SCIENCE CHINA
Life Sciences

• RESEARCH PAPER •

May 2017 Vol.60 No.5: 536–544
doi: 10.1007/s11427-017-9020-1

Phylogenetic constrains on mycorrhizal specificity in eight *Dendrobium* (Orchidaceae) species

Xiaoke Xing*, Xueting Ma, Jinxin Men, Yanhong Chen & Shunxing Guo*

Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China

Received January 9, 2017; accepted February 17, 2017; published online March 13, 2017

Plant phylogeny constrains orchid mycorrhizal (OrM) fungal community composition in some orchids. Here, we investigated the structures of the OrM fungal communities of eight *Dendrobium* species in one niche to determine whether similarities in the OrM fungal communities correlated with the phylogeny of the host plants and whether the *Dendrobium*-OrM fungal interactions are phylogenetically conserved. A phylogeny based on DNA data was constructed for the eight coexisting *Dendrobium* species, and the OrM fungal communities were characterized by their roots. There were 31 different fungal lineages associated with the eight *Dendrobium* species. In total, 82.98% of the identified associations belonging to Tulasnellaceae, and a smaller proportion involved members of the unknown Basidiomycota (9.67%). Community analyses revealed that phylogenetically related *Dendrobium* tended to interact with a similar set of Tulasnellaceae fungi. The interactions between *Dendrobium* and Tulasnellaceae fungi were significantly influenced by the phylogenetic relationships among the *Dendrobium* species. Our results provide evidence that the mycorrhizal specificity in the eight coexisting *Dendrobium* species was phylogenetically conserved.

orchid mycorrhiza, mycorrhizal network, fungal community composition, phylogenetic conservatism

Citation: Xing, X., Ma, X., Men, J., Chen, Y., and Guo, S. (2017). Phylogenetic constrains on mycorrhizal specificity in eight *Dendrobium* (Orchidaceae) species. Sci China Life Sci 60, 536–544. doi: 10.1007/s11427-017-9020-1

INTRODUCTION

Orchidaceae is a large plant family, with more than 26,000 species, that contributes greatly to tropical biodiversity. Most orchid species depend on interactions with mycorrhizal fungi for seed germination and subsequent seedling development because of the tiny size and lack of carbohydrate reserves in seeds (Rasmussen, 2002; Smith and Read, 2008). Some adult photosynthetic orchid species still heterotrophically obtain part of their carbon resources through mycorrhizal fungi (Bidartondo et al., 2004). In recent decades, because of human-induced habitat loss and the lack of effective protection in the face of increasing commercial demand, many orchid species have suffered dramatic declines in distribution and have become rare and endangered (Fu, 1992; Swarts and Dixon, 2009). Fully understanding the mycorrhizal symbiosis associated with orchids is necessary and important for orchid propagation, reintroduction and conservation (Brundrett, 2007; Dearnaley et al., 2012).

Mycorrhizal fungi are increasingly recognized as important biological factors influencing orchid distribution and abundance, and they have been linked to the niche partitioning of orchid species (Jacquemyn et al., 2012; Jacquemyn et al., 2014). Orchids may be more dependent on the symbiosis than their fungal partner, and the appropriate fungi is a main determinant for orchid recruitment (Rasmussen and Rasmussen, 2009; Jacquemyn et al., 2014; Rasmussen et al., 2015; Waud

* Corresponding authors (Xiaoke Xing, email: xkxing2009@hotmail.com; Shunxing Guo, email: sxguo@implad.ac.cn)

et al., 2016). However, in the mutualistic relationship between the orchid and mycorrhizal fungi, it is difficult to explain the mutual selection. Unlike the arbuscular mycorrhizal (AM) fungi, orchid mycorrhizal (OrM) fungi can grow without orchids and are probably independently distributed. Most photosynthetic orchids are not limited to one certain fungal partner at a time (Tupac Otero et al., 2002; McCormick et al., 2004; Shefferson et al., 2008; Jacquemyn et al., 2010; Jacquemyn et al., 2012). Thus, more than one fungus interacts with an individual orchid plant, and multiple host plants can tap into a mycorrhizal network (Jacquemyn et al., 2015), making it difficult to understand the mechanisms of partner selection in the symbiont's establishment.

In recent years, phylogenetic signals have been found in mutualistic and antagonistic host associations, indicating that there is a tendency for close relatives to resemble each other more closely in their characteristics than expected by chance (Wiens et al., 2010). Phylogenetic signals have been observed both in antagonistic interactions, such as those of insect herbivores (Weiblen et al., 2006) and plant pathogens (Gilbert and Webb, 2007), and in mutualistic interactions, such as those of AM (Wang and Qiu, 2006; Reinhart et al., 2012). In OrM, the diversified mycorrhizal fungi with which an orchid species can associate tend to be phylogenetically related (Shefferson et al., 2010; Jacquemyn et al., 2011; Martos et al., 2012). Phylogenetic conservation in the mycorrhizal symbiont has been well addressed. However, phylogenetic conservatism in plant-OrM fungal interactions in other orchid genera remain largely unexplored.

To gain better insights into fungal partner selection in the evolution of orchid species, we investigated the mycorrhizal association in the genus *Dendrobium*, which is a large genus of tropical epiphytic orchids. Some species in *Dendrobium* are used as traditional Chinese herbs, such as *D. officinale*, *D. fimbriatum*, *D. nobile* and *D. chrysanthum*. At present, many *Dendrobium* species across China are in danger of extinction. *Dendrobium* species are distributed throughout many provinces in southern China; however, under geographic isolation or in different ecological niches, genetic drift or ecological adaptation, respectively, could affect the mycorrhizal fungal community composition in orchids. To eliminate the effects of these factors, we focused our research on one niche. Here, we collected eight *Dendrobium* species from one niche in Xishuangbanna, Yunnan Province, China. Our specific aims were to address the following: (i) whether mycorrhizal fungal communities differed among these coexisting *Dendrobium* species; and (ii) whether mycorrhizal fungal communities associated with the *Dendrobium* species were phylogenetically conserved.

RESULTS

We observed signs of OrM colonization in all of the plants investigated. Over 1,100 high-quality internal transcribed spacer (ITS) sequences were obtained and most corresponded to basidiomycete sequences. Based on a 97% similarity sequence cut-off value, 31 different basidiomycete OTUs were distinguished. According to a BLAST algorithm-based analysis, 22 OTUs (OTU 1–22) were associated with members of the Tulasnellaceae family. Additionally, two OTUs (OTU 30 and 31) belonged to Sebacinaceae. OTUs 23 and 24, and OTUs 25 and 26, were related to the Auriculariales and Atractiellales, respectively. Three OTUs (OTU 27–29) of unknown Basidiomycota were also detected (Table 1). Among these fungal OTUs, only four, OTU1, OTU3, OTU5 and OTU18, were shared by two to four *Dendrobium* species. Representative sequences for each OTU were deposited in GenBank (accession numbers KX587473–KX587503).

The basidiomycete community associated with *Dendrobium* species consisted predominantly of fungal OTUs related to Tulasnellaceae (82.98%) and, to a lesser extent, to unknown Basidiomycota (9.67%). OTUs related to Sebacinaceae, which are known to associate with orchids, and other fungal families were only occasionally detected (Figure 1).

For each *Dendrobium* species, the number of associated fungal OTUs varied between one and seven. Four species, *D. nobile*, *D. fimbriatum*, *D. chrysotoxum* and *D. exile*, were associated with at least five fungal OTUs (Table 1). The maximum number of Tulasnellaceae OTUs was associated with *D. nobile*, while the minimum was associated with *D. salaccense* and *D. exile*. The non-metric multidimensional scaling clearly separated the Tulasnellaceae fungal communities in these *Dendrobium* species, except for those of *D. fimbriatum* and *D. salaccense*, which overlapped (Figure 2). Tulasnellaceae members are the predominant species associated with all of the *Dendrobium* species, except for *D. exile*, and their relative abundance levels varied between 61% and 100% (Figure S1 in Supporting Information). Fungal communities in four species, *D. nobile*, *D. cariniferum*, *D. salaccense* and *D. strongylanthum*, were only composed of Tulasnellaceae members (Figure S1 in Supporting Information). Unlike other *Dendrobium* species, the dominant OrM fungal species in *D. exile* were unknown Basidiomycota (relative abundance, 81%), and only one Tulasnellaceae OTU (relative abundance, 15%) was detected (Figure S1 in Supporting Information).

When the similarity level of the Tulasnellaceae OTUs associated with each *Dendrobium* species was examined, a simple Mantel test showed a marginally significant positive correlation between the phylogenetic and ecological distance matrices of the *Dendrobium* species (r=0.3896, P=0.0522). However, when differences in the number of interactions per *Dendrobium* species was controlled by performing a partial Mantel test, this positive correlation became significant (r=0.3617, P=0.0339), indicating that phylogenetically related *Dendrobium* species tend to interact with a similar

Table 1 List of fungal operational taxonomic units (OTUs) identified using cloning techniques, and the relative abundance of each OTU in the *Dendrobium* species[a)]

OTU	Phylogenetic relationship					*Dendrobium* species							
	Accession number	Taxonomic affiliation	Closest match in GenBank (accession number)	Identity	*S*-value	nobi	aphy	fimb	sala	chry	exil	stro	cari
OTU1	KX587473	Tulasnellaceae	Uncultured Tulasnellaceae (KF574228)	100%	1162								
OTU2	KX587474	Tulasnellaceae	*Uncultured Tulasnella* (EU909247)	96%	950								
OTU3	KX587475	Tulasnellaceae	*Tulasnella calospora* (GU166410)	97%	1122								
OTU4	KX587476	Tulasnellaceae	Uncultured Tulasnellaceae (JQ994399)	99%	1009								
OTU5	KX587477	Tulasnellaceae	*Tulasnella* sp.(AY373281)	98%	1110								
OTU6	KX587478	Tulasnellaceae	*Uncultured Tulasnella* (HM230649)	96%	1033								
OTU7	KX587479	Tulasnellaceae	*Epulorhiza sp.* (KU296050)	99%	1038								
OTU8	KX587480	Tulasnellaceae	*Tulasnella calospora* (GU166419)	98%	1155								
OTU9	KX587481	Tulasnellaceae	Uncultured Tulasnellaceae (JX998970)	97%	1038								
OTU10	KX587482	Tulasnellaceae	Uncultured Tulasnellaceae (GQ241802)	93%	939								
OTU11	KX587483	Tulasnellaceae	Uncultured Tulasnellaceae (GQ241802)	94%	957								
OTU12	KX587484	Tulasnellaceae	Uncultured Tulasnellaceae (GQ241802)	99%	1131								
OTU13	KX587485	Tulasnellaceae	Uncultured Tulasnellaceae (JF691403)	99%	791								
OTU14	KX587486	Tulasnellaceae	*Tulasnella irregularis* (GU166413)	97%	1038								
OTU15	KX587487	Tulasnellaceae	Uncultured Tulasnellaceae (DQ925494)	87%	699								
OTU16	KX587488	Tulasnellaceae	Uncultured Tulasnellaceae (DQ925494)	90%	809								
OTU17	KX587489	Tulasnellaceae	Uncultured Tulasnellaceae (DQ925494)	90%	904								
OTU18	KX587490	Tulasnellaceae	Uncultured Tulasnellaceae (JX545218)	99%	1175								
OTU19	KX587491	Tulasnellaceae	Uncultured Tulasnellaceae (JX545218)	95%	1075								
OTU20	KX587492	Tulasnellaceae	Uncultured Tulasnellaceae (JX998901)	91%	798								
OTU21	KX587493	Tulasnellaceae	*Tulasnella violea* (DQ520097)	86%	638								
OTU22	KX587494	Tulasnellaceae	Uncultured Tulasnellaceae (JX998901)	80%	433								
OTU23	KX587495	Auriculariales	Uncultured Auriculariales (JF691277)	93%	739								
OTU24	KX587496	Auriculariales	Uncultured Auriculariales (JQ684854)	92%	889								
OTU25	KX587497	Atractiellales	Uncultured Atractiellales (GU079590)	82%	520								
OTU26	KX587498	Atractiellales	Uncultured Atractiellales (GU079590)	85%	601								
OTU27	KX587499	Basidiomycota	Uncultured Basidiomycota (GU256215)	94%	970								
OTU28	KX587500	Basidiomycota	Uncultured Basidiomycota (JX998753)	86%	702								
OTU29	KX587501	Basidiomycota	Uncultured Basidiomycota (JX998764)	95%	981								
OTU30	KX587502	Sebacinales	Uncultured Sebacina (JX317449)	91%	854								
OTU31	KX587503	Sebacinales	Uncultured Sebacinaceae (KF574233)	99%	1223								

a) Fungi were grouped into OTUs based on a 97% internal transcribed spacer sequence similarity. Tulasnellaceae OTUs are marked in grey. Relative abundance (%): >80 ■; 50–80 ■; 30–50 ■; 10–30 ■; <10 ■. nobi, *D. nobile*; aphy, *D. aphyllum*; fimb, *D. fimbriatum*; sala, *D. salaccense*; chry, *D. chrysotoxum*; exil, *D. exile*; stro, *D. strongylanthum*; cari, *D. cariniferum*.

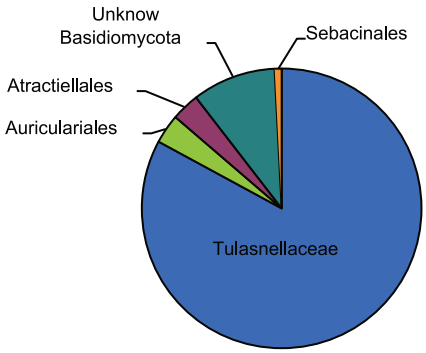

Figure 1 Frequency distribution of fungal taxa associated with eight species of the genus *Dendrobium*.

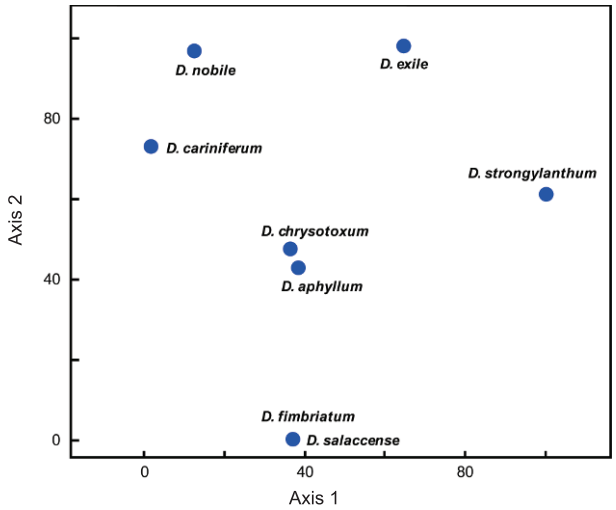

Figure 2 A nonmetric multidimensional scaling (NMDS) plot of mycorrhizal fungi associated with the eight *Dendrobium* species.

set of Tulasnellaceae OTUs.

To further understand which phylogeny influenced the *Dendrobium*-Tulasnellaceae interactions, we incorporated the identities of the interacting taxa into the network (Figure 3). We measured a moderate but significant phylogenetic signal on the *Dendrobium* phylogeny, both when considering the maximum likelihood (ML) phylogeny (d_D=0.3763; 95% CI=0.1321–0.8503) and when considering the Bayesian relaxed clock (BRC) phylogeny (d_D=0.2748; 95% CI=0.0152–0.7568). The phylogenetic signal of the *Tulasnellaceae* phylogenies was close to zero and not significant: for the ML tree, d_T=0.01786 (95% CI=0–0.0655) and for the BRC tree, d_T=0.01218 (95% CI=0–0.0688). The overall strength of the phylogenetic signal for the linear model that fit the actual data (MSE_d=0.0656 and MSE_d=0.0615 for the ML and BRC tree sets, respectively) was closer to that found under the assumption of no phylogenetic covariances (MSE of the star phylogeny=0.0202) than for the assumption of maximum phylogenetic signal (MSE_b=0.1716 and MSE_b=0.1717 for the ML and BRC tree sets, respectively). Thus, only the phylogenetic relationships among the *Dendrobium* species, not among the *Tulasnellaceae* fungi, drive the interaction structure.

DISCUSSION

Mycorrhizal diversity and specificity of *Dendrobium* species

The diversity of fungal symbionts associated with *Dendrobium* species was determined in this research. Among the eight *Dendrobium* species, Tulasnellaceae were associated with all of the plant species, and were the dominant fungal symbionts in seven species. These findings corroborate that the dominate fungi associated with *Dendrobium* plants belong to Tulasnellaceae (Xing et al., 2013). The Tulasnellaceae family maintains important associates with terrestrial orchids worldwide (McCormick et al., 2004; Shefferson et al., 2008; Jacquemyn et al., 2011, Jacquemyn et al., 2015; Bailarote et al., 2012). In addition to Tulasnellaceae fungi, Ceratobasidiaceae and Sebacinales fungi also associate with many orchid species. For example, the *Orchis* species is predominantly associated with Ceratobasidiaceae (Jacquemyn et al., 2015). However, in this research, Sebacinaceae fungi were only detected sporadically.

Mycorrhizal specificity includes both the number of fungal species that a plant can be associated with and the phylogenetic breadth of the symbionts (McCormick et al., 2004; Shefferson et al., 2007). This has been an important issue in OrM ecology because specific fungi may be needed for the promotion of seed germination and for ecological population restoration purposes. Among the 22 Tulasnellaceae OTUs detected in this research, 18 were only found to be associated with one *Dendrobium* species, while four others were associated with two to four *Dendrobium* species. Thus, most of the Tulasnellaceae fungi are of high host specificity and this supports the hypothesis that different fungal partners are needed for different orchid species to survive (McCormick and Jacquemyn, 2014). While the *Dendrobium* species showed a low specificity towards mycorrhizal fungi, almost all *Dendrobium* species were associated with at least two Tulasnellaceae OTUs, except for *D. exile*, which was associated with one specific Tulasnellaceae OTU. One orchid plant can have a symbiotic relationship with more than one fungal partner at the same time, as reported in many orchid species (Tupac Otero et al., 2002; McCormick et al., 2004; Shefferson et al., 2008; Jacquemyn et al., 2010; Martos et al., 2012).

Phylogenetic conservatism in the *Dendrobium*-Tulasnellaceae interactions

Phylogenetic conservatism has been detected in mutualist and host interactions, such as those of AM and ectomycorrhizae

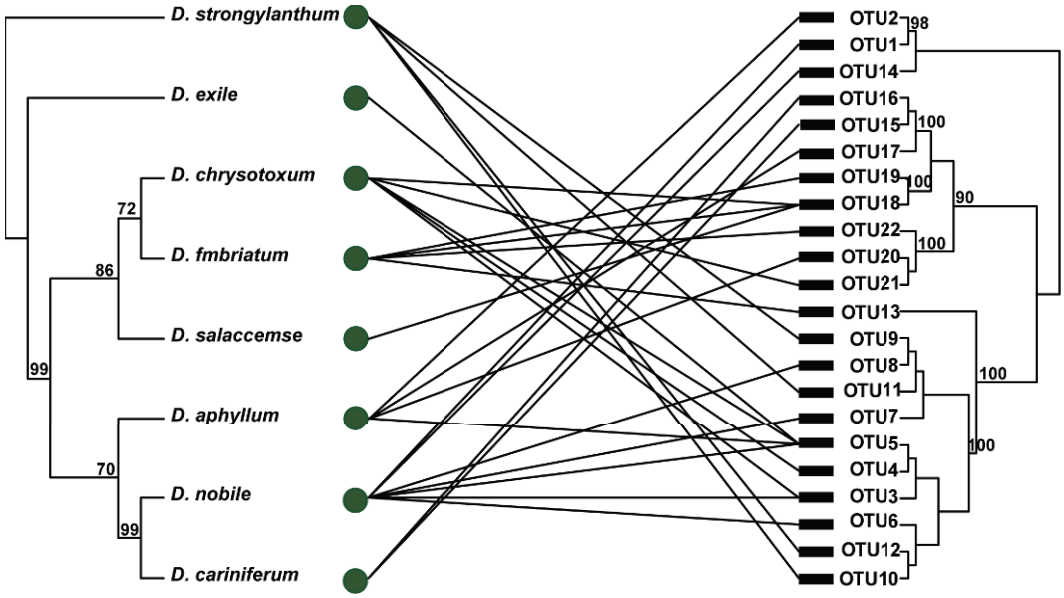

Figure 3 Network of *Dendrobium*-Tulasnellaceae interactions. Lines represent pairwise interactions. Maximum clade credibility trees from Bayesian relaxed clock analyses are shown for *Dendrobium* and Tulasnellaceae. Branch support values (⩾70) above the branches are maximum likelihood nonparametric bootstrap percentages.

(Reinhart et al., 2012; Tedersoo et al., 2013), indicating the tendency for related host plant species to have more similar interactions with mycorrhizae than expected by chance. However, some findings in AM symbiosis contradict this view, suggesting that closely related plants host more dissimilar AM fungal communities (Veresoglou and Rillig, 2014; Reinhart and Anacker, 2014). In fact, it is quite difficult to verify the phylogenetic signal in mutualist and host interactions because multiple factors often mediate species interactions, and the effects of selection on these interactions may be highly variable.

In OrM symbiosis, limited research has focused on detecting the phylogenetic signal in orchid and fungal partner interactions. Interestingly, the reported findings all presented positive results. Jacquemyn et al. investigated the fungal community differences in the genus *Orchis* and found that the interactions between *Orchis* and Tulasnellaceae fungi were significantly influenced by the phylogenetic relationships between *Orchis* species (Jacquemyn et al., 2011). Martos et al. evaluated the phylogenetic signal in the angraecoid orchid species and found that closely related orchids tend to associate with the same or closely related fungi (Martos et al., 2012). Consistent with these findings, we found that closely related *Dendrobium* species hosted more similar OrM fungal communities. A significant phylogenetic signal of *Dendrobium* on the *Dendrobium*-Tulasnellaceae interaction network's constitution revealed that Tulasnellaceae fungi were unlikely to have evolved substantially in response to the *Dendrobium* species. However, *Dendrobium*-Tulasnellaceae interactions are significantly influenced by the phylogenetic relationships between the *Dendrobium* species. This asymmetric relationship may be caused by the ability of OrM fungi to survive independent of orchids (as either saprophytes or parasites). Therefore, their distribution and reproduction are unlikely to be correlated to the orchids. That the OrM fungal community is constrained by plant phylogeny can be explained by natural selection during the evolutionary process or by trait conservatism (Losos, 2008).

Because OrM fungal community composition is influenced by many ecological factors, we limited our sampling site to one habitat to eliminate these other effects. Here, only eight *Dendrobium* species were investigated. We detected a phylogenetic conservatism in *Dendrobium*-Tulasnellaceae fungi interactions, which indicated that phylogenetically related *Dendrobium* species tended to interact with similar sets of mycorrhizal fungi. However, *Dendrobium* is a large genus of tropical epiphytic orchids, distributed in most Asian countries and Australia (Baker and Charles, 1996). Therefore, further studies still need to be performed to verify this conclusion in more *Dendrobium* species and in a larger geographic scope. Additionally, the mechanism of OrM fungal community composition is complex and unexplored. This emphasizes the need to investigate and compare the ecological functions of the fungal partners of each *Dendrobium* species. Comparisons of fungal community composition based on ecological function may explain the evolution of fungal partner selection in orchids.

MATERIALS AND METHODS

Sampling

In this study we focused on the genus *Dendrobium*. Eight

species of this genus, *D. cariniferum*, *D. chrysotoxum*, *D. exile*, *D. aphyllum*, *D. fimbriatum*, *D. strongylanthum*, *D. nobile* and *D. salaccense* were collected from Mengla County (101°25′E, 21°41′N), Xishuangbanna, Yunnan Province, China. For each *Dendrobium* species, 10 individual plants growing 30–50 m apart were sampled. For each individual plant, we collected five root fragments (2–5 cm) whenever possible, without dislodging the plant, yielding 80 sampled individuals from the eight *Dendrobium* species.

Molecular analysis of OrM fungal communities

Sampled roots were surface sterilized with ethanol (70%) for 30 s and rinsed three times in sterile water to avoid unnecessary contaminants from the velamen of the roots and the surface of the root epidermis. Then, the root fragments were checked for the presence of OrM by assaying intracellular hyphal pelotons (Rasmussen, 1995). Five 5-mm-long root sections harboring pelotons were sampled for each root fragment and stored at −20°C for DNA extraction.

The genomic DNA of each root sample was extracted using the DNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's instructions. Because we assessed the fungal community in each *Dendrobium* species, we pooled the DNA of each plant species before further analyses. To describe the basidiomycetes' mycorrhizal communities, we tested the effectiveness of several broad-spectrum basidiomycete-specific primer pairs, including ITS1-OF/ITS4-OF (Taylor and McCormick, 2008), ITS1-OF/ITS4 (White et al., 1990) and ITS1-OF/ITS4-Tul (Taylor and McCormick, 2008). ITS1-OF and ITS4-OF produced the most consistent amplification having a high yield. Clone libraries were constructed following PCR amplification with the primers ITS1-OF and ITS4-OF. PCR conditions were as follows: 94°C for 3 min, followed by 32 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 55 s. The final cycle was followed by a 7 min extension at 72°C. Clone libraries were constructed for each sample using the following procedure: PCR products were purified using the QIAquick PCR purification kit (Qiagen) and cloned using the pGEM-T Easy vector (TaKaRa, Japan) and competent *Escherichia coli* DH5α cells. Then, 96 clones were randomly selected from each library and sequenced using the M13 forward primer. Our previous studies had determined that this was a large enough clonal pool for assessing total species diversity and sampling completeness (Xing et al., 2013; Xing et al., 2015). DNA sequences from all of the samples were aligned using the MEGA5 software (Tamura et al., 2011) followed by manual editing. The sequences were grouped into operational taxonomic units (OTUs) by UPARSE (Edgar, 2013) in which sequences exceeding 97% homology were clustered into the same OUT. This threshold is the usual proxy for the species delimitation among basidiomycetes (Jacquemyn et al., 2011; Martos et al., 2012). To identify the different OTUs, representative sequences for each OTU (as indicated by UPARSE) were queried against GenBank using the BLAST algorithm. Representative sequences for each OTU were deposited in GenBank (Table 1).

Plant ITS amplification and sequencing

From each *Dendrobium* species, we selected one healthy leaf for the genomic DNA extraction. Plant DNA was also extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions. Primers ny43 and ny47 (Cameron, 2005) were selected to amplify the rDNA's ITS region. The PCR conditions were as follows: 94°C for 3 min, followed by 32 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 55 s. The final cycle was followed by a 7 min extension at 72°C. Amplification products were electrophoresed on a 1.0% agarose gel and checked to ensure that a single DNA band of the expected size was produced. For sequencing, a QIAquick PCR purification kit (Qiagen, Germany) was used to purify PCR products from unincorporated nucleotides, excess primer and salts, as well as primer dimers. Sequencing reactions were performed on an ABI-310 capillary sequencer using an ABI dye-terminator kit (ABI/Perkin-Elmer, USA) following the protocol supplied by the manufacturer.

Data analysis

The OrM fungi are usually basidiomycetes (Dearnaley, 2007), mainly members of the *Ceratobasidium*, *Sebacina* and *Tulasnella* genera (Smith and Read, 2008). Here, most of the detected fungi are members of Tulasnellaceae; however, the ITS sequences were too variable to construct a phylogenetic tree spanning all of the fungi detected, so the following analysis was limited to the Tulasnellaceae OTUs.

Based on relative abundance data of the observed OrM fungi in each of the *Dendrobium* species, the fungal community compositions associated with the different *Dendrobium* species were visualized by non-metric multidimensional scaling using the Bray-Curtis coefficient as a distance measure. All of the analyses were performed using the program PC-ORD version 6 (McCune and Mefford, 2011).

To test whether the phylogenetic relatedness of *Dendrobium* species correlated with a similar set of OrM fungi, phylogenetic signal strength was used. This approach has been employed to analyze orchid and OrM fungi interactions (Jacquemyn et al., 2011; Martos et al., 2012). Because phylogenetic signal measurements are based directly on evolutionary rates (branch lengths) estimated by phylogenetic inferences, we first constructed ML and BRC trees for the *Dendrobium* plants and the Tulasnellaceae fungi, respectively. Branch lengths were estimated without a molecular clock assumption in the ML trees, while in BRC trees they were estimated under a relaxed molecular clock assumption and represent time. The ITS sequences of 22 Tulasnellaceae OTUs and eight *Dendrobium* species were aligned using Clustal X version 2.0 (Larkin et al., 2007). The GTR+G

and T92+G+I models of evolution were identified as the best-fit models for the *Dendrobium* and Tulasnellaceae data sets, respectively, using the Akaike Information Criterion implemented in jModelTest 2 (Darriba et al., 2012). For both data sets, an ML phylogeny was constructed with RAxML 7.2.8 (Stamatakis et al., 2008). Clade support was estimated with RAxML through a nonparametric bootstrap analysis of 1,000 pseudo-replicate data sets. In addition to the ML trees, we constructed ultrametric trees with a BRC analysis using BEAST 1.5.4 (Drummond and Rambaut, 2007). The uncorrelated lognormal clock model (Drummond et al., 2006) was selected and a pro forma calibration point was enforced. The root height was fixed at 1.0. Posterior distributions of parameters were approximated using two independent Markov chain Monte Carlo analyses of 2.0×10^7 generations followed by a discarded burn-in of 2.0×10^6 generations (10%).

A simple Mantel test implemented in ZT 1.1 (Bonnet and Van de Peer, 2002) was used to compare phylogenetic distance matrices of *Dendrobium* species with matrices of ecological distances between *Dendrobium* species. The phylogenetic distances for *Dendrobium* species were calculated using the "distance" option in Geneious based on the highest likelihood tree from the ML analysis. The ecological similarity (S) (Rezende et al., 2007) between any two *Dendrobium* species was defined as the number of fungal OTUs with which they both interact divided by the total number of fungal OTUs with which either one interacts. Ecological distances were calculated as 1−S. Simple Mantel tests were run with 10,000 randomizations. Because differences in the number of associated mycorrhizal taxa affect S estimates, we also performed partial Mantel tests in which the number of associated fungal taxa was controlled (the pairwise distance for the number of associated taxa was calculated as the absolute difference in the number of associated taxa between two *Dendrobium* species). This partial Mantel test can discern whether phylogeny strictly affects the identity of fungal OTUs with which *Dendrobium* species interact, independently of the total number of fungi associated with each *Dendrobium* species (Rezende et al., 2007).

In addition to the phylogenetic relatedness of *Dendrobium* species and associated Tulasnellaceae fungi, we further evaluated the strength of the phylogenetic signals of the two phylogenies on the *Dendrobium*-Tulasnellaceae fungi interactions using a linear model approach that fit the phylogenetic variance-covariance matrix to the plant-fungi interaction matrix (Ives and Godfray, 2006). We applied the phylogenetic bipartite linear model of Ives and Godfray (Ives and Godfray, 2006). The structure of the association matrix was decomposed into a phylogenetically corrected mean association strength and a vector of residuals that depended on the phylogenies using an estimated general least square analysis. The reference evolution model used to calculate the phylogenetic structure was based on the Ornstein-Uhlenbeck process that can incorporate stabilizing selection (Blomberg et al., 2003). We calculated the independent phylogenetic signals of the *Dendrobium* (d_D) and Tulasnellaceae (d_T) phylogenies on the interaction matrix and the strength of the signal of both phylogenies combined (MSE_d). The significance of the phylogenetic structure was determined by comparing the mean square error (MSE) of this model of evolution (MSE_d) with the MSE derived under the assumption of no phylogenetic signals (i.e., a star phylogeny) and with the MSE derived under the assumption of a maximum phylogenetic signal (i.e., Brownian motion evolution, MSE_b). The model minimizing the MSE was considered the best fit. Bipartite linear models were performed using the *pblm* function in the *picante* R package (Kembel et al., 2010) and were carried out on the ML and BRC *Dendrobium*-Tulasnellaceae phylogeny sets.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *We thank Dr. Qiang Liu from Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science and Dr. Yu Zhang from Beijing Botanical Garden for their kind help in sample collection. This work was supported by the National Natural Sciences Foundation of China (81274197).*

Bailarote, B.C., Lievens, B., and Jacquemyn, H. (2012). Does mycorrhizal specificity affect orchid decline and rarity? Am J Bot 99, 1655–1665.
Baker, M.L.B, and Charles, O. (1996). Orchid Species Culture: Dendrobium. (Portland: Timber Press).
Bidartondo, M.I., Burghardt, B., Gebauer, G., Bruns, T.D., and Read, D.J. (2004). Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. Proc R Soc B 271, 1799–1806.
Blomberg, S.P., Garland, T., and Ives, A.R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution 57, 717–745.
Bonnet, E., and Van de Peer, Y. (2002). ZT: a software tool for simple and partial Mantel tests. J Stat Softw 7: 1–12.
Brundrett, M.C. (2007). Scientific approaches to Australian temperate terrestrial orchid conservation. Aust J Bot 55, 293.
Cameron, K.M. (2005). Leave it to the leaves: a molecular phylogenetic study of Malaxideae (Epidendroideae, Orchidaceae). Am J Bot 92, 1025–1032.
Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9: 772.
Dearnaley, J.D.W. (2007). Further advances in orchid mycorrhizal research. Mycorrhiza 17, 475–486.
Dearnaley, J.D.W., Martos, F., and Selosse, M.A. (2012). Orchid mycorrhizas: molecular ecology, physiology, evolution and conservation aspects. In: Fungal Association, 2nd ed. B, Hock. (Berlin: Springer-Verlag), pp. 207–230.
Drummond, A.J., Ho, S.Y.W., Phillips, M.J., and Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. PLoS Biol 4, e88.
Drummond, A.J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7, 214.
Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Meth 10, 996–998.
Fu, L.K. (1992). China Plant Red Data Book: Rare and Endangered Plants.

(Beijing: Science Press).
Gilbert, G.S., and Webb, C.O. (2007). Phylogenetic signal in plant pathogen-host range. Proc Natl Acad Sci USA 104, 4979–4983.
Ives, A.R., and Godfray, H.C.J. (2006). Phylogenetic analysis of trophic associations. Am Natist 168, E1–E14.
Jacquemyn, H., Brys, R., Merckx, V.S.F.T., Waud, M., Lievens, B., and Wiegand, T. (2014). Coexisting orchid species have distinct mycorrhizal communities and display strong spatial segregation. New Phytol 202, 616–627.
Jacquemyn, H., Brys, R., Waud, M., Busschaert, P., and Lievens, B. (2015). Mycorrhizal networks and coexistence in species-rich orchid communities. New Phytol 206, 1127–1134.
Jacquemyn, H., Honnay, O., Cammue, B.P.A., Brys, R., and Lievens, B. (2010). Low specificity and nested subset structure characterize mycorrhizal associations in five closely related species of the genus Orchis. Mol Ecol 19, 4086–4095.
Jacquemyn, H., Merckx, V., Brys, R., Tyteca, D., Cammue, B.P.A., Honnay, O., and Lievens, B. (2011). Analysis of network architecture reveals phylogenetic constraints on mycorrhizal specificity in the genus *Orchis* (Orchidaceae). New Phytol 192, 518–528.
Jacquemyn, H., Brys, R., Lievens, B., and Wiegand, T. (2012). Spatial variation in below-ground seed germination and divergent mycorrhizal associations correlate with spatial segregation of three co-occurring orchid species. J Ecol 100, 1328–1337.
Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P., and Webb, C.O. (2010). Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26, 1463–1464.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., and Higgins, D.G. (2007). Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.
Losos, J.B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol Lett 11: 995–1007.
Martos, F., Munoz, F., Pailler, T., Kottke, I., Gonneau, C., and Selosse, M.A. (2012). The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. Mol Ecol 21, 5098–5109.
McCormick, M.K., and Jacquemyn, H. (2014). What constrains the distribution of orchid populations? New Phytol 202, 392–400.
McCormick, M.K., Whigham, D.F., and O'Neill, J. (2004). Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol 163, 425–438.
McCune, B., and Mefford, M.J. (2011). PC-ORD. Multivariate analysis of ecological data. Version 6. Gleneden Beach (USA): MjM Software Design.
Poulin, R. (2010). Decay of similarity with host phylogenetic distance in parasite faunas. Parasitology 137, 733.
Rasmussen, H.N., and Rasmussen, F.N. (2009). Orchid mycorrhiza: implications of a mycophagous life style. Oikos 118, 334–345.
Rasmussen, H.N. (1995). Terrestrial Orchids: From Seed to Mycotrophic Plant. (Cambridge: Cambridge University Press).
Rasmussen, H.N. (2002). Recent developments in the study of orchid mycorrhiza. Plant Soil 244, 149–163.
Rasmussen, H.N., Dixon, K.W., Jersáková, J., and Těšitelová, T. (2015). Germination and seedling establishment in orchids: a complex of requirements. Ann Bot 116, 391–402.
Reinhart, K.O., and Anacker, B.L. (2014). More closely related plants have more distinct mycorrhizal communities. AoB Plants 6, plu051–plu051.
Reinhart, K.O., Wilson, G.W.T., and Rinella, M.J. (2012). Predicting plant responses to mycorrhizae: integrating evolutionary history and plant traits. Ecol Lett 15, 689–695.
Rezende, E.L., Lavabre, J.E., Guimarães, P.R., Jordano, P., and Bascompte, J. (2007). Non-random coextinctions in phylogenetically structured mutualistic networks. Nature 448, 925–928.
Shefferson, R.P., Cowden, C.C., McCormick, M.K., Yukawa, T., Ogura-Tsujita, Y., and Hashimoto, T. (2010). Evolution of host breadth in broad interactions: mycorrhizal specificity in East Asian and North American rattlesnake plantains (*Goodyera* spp.) and their fungal hosts. Mol Ecol 19, 3008–3017.
Shefferson, R.P., Kull, T., and Tali, K. (2008). Mycorrhizal interactions of orchids colonizing Estonian mine tailings hills. Am J Bot 95, 156–164.
Shefferson, R.P., Taylor, D.L., Weiss, M., Garnica, S., McCormick, M.K., Adams, S., Gray, H.M., McFarland, J.W., Kull, T., Tali, K., Yukawa, T., Kawahara, T., Miyoshi, K., and Lee, Y.I. (2007). The evolutionary history of mycorrhizal specificity among lady's slipper orchids. Evolution 61, 1380–1390.
Smith, S.E., and Read, D.J. (2008). Mycorrhizal Symbiosis. 3rd ed. (London: Academic Press).
Stamatakis, A., Hoover, P., and Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. Syst Biol 57, 758–771.
Swarts, N.D., and Dixon, K.W. (2009). Perspectives on orchid conservation in botanic gardens. Trends Plant Sci 14, 590–598.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28, 2731–2739.
Taylor, D.L., and McCormick, M.K. (2008). Internal transcribed spacer primers and sequences for improved characterization of basidiomycetous orchid mycorrhizas. New Phytol 177, 1020–1033.
Tedersoo, L., Mett, M., Ishida, T.A., and Bahram, M. (2013). Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. New Phytol 199, 822–831.
Tupac Otero, J., Ackerman, J.D., and Bayman, P. (2002). Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. Am J Bot 89, 1852–1858.
Veresoglou, S.D., and Rillig, M.C. (2014). Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. Plant Soil 377, 395–406.
Wang, B., and Qiu, Y.L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16, 299–363.
Waud, M., Wiegand, T., Brys, R., Lievens, B., and Jacquemyn, H. (2016). Nonrandom seedling establishment corresponds with distance-dependent decline in mycorrhizal abundance in two terrestrial orchids. New Phytol 211, 255–264.
Weiblen, G.D., Webb, C.O., Novotny, V., Basset, Y., and Miller, S.E. (2006). Phylogenetic dispersion of host use in a tropical insect herbivore community. Ecology 87: S62–S75.
White, T.J., Bruns, T.D., Lee, S.B., and Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A Guide to Methods and Applications, M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, eds. (San Diego: Academic).
Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V., Damschen, E.I., Jonathan Davies, T., Grytnes, J.A., Harrison, S.P., Hawkins, B.A., Holt, R.D., McCain, C.M., and Stephens, P.R. (2010). Niche conservatism as an emerging principle in ecology and conservation biology. Ecol Lett 13, 1310–1324.
Xing, X., Gai, X., Liu, Q., Hart, M.M., and Guo, S. (2015). Mycorrhizal fungal diversity and community composition in a lithophytic and epiphytic orchid. Mycorrhiza 25, 289–296.
Xing, X., Ma, X., Deng, Z., Chen, J., Wu, F., and Guo, S. (2013). Specificity and preference of mycorrhizal associations in two species of the genus *Dendrobium* (Orchidaceae). Mycorrhiza 23, 317–324.

SUPPORTING INFORMATION

Figure S1 Frequency distribution of fungal taxa associated with each *Dendrobium* species.

The supporting information is available online at life.scichina.com and www.springerlink.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.