Mycorrhiza (2015) 25:289–296
DOI 10.1007/s00572-014-0612-5

ORIGINAL PAPER

Mycorrhizal fungal diversity and community composition in a lithophytic and epiphytic orchid

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Received: 9 July 2014 / Accepted: 7 October 2014 / Published online: 17 October 2014

Abstract Some orchid species are present as epiphytes and lithophytes in the same habitat, but little is known about the differences of their mycorrhizal fungal communities. We used *Coelogyne viscosa*, which occurs both as an epiphyte and a lithophyte, as a study system to investigate orchid mycorrhizal fungal communities in lithophytes and epiphytes in Xishuangbanna National Nature Reserve (Yunnan Province, China). Twenty-three fungal operational taxonomic units (OTUs) from 18 sampling sites were identified. Results indicated that mycorrhizal fungal community composition was different between epi- and lithophytes. When we analyzed the *Tulasnellaceae* and *Sebacinales* communities separately, we found that the *Sebacinales* fungal communities were significantly different in the two growth habitats, but the *Tulasnellaceae* fungal communities were not. Our results provide evidence for distinct orchid mycorrhiza fungal communities depending on the growth habitat of the orchid. Consistent with some recent investigations of mycorrhizal fungus community composition, this study suggests that for one orchid, growth habitat affects mycorrhizal symbioses.

Keywords *Coelogyne viscosa* · Orchid mycorrhizal symbiosis · Fungal diversity · Lithophyte · Epiphyte

Introduction

Orchidaceae is one of the largest families of flowering plants with an estimated 26,000 species (Joppa et al. 2011). Most orchid species are dependent on mycorrhizal fungi for seed germination and growth during early stages of plant development (Rasmussen and Rasmussen 2009) as well as in some adult orchid species (Cameron et al. 2006; Rasmussen and Rasmussen 2009; Waterman et al. 2011). The fungi that form orchid mycorrhizas largely belong to the basidiomycetes (Dearnaley 2007) and are called *Rhizoctonias* for convenience (Dearnaley et al. 2012). This group usually includes members of the *Tulasnellaceae*, *Ceratobasidiaceae*, and *Sebacinales*. Dependency on mycorrhizas is increasingly recognized as one of the most important ecological traits influencing plant distributions and abundance and has been linked to niche partitioning in orchid species (Jacquemyn et al. 2012b, 2014). Understanding how fungal associations vary between habitats is important for orchid recovery, as mycorrhizal fungi could be used in orchid propagation and reintroduction (Brundrett 2007).

Mycorrhizal preference is highly variable among orchids. Non-photosynthetic orchids typically exhibit very specific associations to non-"rhizoctonia" saprotrophic partners, including either saprotrophic fungi (on litter or dead wood) or ectomycorrhizal fungi (Dearnaley et al. 2012). In photosynthetic orchids, levels of specificity vary considerably, ranging from narrow to broad specificity (McCormick et al. 2004; Barrett et al. 2010; Shefferson et al. 2010; Jacquemyn et al. 2010; Pandey et al. 2013). For orchid seed germination and protocorm development,

Electronic supplementary material The online version of this article (doi:10.1007/s00572-014-0612-5) contains supplementary material, which is available to authorized users.

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specificity may be due to ecological pressures in different environments (Taylor et al. 2002). Therefore, the availability of appropriate fungal associations may play an important role in determining which habitats allow orchid growth and affect the geographic distribution and ecological niche of some orchid species (McCormick et al. 2009, 2012; Swarts et al. 2010; Jacquemyn et al. 2012a).

Orchids encompass great diversity and considerable variation in life forms, with the majority growing as epiphytes (plant surface dwelling) or lithophytes (rock surface dwelling) (Gravendeel et al. 2004). Some species, especially in tropical areas, are present as both epiphytes and lithophytes, e.g., *Bulbophyllum drymoglossum* (Chung et al. 2007), *Sarcanthus scolopendrifolius* (Chung et al. 2007), *Lieanthes rupestris* (Gómez et al. 2006), *Holcoglossum* spp. (Jin 2005), and *Lepanthes rubipetala* (Schödelbauerová et al. 2010). But do orchids that exist both as epiphytes and lithophytes host divergent mycorrhizal fungus communities? Surprisingly, little research has been carried out to examine divergence in mycorrhizal symbionts between different growth habitats (epiphytic and lithophytic) of the same species.

While these orchids would share any phylogenetic symbiont preferences (Shefferson et al. 2005; Jacquemyn et al. 2011), differences in fungal symbionts may occur due to ecological or dispersal constraints. Compared to epiphytic orchids, lithophytes experience relative extreme growth conditions in terms of nutrient and moisture availability. It is possible that such extremes in growing conditions trump phylogenetic preferences, and ecological constraints are more important for determining mycorrhizal community structure. Alternatively, differences among orchids in their mycorrhizas may have little to do with phylogenetic preferences or ecological functioning. Because orchid fungi are not obligate biotrophs and exhibit broad dispersal patterns in a wide variety of habitats, orchids may be at the mercy of whichever fungi happen to disperse where their seeds germinate. In this case, mycorrhizal fungus communities may be due to factors that influence the distribution of the fungi themselves and not due to any host specificity.

In this research, we ask whether host growth habitat (epi- or lithophytic) affects the mycorrhizal fungus community associated with a single orchid species, *Coelogyne viscosa*. We expect lithophytes to exhibit more specificity than epiphytes because they are subjected to more stressful growth conditions. At the same time, we predict that lithophytic fungal communities will be more phylogenetically conserved. This is because we expect that the stressful lithophytic growth habitat imposes a strong filter on the types of fungi able to dwell in these environments. This should also manifest as a phylogenetic filter (i.e., trait conservatism) (Losos 2008; Peay et al. 2010).

Materials and methods

Study species and sampling

C. viscosa is an epiphytic plant with a stout, creeping rhizome 6–8 mm thick, pseudobulbs 1–1.5 cm apart on the rhizome, 2 leaved at apex. *C. viscosa* is found in forests in Southwest China (Yunnan province), Northeast India, Myanmar, Vietnam, Laos, Thailand, and Malaysia. In Xishuangbanna National Nature Reserve (101° 25′ E, 21° 41′ N), Yunnan Province, China, a population of *C. viscosa* exists both as epiphytes and lithophytes (Fig. 1). Within the reserve, we sampled from 18 sites (containing 9 epiphytes and 9 lithophytes) in a 70×60-m plot in September 2013 (see Appendix S1 for the location of each site in the plot). From each site, five individual plants were randomly selected. In total, 90 plant root samples were collected. Root fragments (about 2 cm long) were collected from each plant and placed in plastic bags. In order to test the differences of mycorrhizal fungal communities composition between the epiphytic and lithophytic habitats, we regarded the different sites of each habitat as one sample composed of roots from five individual plants in that site. Samples were refrigerated until processing (within 3 days of sampling).

Molecular analysis of orchid mycorrhizal fungal communities

From each plant, we selected five root sections (5 mm) harboring pelotons. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions. Because we treated the different sites of each habitat as one sample, we pooled the five plant DNAs from each site before further analysis.

To describe the basidiomycete mycorrhizal community, we tested the effectiveness of several broad-spectrum basidiomycete primer pairs, including ITS1-OF/ITS4-OF (Taylor and McCormick 2008), ITS1-OF/ITS4 (White et al. 1990), and ITS1-OF (White et al. 1990)/ITS4-Tul (Taylor and McCormick 2008). ITS1-OF and ITS4-OF gave the most consistent amplification with high yields. 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 high DH5α. Ninety-six clones were randomly selected from each library and sequenced using the M13 forward primer. DNA sequences from all samples were aligned using MEGA5 software (Tamura et al. 2011) followed by manual editing.

Fig. 1 Population of *Coelogyne viscosa* in Xishuangbanna National Nature Reserve, Yunnan Province, China. **a** Orchid dwelling on a plant surface (epiphyte). **b** Orchid dwelling on a rock surface (lithophyte)

Sequences sharing at least 97 % similarity were grouped as operational taxonomic units (OTUs), which is the usual proxy for species delimitation among basidiomycetes (Jacquemyn et al. 2011; Martos et al. 2012). To identify the different OTUs, representative sequences for each OTU were queried against GenBank using BLAST. Representative sequences for each OTU were deposited in GenBank (accession numbers KF574225-KF574247, Table 1).

Data analysis

Alpha diversity Alpha diversity for each sampling site was calculated using both species richness (the number of OTUs) as well as the reciprocal of Simpson indices (Magurran 1988). To detect differences between epi- and lithophytes, we performed a one-way ANOVA for both species richness and Simpson indices.

Fungal community composition To visualize differences in mycorrhizal communities between sampling sites, non-metric multidimensional scaling (NMDS) ordination techniques were applied using the Bray–Curtis coefficient as distance measure. All analyses were performed using the program PC-ORD version 6 (McCune and Mefford 2011). Then the effect of growth form on fungal community composition was tested with PERMANOVA (Anderson et al. 2008) with "growth habitat" as the only (fixed) factor. We calculated the Bray–Curtis (Bray and Curtis 1957) similarity for each pair of samples and tested for significance using 9999 unrestricted permutations (Type III, partial sum of squares).

Phylogenetic analysis In order to discern the compositional differences, we used UniFrac (Lozupone and Knight 2005) to measure the phylogenetic distance among epi- and lithophytes. As the internal transcribed spacer (ITS) sequences were too variable to enable the construction of a phylogenetic tree spanning of all the detected fungi in this research, we constructed the phylogenetic trees of the three fungal taxa, that is, *Tulasnellaceae*, *Sebacinales*, and *Ceratobasidiaceae* separately. This was accomplished through separate alignments in Clustal X version 2.0 (Larkin et al. 2007) with minor manual adjustments for each fungal taxon. Published orchid mycorrhizal fungal sequences were downloaded from GenBank and added to our data to generate the trees. Phylogenetic relationships among the fungi in each taxon were estimated by determining the best-fit substitution models according to AIC criteria in jModelTest version 2.1 (Darriba et al. 2012) and then using the most similar available model to construct maximum-likelihood trees using MEGA version 5 (Tamura et al. 2011). Bootstrap values were estimated via 1000 replicates. UniFrac tests (weighted and unweighted for relative abundance) were performed using 1000 permutations and calculated with the UniFrac web application (http://bmf2.colorado.edu/unifrac) (Lozupone et al. 2006). UniFrac and *p* test significant values were corrected by using the Bonferroni correction for multiple comparisons.

Results

Identity of mycorrhizal fungi

Examination of all *C. viscosa* individuals revealed fungal colonization (hyphal pelotons) in their root cortical cells. From the 18 sampling sites, we obtained a total of 726 sequences: 245 sequences of *Sebacinales*, 225 sequences of *Tulasnellaceae*, 41 sequences of *Ceratobasidiaceae*, and 215 sequences of other basidiomycetes. We identified 8 OTUs of *Sebacinales*, 5 OTUs of *Tulasnellaceae*, and 3 OTUs of *Ceratobasidiaceae* at the 97 % sequence similarity threshold (see Appendix S2 for the figure showing maximum-likelihood trees constructed with each OTU and reference sequences from GenBank). The three most frequently recorded OTUs (OTUs 1, 13, and 15) accounted for 65 % of all sequences. OTU1 and OTU15 were found in 83 % and 77 % of *C. viscosa* samples, respectively.

In the 16 *Rhizoctonia* OTUs, 13 were found only in either epiphytic (8 OTUs) or lithophytic (5 OTUs) orchid, while only 3 (OTUs 1, 13, and 15) were found in both (Fig. 2). The 8 epiphytic-specific OTUs included 2 in *Tulasnellaceae* (OTUs 3 and 4), 2 in *Ceratobasidaceae* (OTUs 10 and 12) and 4 in *Sebacinales* (OTUs 14, 18, 19, and 20). The 5 lithophyte-specific OTUs included 2 in

Table 1 List of fungal operational taxonomic units (OTUs) identified using cloning techniques

			Phylogenetic relationship				
OTU	Representative sequences	Sequence length	Taxonomic affiliation	Closest match in GenBank (accession number)	Sequence identity	*S* value	*E* value
OTU1	KF574225	706	*Tulasnellaceae*	Uncultured *Tulasnellaceae* clone PD563 (GQ241838)	99	1160	0.0
OTU2	KF574226	708	*Tulasnellaceae*	Uncultured *Tulasnellaceae* clone PD567 (GQ241845)	94	968	0.0
OTU3	KF574227	731	*Tulasnellaceae*	Uncultured *Tulasnellaceae* clone PM438(GQ241820)	87	797	0.0
OTU4	KF574228	672	*Tulasnellaceae*	*Tulasnella* sp. C2-DT-TC-1 (GU166427)	88	763	0.0
OTU5	KF574229	707	*Tulasnellaceae*	Uncultured *Tulasnellaceae* clone FM372.1(JF691399)	95	992	0.0
OTU6	KF574241	706	Unknown Basidiomycete	Uncultured Basidiomycete isolate dfmo0690 (AY969526)	93	843	0.0
OTU7	KF574242	719	Unknown Basidiomycota	Uncultured Basidiomycota clone man41 (GU328562)	85	725	0.0
OTU8	KF574243	725	Unknown *Trechisporales*	Uncultured *Trechisporales* clone FM311.2 (JF691365)	99	1205	0.0
OTU9	KF574244	700	Unknown Basidiomycota	Uncultured ectomycorrhizal fungus (FM999581)	82	569	8e–159
OTU10	KF574230	690	*Ceratobasidiaceae*	Uncultured *Ceratobasidiaceae* clone TP362.1 (FJ691537)	98	699	0.0
OTU11	KF574231	766	*Ceratobasidiaceae*	*Ceratobasidium* sp. AG-F isolate Str52 (DQ102436)	95	992	0.0
OTU12	KF574232	722	*Ceratobasidiaceae*	Uncultured *Ceratobasidiaceae* clone TP362.1 (JF691537)	98	1182	0.0
OTU13	KF574233	712	*Sebacinaceae*	Uncultured *Sebacina* clone 9597 (EU909227)	96	995	0.0
OTU14	KF574234	718	*Sebacinaceae*	Uncultured *Sebacina* isolate TUB 019432 (HQ154340)	91	918	0.0
OTU15	KF574235	715	*Sebacinaceae*	Uncultured *Sebacina* clone 9761 (EU909216)	96	1158	0.0
OTU16	KF574236	733	*Sebacinaceae*	Uncultured mycorrhiza (*Sebacinaceae*) 4078 (AY634132)	89	870	0.0
OTU17	KF574237	615	*Sebacinaceae*	Uncultured *Sebacina* isolate TUB 019445 (HQ154358)	97	822	0.0
OTU18	KF574238	663	*Sebacinaceae*	Uncultured *Sebacina* isolate TUB 019459 (HQ154386)	99	832	0.0
OTU19	KF574239	710	*Sebacinales*	Uncultured *Sebacinales* isolate 601, clone K402 (FN663855)	90	928	0.0
OTU20	KF574240	653	*Sebacinales*	Uncultured *Sebacinales* clone 3EC2_7 (HM451809)	94	676	0.0
OTU21	KF574245	807	*Marasmiaceae*	Uncultured *Marasmiaceae* clone FM150.1 (JF691275)	99	1373	0.0
OTU22	KF574246	736	*Thelephoraceae*	Uncultured *Tomentella* type OTU: LH123 (GQ268670)	95	1099	0.0
OTU23	KF574247	725	*Ganodermataceae*	*Ganoderma gibbosum* isolate XSD-34 (EU273513)	99	1203	0.0

Fungi were grouped into OTUs defined by 97 % internal transcribed spacer (ITS) sequence similarity

Tulasnellaceae (OTUs 2 and 5), 1 in *Ceratobasidaceae* (OTU11), and 2 in *Sebacinales* (OTUs 16 and 17).

Rhizoctonia fungal community associated with *C. viscosa* consisted predominantly of *Sebacinales* (47.95 %) and *Tulasnellaceae* (44.03 %). When considering epiphytic and lithophytic orchids separately, epiphytes contained fewer *Tulasnellaceae* (32.61 %) compared with *Sebacinales* (54.35 %) (p=0.031), whereas lithophytes contained more *Tulasnellaceae* (51.72 %) compared to *Sebacinales* (44.83 %), but was not significant (p>0.05).

We also identified 7 other basidiomycete OTUs in the remaining 215 sequences, including *Marasmiaceae*, *Thelephoraceae*, *Ganodermataceae*, and unknown Basidiomycota (Table 1, also see Appendix S3 for the figure showing the frequency distribution of identified OTUs in epiphytes and lithophytes). In order to test the robustness of our result, we also used the ITS sequence similarity cutoff of 95 %; however, the fungi still could be grouped into 23 OTUs.

Mycorrhizal fungal community composition in epiphytic and lithophytic orchid

The remainder of our analysis involved *Tulasnellaceae*, *Ceratobasidiaceae*, and *Sebacinales* OTUs, since the fungi forming orchid mycorrhizas are primarily these three fungal taxa (Dearnaley et al. 2012).

Alpha diversity In total, we detected 11 and 8 OTUs in epiphytes and lithophytes, respectively. Similarly, Simpson indices were not significantly (p>0.05) higher in epiphytes (5.70) than in lithophytes (5.54). Although epiphytes associated with more OTUs in total, the average number of OTUs per sampling site did not differ significantly (p>0.05) between lithophytes (3.22±1.09) and epiphytes (2.88±0.60).

Community composition In terms of community composition, results of NMDS clearly separated epiphyte and lithophyte

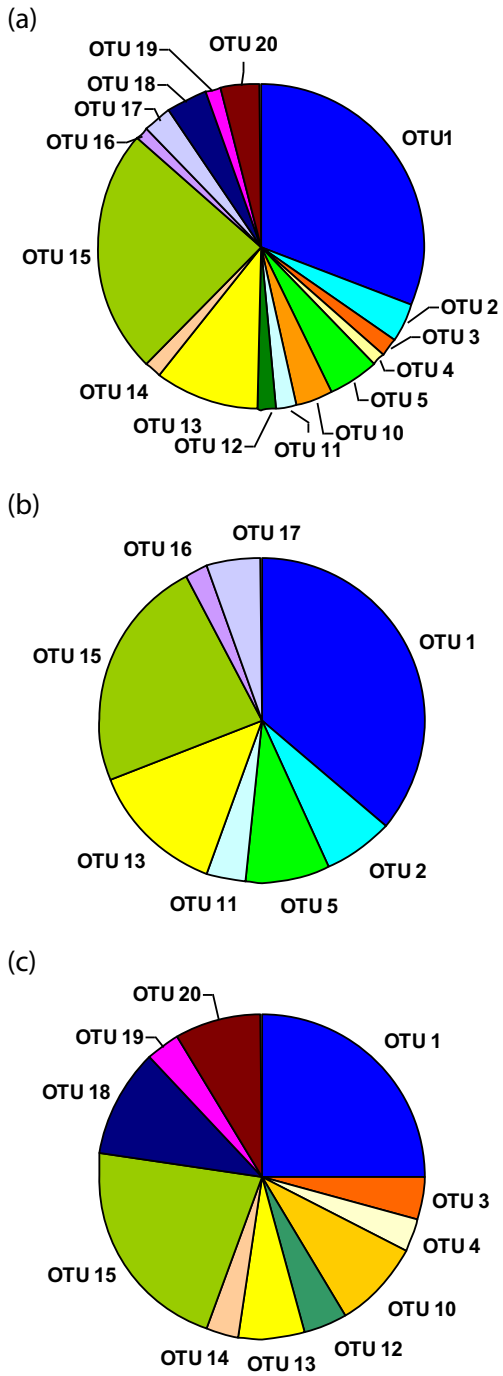

Fig. 2 Frequency distribution of identified operational taxonomic units (OTUs) of *Tulasnellaceae*, *Ceratobasidiaceae*, and *Sebacinales* in *Coelogyne viscosa*. **a** All plants. **b** Lithophytes only. **c** Epiphytes only

(Fig. 3). Additionally, lithophyte- and epiphyte-associated fungal communities were marginally significantly different (Bray–Curtis; Pseudo $F=1.989$, $p=0.048$) in the PERMANOVA. This was also true when we used the presence/absence of taxa, irrespective of abundance (Pseudo $F=2.169$, $p=0.036$). When we analyzed the *Tulasnellaceae* and *Sebacinales* separately, we found that the *Sebacinales* fungal communities were significantly different between the two growth habitats (Pseudo $F=2.791$, $p=0.030$), while the *Tulasnellaceae* fungal communities were not (Pseudo $F=0.551$, $p=0.631$). When using the presence/absence data, the results did not change (Pseudo $F=3.436$, $p=0.017$ for *Sebacinales*; Pseudo $F=0.656$, $p=0.547$ for *Tulasnellaceae*).

We also looked at differences between fungal communities in terms of phylogenetic distance (UniFrac). Because both OTUs and overall abundance were lower for sequences belonging to the *Ceratobasidaceae*, our analysis was limited to the *Tulasnellaceae* and *Sebacinales*. Phylogenetic comparisons of *Sebacinales* revealed significant community differences between fungal communities associated with epi- or lithophytes, regardless of whether comparisons were based on the presence/absence ($p=0.02$) or relative abundance of individuals ($p=0.03$). Conversely, the *Tulasnellaceae* fungal communities were not significantly different between epi- and lithophytes, regardless of weighting.

Discussion

Mycorrhizal fungal community composition

Overall, there was little overlap in the fungal taxa that occurred on epi- and lithophytes. *C. viscosa* associated with fungal OTUs related to the three mycorrhizal fungal clades, *Tulasnellaceae*, *Ceratobasidiaceae*, and *Sebacinales*. Among them, *Sebacinales* and *Tulasnellaceae* were dominant fungal symbionts in both growth habitats. These fungal families were known as important associates in species of the terrestrial temperate orchid, i.e., *Sebacinales* in *Caladenia huegelii* (Swarts et al. 2010), *Ceratobasidiaceae* in *Goodyera* spp. (Shefferson et al. 2010), and *Tulasnellaceae* in worldwide terrestrial orchids (Kristiansen et al. 2004; McCormick et al. 2004; Shefferson et al. 2008; Yuan et al. 2010; Jacquemyn et al. 2010, 2012c). In addition to OTUs associated with *Rhizoctonia* fungi, OTUs related to members of *Marasmiaceae*, *Thelephoraceae*, and *Ganodermataceae* were observed very sporadically. *Marasmiaceae* and *Thelephoraceae* fungi have also been found in other epiphytic or terrestrial orchids, i.e., *Marasmiaceae* in epiphytic orchids (Martos et al. 2012) and *Thelephoraceae* in terrestrial orchids (Jacquemyn et al. 2011), which were only found in lithophytes in this research.

Mycorrhizal specificity has been defined by both the number of fungal species that a plant can be associated with and the phylogenetic breadth of symbionts (McCormick et al. 2004; Shefferson et al. 2007). We detected a low specificity in mycorrhizal associations of *C. viscosa*, whatever their growth habitats are, because almost all of the *C. viscosa* plants here interacted with more than one mycorrhizal fungus associate. Similar results have been reported in the literature

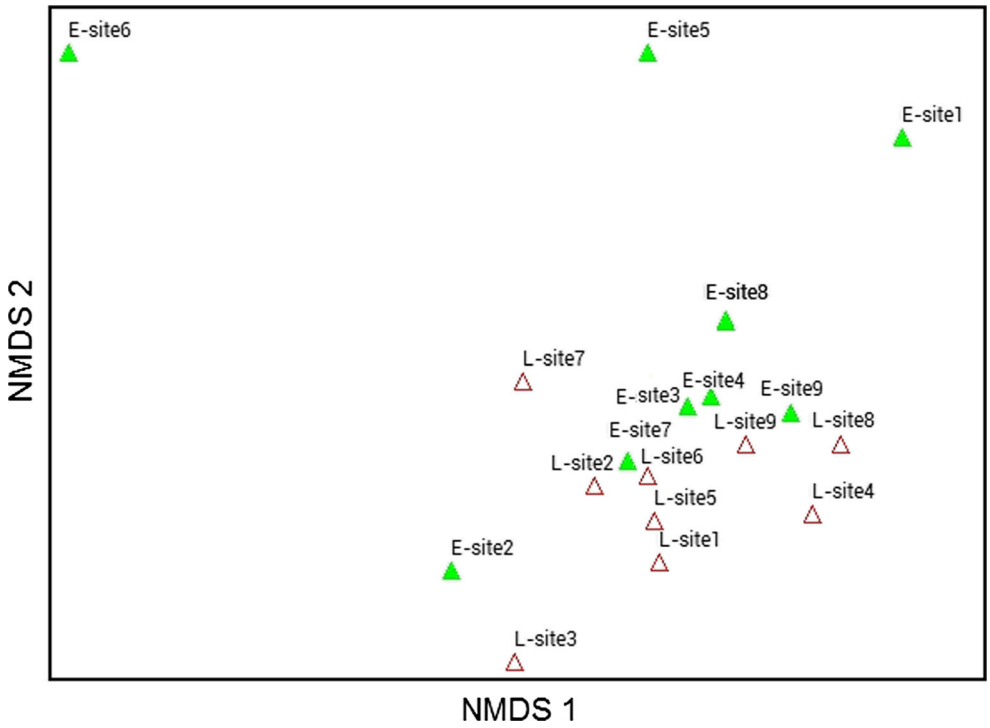

Fig. 3 A nonmetric multidimensional scaling (NMDS) plot of mycorrhizal fungi detected in lithophyte and epiphyte of the orchid *Coelogyne viscosa*: lithophyte (*L*), *red*; epiphyte (*E*), *green*

(Otero et al. 2002; McCormick et al. 2004; Shefferson et al. 2008; Jacquemyn et al. 2010, 2012c; Martos et al. 2012). Additionally, by comparing the dominance of various fungal families in the *C. viscosa* population, our data also show that the dominant fungal family differs between epiphytes and lithophytes. This result is consistent with the observations that some orchid species may be able to switch to different fungal partners under adverse growth conditions (McCormick et al. 2006), and the dominance of fungal families can be influenced by habitat preferences of mycorrhizal partners (Pandey et al. 2013).

Comparison of fungal communities in epiphytic and lithophytic growth habitats

As we predicted, we found that mycorrhizal fungal community composition differed between epiphytic and lithophytic growth habitats, although the difference was weak. When we analyzed the *Tulasnellaceae* and *Sebacinales* fungal communities separately, we found some differences in the fungal communities between the two growth habitats. The *Sebacinales* fungal communities between the two growth habitats were significantly different, but the *Tulasnellaceae* fungal communities were not.

The number of OTUs per sampling site did not differ significantly between epiphytes and lithophytes. However, we detected more fungal OTUs in epiphytes than in lithophytes in total. This result may indicate that fungal assemblages on tree bark are more diverse than those occurring on rock surfaces. Lithophytes are exposed to relatively extreme conditions compared to epiphytes, i.e., low water and nutrient availability, which may lead to a higher dependency on fungal symbionts in lithophytes than in epiphytes. It may be that orchids select the best partner from the species pool to meet their nutritional demands (Otero et al. 2005; Rasmussen and Rasmussen 2009). Thus it may be that there are fewer fungi that are able to fulfill these stringent needs compared to those associating with the more moderate growing conditions of the epiphytes. It has been suggested that phylogenetically conserved fungi may have similar ecological traits (Maherali and Klironomos 2007; Peay et al. 2010), so it is possible that the fungi which are able to persist in lithophytes may possess different traits than those on tree surfaces, and as a consequence, be more closely related. In fact, we found that in the *Sebacinales*, fungal communities associating with lithophytes were less phylogenetically distant than those associating with epiphytes.

It is also possible that fungi dwelling on rock surfaces represent a subset of fungi at each site simply due to dispersal constraints of the fungi themselves. Unlike AM and ectomycorrhizal fungi, orchid fungi are not thought to be obligate biotrophs, so the distribution of orchid mycorrhizal fungi is somewhat independent of the distribution of orchids (McCormick et al. 2012). On the other hand, epiphytic or lithophytic orchids are less well studied with regard to their mycorrhizal associations in comparison with terrestrial orchids (Martos et al. 2012; Dearnaley et al. 2012). However, recent molecular identification reveals mycobionts of epiphyte as the typical rhizoctonias of green orchids (Martos et al. 2012) and which may also make an important role in

their life cycle (Dearnaley et al. 2012). Thus, we expect fungal communities on rock surfaces or tree bark to reflect ecological pressures on fungi and that the fungal community composition may determine whether the plants develop as epiphytes or lithophytes.

Whatever the mechanism is, active host selection for fungal traits, or the passive acquisition of available fungi on rock surfaces, our results reflect certain ecological isolation of fungal partners between these two contrasting growth habitats. Although we know little about the ecological function of fungi in epiphytic and lithophytic growth habitats, the obligate fungal dependence of early orchid life history stages indicates that orchids are likely to be strongly affected by the distribution of particular fungi (McCormick et al. 2012). This emphasizes the need to investigate and compare the ecological function of fungal partners in different habitats or growth niches—for example, using the in situ seed baiting method (Brundrett et al. 2003) to identify the fungi capable of supporting seed germination.

Acknowledgments This research was financially supported by the National Natural Sciences Foundation of China (No. 81274197). We thank Randy Molina and the two anonymous referees for the useful comments on this manuscript.

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