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Highly diverse and spatially heterogeneous mycorrhizal symbiosis in a rare epiphyte is unrelated to broad biogeographic or environmental features

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Abstract

Symbiotic interactions are common in nature. In dynamic or degraded environments, the ability to associate with multiple partners (i.e. broad specificity) may enable species to persist through fluctuations in the availability of any particular partner. Understanding how species interactions vary across landscapes is necessary to anticipate direct and indirect consequences of environmental degradation on species conservation. We asked whether mycorrhizal symbiosis by populations of a rare epiphytic orchid (*Epidendrum firmum*) is related to geographic or environmental heterogeneity. The latter would suggest that interactions are governed by environmental conditions rather than historic isolation of populations and/or mycorrhizal fungi. We used DNA-based methods to identify mycorrhizal fungi from eleven *E. firmum* populations in Costa Rica. We used molecular and phylogenetic analyses to compare associations. *Epidendrum firmum* exhibited broad specificity, associating with diverse mycorrhizal fungi, including six Tulasnellaceae molecular operational taxonomic units (MOTUs), five Sebacinales MOTUs and others. Notably, diverse mycorrhizal symbioses formed in disturbed pasture and roadside habitats. Mycorrhizal fungi exhibited significant similarity within populations (spatial and phylogenetic autocorrelation) and significant differences among populations (phylogenetic community dissimilarity). However, mycorrhizal symbioses were not significantly associated with biogeographic or environmental features. Such unexpected heterogeneity among populations may result from complex combinations of fine-scale environmental factors and macro-evolutionary patterns of change in mycorrhizal specificity. Thus, *E. firmum* exhibits broad specificity and the potential for opportunistic associations with diverse fungi. We suggest that these characteristics could confer symbiotic assurance when mycorrhizal fungi are stochastically available, which may be crucial in dynamic or disturbed habitats such as tropical forest canopies.

Keywords: epiphyte, mycorrhizal symbiosis, Neotropics, Orchidaceae, phylogenetic community dissimilarity

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Introduction

During a time of rapid biodiversity loss and environmental change, understanding the role of species interactions in the maintenance of biodiversity is a key objective for ecologists. Species interactions have an evolutionary basis and can be influenced by ecological factors that vary over space and time (Thompson 2005). Environmental degradation can cause conservation challenges for species that rely on these interactions, such as parasites and mutualists (Dunn *et al.* 2009). For example, coral bleaching occurs when distressed reef-building corals expel their intracellular symbiotic

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partners (*Symbiodinium* spp.), and differential mortality of hosts is related to the sensitivity of their partner to changing conditions (Sampayo *et al.* 2007). Some species may strategically specialize on a symbiotic partner. Species specializing on a single partner often interact with generalist species (i.e. species capable of interacting with a broad number of partners), because the latter may be less susceptible to environmental stochasticity and may have broader ranges than related specialists. Broad spatial and temporal availability of generalists may stabilize ecological interaction networks that include their more specialized partners when the environment fluctuates (Bascompte & Jordano 2007). Better understanding of heterogeneity in species interactions is needed to anticipate direct and indirect consequences of environmental degradation on species conservation.

Tropical deforestation isolates and degrades habitat fragments, reducing biodiversity both directly and indirectly (Gardner *et al.* 2009). Rare species that historically occupy a small portion of their habitat are more likely to go extinct due to habitat fragmentation (Hanski 1998), particularly if they require specific habitats or species interactions that are subject to disturbance (Davies *et al.* 2004; Dunn *et al.* 2009). Thus, the evolutionary and ecological histories of species interactions shape the fate of populations in degraded landscapes.

The Orchidaceae is among the largest plant families (≥26 000 species), contributing greatly to tropical biodiversity (WCSP 2013). Symbiotic mycorrhizal fungi are critical for orchids because orchid seeds heterotrophically extract carbon and nutrients from fungi during establishment, and mature plants often continue mycorrhizal symbiosis (Dearnaley *et al.* 2012). Mycorrhizal symbiosis by green orchids differs from other mycorrhizal symbioses (e.g. arbuscular, ecto-, ericoid or achlorophyllous orchid mycorrhizal symbioses) because the fungi are taxonomically and ecologically distinct (typically free-living saprotrophs), because seedlings heterotrophically extract nutrients from fungi and because it is unclear how much, if any, photosynthetically derived carbon is mutualistically transferred from orchids to fungi (Smith & Read 2008). Orchid rarity may be caused by mycorrhizal specialization if the required fungus is itself rare or of limited availability (Swarts *et al.* 2010). However, ecological context strongly influences this relationship, because mycorrhizal specialization does not always lead to rarity (Phillips *et al.* 2011), and some rare orchids exhibit broad interactions (Pandey *et al.* 2013). Rare epiphytic plants that grow on trees rather than on the ground are highly susceptible to environmental degradation due to losses of host trees and microclimatic changes (Sodhi *et al.* 2008). Thus, epiphytic orchids in fragmented forests require both the right trees and the right mycorrhizal fungi to persist. Yet while >70% of orchid species are epiphytes, epiphytic orchid mycorrhizal symbioses receive relatively little attention (Otero *et al.* 2004; Suárez *et al.* 2008; Martos *et al.* 2012).

The evolution of species interactions is of central importance to the conservation of rare species. That many orchids are mycorrhizal specialists suggests that their fungal associates strongly influence orchid evolution and population dynamics (Dearnaley *et al.* 2012). The maintenance of species interaction networks (e.g. plant–pollinator networks) is less robust to disturbance when phylogenetically related species are lost together, as often occurs in nature (Bascompte & Stouffer 2009). The total diversity of mycorrhizal fungi with which an orchid species can associate tends to have a phylogenetic basis, such that related orchids associate with similar ranges of fungi (Shefferson *et al.* 2010; Martos *et al.* 2012). However, under geographic isolation or different ecological conditions, genetic drift or local adaptation could lead related orchids to different mycorrhizal symbioses (Waterman & Bidartondo 2008).

Ecological variation may also influence species interactions. That relevant fungal distributions vary with resources such as water or organic matter is centrally important to orchid mycorrhizal symbiosis (Dearnaley 2007; Diez 2007). The diversity limiting process in which certain taxa occur only in certain environments is known as environmental filtering (Keddy 1992). If orchid species depend on a unique symbiotic fungus, the same fungus would be required across space and time, and its absence would be an environmental filter. Conversely, if orchid species associate with multiple mycorrhizal fungi, fungi may be heterogeneously available, but the need for a particular fungal species would be lessened, at least in principle.

We investigated mycorrhizal associations of the rare epiphytic orchid, *Epidendrum firmum*, which occurs in multiple geographically discrete mountain habitats that are increasingly fragmented by human activities in Costa Rica. We consider this species rare due to its historically limited environmental and geographic range (Rabinowitz 1981). However, its ability to secondarily colonize small, cultivated trees in disturbed areas provides a valuable opportunity to investigate the mycorrhizal fungi available to epiphytic orchids in fragmented tropical habitats. We asked (i) Does this rare orchid associate with one or multiple mycorrhizal fungi? And (ii) does variation in the suite of fungal partners of *E. firmum* correspond to biogeographic or environmental factors? We used DNA-based methods to test the hypotheses that (i) orchid populations associate with a diversity of mycorrhizal fungi and that (ii) variation between mycorrhizal fungi is related to geographic isolation and/or environmental differences across elevation. Diverse suites of mycorrhizal fungi

across mountain ranges, although not within them, would suggest that populations are geographically isolated in terms of their mycorrhizal associates. Alternatively, suites of mycorrhizal fungi may vary with broad environmental features such as temperature or seasonal precipitation, rather than mountain range. This latter trend would suggest that interactions are governed by environmental filtering rather than the historic isolation of populations and/or their mycorrhizal fungi.

Methods

We tested the hypotheses that diverse mycorrhizal fungi vary with biogeographic and/or environmental differences. We (i) identified fungi from *Epidendrum firmum* roots using DNA barcodes, (ii) tested whether fungi are more or less similar within populations than expected by chance and (iii) compared suites of fungi associated within populations with geographic and environmental factors. We analysed related genetic data: (i) long sequence alignments that maximize taxonomic resolution and (ii) shorter alignments that characterize phylogenetic relationships.

Study species and sampling

The epiphytic orchid *E. firmum* Rchb.f. 1866, subtribe Laeliinae, is a part of the *E. difforme* species complex (Dressler 1993). It occurs on the Pacific slope of Central American mountains from Nicaragua to Panama, specializing on narrow mid-elevation habitats classified as humid premontane (bmh-P) and humid tropical premontane transition forest (bmh-T12) by the Holdridge life zone system (T.R.K., pers. obs.; herbarium records). Populations occur in undisturbed forests canopies and in shrubs or trees from disturbed areas. Little genetic structure was found among *E. firmum* populations in Costa Rica, but genetic analyses revealed distinct population histories of populations in the Guanacaste, Tilarán and Talamanca mountain ranges, as well as northern and southern Tilarán regions (Fig. 1a; Kartzinel *et al.*, in press).

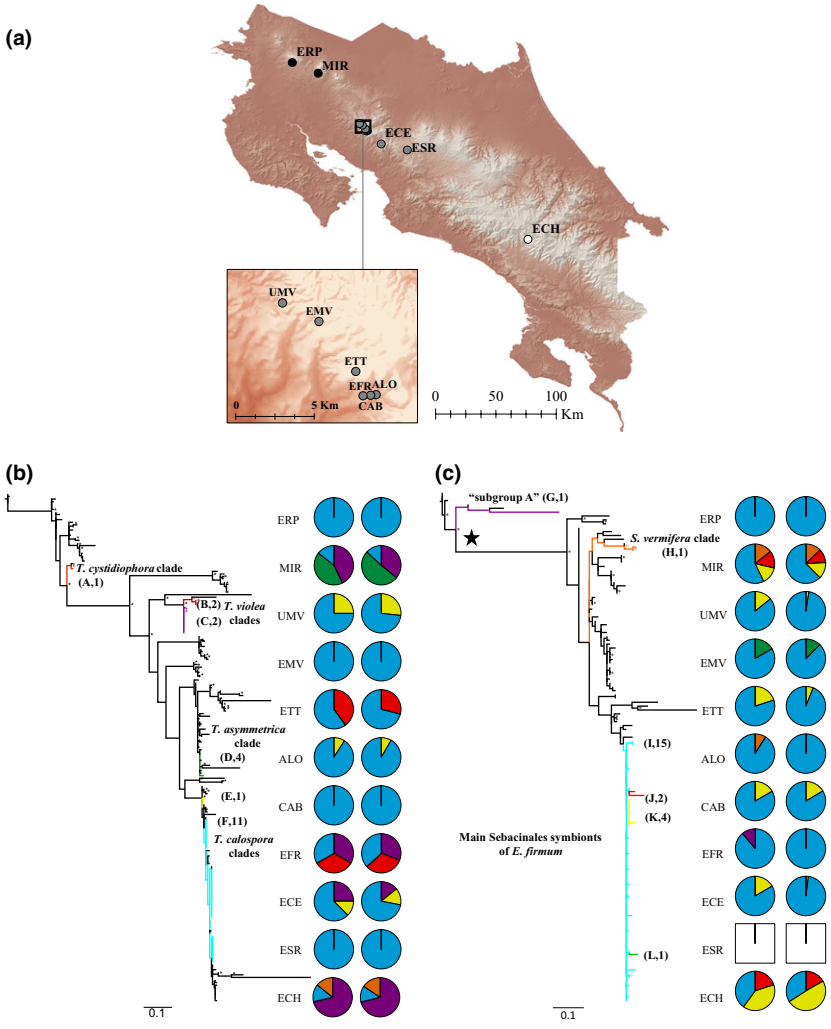

Fig. 1 Geographic differences in Tulasnellaceae and Sebacinales. (a) Map shows populations in Costa Rica. Shaded points correspond to different mountain ranges (black = Guanacaste; grey = Tilarán; white = Talamanca). Northern Tilarán populations are in the inset. Phylogeny with (b) Tulasnellaceae and (c) Sebacinales branches coloured to correspond to MOTUs. Black branches correspond to similar sequences from GenBank. In parenthesis, MOTU names (A-F and H-L) are followed by number of haplotypes. Nodes with * obtained ≥50% bootstrap support, and scale bars represent nucleotide substitutions. The star indicates the node separating Sebacinales subgroup A (above) and B (below), as previously denoted (Weiß *et al.* 2011). Detailed phylogenies appear in Figs S2-S4 (Supporting information). Pie chart colours correspond to phylogeny branches. The first pie chart columns indicate observed MOTU proportions within populations, while the second presents $MOTU_{rarefied}$ proportions. MOTU G was excluded from rarefaction.

Eleven populations were sampled from January 2009 to July 2011 in a manner designed to capture local, regional and species-level variation (Table S1, Supporting information). Populations are defined as all *E. firmum* on trees within a site (~1–2 ha). Populations spanned ~900–1400 mASL, including one primary forest, five roadside and five pasture sites (Fig. 1a; Table S1, Supporting information). Given the difficulty in locating and sampling populations of this rare species in remote locations, populations were opportunistically sampled when first encountered. Thus, some populations were sampled in different months and years (Table S1, Supporting information). It was possible to revisit some sites, while avoiding resampling unmarked individuals, to increase sample size (Table S1, Supporting information). Adult plants identified by the presence of ≥1 reproductive stem were sampled in all populations. In total, we identified fungi in roots of 6–16 orchids/population (mean = 10.8) from multiple host trees and tree species to capture the broadest mycorrhizal fungal diversity at a site (Table S1, Supporting information). The total possible host tree diversity for *E. firmum* is unknown, but hosts in most study sites included cultivated *Sapium glandulosum*, *Acnistus arborescens, Erythrina berteroana* and *Cupressus lusitanica*. Samples were kept at ~4 °C and processed within 24–48 h. Roots were surface sterilized in 10% bleach and examined for mycorrhizal pelotons under a microscope. One to six root samples (~10 mm long; 2–5 mm diameter) were saved for molecular analysis when mycorrhizal pelotons were observed in a corresponding cross-section. Samples were snap-frozen in liquid nitrogen and transported to the University of Georgia for analysis.

Genetic data acquisition

We employed multiple primers and molecular cloning to thoroughly characterize mycorrhizal diversity. DNA was extracted using Qiagen Plant DNeasy mini kits (Qiagen, Valencia, CA, the USA) or CTAB (Doyle & Doyle 1990). The nuclear ribosomal ITS1-5.8S-ITS2 (ITS) region was amplified beginning with the fungal primers ITS1F/ITS4 (White *et al.* 1990). If sequencing failed, we employed alternative primers including ITS1OF/ITS4OF (Taylor & McCormick 2008), ITS1/ITS4-TUL (Taylor & McCormick 2008), ITS1/ITS4-SEB (Tendersoo *et al.* 2011), ITS1/TW14 (Cullings 1994) and ITS1OF/TW14. To corroborate taxonomic inference based on ITS, we amplified the mitochondrial large subunit (mtLSU) with primers ML5/ML6 (Bruns *et al.* 1998). When multiple PCR product bands were seen on an agarose gel, we employed molecular cloning by ligating PCR products into pDrive (Qiagen) with transformation in StrataGene XL-10 Gold Ultracompetent cells (StrataGene, Agilent Technologies, Santa Clara, CA, the USA). Cloned DNA was amplified using the original PCR primers and sequenced (1–12 colonies/sample). PCR products were purified with ExoSAP-IT (Sigma, St. Louis, MO, the USA) and sequenced with BIGDYE, version 3.1, on an ABI3730 (Applied Biosystems, Inc.) at the Georgia Genomics Facility. Sequences were edited and assembled in Sequencher, version 4.9 (GeneCodes Corp., Ann Arbor, MI, the USA).

Fungal identification

Fungal identities were inferred with BLAST searches of the NCBI database. Sequences were considered mycorrhizal if they corresponded to known orchid mycorrhizal taxa (Dearnaley *et al.* 2012). All basidiomycete and all ascomycete sequences were aligned in ClustalW (Larkin *et al.* 2007) and grouped into molecular operational taxonomic units (MOTUs) using OPTSIL with complete linkage clustering (Göker *et al.* 2009; Setaro & Kron 2011) and a 3% sequence difference threshold (Nilsson *et al.* 2008). Care must be taken with community diversity estimates from sequencing, as errors artificially inflate diversity metrics (Dickie 2010). We adopted a balanced strategy to minimize potential for such bias, without precluding the biologically meaningful possibility that closely related taxa vary within and among populations. Thus, prior to further analysis, mycorrhizal taxa were clustered into 'haplotypes' using 1% sequence similarity to identify and remove duplicate sequences from fungi within the same plant. These sequences were identical or different slightly, possibly due to sequencing errors, originating from assaying multiple root sections per plant, molecular cloning or employing multiple primer pairs on the same sample. Removing putatively duplicate sequences allowed us to make conservative inferences in this taxonomically difficult group, incorporating potentially meaningful variation below the 3% level into analyses without overestimating diversity. Full alignment sizes were as follows: 508 bp for 70 Tulasnellaceae ITS, 468 bp for 71 Sebacinales ITS and 340 bp for 30 Tulasnellaceae mtLSU. Phylogenetic analyses included GenBank reference sequences: 166 bp of 197 Tulasnellaceae 5.8S, 192 bp of 132 Sebacinales 5.8S and 235 bp of 62 Tulasnellaceae mtLSU. Shorter alignments of conserved regions (e.g. 5.8S) are often required to minimize dubious taxonomic placement of these fungi (e.g. Suárez *et al.* 2006; Taylor & McCormick 2008). Maximum-likelihood trees were produced in PhyML-aBayes with 1000 bootstrap replicates (Guindon *et al.* 2010; Anisimova *et al.* 2011). Phylogenetic analysis revealed Sebacinales corresponding to ecologically distinct clades

(subgroup A and subgroup B, see results). Both subgroups include orchid mycorrhizal fungi; however, subgroup A contains mycorrhizal fungi of achlorophyllous orchids, while subgroup B contains mycorrhizal fungi of green orchids (Weiß *et al.* 2011). Thus, only subgroup B is included in analyses.

Mycorrhizal diversity and local similarity

Prior to analysis, resampling procedures were used to assess sampling sufficiency. Briefly, sequences were bootstrapped to produce sample-based rarefaction curves of nucleotide diversity (π) within the best-sampled populations, as well as haplotype and phylogenetic diversity across populations (see Appendix S1 for details). To compare Tulasnellaceae and Sebacinales MOTU richness, we accounted for heterogeneity in sample size by rarefaction to the smallest sample size per taxon per population ($N = 2$) in R, version 2.15.1 (R Core Development Team 2010).

The mycorrhizal diversity of orchid populations was evaluated. We calculated nucleotide diversity ignoring pairwise alignment positions with missing data (π; Nei 1987), accounting for heterogeneity in sample size through rarefaction to the smallest sample size per taxon per population ($N = 2$). We standardized effect size of mean pairwise phylogenetic distance (SES_{MPD}) among fungi within groups (Webb *et al.* 2002). Standardized effect size was necessary due to different sample sizes and was calculated by comparing observed mean phylogenetic distance (MPD) to expected values in 999 null groups generated by random draws from the sample pool, standardized by the standard deviation (Kembel *et al.* 2010). Positive values indicate phylogenetic evenness, while negative values indicate clustering. All phylogenetically distinguishable taxa were considered in phylogenetic analyses, although some closely related haplotypes were necessarily aggregated. If the evolutionary history of fungi, as characterized by a phylogeny, has an important influence on the tendency of different fungi to form symbioses with *E. firmum*, then aggregating fungal taxa at the tips of the phylogeny should not obscure this relationship. Groups with only one distinguishable taxon were assumed to have zero phylogenetic diversity. Analyses employed *pegas* (Paradis 2010) and *picante* (Kembel *et al.* 2010) in R, version 2.15.1 (R Core Development Team 2010).

We tested whether populations associate with a subset of mycorrhizal fungi via spatial autocorrelation analysis. Pairwise Tamura–Nei distance among sequences was calculated in Arlequin, version 3.1 (Excoffier *et al.* 2005), to determine sequence relatedness (r_{ij}) within populations exceeded the expectations from 999 random permutations in GenAlEx, version 6.2 (Peakall & Smouse 2006). Phylogenetic autocorrelation was assessed using Π_{ST} to measure the extent that phylogenetic similarity within groups exceeds similarity among groups, testing for significant local similarity by randomly shuffling communities among phylogeny tips 1000 times with model *1s* in *spacodiR* (Eastman *et al.* 2001; Hardy 2008). By default, populations with only one distinguishable taxon were excluded.

We tested whether diversity of mycorrhizal fungi varied with environmental factors via Kendall's τ test. Response variables were $\pi_{rarefied}$ and SES_{MPD}, and explanatory variables included latitude, elevation and bioclimatic variables from high-resolution WorldClim, version 1.4 data (http://www.worldclim.org/), that characterize temperature (BIO1, annual mean temperature) and seasonal precipitation (BIO15, precipitation seasonality) (Hijmans *et al.* 2005). Elevation, temperature and precipitation seasonality were not highly cross-correlated (Pearson $r = 0.132$–0.679, absolute value range), although latitude and elevation were ($r = -0.750$). Nonparametric Kendall's τ was used due to small sample size, non-normal distributions and tied ranks of some ecological variables. Correlations were tested with *Kendall* (McLeod 2011) in R (R Core Development Team 2010).

Mycorrhizal comparisons

We tested for differences in mycorrhizal fungi among orchid populations, evaluating the expectation that differences correspond to population groups separated into (i) three geographically discrete mountain ranges or (ii) four regions defined by different *E. firmum* population histories. The three mountain ranges are the cordilleras Guanacaste, Tilarán and Talamanca, which have distinct geologic histories (shown in Fig. 1a). The four regions in which *E. firmum* has different population histories were identified in a genetic study of this species, justifying subdivision of populations within Tilarán (Kartzinel *et al.* in press; Table S1, Supporting information).

We used the phylogenetic community dissimilarity (PCD) metric to test whether mycorrhizal fungi significantly differed among populations (Ives & Helmus 2010). PCD allows the total dissimilarity between two groups (i.e. PCD) to be expressed in terms of the distinct ecological (i.e. compositional, PCDc) and evolutionary (i.e. phylogenetic, PCDp) differences among groups through the relationship: PCD = PCDc * PCDp (Ives & Helmus 2010). The metric PCDc is related to Sørensen's index (1- Sørensen's index, modified to remove dependence on community size), and the metric PCDp uses a phylogeny to measure variation between the unshared taxa. To compare groups varying in

species richness, PCD is standardized by 10 000 random draws from a null community under the assumption that species are drawn at random from the species pool. Thus, if PCD = 1, the phylogenetic composition of two communities is not different than expected by random draws of a phylogeny. Communities are more similar or more different than expected by chance if PCD <1 or PCD >1, respectively. The random expectation for compositional (PCDc) and phylogenetic (PCDp) components of this metric is also 1. A major advantage of this metric is that it accounts for the possibility that groups sharing no fungal sequences may still be similar if they contain phylogenetically related taxa. Nonmetric multidimensional scaling (NMDS) was used to visualize PCD, expecting populations to cluster by mountain ranges or population history. Ordination was considered reliable with stress values ≤0.2. We tested for significant associations between suites of mycorrhizal fungi and differences in pairwise distances (log km), elevation and bioclimatic variables (above). Mantel tests were used to correlate each pairwise PCD matrix with the matrix of absolute differences in each predictor, and significance was evaluated with 9999 permutations. Analyses used *picante* (Kembel *et al.* 2010) and *vegan* (Oksanen *et al.* 2012) in R (R Core Development Team 2010).

Results

Epidendrum firmum associates with diverse mycorrhizal fungi, although populations tend to associate with only a subset of the total mycorrhizal diversity observed throughout Costa Rica. Unexpectedly, fungal diversity and dissimilarity did not vary in a manner consistent with the influence of ecological factors or the history of plant populations in different mountain regions.

Fungal identification

Fungi from 119 plants were identified, including 30 MOTUs from 13 basidiomycete orders and 20 MOTUs from nine ascomycete orders (Appendix S2; Table S2, Supporting information). From 111 plants (94%), we identified six basidiomycete orders known to include orchid mycorrhizal fungi. Most plants (109; 92%) formed mycorrhizal symbiosis with the Cantharellales family Tulasnellaceae (6 MOTUs, 21 haplotypes, 73 plants; Fig. 2a) and/or the Sebacinales (6 MOTUs, 24 haplotypes, 59 plants, including one plant associating with Sebacinales subgroup A; Fig. 2b). Co-occurrence of Tulasnellaceae and Sebacinales was observed in 23 plants (21%). These co-occurrences ranged from 0 to 50% of plants that associated with at least one of these taxa per population. The number of plants for which only Tulasnellaceae or Sebacinales were detected was greater than random expectations from the chi-squared distribution ($\chi^2 = 22.84$, $d.f. = 1$, $P = 1.76 * 10^{-6}$). Two plants associated with two Tulasnellaceae MOTUs, and seven associated with two Sebacinales MOTUs. One plant contained multiple Tulasnellaceae haplotypes belonging to one MOTU, and seven plants contained multiple Sebacinales haplotypes belonging to one MOTU. Basidiomycete taxa containing fungi that may form orchid mycorrhizal symbioses, but rarely associate with *E. firmum*, include other Cantharellales (Ceratobasidiaceae), Agaricales, Hymenochaetales, Thelephorales and Russulales (Fig. 2a). Ascomycetes occurring as orchid endophytes with potential for beneficial or deleterious associations include Hypocreales, Capnodiales, Pleosporales and Helotiales (Fig. 2). Other taxa with uncertain function were detected and may have withstood surface sterilization as endophytes, pathogens or saprotrophs that entered the roots. These taxa include Trechisporales, Boletales, Polyporales, Corticales, Tremellales, Erythrobasidiales, Auriculariales, Trichosphaeriales, Saccharomycetales, Pertusariales, Xylariales and a mitosporic ascomycete (Appendix S2; Fig. 2).

Phylogenetic analyses support the designation of Tulasnellaceae MOTUs, but four Sebacinales MOTUs form a poorly supported clade (Figs 1b,c, S2 and S3, Supporting information). Some Tulasnellaceae were widespread (MOTU F; Fig. 1), while others only associate with populations in one mountain range (A, B, D, E; Fig. 1). Two MOTUs form a clade with *T. calospora*, where one (F) is the most common Tulasnellaceae, and the other (E) only associated with three plants in different Tilarán populations. Similarly, two MOTUs form a clade with *T. violea*, with one (C) occurring in each mountain range, while the other (B) was only detected in three plants from two Tilarán populations. One MOTU (D), found in four MIR plants, formed a clade with *T. asymmetrica*. Another MOTU (A), found only once in ECH, forms a clade with *T. cystidiophora*. Populations MIR and ECH are lowest and highest elevation populations, respectively. Sebacinales include many taxa of mycorrhizal fungi that are phylogenetically attributed to two subgroups (A and B). Most Sebacinales MOTUs (I – L) are similar to subgroup B, which forms mycorrhizal symbioses with photosynthetic orchids (Fig. 1). Taxa within this group are so closely related that five of the eight sequences in MOTU K (based on 3% clustering of 5.8S) were aggregated into the most frequent haplotype within MOTU I (based on 1% clustering of ITS). One MOTU (H), found once in Guanacaste (MIR) and once in Tilarán (ALO), is similar to other common mycorrhizal fungi of photosynthetic orchids in Sebacinales subgroup B. An MOTU (G), detected once in Tilarán (EFR), is similar to mycorrhizal fungi from Sebacinales subgroup A that associate with achlorophyllous orchids.

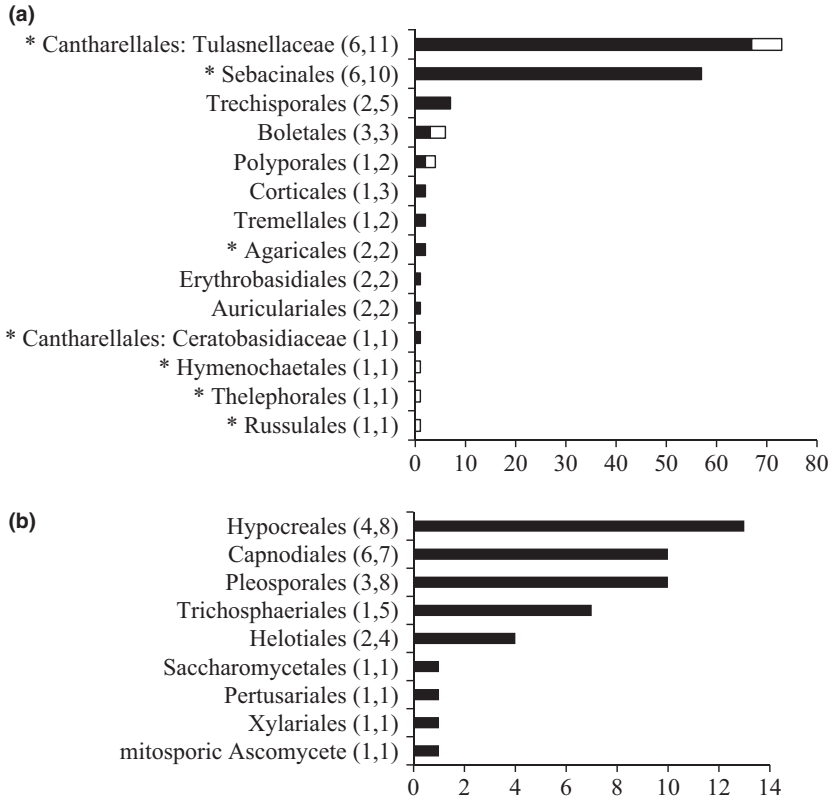

Fig. 2 Number of plants associating with fungal taxa. Basidiomycetes (a) and ascomycetes (b) were detected. Numbers in parentheses indicate the number of MOTUs and populations in which taxa were observed. Asterisks denote groups known to contain orchid mycorrhizal fungi. Black bars represent the number of plants for which at least one ITS sequence was obtained. White bars represent additional plants for which only mtLSU sequences were obtained. We list the minimum potential number of MOTUs when both ITS and mtLSU can contribute to counts.

Mycorrhizal diversity and local similarity

We confirmed adequate sampling of most populations as resampling indicated that ≥5 sequences should provide robust diversity estimates (Fig. S1, Supporting information). New haplotypes would likely emerge with additional sampling, but would not likely add phylogenetic diversity (Fig. S1, Supporting information).

The diversity of Tulasnellaceae and Sebacinales associating with *E. firmum* differed. Tulasnellaceae occurred in all populations, with 1–3 MOTUs/population ($mean_{rarefied}$ = 1.415; Fig. 1b), 2–6 haplotypes/population ($mean_{rarefied}$ = 1.737; Table S3, Supporting information) and broad diversity ranges ($0.030 \geq \pi_{rarefied} \geq 0.228$, mean = 0.116; $-2.321 \geq SES_{MPD} \geq 1.511$, mean = −0.702; Table S3, Supporting information). Significant autocorrelation in sequence (r_{ij} = 0.368; $P \leq 0.001$) and phylogenetic (Π_{ST} = 0.186; $P \leq 0.008$) analyses similar to Tulasnellaceae occur among plants within populations. Eight populations had negative SES_{MPD} values, indicating a tendency for phylogenetic clustering, although only two were significantly clustered (Table S3, Supporting information). Sebacinales occurred in all populations except one (ESR), with 1–4 MOTUs/population ($mean_{rarefied}$ = 1.309; Fig. 1c), 2–8 haplotypes/population ($mean_{rarefied}$ = 1.834; Table S3, Supporting information) and low diversity ($0.005 \geq \pi_{rarefied} \geq 0.041$, mean = 0.014, $-0.914 \geq SES_{MPD} \geq 1.397$, mean = −0.180; Table S3, Supporting information). Sebacinales sequences co-occurring within populations are not more similar than random expectations (r_{ij} = 0.027; $P \leq 0.120$), and there was no significant excess phylogenetic similarity (Π_{ST} = 0.083; $P \leq 0.187$). Six populations had negative SES_{MPD} values, indicating a tendency for clustering, although only two were significantly clustered (Table S3, Supporting information).

No environmental variable was correlated with mycorrhizal diversity. Tulasnellaceae π was marginally correlated with seasonal precipitation, where diversity decreased with greater seasonal precipitation changes (Table 1). Imprecision in diversity estimates may result from small sample sizes, so analyses were repeated for populations with ≥5 sequences/taxon. No qualitative changes occurred, but there was a marginally significant relationship between Tulasnellaceae SES_{MPD} and mean annual temperature ($\tau = -0.690$; $P \leq 0.085$).

Mycorrhizal comparisons

Spatial patterns of variation between mycorrhizal fungi differed between Tulasnellaceae and Sebacinales, but neither varied with ecological or geographic differences.

Table 1 Environmental correlates of mycorrhizal diversity (Kendall's τ) or dissimilarity (Mantel's r)

Model	$\pi_{rarefied}$	SES_{MPD}	PCD	PCDc	PCDp
Tulasnellaceae					
Location	0.018	−0.222	−0.065	0.118	−0.013
Elevation (metres above sea level)	−0.018	0.278	−0.130	0.063	−0.106
Annual mean temperature (°C)	−0.245	−0.412	−0.122	0.141	−0.145
Precipitation seasonality (mm, coefficient of variation)	−0.434*	−0.295	−0.041	−0.177	0.135
Sebacinales					
Location	0.022	0.135	0.098	0.040	−0.006
Elevation (metres above sea level)	0.067	−0.405	0.094	0.035	0.129
Annual mean temperature (°C)	−0.303	0.283	0.163	0.070	0.129
Precipitation seasonality (mm, coefficient of variation)	−0.349	−0.047	−0.148	−0.005	−0.072

SES_{MPD}, standardized effect size of mean phylogenetic diversity; PCD, phylogenetic community dissimilarity; PCDc, the composition component of community dissimilarity; PCDp, the phylogenetic component of community dissimilarity.
Model = explanatory variable or matrix. Population locations are described by latitude for correlation with mycorrhizal diversity or a pairwise distance matrix for correlation with PCD matrices. Response variables are $\pi_{rarefied}$, rarefied nucleotide diversity.
*Marginally significant ($P \leq 0.10$).

Tulasnellaceae and Sebacinales PCD showed distinct clusters among populations by NMDS that do not correspond to mountain ranges or plant population with different histories (Fig. 3). Many population pairs did not have dissimilar compositions of either Tulasnellaceae or Sebacinales (PCDc ≤1.0), but patterns of phylogenetic dissimilarity (PCDp) differed for Tulasnellaceae and Sebacinales (Fig. 3). Tulasnellaceae PCD clusters are strongly influenced by whether groups only include MOTUs affiliated with *T. calospora* or also include a broader diversity of Tulasnellaceae, and these affiliations do not reflect a geographic pattern (Figs 1b and 2a). A population from southern Tilarán (ESR) was the only population to associate with only one distinguishable Tulasnellaceae haplotype and thus does not cluster with other populations. In contrast, even though most Sebacinales occur in the clade comprising MOTUs I-L (Fig. 1c), most haplotypes from this clade occurred only in one population (77%). Thus, private haplotypes reflect a substantial proportion of the phylogenetic breadth of compatible Sebacinales. Two populations (ERP and EFR) had identical Sebacinales composition (i.e. PCDc = 0), rendering pairwise PCDp and PCD inapplicable because phylogenetic variation is undefined among identical groups. Undefined values were assumed to approach zero ($=10^{-7}$) for ordination and statistical analysis. Distantly related MOTU H occurs only in populations ALO and MIR, causing them to cluster with one another phylogenetically (PCD and PCDp) despite nonidentical compositions (PCDc). Neither the compositional nor the phylogenetic affiliations of mycorrhizal fungi reflect geographic patterns or ecological variables that we evaluated (Figs 1c and 3b; Table 1).

Discussion

Consistent with expectations, diverse mycorrhizal fungi of *Epidendrum firmum* varied between populations. However, associations with dissimilar fungi cannot be attributed to differences among geographic regions or environments. We suggest that patterns of *E. firmum* mycorrhizal symbiosis reflect more complex combinations of environmental factors and evolutionary history.

Epidendrum firmum exhibits broad mycorrhizal specificity relative to many orchids. Mycorrhizal specificity (π) of photosynthetic orchids in the literature ranged from 0.001 to 0.325 for Tulasnellaceae and 0.014–0.076 for Sebacinaceae (Pandey *et al.* 2013). Compared with other orchids, *E. firmum* thus exhibits broad overall specificity, with broad Tulasnellaceae specificity and narrower Sebacinaceae (subgroup B) specificity. Few orchids are known to associate with each major family of orchid mycorrhizal fungi as *E. firmum* does (Tulasnellaceae, Sebacinaceae, Ceratobasidiaceae), and epiphytes tend to be relatively constrained in their symbioses with these families (Martos *et al.* 2012; Pandey *et al.* 2013). Finally, associations between *E. firmum* and possible ectomycorrhizal fungi (e.g. Thelephorales and Russulales) merit further investigation as this could render *E. firmum* an orchid with unusually broad associations (Dearnaley *et al.* 2012).

We hypothesized that diverse mycorrhizal fungi vary with biogeographic and/or environmental factors. When populations exhibit local mycorrhizal specificity, for reasons that could include local adaptation, regional distributions and context dependency (Egger & Hibbert 2004), a pattern should emerge in which symbioses are similar within the same region or environment, but

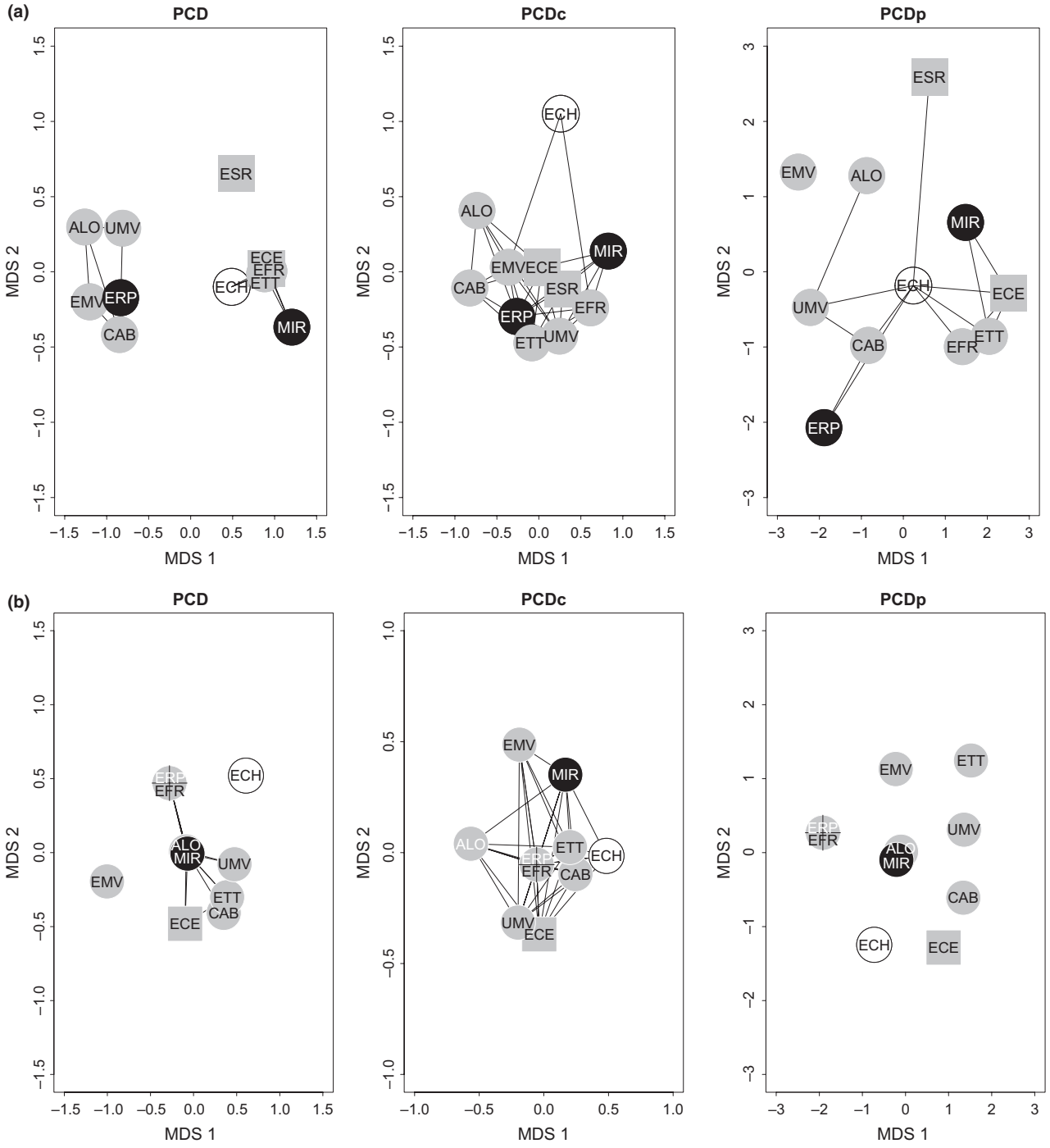

Fig. 3 Nonmetric multidimensional scaling (NMDS) of mycorrhizal fungi. PCD, PCDc and PCDp show (a) Tulasnellaceae and (b) Sebacinales do not cluster geographically. Populations are shaded by mountain range (black = Guanacaste; grey = Tilarán; white = Talamanca). Southern Tilarán populations are indicated as squares. In (b) two populations, ERP and EFR have identical compositions and are superimposed. Lines connecting populations indicate that they are not significantly dissimilar (i.e. PCD, PCDc or PCD$p \leq 1.0$). Stress values <0.2 indicated valid PCD, PCDc and PCDp plots for the Tulasnellaceae (0.039, 0.086 and 0.181, respectively) and Sebacinales (0.082, 0.105 and 0.139, respectively).

dissimilar across regions or environments (Waterman & Bidartondo 2008). This is not what we found. Mycorrhizal fungi exhibited significant similarity within populations and significant dissimilarity among neighbouring populations (*e.g.* ALO, CAB and EFR separated by <1.0 km), yet differences did not reflect region or environment.

We suggest three interrelated hypotheses about this pattern that merit further investigation: (i) environmental determinants of mycorrhizal specificity principally occur at different spatial or temporal scales than we evaluated, (ii) evolutionary change in specificity for *E. firmum*, and perhaps its relatives, principally occurs at higher taxonomic levels, and (iii) as a species with broad mycorrhizal specificity, *E. firmum* opportunistically associates with diverse fungi that are stochastically available. We consider each hypothesis in detail below.

Environmental factors may often influence mycorrhizal symbioses, but a lack of correlation between *E. firmum* mycorrhizal fungi and environmental factors suggests that it is not consistently influenced by fungal habitat preference at the population level. Mycorrhizal fungi associating with populations of the terrestrial, photosynthetic orchid *Piperia yadonii* differ among forest types (Pandey *et al.* 2013). Andean epiphytic orchid communities may exhibit a gradient in which lower mycorrhizal diversity occurs in higher elevation populations, perhaps indicating fewer mycorrhizal fungi are available, although these orchid communities also vary elevation (Suárez *et al.* 2006, 2008). Given such findings for other orchids, it is possible that relationships between *E. firmum* mycorrhizal fungi and the environment are subtle (e.g. weak trend between Tulasnellaceae π_{rarefied} and precipitation seasonality; Table 1) and may become clearer with more studies and higher-resolution climate data. Complex patterns may also occur if symbiotic variation increases in extreme environments, as suggested by uncommon *E. firmum* mycorrhizal fungi at elevation extremes (e.g. MIR and ECH; Fig. 1b,c; Tables S1, Supporting information). Alternatively, because most populations occurred in disturbed areas, which may alter fungal communities (e.g. Sebacinales; Garnica *et al.* 2012), historic trends may have been obscured by disturbance. Phenological and/or orchid developmental stage differences also conceivably obscured more meaningful geographic or ecological patterns. Indeed, orchid mycorrhizal change can occur with season (Dixon & Tremblay 2009), drought (McCormick *et al.* 2006) and orchid developmental stage (Bidartondo & Read 2008). While such determinants of orchid mycorrhizal symbiosis could act at different spatial or temporal scales than we considered, difficulty in locating this rare species in remote locations rendered tests of this prohibitive.

For *E. firmum* and its relatives, evolutionary change in mycorrhizal specificity may follow a process that does not produce significant differences among relatively recently diverged populations. Neotropical forest canopies harbour a broad diversity of mycorrhizal fungi for epiphytic orchids, including many that were not found in *E. firmum* (Otero *et al.* 2004; Suárez *et al.* 2008), suggesting that some unutilized mycorrhizal fungi were available. The availability of unutilized partners suggests that the breadth of fungi compatible with *E. firmum* is influenced by macro-evolutionary history. Because many *Epidendrum* spp. also form mycorrhizal symbioses with related fungi (Hadley 1970; Dearnaley 2007), these patterns are consistent with the hypothesis that mycorrhizal associations in *Epidendrum* spp. evolve via Brownian motion (Shefferson *et al.* 2010; Martos *et al.* 2012). Brownian motion is a pattern of evolutionary random walks, in which the identity and taxonomic breadth of compatible mycorrhizal fungi may vary, but in which major evolutionary jumps via adaptation or stasis via constraint are unlikely. This evolutionary process potentially rendered *E. firmum* a species broadly capable of diverse associations with fungi throughout its range. A 'snapshot' of mycorrhizal fungi within *E. firmum* populations, and perhaps other species with broad specificity, may not portray the full breadth of potential mycorrhizal fungi. Yet the associations diagnostic of such broad specificity (i.e. $\geq$2 unrelated taxa; Dearnaley *et al.* 2012) are evident in most populations.

Because mycorrhizal fungi do not deterministically vary between *E. firmum* populations in different regions or environments and because *E. firmum* exhibits broad specificity, we hypothesize that *E. firmum* opportunistically associates with heterogeneously available fungi. Although distinguishing between the hypotheses that particular fungi are excluded from symbiosis or are simply absent requires knowledge of fungal distributions, heterogeneously available mycorrhizal fungi may result from limited spatial distributions, local environments or competition. At least some *E. firmum* mycorrhizal fungi may be geographically widespread. Identical Sebacinales sequences are known to span continents (Selosse *et al.* 2007; Weiß *et al.* 2011). The most common *Sebacina* MOTU (I) associating with *E. firmum* has recently been reported in accessions from temperate orchids, suggesting that this taxon is indeed widespread (Table S2, Supporting information; Pandey *et al.* 2013). Global phylogenetic analyses of Tulasnellaceae are unavailable, but broad distributions are also probable considering some sequences from *E. firmum* correspond to sequences from Asian orchids (e.g. Figs S2 and S4, Supporting information). Nevertheless, ecological factors varying at scales as local as neighbouring roots may strongly influence these symbioses. For example, Tulasnellaceae and Sebacinales occurred together in most populations, but infrequently occurred in the same plant. Thus, plants could selectively associate with Tulasnellaceae and Sebacinales in different microenvironments, these fungi could compete within or around orchid roots, or external environmental factors could indirectly influence mycorrhizal symbiosis via direct impacts on fungi. Even when mycorrhizal fungi occur

near orchid roots, they must be sufficiently abundant for symbioses to develop (McCormick *et al.* 2012). Our sampling strategy was too broad to evaluate these possibilities, which may occur at microscopic scales.

Finally, as a further hypothesis, broad mycorrhizal specificity could indicate that some mycorrhizal fungi are, in effect, functionally redundant for *E. firmum*. Functional redundancy does not imply complete lack of unique qualities among species, but a degree of overlap in the part of the fungal niche upon which orchids rely. Such redundancy may confer symbiotic assurance to species with broad specificity in changing environments, contrasting with specialization that increases risk of co-extinction (Dunn *et al.* 2009). Local fluctuations in the abiotic environment or colonization of disturbed sites are two scenarios in which epiphytic orchids may encounter a narrow subset of their total mycorrhizal fungi (e.g. McCormick *et al.* 2006; Shefferson *et al.* 2008). Such site-specific factors can give rise to differences among populations without broad-scale geographic or climatic trends. Thus, better knowledge of canopy fungal distributions would strengthen insights into epiphytic orchid mycorrhizal symbiosis (Dearnaley *et al.* 2012; Martos *et al.* 2012). Although rarely tested experimentally, only mixed and species-specific support exists for the hypothesis that mycorrhizal specialization constrains rare orchid distributions (Swarts *et al.* 2010; Phillips *et al.* 2011). We do not conversely suggest that broad specificity enables widespread orchids, as many rare and endemic orchids, including *E. firmum*, exhibit broad specificity (e.g. Pandey *et al.* 2013 and references therein). Instead, broad specificity could simply be one part of a trait syndrome, mitigating challenges posed by environmental changes through symbiotic assurance. Redundant symbiotic networks could be particularly important for species that are historically rare, occur in spatially structured or unpredictable environments and persist in disturbed habitats (Bascompte & Jordano 2007; Phillips *et al.* 2011). Functional differences among orchid mycorrhizal fungi have been identified through *in vitro* germination and growth assays (Otero *et al.* 2007; Porras-Alfaro & Bayman 2007), which could guide tests of hypotheses about functional redundancy.

Ecologists and land managers need better understanding of how ecological change affects symbioses to better protect the exceptional orchid diversity. We demonstrated that a rare epiphytic orchid forms diverse mycorrhizal symbioses, even in highly disturbed areas. Although much spatial variation in these symbioses requires further explanation, we suggest that some variation reflects the availabilities of different fungi. This should be a focus of research where spatial and temporal availability of symbiotic partners may often change.

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References

Anisimova M, Gil M, Dufayard JF, Dessimoz C, Gascuel O (2011) Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. *Systematic Biology*, **60**, 685–699.

Bascompte J, Jordano P (2007) Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology Evolution and Systematics*, **38**, 567–593.

Bascompte J, Stouffer DB (2009) The assembly and disassembly of ecological networks. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **364**, 1781–1787.

Bidartondo MI, Read DJ (2008) Fungal specificity bottlenecks during orchid germination and development. *Molecular Ecology*, **17**, 3707–3716.

Bruns TD, Szaro TM, Gardes M *et al.* (1998) A sequence database for the identification of ectomycorrhizal basidiomycetes by phylogenetic analysis. *Molecular Ecology*, **7**, 257–272.

Cullings KW (1994) Molecular phylogeny of the Monotropoideae (Ericaceae) with a note on the placement of the Pyroloideae. *Journal of Evolutionary Biology*, **7**, 501–516.

Davies KF, Margules CR, Lawrence JF (2004) A synergistic effect puts rare, specialized species at greater risk of extinction. *Ecology*, **85**, 265–271.

Dearnaley J (2007) Further advances in orchid mycorrhizal research. *Mycorrhiza*, **17**, 475–486.

Dearnaley JDW, Martos F, Selosse M-A (2012) Orchid mycorrhizas: Molecular ecology, physiology, evolution and conservation aspects. In: *Fungal Associations*, 2nd edn (ed. Hock B), pp. 207–230. Springer-Verlag, Berlin.

Dickie IA (2010) Insidious effects of sequencing errors on perceived diversity in molecular surveys. *New Phytologist*, **188**, 916–918.

Diez JM (2007) Hierarchical patterns of symbiotic orchid germination linked to adult proximity and environmental gradients. *Journal of Ecology*, **95**, 159–179.

Dixon K, Tremblay RL (2009) Biology and natural history of *Caladenia*. *Australian Journal of Botany*, **57**, 247–258.

Doyle J, Doyle J (1990) Isolation of plant DNA from fresh tissue. *Focus*, **12**, 13–15.

Dressler RL (1993) *Field Guide to the Orchids of Costa Rica and Panama*. Cornell University Press, New York, NY.

Dunn RR, Harris NC, Colwell RK, Koh LP, Sodhi NS (2009) The sixth mass (co) extinction - Are most endangered species

parasites and mutualists? *Proceedings of the Royal Society B-Biological Sciences*, **276**, 3037–3045.
Eastman JM, Paine CET, Hardy OJ (2001) spacodiR: structuring of phylogenetic diversity in ecological communities. *Bioinformatics*, **17**, 2437–2438.
Egger KN, Hibbert DS (2004) The evolutionary implications of exploitation in mycorrhizas. *Canadian Journal of Botany*, **82**, 1110–1121.
Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, **1**, 47–50.
Gardner TA, Barlow J, Chazdon RL *et al.* (2009) Prospects for tropical forest biodiversity in a human-modified world. *Ecology Letters*, **12**, 561–582.
Garnica S, Riess K, Bauer R, Oberwinkler F, Weiß M (2012) Phylogenetic diversity and structure of sebacinoid fungi associated with plant communities along an altitudinal gradient. *FEMS Microbiology Ecology*, **83**, 265–278.
Göker M, García-Blázquez G, Voglmayr H, Tellería MT, Martín MP (2009) Molecular taxonomy of phytopathogenic fungi: a case study in *Peronospora*. *PLoS ONE*, **4**, e6319.
Guindon S, Dufayard JF, Lefort V *et al.* (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, **59**, 307–321.
Hadley G (1970) Non-specificity of symbotic infection in orchid mycorrhiza. *New Phytologist*, **69**, 1015–1023.
Hanski I (1998) Metapopulation dynamics. *Nature*, **396**, 41–49.
Hardy OJ (2008) Testing the spatial phylogenetic structure of local communities: statistical performances of different null models and test statistics on a locally neutral community. *Journal of Ecology*, **96**, 914–926.
Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.
Ives AR, Helmus MR (2010) Phylogenetic metrics of community similarity. *The American Naturalist*, **176**, E128–E142.
Kartzinel TR, Shefferson RP, Trapnell DW (in press) Relative importance of pollen and seed dispersal across a Neotropical mountain landscape for an epiphytic orchid. *Molecular Ecology*, doi:10.1111/mec.12551.
Keddy PA (1992) Assembly and response rules - 2 goals for predictive community ecology. *Journal of Vegetation Science*, **3**, 157–164.
Kembel SW, Cowan PD, Helmus MR *et al.* (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463–1464.
Larkin MA, Blackshields G, Brown NP *et al.* (2007) Clustal W and clustal X version 2.0. *Bioinformatics*, **23**, 2947–2948.
Martos F, Munoz F, Pailler T *et al.* (2012) The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. *Molecular Ecology*, **21**, 5098–5109.
McCormick MK, Whigham DF, Sloan D, O'Malley K, Hodkinson B (2006) Orchid-fungus fidelity: a marriage meant to last? *Ecology*, **87**, 903–911.
McCormick MK, Taylor DL, Juhaszova K, Burnett RK Jr, Whigham DF, O'Neill JP (2012) Limitations on orchid recruitment: not a simple picture. *Molecular Ecology*, **21**, 1511–1523.
McLeod AI (2011) *Kendall: Kendall Rank Correlation and Mann-Kendall Trend Test*. R package version 2.2. Available from http://CRAN.R-project.org/package=Kendall.
Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY.
Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H (2008) Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification procedures. *Evolutionary Bioinformatics*, **4**, 193–201.
Oksanen J, Blanchet FG, Kindt R *et al.* (2012) vegan: Community Ecology Package. Available from http://CRAN.R-project.org/package=vegan.
Otero JT, Ackerman JD, Bayman P (2004) Differences in mycorrhizal preferences between two tropical orchids. *Molecular Ecology*, **13**, 2393–2404.
Otero JT, Flanagan NS, Herre EA, Ackerman JD, Bayman P (2007) Widespread mycorrhizal specificity correlates to mycorrhizal function in the Neotropical, epiphytic orchid *Ionopsis utricularioides* (Orchidaceae). *American Journal of Botany*, **94**, 1944–1950.
Pandey M, Sharma J, Taylor DL, Yadon VL (2013) A narrowly endemic photosynthetic orchid is non-specific in its mycorrhizal associations. *Molecular Ecology*, **22**, 2341–2354.
Paradis E (2010) pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, **26**, 419–420.
Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.
Phillips RD, Barrett MD, Dixon KW, Hopper SD (2011) Do mycorrhizal symbioses cause rarity in orchids? *Journal of Ecology*, **99**, 858–869.
Porras-Alfaro A, Bayman P (2007) Mycorrhizal fungi of *Vanilla*: diversity, specificity, and effects on seed germination and plant growth. *Mycologia*, **99**, 510–525.
R Core Development Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org/.
Rabinowitz D (1981) Seven forms of rarity. In: *The Biological Aspects of Rare Plant Conservation*(ed. Synge H), pp. 205–217. Wiley, New York, NY.
Sampayo EM, Ridgway PB, Hoegh-Guldberg O (2007) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 10444–10449.
Selosse MA, Setaro S, Glatard F, Richard F, Urcelay C, Weiß M (2007) Sebacinales are common mycorrhizal associates of Ericaceae. *New Phytologist*, **174**, 864–878.
Setaro SD, Kron KA (2011) Neotropical and North American Vaccinioideae (Ericaceae) share their mycorrhizal Sebacinales - An indication for concerted migration? *PloS Currents*, **3**, RRN1227.
Shefferson RP, Kull T, Tali K (2008) Mycorrhizal interactions of orchids colonizing Estonian mine tailings hills. *American Journal of Botany*, **95**, 156–164.
Shefferson RP, Cowden CC, McCormick MK *et al.* (2010) Evolution of host breadth in broad interactions: mycorrhizal specificity in East Asian and North American rattlesnake

plantains (*Goodyera spp.*) and their fungal hosts. *Molecular Ecology*, **19**, 3008–3017.
Smith SE, Read DJ (2008) *Mycorrhizal Symbiosis*, 3rd edn. Academic Press, San Diego, CA.
Sodhi NS, Koh LP, Peh KSH *et al.* (2008) Correlates of extinction proneness in tropical angiosperms. *Diversity and Distributions*, **14**, 1–10.
Suárez JP, Weiß M, Abele A *et al.* (2006) Diverse tulasnelloid fungi form mycorrhizas with epiphytic orchids in an Andean cloud forest. *Mycological Research*, **110**, 1257–1270.
Suárez JP, Weiß M, Abele A, Oberwinkler F, Kottke I (2008) Members of Sebacinales subgroup B form mycorrhizae with epiphytic orchids in a Neotropical mountain rain forest. *Mycological Progress*, **7**, 75–85.
Swarts ND, Sinclair EA, Francis A, Dixon KW (2010) Ecological specialization in mycorrhizal symbiosis leads to rarity in an endangered orchid. *Molecular Ecology*, **19**, 3226–3242.
Taylor DL, McCormick MK (2008) Internal transcribed spacer primers and sequences for improved characterization of basidiomycetous orchid mycorrhizas. *New Phytologist*, **177**, 1020–1033.
Tendersoo L, Bahram M, Jairus T *et al.* (2011) Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rain forests of Continental Africa and Madagascar. *Molecular Ecology*, **20**, 3071–3080.
Thompson J (2005) *The Geographic Mosaic of Coevolution*. The University of Chicago Press, Ltd., Chicago, IL.
Waterman RJ, Bidartondo MI (2008) Deception above, deception below: linking pollination and mycorrhizal biology of orchids. *Journal of Experimental Botany*, **59**, 1085–1096.
WCSP (2013) *World Checklist of Selected Plant Families*. Facilitated by the Royal Botanic Gardens, Kew. Available from http://apps.kew.org/wcsp/.
Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. *Annual Review of Ecology and Systematics*, **33**, 475–505.
Weiß M, Sýkorová Z, Garnica S *et al.* (2011) Sebacinales everywhere: previously overlooked ubiquitous fungal endophytes. *PLoS ONE*, **6**, e16793.
White TJ, Bruns TD, Taylor LS (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols. A Guide to Method and Application* (eds McInnes MA, Gelfand DH, Snisky JJ & White TJ), pp. 315–322. Academic Press, San Diego, CA.

T.R.K. designed the study, collected samples, conducted laboratory work, analysed data and wrote the manuscript. All authors participated intellectually, contributed resources and refined the manuscript.

Data accessibility

All DNA sequences used in the present analysis: GenBank Accession nos JX998685-JX999045, KC140565-KC140566, KC440181.

Sequence alignments and tree files: Dryad doi:10.5061/dryad.c6 g84

Supporting information

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

Fig. S1 Assessing sampling sufficiency with resampling procedures.

Fig. S2 Phylogeny of Tulasnellaceae based on 5.8S.

Fig. S3 Phylogeny of Sebacinales based on 5.8S.

Fig. S4 Phylogeny showing Tulasnellaceae based on mtLSU.

Table S1 Geographic locations of 11 sampled study populations.

Table S2 Top BLAST hits for sequences generated in this study.

Table S3 The diversity of Tulasnellaceae and Sebacinales based on ITS.

Appendix S1 Assessing sampling sufficiency.

Appendix S2 Detailed Summary of Fungal Identification.