fabaceae subfamily Caesalpinioideae) with scattered canopy trees of *A. insignis* (Benth.) Endl. (Fabaceae subfamily Papilionoideae). *Dicymbe altsonii* and *A. insignis* have typical arboreal architecture with a single bole and horizontal crown, although *A. insignis* is more strongly buttressed. By contrast, *D. corymbosa* has a reiterative physiognomy including a vertically layered crown, multiple stems which vary in size, and adventitious roots, resulting in a large litter-trapping capacity (Woolley *et al.*,

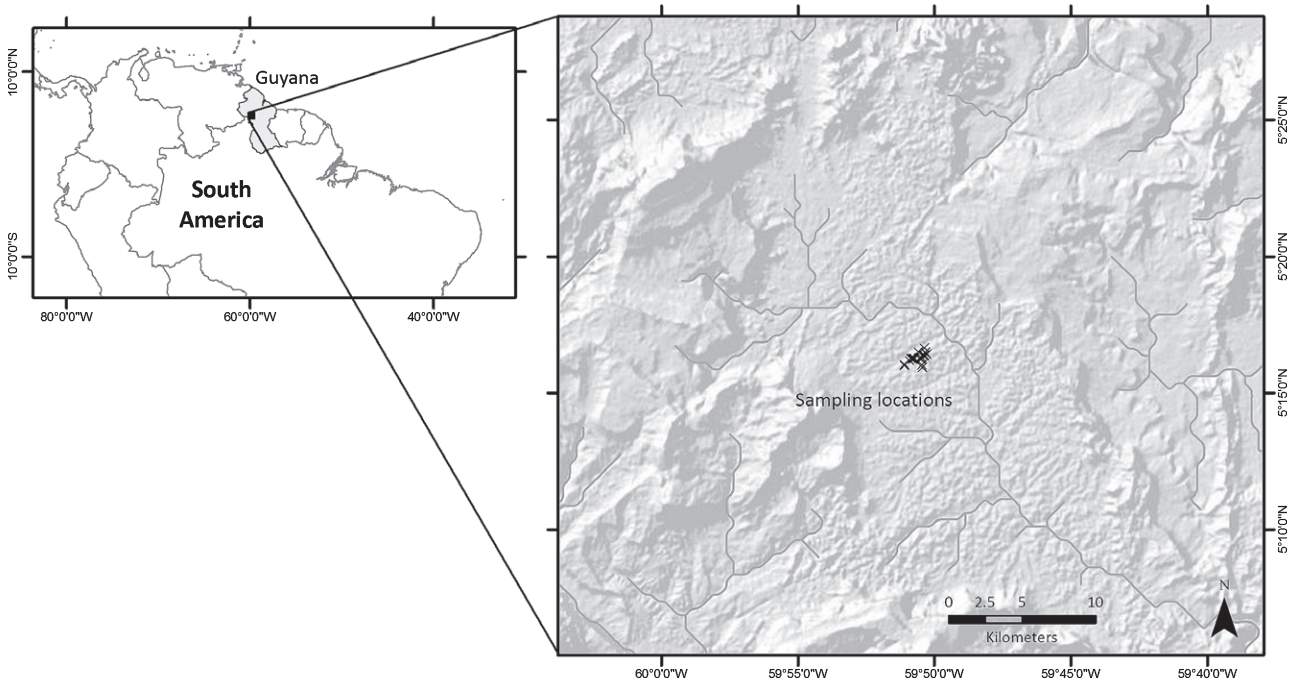

Fig. 1 Map showing general area of study site in western Guyana and distribution of sampling sites in the Upper Potaro River Basin.

2008). The base camp was located at 5°16′14.5″ N; 59°50′39.1″ W and the surrounding terrain is characterized by low, rolling hills with red loam lateritic soils, ranging in elevation from 650 to 800 m above sea level (asl). Details on the climate, geology, soils, forest structure and distribution of forest types have been published previously (Henkel *et al.*, 2002, 2005a; Henkel, 2003; Degagne *et al.*, 2008).

Ectomycorrhizal root sampling

We identified 19 sites ('trios') where individual trees > 20 cm diameter at breast height (dbh) of each of the three host plant species occurred at ≤ 25 m distance from each other. This yielded 57 total trees (19 per species) with a mean dbh of 94.5 cm. The trio sampling sites were distributed along several adjacent ridges with uniform soils and forest composition within 1 km of camp. Distances between site pairs ranged from 28 to 1320 m (mean = 689 m). Four lateral roots from each sampled tree were traced 1–5 m from the base to the fine roots, where roots, soil and litter were excavated and pooled until *c.* 1000 cm^3 of material rich in ECM roots was obtained. Roots were harvested only when unequivocably traced back to the sample tree. These composite root samples were rinsed in water and inspected under a dissecting microscope. Twenty ECM roots were selected from each of the 57 trees. We attempted to maximize ECM diversity for each sample by choosing as many different morphotypes as possible up to a total of 20. A total of 1140 individual ECM roots were preserved in cetyltrimethylammonium bromide (CTAB) buffer and transported to the laboratory. Vouchers of representative trees were obtained and healthy leaves were silica-dried for DNA analysis (see the Supporting Information, Table S2).

Fungal identification

Sporocarps of putative ECM fungi were collected within the tree sampling area in December 2009 and at a nearby site on the Upper Potaro River during 2000–2010. For information on site, specimen identification and herbaria see Henkel *et al.* (2002, 2005b, 2011) and references therein. Sporocarp DNA was extracted using a CTAB protocol (Gardes & Bruns, 1993) or an Extract-N-Amp Plant kit (Sigma-Aldrich, St Louis, MO, USA). Undescribed species were identified to the genus based on morphological data and rDNA sequences. Taxa detected only on roots were identified to genus and their uniqueness at the species level determined using BLAST comparisons against our in-house ribosomal DNA sporocarp database and GenBank. Internal transcribed spacer (ITS) sequences were considered conspecific if they differed by < 3% across the ITS region (Smith *et al.*, 2007; Hughes *et al.*, 2009). Taxa were assigned to the phylogenetically defined ECM fungal lineages recognized by Tedersoo *et al.* (2010a). These lineages constitute monophyletic groups that have independently evolved the ability to form ECM symbioses with plants. Any ECM root sequences that did not fit into these groups were putatively considered representatives of new ECM lineages.

Molecular protocols for fungi

The ECM roots were rinsed in water, dried on paper towels, and crushed with forceps in tubes containing 25 μl of extraction buffer from an Extract-N-Amp PCR kit (Sigma-Aldrich). Roots were then incubated and mixed with 25 μl of dilution buffer. Fungal internal transcribed spacer (ITS)-28S rDNA was PCR-amplified with forward primer ITS1F in combination with ITS4. When amplification with ITS4 was unsuccessful, we used either ITS2, ITS4B, or LR3 (Vilgalys & Hester, 1990; White *et al.*, 1990; Gardes & Bruns, 1993). The PCR protocols followed Smith *et al.* (2007) with modifications in annealing temperature for different primers. Amplicons were visualized on 1.5% agarose gels stained with SYBR Green I (Molecular Probes, Eugene, OR, USA). Amplicons were cleaned with EXO and SAP enzymes (Glenn & Schable, 2005). Sequencing was performed with the same primers using Big Dye Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA, USA). Sequences were edited with SEQUENCHER v.4.1 (Gene Codes Inc., Ann Arbor, MI, USA).

Molecular protocols and phylogenetic analysis of host plants

Plant DNA was extracted from leaves of *D. corymbosa*, *D. altsonii* and *A. insignis* using the DNeasy Plant Kit (Qiagen). Three DNA regions were PCR amplified using published primers and protocols: ITS rDNA and chloroplast Maturase K (*MatK*) gene and the *trnL* intron (*trnL*). The *MatK* region was amplified and sequenced with primers trnK685F, trnK2R* and matK1100L (Wojciechowski *et al.*, 2004), ITS was amplified and sequenced with NS7 and ITS4 (White *et al.*, 1990), and *trnL* was amplified and sequenced with trnC and trnD (Taberlet *et al.*, 1991). The *trnL* region was also PCR-amplified from randomly selected roots of each species to verify host plant identity.

To estimate the number of times the ECM habit has evolved in different lineages of Fabaceae, we compiled a main dataset of *MatK* and *trnL* sequences of representative taxa from across the family. This dataset included at least one taxon for each of the known ECM genera of Fabaceae and a diverse range of nonECM species from different subfamilies (Brundrett, 2009). Current phylogenetic concepts in Fabaceae followed Wojciechowski *et al.* (2004) and Bruneau *et al.* (2008) (Table S2). Attempts to analyse a more taxon-rich dataset with distant outgroups decreased our ability to infer relationships among the ECM Fabaceae lineages because alignments became progressively more ambiguous; we therefore restricted the number of taxa used in the analysis. We also compiled and analysed a second, three-gene (*MatK*, *trnL*, ITS) dataset consisting mostly of Caesalpinioideae and a few Papilionoideae to potentially improve phylogenetic resolution among ECM Caesalpinioideae.

Plant sequences were assembled in MESQUITE v. 2.73 (Maddison & Maddison, 2010) and aligned with the help of MUSCLE (Edgar, 2004). The main dataset included 73 taxa. Both genes were available for all but the Australian ECM genera in the *Mirbelieae* for which only *trnL* was available. The alignment included 1034 bp of *trnL* and a 2019 bp of *MatK*. Once ambiguously aligned regions were excluded, 524 bp of *trnL* and 1679 of *MatK* were used for analysis. The second, three-gene dataset included 51 species and an additional 1881 bp alignment of ITS rDNA. Once ambiguously aligned regions were excluded, 2720 bp from the three genes were used for analysis. For both datasets *Dialium guianense* was selected as the outgroup taxon. This caesalpinioid taxon is a member of the basal tribe *Dialinae* and is not closely related to any of the other species in the analyses (Bruneau *et al.*, 2008).

Preliminary analyses did not reveal strong phylogenetic discordance, so the genes were combined for all analyses. Maximum parsimony analyses (MP) were performed with PAUP* (Swofford, 2002) and maximum likelihood (ML) analyses were performed with GARLI (Zwickl, 2006). MR MODELTEST was used to determine that the GTR + I + G model was the best model for all analyses (Nylander, 2004). Bootstrap support was assessed with 1000 MP boostrap replicates using the 'fast stepwise addition' option in PAUP* whereas ML bootstrap analysis was performed with 500 bootstrap replicates using default settings in GARLI. Bayesian analyses were conducted with MR BAYES v. 3.12 (Huelsenbeck & Ronquist, 2001). Posterior probabilities were obtained by running four Markov–Monte Carlo (MCMC) chains for 20 000 000 generations, sampling every 1000 generations and discarding the initial 15% of trees as the burn in.

Analysis of ectomycorrhizal diversity, host preferences and spatial patterns

The Shannon–Wiener diversity index (H') and the Simpson's diversity index (1-D) were calculated for ECM fungi using PC-ORD 6 (McCune & Mefford, 2011). To compare the effect of site vs host tree species on the ECM fungal community structure, we calculated MRPP (Multiple Response Permutation Procedure) and perMANOVA (permutational multivariate analysis of variance) using PC-ORD 6 (McCune & Mefford, 2011). Both MRPP and perMANOVA were performed for each grouping variable comparison using 999 randomized runs and four different distance measures (Euclidean, relative Euclidean, Jaccard and Sørensen) to ensure that results were consistent across distance measures. The MRPP is a nonparametric, multivariate procedure that tests the null hypothesis of no difference between groups (site and host species). The test statistic (T) explains the separation between groups in multidimensional 'species' space; the more negative the T-value, the greater the separation between groups. The

P-value quantifies separation between groups when compared with what is expected by chance and *A* represents the chance-corrected within group agreement and is a measure of effect size (McCune & Grace, 2002). Similar to conventional ANOVA, perMANOVA calculates an *F*-value statistic by comparing the ratio of among-group sums of squares with within-group sums of squares and does not assume multivariate normality. In perMANOVA, probabilities were calculated with Monte Carlo randomization procedures (999 runs), where *P*-values are the probability of obtaining an *F*-value larger than expected based on random permutations of the original data (McCune & Grace, 2002). To test for statistically significant differences in frequency of individual ECM fungal species, we used the software package R (R Core Development Team, 2007) to perform a Fischer's exact test for each fungal species across each of the three pairwise tree comparisons. The Fisher exact test is a significance test used in the analysis of contingency tables with low sample sizes. We applied a Bonferroni correction to determine significance at $P = 0.01667$ ($P = 0.05/3$).

Biodiversity and biogeography of the /clavulina lineage

The monophyletic /clavulina lineage is essentially equivalent to the coral mushroom genus *Clavulina* (Cantharellales) (Tedersoo *et al.*, 2010a; J. K. Uehling *et al.*, unpublished). The high species diversity of this lineage in tropical South America based on sporocarps (Henkel *et al.*, 2011) and ECM roots in this study prompted us to examine global /clavulina diversity and biogeographical patterns. All /clavulina ITS sequences in GenBank were identified and downloaded using the Emerencia website (Ryberg *et al.*, 2009). This search produced 535 sequences supplemented by 53 unpublished sequences obtained from L. Tedersoo (Tartu University, Estonia). The ITS barcode was selected because it was the only DNA region with global representation of /clavulina sequences. All sequences were examined in a preliminary alignment and with BLAST searches. Sequences that were putatively chimeric, were < 300 bp long, had many ambiguities or aberrant 5.8 s sequences, or that had BLAST matches to genera other than *Clavulina* were discarded. The 588 ITS sequences were combined with 44 unique /clavulina sequences from Guyana (23 from sporocarps and 21 from roots). The resulting 632 sequences were partitioned into operational taxonomic units (OTUs) meant to approximate fungal species by grouping sequences at the ≥ 97% similarity level using the 'clean data' algorithm in SEQUENCHER (Smith *et al.*, 2007). For each OTU, one high-quality sequence was included in the final analysis. Frequent indels in /clavulina ITS sequences prevented reliable phylogenetic analysis so two alternative methods were used to analyse diversity: comparison of ITS length variation in PAUP*; and calculation of uncorrected pairwise genetic distances between all ITS sequences using MOTHUR (Schloss *et al.*, 2009). In MOTHUR, each of the 143 global OTUs was compared with every other OTU using a 'local alignment'. As each pair of sequences was independently aligned, this provided more accurate comparisons than those obtained with a global alignment. The 'pairwise.seqs' command was used with the BLAST algorithm and the following settings: +1 reward for matching bases, −2 penalty for mismatching bases, terminal gaps not penalized, and gap costs = linear. To assess the diversity of /clavulina in different biogeographical regions, /clavulina OTUs were sorted into four areas of origin: temperate zones (including Northern and Southern Hemispheres); tropical zones; tropical South America and global. For each region, mean genetic distances and standard deviations for the /clavulina sequences were calculated and compared. We also examined how two important morphological features, sporocarp morphology and sterigmata (slender projections where the basidiospores are produced) per basidium, were distributed in the different world regions.

Results

Diversity and structure of the ectomycorrhizal fungal community

Internal transcribed spacer sequences of ECM fungi were successfully generated from *c.* 90% (1020/1140) of the roots sampled from the 57 host trees. We detected 118 distinct OTUs that corresponded with ECM fungal species. These represent 17 different phylogenetic lineages (Table S1). Approx. 41% of the fungal OTUs from roots were matched to sporocarps (49/118). Sixty-eight fungal OTUs were detected three times or more, along with 20 doubleton and 32 singleton OTUs. We detected 9–18 fungal OTUs per individual tree (mean = 13.8, SD = 2.3). The high species richness, high Shannon–Wiener Index value of $H' = 2.648$, and the high Simpson's Diversity Index value of 1-D = 0.925 indicate that ECM fungal diversity was comparable to that found in a number of species-rich temperate and tropical sites (Peay *et al.*, 2010b; L. Tedersoo *et al.*, unpublished data). The most OTU-rich lineages were /clavulina (21), /russula-lactarius (21), /boletus (19) and /tomentella-thelephora (15). The fungal community was dominated by *Basidiomycota* with only three *Ascomycota* OTUs from the /elaphomyces and /sordariales lineages.

We detected five fungal OTUs that could not be assigned to any of the ECM lineages defined by Tedersoo *et al.* (2010a). The closest sequence matches for four of these OTUs were ECM fungi from Bornean dipterocarp roots (Table 1) (Peay *et al.*, 2010b). These include one OTU in Atheliales, two OTUs within a lineage of Polyporales and two OTUs in separate lineages of Agaricales (Tables 1, S1).

Table 1 Closest BLAST matches and frequency of occurrence of five root-inhabiting ectomycorrhizal (ECM) fungal operational taxonomic units (OTUs) that could not be assigned to any previously known ectomycorrhizal fungal lineages

ECM taxon name	ECM lineage	Closest BLAST match	% Similarity	GenBank no.	Frequency on roots			
					All hosts	*Aldina insignis*	*Dicymbe corymbosa*	*Dicymbe altsonii*
Agaricales (Tricholomataceae) ECM926	Agaricales1	Tricholomataceae ECM LH136 (Malaysia) GQ268683	602/638 (94%)	JN168774	1	0	0	1
Agaricales ECM268	Agaricales2	Agaricales ECM LH134 (Malaysia) GQ268681	397/439 (90%)	JN168676, JN168662	2	0	1	1
Atheliales ECM644	Atheliales1	Atheliales ECM LH25 (Malaysia) GQ268572	549/666 (82%)	JN168682	9	3	4	2
Polyporales ECM259[1]	Polyporales1	*Phlebiella vaga* EU118660	617/639 (97%)	JN168733	1	0	1	0
Polyporales ECM287	Polyporales1	Polyporales ECM LH78 (Malaysia) GQ268625	670/714 (94%)	JN168734	1	1	0	0

[1]Sequences of 28S rDNA from ECM259 matched closest with *Phlebiella* spp. and *Polyporales* ECM LH80 (Malaysia) GQ268627.

Although ITS sequences resolve these ECM fungi at the ordinal level, their closest relatives are not completely clear. ITS sequences of the two *Polyporales* OTUs are *c.* 90% similar to the ITS sequence from vouchered ECM roots of *D. corymbosa* (TH70815). Microscopic examination of ECM collection TH70815 revealed normal ECM formation, including a fungal mantle, Hartig net and otherwise healthy root tissue (Fig. S1).

Spatial patterns and host associations

Most ECM fungal OTUs, including all of the 28 most frequently detected fungi, were generalists associated with all three of the host plant species (Fig. 2). Similar richness was detected on the three hosts (*D. corymbosa* = 87 OTUs, *D. altsonii* = 89 OTUs and *A. insignis* = 82 OTUs). Sampling effort curves indicate that the community was not exhaustively sampled, but the three hosts were equitably sampled (Fig. 3). We directly sequenced *trnL* from 33 randomly selected ECM roots to corroborate host plant identifications. Sequences from each of these matched that obtained from leaves of the expected host species (data not shown).

There was no evidence of host specialization among ECM fungi (Fig. 2). Among the 88 nonsingleton OTUs, 73 (83%) were detected on all three of the host plant species and 81 (92%) were found on at least two of the host species. Thus, only seven nonsingleton OTUs were restricted to a single host species and all of these fungi were rare (i.e. found on only two trees). The Fisher's exact tests showed that 117 out of 118 ECM species were not statistically different in their associations with the three host plant species. Only one ECM fungus, *Tomentella* MES348, was statistically more frequent on *D. corymbosa* than on *D. altsonii*.

Both MRPP and perMANOVA tests indicated that variance in the fungal community was not significantly partitioned by host species regardless of the distance measure used. The sampling site of a trio, however, was a significant explanatory variable (Table 2). Although some of the host trios had ECM communities that were statistically different from other trios, the variance explained by site was, nonetheless, low based on MRPP (Euclidean $T = -2.45$, Sørensen $T = -2.41$) and perMANOVA (variability explained, Euclidean = 3.7%, Sørensen = 7.4%). These measures imply that a modest degree of geographical structure is present in the ECM fungal community at sampling distances of < 1 km. The local spatial patterning in the ECM fungal community was probably influenced by several cases where uncommon fungal OTUs were present on two or three trees in a given trio, but were rare or absent from other nearby trios.

Biodiversity and biogeography of the */clavulina lineage

The pruned dataset of 143 global */clavulina OTUs consisted of 58 OTUs from temperate zones and 85 OTUs

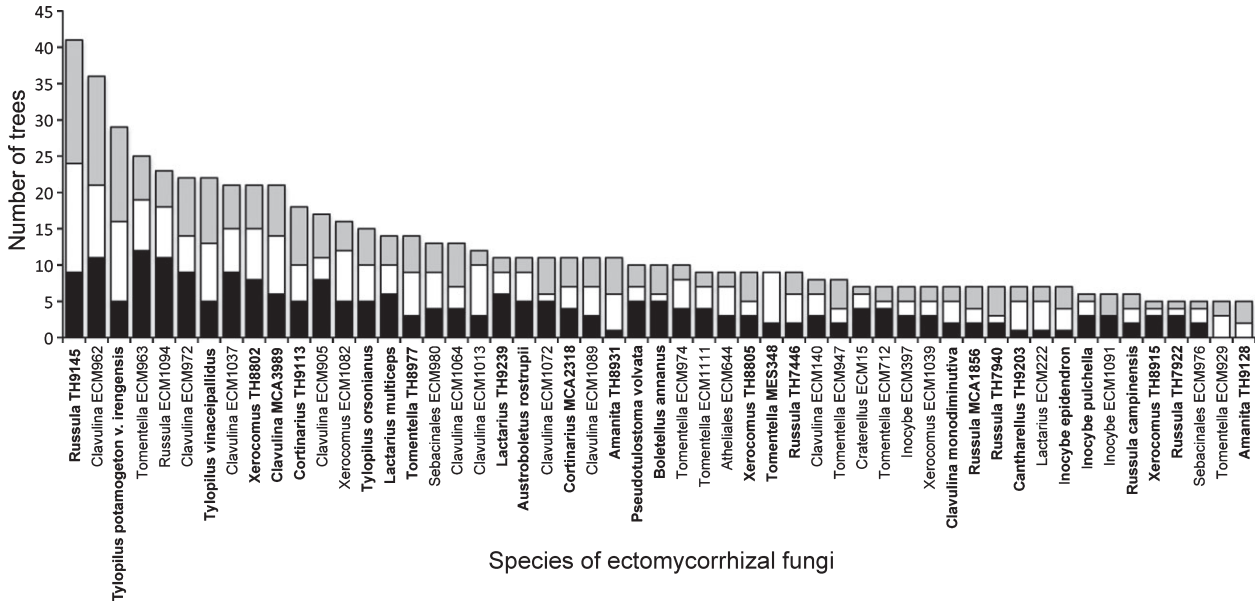

Fig. 2 Frequency of the 45 most common ectomycorrhizal (ECM) fungi on the roots of the three host plants, *Aldina insignis* (black bars), *Dicymbe corymbosa* (white bars), and *Dicymbe altsonii* (grey bars). Each of these common fungal species occurred on five or more trees; 41 of these operational taxonomic units (OTUs) were detected on the roots of all three host plant species. Names in bold designate named fungal species whereas species with TH, MCA, or MES numbers matched voucher specimens of undescribed species. The ECM numbers correspond to fungal OTUs known only from ECM root sequences.

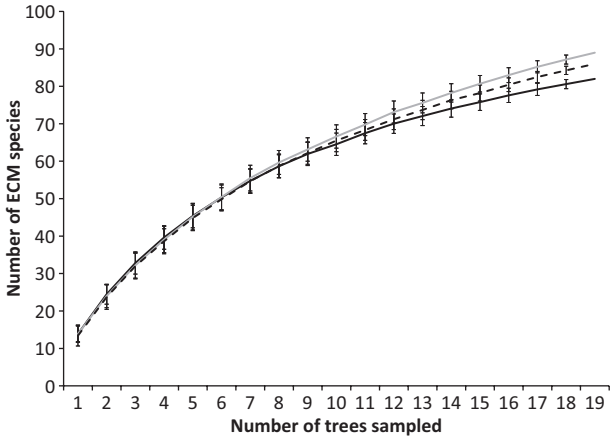

Fig. 3 Sampling effort curves for ectomycorrhizal (ECM) fungal species on the roots of the three leguminous host trees, *Aldina insignis* (solid black line), *Dicymbe corymbosa* (dashed black line) and *Dicymbe altsonii* (solid grey line). Error bars depict standard deviations.

from the tropics. The tropical South American /clavulina assemblage accounted for 48 of 143 OTUs, *c.* 34% of the global total (Table 3). The average genetic distance for tropical South American /clavulina was 0.51, only slightly less than the global average genetic distance of 0.52. Tropical South American /clavulina were also more variable in the full ITS length than those from the temperate regions and were almost as variable in length as the /clavulina sequences from the entire world. There was a similar pattern when ITS1 and ITS2 were analysed separately. Furthermore, there is greater morphological variation in *Clavulina* species from tropical South America than in those from other geographical regions (Table 3).

Phylogenetic analysis of ectomycorrhizal Fabaceae

For the two-gene plant dataset (*MatK* and *trnL*), the final alignment of 2203 bp yielded 604 parsimony-informative characters. Parsimony analysis resulted in 19 368 equally parsimonious trees of 3163 steps (CI = 0.592, RI = 0.821). Both MP and ML bootstrap analyses and Bayesian posterior probabilities supported relationships shown by published Fabaceae phylogenies. These relationships include several monophyletic clades within Caesalpinioideae as well as monophyly of Papilionoideae and Mimosoideae (Wojciechowski *et al.*, 2004; Bruneau *et al.*, 2008). The overall topology of the consensus tree (Fig. 4) was similar to the best ML tree ($-\log_e$ 10 124.98, phylogram not shown). The phylogeny identified four distinct lineages of ECM-forming Fabaceae: the *Berlinia* + *Intsia* clade (Caesalpinioideae); the Australian *Acacia* clade (Mimosoideae); the Australian Mirbelieae clade (Papilionoideae); and the genus *Aldina* (Papilionoideae). All analyses showed that each of these ECM lineages was nested within a larger nonECM clade, suggesting that the ECM habit has evolved at least four times. *Dicymbe* spp. were nested within the *Berlinia* + *Intsia* clade whereas *A. insignis* was not closely related to any other ECM taxa.

The three-gene dataset included fewer taxa but used an additional locus (ITS rDNA). Analysis of this dataset generated

Table 2 Results of two statistical tests, MRPP (Multiple Response Permutation Procedure) and perMANOVA (permutational MANOVA), used to examine potential impact of host tree species and sampling site on the structure of the ectomycorrhizal fungal community on three leguminous tree species (*Dicymbe corymbosa*, *Dicymbe altsonii* and *Aldina insignis*) in a tropical rainforest in Guyana. The results of each test are shown using both Euclidean and Sørensen distance measures

	Distance measure	*A*	*T*	*P* <
(a) MRPP				
Host tree species	Euclidean	0.0005	−0.25	0.38
	Sørensen	0.009	−0.42	0.32
Site	Euclidean	0.020	−2.45	0.009
	Sørensen	0.038	−2.41	0.009
	Distance measure	Variability explained (%)	*F*	*P* <
(b) perMANOVA				
Host tree species	Euclidean	0.21	1.04	0.35
	Sørensen	0.77	1.15	0.25
Site	Euclidean	3.7	1.1	0.013
	Sørensen	7.4	1.2	0.011

T, the separation between groups in multidimensional 'species' space; the more negative the T-value, the greater the separation between groups.
A, the chance-corrected within group agreement and is a measure of effect size (McCune & Grace, 2002).

108 equally parsimonious trees (2312 steps, CI = 0.668, RI = 0.802) and an optimal ML tree with likelihood of $\log_e$ −16 743.28171 (Fig. S2). The ML tree and the strict MP consensus were similar overall and showed the same basic relationships with only minor arrangements in the *Berlinia* clade. The *Intsia* + *Afzelia* clade was resolved as phylogenetically distinct but sister to the *Berlinia* clade. Also included within this crown *Berlinia* + *Intsia* group of mostly ECM-forming Caesalpinioideae are putatively nonECM taxa *Brownea coccinea*, *Hymenostegia afzelii* and *Tamarindus indica*. In the *Berlinia* group, all genera and species are known to form ECM except for *Humboldtia vahliana* (Brundrett, 2009).

Discussion

Diversity and structure of the ectomycorrhizal fungal community

Trees in the genera *Dicymbe* and *Aldina* are known to form ECM symbioses (Singer & Araujo, 1979; Henkel *et al.*, 2002) but this is the first in-depth molecular study of fungal ECM symbionts of leguminous trees in the Neotropics. At a single site we detected 118 ECM fungal OTUs on the roots of *D. corymbosa*, *D. altsonii*, and *A. insignis*, each of which harbored 82–89 OTUs. The diverse ECM assemblage included members of 17 fungal lineages and, similar to other tropical studies, we found a high species richness in the /russula-lactarius, /boletus, and /tomentella-thelephora lineages. Unique to this study was the exceptional diversity in the /clavulina lineage. The high overall fungal species richness and the concurrently high Shannon–Wiener and Simpson's diversity estimates indicate that the ECM fungal diversity of this Guiana Shield site is on a par with dipterocarp forests of Borneo and many ECM-rich forests from temperate and boreal zones (Peay *et al.*, 2010b; Tedersoo & Nara, 2010; L. Tedersoo *et al.*, unpublished data).

Despite the phylogenetic differences and independent evolution of the ECM symbiosis in *Dicymbe* and *Aldina*, we found almost no evidence of ECM fungal host preferences at any taxonomic level (Table 2). Nearly 92% of fungal OTUs were multihost generalists detected on the roots of

Table 3 Global diversity patterns in the /clavulina lineage based on morphology and on internal transcribed spacer (ITS) sequence data from GenBank and this study

World region	Operational taxonomic units (OTUs, % of total)	Mean genetic distance[1] (SD)	Sporocarp Morphology[2]	Sterigmata per basidium[3]	Mean full ITS length[4] (range)	Mean ITS1 length[4] (range)	Mean ITS2 length[4] (range)
Temperate	58 (41)	0.48 (0.20)	C, R	2	608.8 (548–655)	205.9 (163–239)	248.5 (236–264)
Tropical	85 (59)	0.50 (0.21)	C, R, I, M	2 (3–6)	610.6 (510–661)	207.1 (106–247)	248.7 (207–280)
Tropical South America	48 (34)	0.51 (0.21)	C, R, I, M	2 (3–6)	608.8 (510–647)	206.6 (106–233)	247.5 (207–280)
Global	143 (100)	0.52 (0.20)	C, R, I, M	2 (3–6)	610.0 (510–661)	206.6 (106–247)	248.6 (207–280)

[1]Genetic distance based on pairwise ITS comparisons using length-adjusted BLAST scores (see the Materials and Methods section for details).
[2]Morphological diversity includes four main sporocarp types: clavarioid (C), resupinate (R), infundibuliform (I) and monopodial (M).
[3]Sterigmata are the slender projections where the basidiospores are produced. Globally, most *Clavulina* species have two sterigmata per basidium but several tropical South American taxa routinely have 3–4 or 4–6 sterigmata per basidium (Thacker & Henkel, 2004; Henkel *et al.*, 2005b, 2011; J. K. Uehling *et al.*, unpublished).
[4]ITS length calculations based on taxa with complete sequences for each comparison; full ITS = 124 spp., ITS1 only = 134 spp., ITS2 only = 127 spp.

Fig. 4 Maximum parsimony 50% majority rule consensus tree depicting relationships among Fabaceae based on chloroplast *trnL* and maturase K (*MatK*). Ectomycorrhizal (ECM) host plants (bold type) are distributed among four distinct lineages in the three subfamilies Caesalpinioideae, Mimosoideae and Papilionoideae. Among ECM host species from Guyana sequenced for this study, *Aldina insignis* is placed among nonECM Papilionoideae whereas *Dicymbe corymbosa* and *Dicymbe altsonii* are nested within the *Berlinia* + *Intsia* clade of the Caesalpinioideae. Support values for the phylogeny are parsimony bootstrap/maximum likelihood bootstrap/Bayesian posterior probabilities. Closed circles indicate nodes that received maximum support with all three measures.

two or three of the tree species (Fig. 2). Furthermore, Fisher's exact tests illustrated that, at the species level, 117 out of 118 ECM species were not statistically associated with one host tree over another. This situation is similar to that in West African forests of Fabaceae and Phyllanthaceae where ECM fungal symbionts exhibited almost no preferences among five sympatric host tree species (Diédhiou *et al.*, 2010). By contrast, other studies have demonstrated fungal host preference in communities with multiple sympatric ECM hosts in both the temperate zones (Ishida *et al.*, 2007; Tedersoo *et al.*, 2008; Smith *et al.*, 2009) and the lowland Neotropics (Tedersoo *et al.*, 2010c). In an

Ecuadorian rainforest Tedersoo *et al.* (2010c) detected only 38 ECM fungal species from seven lineages, but the fungi exhibited strong preferences among the 26 sympatric host species of *Coccoloba* (Polygonaceae), *Neea* and *Guapira* (Nyctaginaceae). Although the co-occurring host plant species were diverse at the Ecuadorian site, the plant populations consisted of widely dispersed understory plants scattered throughout a forest overwhelmingly dominated by AM canopy trees. Tedersoo *et al.* (2010c) hypothesized that the low ECM fungal diversity and strong host preferences were partly a result of resource and dispersal limitations for the mycobionts. They suggested that these isolated host plants functioned as discrete habitat islands with limited photosynthetic resources and inter-connectivity.

The situation in many Guiana Shield rainforests is quite different. The high ECM fungal diversity on only three sympatric host plant species reported here may be attributable to the large resource pool locally available to mycobionts. The study site is nested within a part of Guyana that is codominated by ECM *Dicymbe* species, along with large, scattered individuals of *A. insignis*, which together can account for 65–85% of the canopy trees (Degagne *et al.*, 2008). *Dicymbe* species dominate these central Guiana Shield forests via a combination of life-history traits including the ECM symbiosis, mast fruiting and resulting high recruitment, large and persistent root biomass, and reiterative growth that yields multistemmed individuals with indeterminate life-spans (Zagt, 1997a,b; Henkel *et al.*, 2005a; Mayor & Henkel, 2005; Woolley *et al.*, 2008). *Dicymbe corymbosa* may also restrict recruitment of other trees by creating highly shaded understory conditions in conjunction with large litter accumulations, rendering it difficult for AM trees to establish (McGuire, 2007). Dominance of closed-canopy forests by *Dicymbe* species is currently documented only for western Guyana and at some locations *Dicymbe* species codominate stands with other canopy host genera such as *Aldina* and *Pakaraimea* (Dipterocarpaceae) (Henkel *et al.*, 2002; T. Henkel *et al.* unpublished data; this study), ECM genera that also occur more widely in the Guiana Shield region (Moyersoen, 2006; Mardegan *et al.*, 2009). In this ecological setting, barriers to ECM fungal success and diversification owing to resource and dispersal limitations should be minimal. We hypothesize that in the central Guiana Shield, local forest dominance of ECM plants along with regional prevalence of several ECM canopy tree genera are major drivers of ECM fungal diversity.

Phylogenetic relationships of ectomycorrhizal Fabaceae

Trees in the Fabaceae are dominant components of the woody flora in much of the Neotropics, West Africa and Australia, and to a lesser extent in Southeast Asia (Newbery *et al.*, 1997, 2002; Torti *et al.*, 2001; Crisp *et al.*, 2004; ter Steege *et al.*, 2006). Although phylogenetic studies have addressed morphology, taxonomy and root nodulation in this ecologically important plant family, the ECM symbiosis is rarely mentioned in the Fabaceae literature and this trait has not been studied in a phylogenetic context (Sprent & James, 2007). This aspect of Fabaceae biology was likely neglected because of conflicting reports on ECM formation in some species and because ECM-forming taxa are primarily tropical or Austral and infrequently collected.

The ECM habit has been thoroughly documented in many species of African and some Asian Caesalpinioideae (Fig. 4) as well in Australian species of *Acacia* subgenus Phyllodinae (Fabaceae subfamily Mimosoideae) (Alexander & Högberg, 1986; Alexander, 1989; Duponnois & Plenchette, 2003; Andre *et al.*, 2005). By contrast, only a handful of Papilionoideae species have been convincingly shown to form ECM. Of these, the genus *Aldina* contains 26 species endemic to nutrient poor habitats in the greater Guiana Shield (Stergios & Aymard, 2008). All of the *Aldina* species that have been examined at sites in Brazil, Venezuela and Guyana form ECM associations (Singer & Araujo, 1979; Singer & Aguiar, 1986; Henkel *et al.*, 2002; Moyersoen, 2006). The phylogenetic position of *Aldina* has been the subject of debate (Doyle *et al.*, 1997; Pennington *et al.*, 2001) but the genus is not closely related to other known ECM Fabaceae (Fig. 4). Several studies have also convincingly shown the ECM status of a clade of Australian papilionoid genera in the tribe Mirbelieae, including *Gastrolobium*, *Gompholobium*, *Jacksonia*, *Mirbelia* and *Oxylobium* (Kope & Warcup, 1986; Brundrett & Abbott, 1991; Brundrett, 2009). The African papilionoid *Pericopsis angolensis* (Baker) Meeuwen has been reported to form ECM in some studies (Alexander & Högberg, 1986; Högberg & Piearce, 1986) but other times this species forms root nodules and arbuscular mycorrhizas without ECM (Högberg, 1990). Other reports of ECM in the papilionoid genera *Robinia* and *Lonchocarpus* have either been refuted or remain unsubstantiated (Frioni *et al.*, 1999; Kovács *et al.*, 2007; Sprent & James, 2007).

Our phylogenetic analysis indicates that the ECM symbiosis has evolved in at least four distinct lineages of Fabaceae (Fig. 4). The phylogeny depicts *A. insignis* among nonECM Papilionoideae and confirms that this species is phylogenetically distant from the caesalpinioid *Dicymbe* species. As shown by Bruneau *et al.* (2008), *Dicymbe* is related to a diverse clade of African ECM Caesalpinioideae (Figs 4, S2). The separation of different ECM lineages (e.g. *Dicymbe*, *Intsia* + *Afzelia* and *Berlinia* clades) in various phylogenetic analyses that have used different data and methods (Bruneau & Forest, 2001; Bruneau *et al.*, 2008; this study) indicates the possibility of multiple, independent ECM origins within the Caesalpinioideae. An alternate

hypothesis is that the ECM symbiosis may have been lost in some caesalpinioid taxa (e.g. *B. coccinea* and *T. indica*; Fig. 4). More phylogenetic data and ECM root sampling of Caesalpinioideae species will be necessary to resolve the evolutionary history of the ECM symbiosis in this group.

Origins and diversification of tropical ECM fungi

Tedersoo & Nara (2010) suggested that ECM fungi reach peak diversity at higher latitudes and that most ECM lineages evolved in the temperate zones. In their analysis, only the /russula-lactarius lineage was more diverse in tropical than in temperate habitats. By contrast, the /inocybe lineage was more diverse in the temperate zones, despite the fact that this group apparently arose in the paleotropics (Matheny *et al.*, 2009; Tedersoo & Nara, 2010). Tedersoo *et al.* (2010a) could find no evidence of endemic tropical ECM fungal lineages and noted that several temperate ECM lineages are apparently absent or species-poor in the tropics (e.g. /albatrellus, /cenococcum, / endogone and /hygrophorus). However, these assessments were based on a limited amount of data from the tropics and additional studies from tropical ecosystems may alter this view of ECM fungal biogeography and biodiversity.

We detected several fungal OTUs in the Agaricales, Polyporales and Atheliales that could not be assigned to any of the monophyletic ECM lineages defined by Tedersoo *et al.* (2010a). The closest BLAST matches for these enigmatic OTUs were mostly sequences from ECM roots of Bornean dipterocarps (Table 1; Peay *et al.*, 2010b). The occurrence of these fungi on healthy ECM roots in two widely disjunct tropical sites suggests that they may represent previously undocumented tropical ECM fungal lineages. Some morphological data also support their ECM status; ITS sequences of the *Polyporales* OTUs were similar to those of vouchered ECM specimens that have a typical mantle and Hartig net (Fig. S1). Future morphological studies of ECM roots in the Guiana Shield and other tropical sites will help clarify the identity and symbiotic functioning of these putatively ECM fungi.

More important was our finding that the /clavulina lineage was highly diverse and abundant at the Guyana site. Several lines of evidence suggest that the /clavulina lineage, which is more-or-less equivalent to the coral mushroom genus *Clavulina* (Cantharellales) (Tedersoo *et al.*, 2010a; J. K. Uehling *et al.*, unpublished), has a previously unrecognized center of diversity in tropical South America. We found 21 distinct /clavulina OTUs on roots of the three host plant species. All but one of the 57 trees hosted at least one /clavulina OTU and each individual tree hosted an average of 3.5 /clavulina OTUs. One individual *D. altsonii* tree hosted seven /clavulina OTUs, compared with the six phylogenetically delimited species documented for the continent of Europe (Olariaga *et al.*, 2009).

Our analysis of global /clavulina ITS sequences also suggests that this lineage is most diverse in tropical habitats. Out of 143 global /clavulina OTUs, 59% were from tropical regions, despite much lower sampling effort in the tropics. Tropical South American sequences accounted for one-third of the world's /clavulina OTUs and the mean genetic distance for tropical South American OTUs was almost as high as that for the global /clavulina dataset (0.51 vs 0.52, Table 3). The wide variability in ITS length among the tropical South American OTUs is an additional indicator of high diversity (Table 3). Furthermore, ECM fungal sporocarp surveys in *Dicymbe* forests have uncovered many new and morphologically divergent species in the /clavulina lineage and shown that /clavulina sporocarp diversity is higher at the Guyana sites than anywhere else in the world (Thacker & Henkel, 2004; J. K. Uehling *et al.*, unpublished; T. Henkel *et al.*, unpublished data). Molecular analyses based on *rpb*2 and 28S rDNA also suggest that the /clavulina lineage is phylogenetically diverse in tropical South America (Thacker & Henkel, 2004; J. K. Uehling *et al.*, unpublished). While the /clavulina lineage is globally distributed (Tedersoo *et al.*, 2010a), the molecular and morphological data presented here suggest that this lineage likely evolved or underwent diversification in tropical South America. As far as we know, this is the most convincing case thus far for an evolutionary radiation among ECM fungi in the tropics.

Conclusions

The high diversity and absence of host preferences for ECM fungi in this Guiana Shield rainforest alter the current view of ECM communities in lowland Neotropical forests. Although host identity and dispersion strongly influences the ECM fungal community in tropical forests with scattered understory hosts that are phylogenetically distant (Tedersoo *et al.*, 2010c), this is not the case in Fabaceae-dominated tropical forests where the hosts are large canopy trees growing in dense stands. A high degree of host sharing by ECM fungi was prevalent whether hosts belonged to the same genus (i.e. *Dicymbe* species) or represented independent evolutionary acquisitions of the ECM symbiosis (i.e. *Dicymbe* vs *Aldina*). The robust diversity of ECM fungi, the discovery of a center of diversity for the /clavulina lineage and presence of unique, putatively tropical-endemic ECM fungal lineages in the Guiana Shield emphasize the need for expanded ECM studies in the tropics.

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References

Alexander IJ. 1989. Systematics and ecology of ectomycorrhizal legumes. In: Stirton CH, Zarucchi JL, eds. *Advances in legume biology*. Monographs in Systematic Botany No. 29. St. Louis, MO, USA: Missouri Botanical Garden, 607–624.

Alexander IJ. 2006. Ectomycorrhizas – out of Africa? *New Phytologist* **172**: 589–591.

Alexander IJ, Högberg P. 1986. Ectomycorrhizas of tropical angiospermous trees. *New Phytologist* **102**: 541–549.

Alexander IJ, Lee S. 2005. Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity. In: Burslem D, Pinard M, Hartley S, eds. *Biotic interactions in the tropics*. Cambridge, UK: Cambridge University Press, 165–203.

Amend A, Seifert K, Samson R, Bruns TD. 2010. Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. *Proceedings of the National Academy of Sciences, USA* **31**: 13748–13753.

Andre S, Galiana A, Le Roux C, Prin Y, Neyra M, Duponnois R. 2005. Ectomycorrhizal symbiosis enhanced the efficiency of inoculation with two *Bradyrhizobium* strains and *Acacia holosericea* growth. *Mycorrhiza* **15**: 357–364.

Aponte C, García L, Marankn T, Gardes M. 2010. Indirect host effect on ectomycorrhizal fungi: leaf fall and litter quality explain changes in fungal communities on the roots of co-occurring Mediterranean oaks. *Soil Biology and Biochemistry* **42**: 788–796.

Arnold EA, Lutzoni F. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* **88**: 549–551.

Bandou E, Lebailly F, Muller F, Dulormne M, Toribio A, Chabrol J, Courtecuisse R, Plenchette C, Prin Y, Duponnois R *et al.* 2006. The ectomycorrhizal fungus *Scleroderma bermudense* alleviates salt stress in seagrape (*Coccoloba uvifera* L.) seedlings. *Mycorrhiza* **16**: 559–565.

Bereau M, Gazel M, Garbaye J. 1997. Mycorrhizal symbioses in the trees of tropical rainforest of French Guiana. *Canadian Journal of Botany* **75**: 711–716.

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.

Brundrett M, Abbott L. 1991. Roots of jarrah forest plants. I. Mycorrhizal associations of shrubs and herbaceous plants. *Australian Journal of Botany* **39**: 445–457.

Bruneau A, Forest F. 2001. Phylogenetic relationships in the *Caesalpinioideae* (*Leguminosae*) as inferred from chloroplast *trnL* intron sequences. *Systematic Botany* **26**: 487–514.

Bruneau A, Mercure M, Lewis G, Herendeen P. 2008. Phylogenetic patterns and diversification in the caesalpinioid legumes. *Botany* **86**: 697–718.

Crisp M, Cook L, Steane D. 2004. Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? *Philosophical Transactions of the Royal Society of London Series B – Biological Sciences* **359**: 1551–1571.

Degagne RS, Henkel TW, Steinberg SJ, Fox L. 2008. Identifying *Dicymbe corymbosa* monodominant forests in Guyana using satellite imagery. *Biotropica* **41**: 7–15.

Dickie I. 2007. Host preference, niches and fungal diversity. *New Phytologist* **174**: 230–233.

Dickie I, Moyersoen B. 2008. Towards a global view of ectomycorrhizal ecology. *New Phytologist* **180**: 263–265.

Diédhiou AG, Selosse M-A, Galiana A, Diabaté M, Dreyfus B, Bâ AM, De Faria SM, Béna G. 2010. Multi-host ectomycorrhizal fungi are predominant in a Guinean tropical rainforest and shared between canopy trees and seedlings. *Environmental Microbiology* **8**: 2219–2232.

Doyle JJ, Doyle JL, Ballenger JA, Dickson EE, Kajita T, Ohashi H. 1997. A phylogeny of the chloroplast gene rbcL in the *Leguminosae*: taxonomic correlations and insights into the evolution of nodulation. *American Journal of Botany* **84**: 541–554.

Duponnois R, Plenchette C. 2003. A mycorrhiza helper bacterium enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species. *Mycorrhiza* **13**: 85–91.

Edgar R. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Fassi B, Fontana A. 1962. Micorize ectotrofiche di *Brachystegia laurentii* e di alcune altre *Cesalpiniaceae* minori del Congo. *Allionia* **8**: 121–131.

Frank AB. 1885. Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. *Berichte der Deutschen Botanischen Gesellschaft* **3**: 128–145.

Frioni L, Minasian H, Volfovicz R. 1999. Arbuscular mycorrhizae and ectomycorrhizae in native tree legumes in Uruguay. *Forest Ecology and Management* **115**: 41–47.

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

Glenn T, Schable N. 2005. Isolating microsatelline DNA loci. In: Zimmer EA, Roalson EH, eds. *Molecular evolution: producing the biochemical data, part B*. San Diego, CA, USA: Academic Press, 202–222.

Henkel TW. 2003. Monodominance in the ectomycorrhizal *Dicymbe corymbosa* (*Caesalpiniaceae*) from Guyana. *Journal of Tropical Ecology* **19**: 417–437.

Henkel TW, Aime MC, Uehling JK, Smith ME. 2011. New species and distribution records of *Clavulina* (*Cantharellales, Basidiomycota*) from the Guiana Shield. *Mycologia* **103**: 883–894.

Henkel TW, Mayor J, Woolley L. 2005a. Mast fruiting and seedling survival of the ectomycorrhizal, monodominant *Dicymbe corymbosa* (Caesalpiniaceae) in Guyana. *New Phytologist* **167**: 543–556.

Henkel TW, Meszaros R, Aime MC, Kennedy A. 2005b. New *Clavulina* species from the Pakaraima Mountains of Guyana. *Mycological Progress* **4**: 343–350.

Henkel TW, Terborgh J, Vilgalys RJ. 2002. Ectomycorrhizal fungi and their leguminous hosts in the Pakaraima Mountains of Guyana. *Mycological Research* **106**: 515–531.

Hillebrand H. 2004. On the generality of the latitudinal diversity gradient. *American Naturalist* **163**: 192–211.

Högberg P. 1990. ^{15}N natural abundance as a possible marker of the ectomycorrhizal habit of trees in mixed African woodlands. *New Phytologist* **115**: 483–486.

Högberg P, Piearce GD. 1986. Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation type and successional patterns. *Journal of Ecology* **74**: 775–785.

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

Hughes KW, Peterson RH, Lickey EB. 2009. Using heterozygosity to estimate a percentage DNA sequence similarity for environmental

species' delimitation across basidiomycete fungi. *New Phytologist* **182**: 795–798.

Ishida T, Nara K, Hogetsu T. 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer–broadleaf forests. *New Phytologist* **174**: 430–440.

Jablonski D, Roy K, Valentine JW. 2006. Out of the tropics: evolutionary dynamics of the latitudinal diversity gradient. *Science* **314**: 102–106.

Kope H, Warcup J. 1986. Synthesized ectomycorrhizal associations of some Australian herbs and shrubs. *New Phytologist* **104**: 591–599.

Kovács GM, Jakucs E, Bagi I. 2007. Identification of host plants and description of sclerotia of the truffle *Mattirolomyces terfezioides*. *Mycological Progress* **6**: 19–26.

Maddison WP, Maddison DR. 2010. *Mesquite: a modular system for evolutionary analysis, version 2.73*. [WWW document]. URL http://mesquiteproject.org [accessed on 3 April 2011].

Mangan SA, Schnitzer SA, Herre EA, Mack KM, Valencia MC, Sanchez EI, Bever JD. 2010. Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* **466**: 752–755.

Mardegan SF, Nardoto GB, Higuchi N, Moreira MZ, Martinelli LA. 2009. Nitrogen availability patterns in white-sand vegetations of Central Brazilian Amazon. *Trees – Structure and Function* **23**: 479–488.

Matheny PB, Aime MC, Bougher NL, Buyck B, Desjardin DE, Horak E, Kropp BR, Lodge DJ, Trappe JM, Hibbett DS. 2009. Out of the Palaeotropics? Historical biogeography and diversification of the cosmopolitan ectomycorrhizal mushroom family Inocybaceae. *Journal of Biogeography* **36**: 577–592.

Mayor JR, Henkel TW. 2005. Do ectomycorrhizas alter leaf-litter decomposition in tropical monodominant forests of Guyana? *New Phytologist* **169**: 579–588.

McCune B, Grace JB. 2002. *Analysis of ecological communities*. Gleneden Beach, OR, USA: MjM Software.

McCune B, Mefford M. 2011. *PC-ORD. Mulitvariate analysis of ecological data, version 6*. Gleneden Beach, OR, USA: MjM Software.

McGuire KL. 2007. Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest. *Ecology* **88**: 567–574.

Molina R, Massicotte H, Trappe JM. 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF, ed. *Mycorrhizal functioning*. London, UK: Chapman & Hall, 357–423.

Morris MH, Perez-Perez MA, Smith ME, Bledsoe CS. 2009. Influence of host species on ectomycorrhizal communities associated with two co-occurring oaks (*Quercus* spp.) in a tropical cloud forest. *FEMS Microbiology Ecology* **69**: 274–287.

Morris MH, Smith ME, Rizzo DM, Rejmanek M, Bledsoe CS. 2008. Contrasting ectomycorrhizal fungal communities on the roots of co-occurring oaks (*Quercus* spp.) in a California woodland. *New Phytologist* **178**: 167–176.

Moyersoen B. 2006. *Pakaraimaea dipterocarpacea* is ectomycorrhizal, indicating an ancient Gondwanaland origin for the ectomycorrhizal habit in Dipterocarpaceae. *New Phytologist* **172**: 753–762.

Newbery DM, Alexander IJ, Rother JA. 1997. Phosphorus dynamics in a lowland African rain forest: the influence of ectomycorrhizal trees. *Ecological Monographs* **67**: 367–409.

Newbery DM, Alexander IJ, Rother JA. 2000. Does proximity to conspecific adults influence the establishment of ectomycorrhizal trees in rain forest? *New Phytologist* **147**: 401–409.

Newbery DM, Chuyong GB, Green JJ, Songwe NC, Tschuenteu F, Zimmermann L. 2002. Does low phosphorus supply limit seedling establishment and tree growth in groves of ectomycorrhizal trees in a central African rainforest? *New Phytologist* **156**: 296–311.

Nylander JAA. 2004. *MrModeltest, version 2*. Computer software distributed by the author. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University.

Olariaga I, Jugo B, García-Etxebarria K, Salcedo I. 2009. Species delimitation in the European species of *Clavulina* (*Cantharellales, Basidiomycota*) inferred from phylogenetic analyses of ITS region and morphological data. *Mycological Research* **113**: 1261–1270.

Peay KG, Bruns TD, Kennedy PG, Bergemann S, Garbelotto M. 2007. A strong species–area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. *Ecology Letters* **10**: 470–480.

Peay KG, Garbelotto M, Bruns T. 2010a. Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. *Ecology* **91**: 3631–3640.

Peay KG, Kennedy PG, Davies SJ, Tan S, Bruns TD. 2010b. Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. *New Phytologist* **185**: 529–542.

Pennington R, Lavin M, Ireland H, Klitgaard B, Preston J, Hu J. 2001. Phylogenetic relationships of basal papilionoid legumes based upon sequences of the chloroplast *trnL* intron. *Systematic Botany* **26**: 537–556.

Potapov P, Yaroshenko A, Turubanova S, Dubinin M, Laestadius L, Thies C, Aksenov D, Egorov A, Yesipova Y, Glushkov I. 2008. Mapping the world's intact forest landscapes by remote sensing. *Ecology and Society* **13**: 51.

R Core Development Team. 2007. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, http://www.r-project.org/.

Redhead JF. 1968. Mycorrhizal associations in some Nigerian forest trees. *Transactions of the British Mycological Society* **51**: 377–387.

Riviere T, Diedhiou A, Diabate M, Senthilarasu G, Natarajan K, Verbeken A, Buyck B, Dreyfus B, Bena G, Ba A. 2007. Genetic diversity of ectomycorrhizal Basidiomycetes from African and Indian tropical rain forests. *Mycorrhiza* **17**: 415–428.

Ryberg M, Kristiansson E, Sjokvist E, Nilsson RH. 2009. An outlook on the fungal internal transcribed spacer sequences in GenBank and the introduction of a web-based tool for the exploration of fungal diversity. *New Phytologist* **181**: 471–477.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ *et al.* 2009. Introducing MOTHUR: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Singer R, Aguiar IA. 1986. Litter decomposing and ectomycorrhizal basidiomycetes in an Igapo Forest. *Plant Systematics and Evolution* **153**: 107–117.

Singer R, Araujo IJG. 1979. Litter decomposition and ectomycorrhiza in Amazonian forests 1. A comparison of litter decomposing and ectomycorrhizal basidiomycetes in latosol terra-firma rain forest and white podzol campinarana. *Acta Amazonica* **9**: 25–42.

Smith ME, Douhan G, Fremier AK, Rizzo DM. 2009. Are true multihost fungi the exception or the rule? Dominant ectomycorrhizal fungi on *Pinus sabiniana* differ from those on co-occurring *Quercus* species. *New Phytologist* **182**: 295–299.

Smith ME, Douhan GW, Rizzo DM. 2007. Intra-specific and intra-sporocarp ITS variation of ectomycorrhizal fungi as assessed by rDNA sequencing of sporocarps and pooled ectomycorrhizal roots from a *Quercus* woodland. *Mycorrhiza* **18**: 15–22.

Smith S, Read D. 2008. *Mycorrhizal symbiosis, 3rd edn*. London, UK: Academic Press.

Sprent JI, James EK. 2007. Legume evolution: where do nodules and mycorrhizas fit in? *Plant Physiology* **144**: 575–581.

ter Steege H, Pitman NC, Phillips OL, Chave J, Sabatier D, Duque A, Molino JF, Prévost MF, Spichiger R, Castellanos H *et al.* 2006. Continental-scale patterns of canopy tree composition and function across Amazonia. *Nature* **443**: 444–447.

Stergios BD, Aymard GA. 2008. A striking new species of *Aldina* (Fabaceae-Swartzieae-Aldininae) from the Venezuelan Guayana highlands. *Harvard Papers in Botany* **13**: 29–33.

Swofford DL. 2002. *PAUP* Phylogenetic analysis using parsimony (*and other methods), version 4.0*. Sunderland, MA, USA: Sinauer Associates.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Koljalg 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.

Tedersoo L, May T, Smith ME. 2010a. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* **20**: 217–263.

Tedersoo L, Nara K. 2010. General latitudinal gradient of biodiversity is reversed in ectomycorrhizal fungi. *New Phytologist* **185**: 351–354.

Tedersoo L, Nilsson R, Abarenkov K, Jairus T. 2010b. 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytologist* **188**: 291–301.

Tedersoo L, Sadam A, Zambrano M, Valencia R, Bahram M. 2010c. Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. *The ISME Journal* **4**: 465–746.

Tedersoo L, Suvi T, Beaver K, Koljalg U. 2007. Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpiniaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). *New Phytologist* **175**: 321–333.

Thacker J, Henkel TW. 2004. New species of Clavulina from Guyana. *Mycologia* **96**: 650–657.

Thomazini LI. 1974. Mycorrhiza in plants of the Cerrado. *Plant and Soil* **41**: 707–711.

Torti SD, Coley PD, Kursar TA. 2001. Causes and consequences of monodominance in tropical lowland forests. *American Naturalist* **157**: 141–153.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

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

Wiens JJ, Graham CH, Moen DS, Smith SA, Reeder TW. 2006. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. *American Naturalist* **168**: 579–596.

Wojciechowski M, Lavin M, Sanderson M. 2004. A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. *American Journal of Botany* **91**: 1846–1862.

Woolley LP, Henkel TW, Sillett SC. 2008. Reiteration in the monodominant tropical tree *Dicymbe corymbosa* (Caesalpiniaceae) and its potential adaptive significance. *Biotropica* **40**: 32–43.

Wright SJ. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* **130**: 1–14.

Zagt RJ. 1997a. Pre-dispersal and early post-dispersal demography, and reproductive litter production, in the tropical tree *Dicymbe altsonii* in Guyana. *Journal of Tropical Ecology* **13**: 511–526.

Zagt RJ. 1997b. Stem sprouting of *Dicymbe altsonii* in the tropical rainforest of Guyana: impact of soil type and potential for regeneration. In: Zagt RJ, ed. *Tree demography in the tropical rainforest of Guyana*. Georgetown, Guyana: Tropenbos Guyana Programme, 55–72.

Zwickl D. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Doctoral Dissertation, University of Texas at Austin, Austin, TX, USA.

Supporting Information

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

Fig. S1 Morphology of vouchered ectomycorrhiza collection TH70815.

Fig. S2 Maximum likelihood phylogeny of representative species of Fabaceae based on *trnL*, *MatK* and internal transcribed spacer (ITS) sequences.

Table S1 Ectomycorrhizal fungi detected on the roots of *Aldina insignis*, *Dicymbe corymbosa* and *Dicymbe altsonii*

Table S2 Sequence data, taxonomic groupings, and ectomycorrhizal (ECM) status of Fabaceae used in the phylogenetic analyses

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