Fungal Ecology 34 (2018) 67–75

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Untangling factors that drive community composition of root associated fungal endophytes of Neotropical epiphytic orchids

Stefania Cevallos [a, b], Paulo Herrera [b], Aminael Sánchez-Rodríguez [b], Stéphane Declerck [a], Juan Pablo Suárez [b, *]

[a] Université catholique de Louvain, Earth and Life Institute, Applied Microbiology, Mycology, Croix du Sud, 2 box L7.05.06, B-1348 Louvain-la-Neuve, Belgium
[b] Departamento de Ciencias Biológicas, Universidad Técnica Particular de Loja, San Cayetano Alto y Paris, C.P. 11 01 608, Loja, Ecuador

ARTICLE INFO

Article history:
Received 23 November 2017
Received in revised form
16 April 2018
Accepted 5 May 2018

Corresponding Editor: Thorunn Helgason

Keywords:
Fungal community composition
Drivers of endophyte communities
Epiphytic orchids
Root-associated endophytes
Next generation sequencing
Fungal community analysis

ABSTRACT

In orchids, most of the root-associated fungal endophytes remain undescribed as well as the drivers that affect their interactions with the plants. We characterized root-associated fungal endophytes of co-existing orchids across sites in two areas of montane rainforest in the southern Ecuadorian Andes. We amplified the nrDNA ITS2 region of 130 orchid individuals with Illumina MiSeq technology and tested whether changes in the structure of fungal communities are associated with hosts' phylogeny or the sites where the orchids grow. We identified 3492 OTUs corresponding to the Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Zygomycota phyla. Fungal communities associated with orchids at the lower geographic areas (between 2050 and 2800 m a.s.l.) showed that host evolution and sites are drivers that could shape distinct fungal communities, while at the highest geographic areas (between 3000 and 3500 m a.s.l.), no distinct fungal communities were found neither between co-existing orchid species nor between sites. These results suggested that among orchid species, abiotic and biotic factors do not influence the composition of fungal communities in the same way.

1. Introduction

Orchid roots harbor a diverse set of fungal species with distinct ecological attributes such as saprobes, latent pathogens or symbionts (Kohout et al., 2013; Ma et al., 2015). Fungi located inside orchid roots in the velamen or in the cortical tissue are collectively termed root-associated endophytes (Brundrett, 2002). However, studies focusing on root-associated endophytes frequently ignore the species present in the velamen because this thick corky epidermis is generally recognized as an adaptive structure for water and nutrient conservation (Zotz and Winkler, 2013). Fungi present in the velamen are thus most often considered as surface contaminants or opportunists associated with roots (e.g. Yuan et al., 2010). Thus, the root cortical tissue is the main target of multiple studies focusing on orchid fungal endophytes because it often harbors fungi that may interact with the plant as symbionts (Brundrett, 2002; Smith and Read, 2008).

Fungal endophytes in root cortical tissues comprise a polyphyletic group (Smith and Read, 2008) that includes mycorrhizal and non-mycorrhizal fungi. While mycorrhizal fungi have been widely investigated across multiple orchid species and with recognized ecological roles in their life cycle (Dearnaley et al., 2012), non-mycorrhizal fungi have been less studied (Ma et al., 2015).

Most studies focusing on community composition of orchid root-associated endophytes (both mycorrhizal and non-mycorrhizal fungi) have been based on culture-dependent methods (Herrera et al., 2010; Novotná et al., 2018). However, the great majority of fungi cannot be grown in artificial conditions and thus culture-independent methods (sequencing and cloning) have been developed to increase the knowledge of fungal diversity (Kristiansen et al., 2001). With the development of powerful molecular methods in recent years (e.g. next generation sequencing), the range of fungi identified to the species level has increased

* Corresponding author. Universidad Técnica Particular de Loja, 11 01 608 Loja, Ecuador.
E-mail address: jpsuarez@utpl.edu.ec (J.P. Suárez).

https://doi.org/10.1016/j.funeco.2018.05.002
1754-5048/

markedly, making more accurate ecological inferences now possible (Smith and Peay, 2014).

Studies conducted on the orchid root-associated fungal endophytes, using culture-independent methods, mostly described fungal diversity and abundance (Kohout et al., 2013). However, how root-associated fungal endophyte communities are assembled in orchids remains poorly investigated (for a review, see Dearnaley et al., 2012) and the information about the abiotic and biotic factors that drive such fungal community composition is even more limited.

Abiotic factors such as temperature and humidity are generally variable across sites but also at the same site, altitudinal gradient could result in peculiar microclimates that shape plant-fungi interactions as was observed in orchid mycorrhizal fungi (Garnica et al., 2013). Similarly, biotic factors such as the host phylogeny or the interactions between co-existing species were suggested to affect fungal distribution patterns (see Waterman et al., 2011). For instance, conforming to the co-existence theory, species are able to cohabit because they use different niches (Götzenberger et al., 2012) determined by the host (Oliveira et al., 2014).

Nowadays, the information about the specific factors that drive the occurrence and variation of orchid root-associated fungal endophytes in natural environments is scarce (e.g. Sudheep and Sridhar, 2012; Bunch et al., 2013) probably because of the limited investigations of orchid species, populations or sites (Ma et al., 2015). Studies on orchid root-associated fungal endophytes were mostly focused on terrestrial orchids in temperate ecosystems (e.g. Jacquemyn et al., 2015; Těšitelová et al., 2015), whereas only a few concerned epiphytic orchids in tropical areas (see Bayman and Otero, 2006; Herrera et al., 2010; Oliveira et al., 2014; Novotná et al., 2018). This fact contrasts with the higher orchid diversity concentrated in Neotropical ecosystems (Dressler, 1990; Pridgeon, 1995). In the Ecuadorian Andes, one of the world's hotspots of biodiversity (Beck et al., 2008), an important variety of basidiomycetes and ascomycetes has been identified associated with epiphytic orchid roots (e.g. Suárez et al., 2006, 2008, 2016; Herrera et al., 2010, 2018; Riofrío et al., 2013; Novotná et al., 2018). However, it is likely that a large number of fungal endophytes remain undescribed due to methodological biases (Tedersoo et al., 2010; Kohout et al., 2013) and also because the target of each study was either mycorrhizal or non-mycorrhizal fungi but not both at the same time.

To fill this gap, the first critical step is to elucidate the diversity and community assemblage of orchid root-associated endophytes of cortical tissues including mycorrhizal and non-mycorrhizal fungi. In the present study, we evaluated root-associated fungal endophytes colonizing the cortical tissues of native epiphytic orchid species: *Cyrtochilum flexuosum, Cyrtochilum myanthum* and *Maxillaria calantha* co-occurring in the Podocarpus National Park (PNP) and *Epidendrum marsupiale* and *Cyrtochilum pardinum* co-occurring in the Cajas National Park (CNP). We used a metagenomic approach based on the analysis of the internal transcribed spacer 2 sequences via the Illumina MiSeq technology. Our objectives were to: (i) characterize root fungal endophytes associated with the aforementioned epiphytic orchids; (ii) compare endophyte communities between co-existing orchid species; (iii) evaluate the root-associated fungal endophyte communities between orchid sites/populations; and (iv) compare global orchid root-associated fungal endophytes between sites at PNP and CNP. We expected distinct fungal endophyte communities between co-existing orchid species if the orchid phylogeny displays an effect on the fungal community composition. We also expected distinct root-associated fungal endophyte communities across sites/populations due to effects of altitude or site over the community considering a habitat dependent community composition hypothesis.

2. Materials and methods

2.1. Sample collection

Roots of orchids were collected in 2012 and 2013, with a total of 130 individual plants sampled along six sites of evergreen upper montane forests in the Southern Ecuadorian Andes. Two sites close to the PNP in Zamora-Chinchipe province were chosen: the first one called 'Curva Misteriosa' (site 1; 3°59′32″S, 79°06′29″W) and the second one 'El Tiro' (site 2; 3°59′20″S, 79°08′38″W), both sites located between 2050 and 2800 m a.s.l. Curva Misteriosa is characterized by a steep slope (51%), with trees 5–8 m high (Riofrío et al., 2007), a mean annual temperature of 20.8 °C and mean annual precipitation of 2193 mm (Bendix et al., 2008). El Tiro has the ridge covered with forests on the slope sides and open grass, bromeliad, or dwarf shrub formations along the crest line (Setaro et al., 2006), the mean annual temperature is 9.8 °C and mean annual precipitation is 3000 mm (Gradstein et al., 2008). At both sites, the characteristic vegetation includes epiphytic plants such as orchids, ferns and bromeliads (Mandl et al., 2010); the climate is cool and prehumid while, the soil is poor and acidic (Gradstein et al., 2008). The other four sites were 'High Mazán' (site 3; 2°52′13″S, 79°7′26″W), 'Low Mazán' (site 4; 2°52′19″S, 79°7′8″W), 'High Llaviucu' (site 5; 2°50′26″S, 79°10′29″W) and 'Low Llaviucu' (site 6; 2°50′36″S, 79°8′37″W), located in the CNP in Azuay province between 3000 and 3500 m a.s.l. The sites at CNP harbor around 300 species of vascular plants (Montesinos, 1996) and Orchidaceae is the second most diverse family (ETAPA, 2005). The climate fluctuates between −2 °C and 18 °C (Minga et al., 2016). The annual precipitation is 1200 mm in average with hail and snow episodes (Sklenár et al., 2011).

The study sites were selected based on the presence of common epiphytic orchid species. At site 1 and site 2, three epiphytic orchid species were sampled, belonging to the tribe Cymbidieae: *C. flexuosum* (species 1), *C. myanthum* (species 2) and *M. calantha* (species 3). At site 3, 4, 5 and 6, the common epiphytic orchid species were *E. marsupiale* (species 4) and *C. pardinum* (species 5), members of Epidendreae and Cymbidieae tribes, respectively. In total, 19, 28, 21, 21, 22 and 19 orchid individuals were collected from sites 1, 2, 3, 4, 5 and 6, respectively.

2.2. Screening of root-associated endophytic fungi

Transverse sections from each root sample were cut with a razor blade. The sections were stained with methyl blue 0.05% solution (C. I. 42,780, Merck) in sterile water for 3 min and observed under a Axiostar plus microscope (Carl Zeiss, Göttingen, Germany) at 40 × magnification to briefly verify the presence of fungal coils, as evidence of colonized root sections. The selected samples (root sections) were then surface-disinfected and since the fungi located in the velamen (dead tissue) are assumed to be surface contaminants (e.g. Yuan et al., 2010), the velamen was eliminated. Only the cortical tissue (alive tissue) was kept for DNA extraction.

2.3. Molecular analysis

For DNA extraction, two/three pieces of colonized roots (1–2 cm long) were used per plant individual. Genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) as described by the manufacturer's instructions. To amplify the fungal internal transcribed spacer 2 (ITS2) region, the primer pair ITS86F (Turenne et al., 1999) and ITS4 (White et al., 1990) was used according to Jacquemyn et al. (2016). One 20 μl polymerase chain reaction (PCR) contained 11.6 μl of sterile water, 4 μl of the 5X Phusion HF Buffer, 0.4 μl of each primer (0.5 μM), 0.4 μl of the

10 mM dNTPs, 0.2 μl of the Phusion High-Fidelity DNA Polymerase (Thermo Scientific, Wilmington, DE, USA) and 3 μl of total DNA extract. PCR conditions were as follows: initial denaturation at 98 °C for 30 s followed by 30 cycles at 98 °C for 10 s, 60 °C for 20 s, 72 °C for 30 s, and a final extension step at 72 °C for 10 min. A negative control reaction without DNA template was included in each PCR. Afterwards, PCR amplification success was tested in 1% agarose gel electrophoresis and amplicons within the appropriate size range purified using Wizard® SV Gel and Clean-up System (Promega, Madison, WI, USA). Concentrations and purity of amplicons were determined using a 2000c Spectrophotometer NanoDrop® (Thermo Scientific, Wilmington, USA). Finally, the target amplicons were sequenced using Illumina MiSeq® technology (IMGM Laboratories GmbH, Martinsried, Germany) that generated 300 bp long paired-end reads (See Supplementary information).

2.4. Bioinformatics analysis

Operational taxonomic units (OTUs) from the raw Illumina data were reconstructed using the UPARSE software (Edgar, 2013). First, single sequences were obtained after the assembly of the overlapping paired reads using the 'fastq_mergepairs' command. Second, a quality filter was applied using the 'fastq_filter' command with a maximum expected error threshold of 0.3 for single sequences. The truncation length of sequences was set to 240 bp to maximize the number of retained sequences after quality filters. Third, to remove singletons the 'derep_fullength' command was used. Finally, sequences with 97% homology were clustered into the same OTU using the 'cluster_otus' command.

Taxonomic assignment of OTUs was performed using the BLASTN algorithm implemented in UNITE database http://unite.ut.ee; (Abarenkov et al., 2010a) through the PlutoF (Abarenkov et al., 2010b) web-based sequence management workbench (2017-06-28 release). The taxonomically defined OTUs were parsed against the FunGuild v1.0 (http://www.stbates.org/guilds/app.php) database to designate putative trophic strategies.

2.5. Data analysis and statistics

Read counts of the root-endophyte-OTUs per sample were converted into presence/absence matrix to evaluate the relationships between root-associated fungal endophyte community and the host orchid species or study site. Accumulation curves were constructed for each site with the sample-based rarefaction method using 100 permutations applied to binary data using EstimateS 9.1.1 software (Colwell, 2013). The observed root-endophyte richness per site was evaluated using the Clench equation ($Sn = a^*n/(1 + b^*n)$) executed in STATISTICA (StatSoft, Tulsa, OK, USA), where a is the rate of new species increment, n is the sampling effort, and b is a parameter related to the shape of the curve (Jiménez-Valverde and Hortal, 2003). In addition, differences in root-associated fungal endophyte communities from co-existing orchid species were evaluated using permutational analysis of variance (PERMANOVA) with 999 permutations using the adonis function of the vegan package (Oksanen et al., 2016) in R (R Development Core Team, 2014). Furthermore, to investigate which fungal communities were more similar to each other, a pairwise assessment using Jaccard index implemented in the statistical software SPSS 22 (IBM Corp., Somers, NY, USA) was performed and implemented as in Cevallos et al. (2017).

Analyses related to root-associated fungal endophyte community composition across study sites-orchid populations and across both areas of montane rainforest (PNP and CNP) were performed based on the binary data (presence/absence matrix) independently per species. Using SPSS 22 non-metric multidimensional scaling (NMDS) plots were generated to visualize differences in root-associated endophytic communities between orchid populations and between the two areas of montane rainforest. The effect of the site-population was also tested for significance using PERMANOVA analysis under the same conditions as aforementioned (999 permutations using the adonis function of the vegan package in R). Although, endophyte communities' comparison between both areas of montane rainforest is biased by local environment condition and by the orchid species, it could give us some insights about the factors that could affect the endophytes communities' composition.

3. Results

3.1. Taxonomic coverage of root-associated fungal endophyte communities and putative life strategy

MiSeq sequencing of the 130 orchid individuals sampled at the six studied sites yielded a total of 76721 quality-filtered sequences with a length of 240 bp. During the OTUs reconstruction from the quality-filtered sequences, singletons, as well as chimeric sequences (5.5% of all reconstructed sequences), were discarded to obtain 3413 OTUs (3% sequence dissimilarity cutoff) assigned to root-associated fungal endophytes. The data were deposited in GenBank (Bioproject PRJNA344001 and PRJNA417757). Endophyte communities identified in association with *C. flexuosum, C. myanthum, M. calantha, E. marsupiale* and *C. pardinum* included members of 103 orders (Table S1) in the phyla Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Zygomycota. Summed over the two areas of montane rainforest (e.g. PNP and CNP), the highest number of OTUs (414) was assigned to the Agaricales, while considering the two areas of montane rainforest separately, Helotiales was the highest at PNP (156 OTUs) and Agaricales at CNP (385 OTUs). On average, individuals from *C. pardinum* harbored more OTUs (Fig. 1). The most frequent OTU was OTU1 belonging to the Xylariales. It was identified in all the individuals sampled at PNP and CNP.

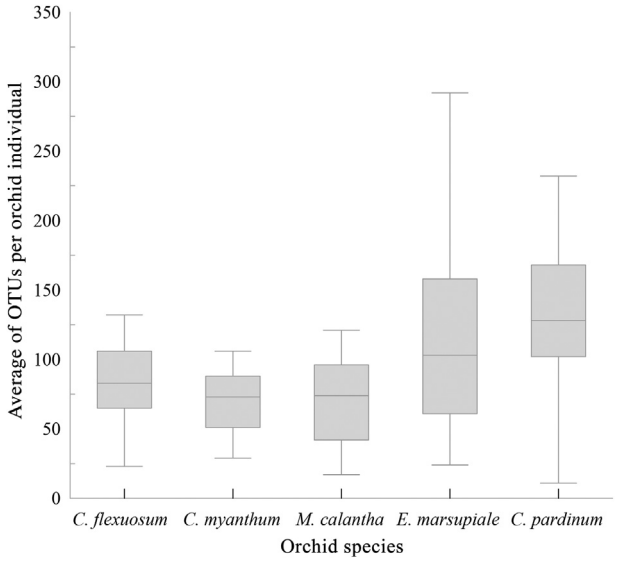

Fig. 1. Richness average of operational taxonomic units (OTUs) identified in association with *Cyrtochilum flexuosum*, *Cyrtochilum myanthum*, *Maxillaria calantha*, *Epidendrum marsupiale* and *Cyrtochilum pardinum*. The whiskers represent the highest and lowest number of OTUs that an orchid individual could have had.

Using FunGuild database, the putative life strategy was assigned only to OTUs with taxonomic assignment at 'species' level (1200 OTUs). Analyzed OTUs were assigned to saprotroph pathotroph-saprotroph, saprotroph-symbiotroph, pathotroph-saprotroph-symbiotroph, pathogen-saprotroph-symbiotroph, symbiotroph, pathotrophs or pathotroph-symbiotroph guilds (Fig. 2). Finally, evaluation of the endophyte communities using accumulation curves showed that the fungal communities did not reach a plateau in any of the study sites (Fig. 3). It was calculated that 48%, 51%, 48%, 46%, 43% and 45% of the estimated richness was observed at site 1, 2, 3, 4, 5 and 6, respectively.

3.2. Root-associated fungal endophyte communities between co-existing orchid species

PERMANOVA analysis revealed that *C. flexuosum, C. myanthum* and *M. calantha,* co-existing at site 1 and site 2 located at PNP, harbored significantly different fungal endophyte communities ($P < 0.0001$ and 0.0003, respectively). Conversely, no significant changes in the fungal endophyte communities were noticed for *E. marsupiale* and *C. pardinum* that co-exist at CNP, specifically at sites 3, 4, 5 and 6 ($P = 0.2521$, 0.6999, 0.4662 and 0.6936, respectively). In addition, the similarity between fungal endophyte communities was assessed in co-existing orchid species pairwisely using Jaccard indexes calculated by contrasting binary vectors that represent the presence/absence of OTUs associated with individuals of each species pair. This analysis could be conducted at PNP. Analyzing the variance of Jaccard indexes (dependent variable) as a function of the host phylogenic distance (factor) by means of a one-way ANOVA, we found that at site 1, the similarity was significantly higher between fungal endophyte communities associated with *C. flexuosum* and *C. myanthum* than endophyte communities associated with either species and with *M. calantha*. In contrast, at site 2 the similarity between the fungal endophyte communities associated with *C. flexuosum* and *M. calantha* were significantly higher than the similarity between endophyte communities associated with the close relatives *Cyrtochilum* species (Table 1).

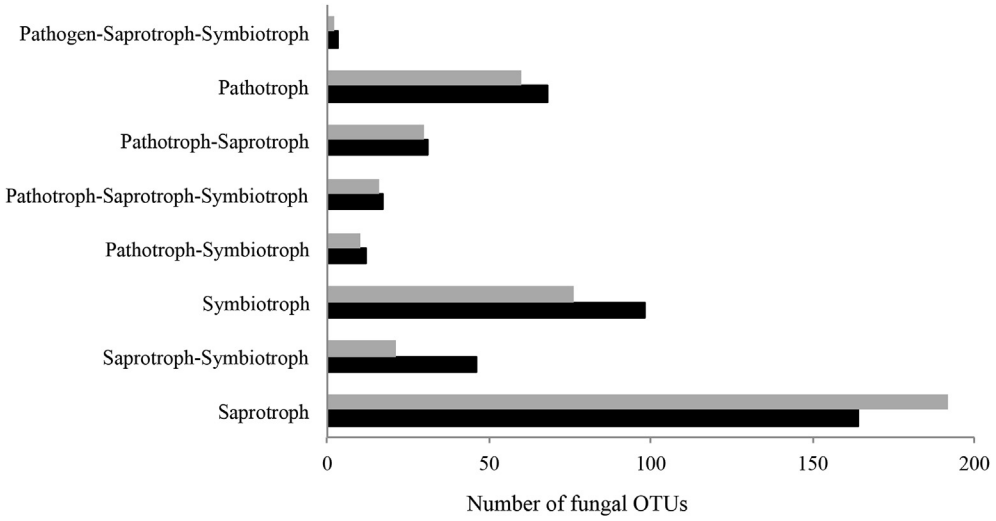

Fig. 2. Frequency distribution displaying the number of operational taxonomic units (OTUs) belonging to the different trophic guilds identified at Podocarpus National Park (gray bars) and Cajas National Park (black bars).

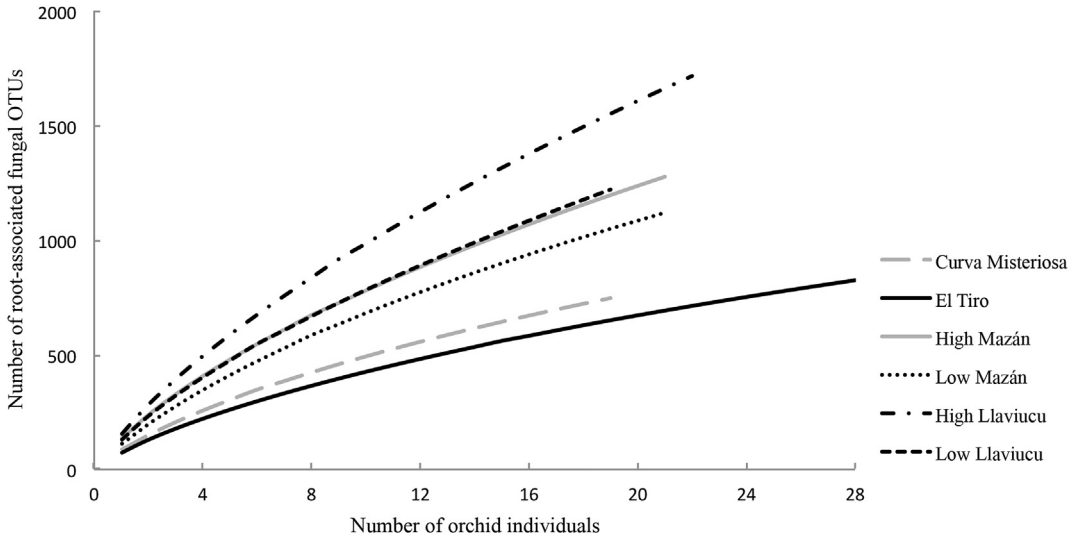

Fig. 3. Species accumulation curves describing the identified number of operational taxonomic units (OTUs) of root-associated fungi as a function of the sampled orchids per studied sites.

Table 1
Analysis of variance of the similarity between root-associated fungal endophytic communities associated with *Cyrtochilum flexuosum, Cyrtochilum myanthum, and Maxillaria calantha*. Similarity between fungal communities is expressed as a Jaccard index.

Comparison	Site 1		Site 2	
	Jaccard indexes mean difference ± std. error	*P* value	Jaccard indexes mean difference ± std. error	*P* value
C. flexuosum-C. myanthum vs. *C. myanthum-M. calantha*	0.04279* ± 0.02314	0.001	−0.00428 ± 0.01602	0.815
C. flexuosum-C. myanthum vs. *C. flexuosum-M. calantha*	0.03200* ± 0.01981	0.001	−0.03839* ± 0.01754	0.001
C. myanthum-M. calantha vs. *C. flexuosum-C. myanthum*	−0.04279* ± 0.02314	0.001	0.00428 ± 0.01654	0.815
C. myanthum-M. calantha vs. *C. flexuosum-M. calantha*	−0.01079 ± 0.02069	0.434	−0.03411* ± 0.01939	0.001
C. flexuosum-M. calantha vs. *C. flexuosum-C. myanthum*	−0.03200* ± 0.01982	0.001	0.03839* ± 0.01754	0.001
C. flexuosum-M. calantha vs. *C. myanthum-M. calantha*	0.01079 ± 0.0207	0.434	0.03411* ± 0.01939	0.001

*Difference between Jaccard indexes considered statistically significant (*P* value ≤ 0.05).

3.3. Root-associated fungal endophytes between orchid populations

The NMDS ordinations did not show clear distinctive patterns in fungal endophyte communities between orchid populations (Supplementary Material Fig. S1). However, the PERMANOVA analysis revealed that endophytes associated with *C. flexuosum, C. myanthum* and *M. calantha* (orchids distributed at PNP) differed significantly between orchid populations ($P = 0.0031$, 0.0029 and 0.0303, respectively). Meanwhile, endophyte communities associated with *E. marsupiale* and *C. pardinum* (orchid distributed at CNP) were not significantly different between orchid populations ($P = 0.6992$ and 0.4948, respectively). The subsequent pairwise Tukey test performed to compare the means showed that the endophyte community associated with *E. marsupiale* at site 3 was less similar to the endophyte communities at sites 4, 5 and 6 (Supplementary Material Table S2), whereas, the root endophyte community associated with *C. pardinum* at site 4 was the most divergent.

Finally, we identified a set of root-associated fungal endophytes that overlapped across populations of all evaluated orchid populations and another set of endophytes identified only at a particular site-population combination (Supplementary Material Table S3 and Fig. S2). Within the overlapped OTUs, members of Helotiales were the more abundant in populations of *C. flexuosum, C. myanthum* and *M. calantha* while Glomerales and Hypocreales were the most abundant in the populations of *E. marsupiale* and *C. pardinum*, respectively.

3.4. Differences in root-associated fungal endophyte communities between the two areas of montane rainforest (PNP vs CNP)

The NMDS ordination of the root-associated fungal endophyte communities between orchid species at PNP versus orchid species at CNP, yielded distinct endophyte communities (Fig. 4). This was confirmed by the PERMANOVA analysis ($P < 0.0001$). However, 45 similar OTUs were found at both areas of montane rainforest and identified in at least one individual per sampled orchid population. The order with the highest number of OTUs was Helotiales with six OTUs, followed by Cantharellales and Sebacinales with four OTUs each; Xylariales, Thelephorales, Polyporales, Pleosporales with three OTUs each; Atractiellales, Chaetothyriales, Agaricales with two OTUs each; Sordariales, Hymenochaetales, Capnodiales, Pezizales, Glomerellales, Lecanorales, Hypocreales, Diaporthales, Gloeophyllales, Ostropales, Malasseziales, Mucorales with one OTU and one undefined Ascomycota OTU.

4. Discussion

4.1. Diversity of root-associated fungal endophytes

Root fungal endophytes are generally considered as favorable inhabitants of plants that may contribute to their productivity and eventually to the maintenance of ecosystem functions (Jumpponen et al., 2017). In orchids, although the ecological implications of these fungal communities are not yet clear, a polyphyletic fungal group has been reported (Kohout et al., 2013). In the present study, we applied Illumina MiSeq sequencing to characterize the diversity of root-associated fungal endophytes of five epiphytic orchid species (i.e. *C. flexuosum, C. myanthum, M. calantha, E. marsupiale* and *C. pardinum*) distributed in montane forests of Southern Ecuador. Out of the 130 collected individuals, 3413 OTUs of endophytic fungi were detected and up to 1718 different OTUs per orchid species. Although an important number of OTUs were identified (3413 OTUs), contrasting with earlier studies (e.g. Herrera et al., 2010; Kottke et al., 2013), the inventories at all sites were still incomplete. However, the use of Illumina MiSeq technology represents a more powerful platform for the identification of the microbial communities, including rare species (Tedersoo et al., 2010) than traditional sequencing methods (e.g. Sanger sequencing).

The detected OTUs belonged to the Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Zygomycota phyla. Based on culture-dependent studies conducted in the same area, Herrera et al. (2010) and Novotná et al. (2018) identified far fewer endophytes. Indeed, Novotná et al. (2018) isolated 49 OTUs (13 orders) from three orchid species, including *C. myanthum,* while Herrera et al. (2010) identified 58 isolates (12 orders) from four orchid species belonging to Pleurothallidinae subtribe. Although, culture-dependent methods are necessary to preserve the fungal diversity in culture collections and to perform *in vitro* studies focused on orchid-fungi interactions (Novotná et al., 2018), these methods largely underestimate the diversity (Waterman et al., 2011) mainly because not all fungi are able to grow under *in vitro* conditions (Kageyama et al., 2008). The culture-independent method used in the present study (e.g. NGS) yielded a much higher diversity, likely to increase our comprehension of fungal endophyte communities associated with orchids. However, this method is destructive and no fungi could be preserved in collection. Both approaches should thus be considered as complementary and useful.

Our results revealed that the Agaricales was the most OTU-rich order (414 OTUs) summed over the six sampling sites. Members of Agaricales have been described as mycobionts of achlorophyllous and green orchids (Bidartondo et al., 2004; Martos et al., 2009) but also as mycorrhizal symbionts of epiphytic orchids (Kartzinel et al., 2013). However, the most frequent OTU was OTU1 (identified in

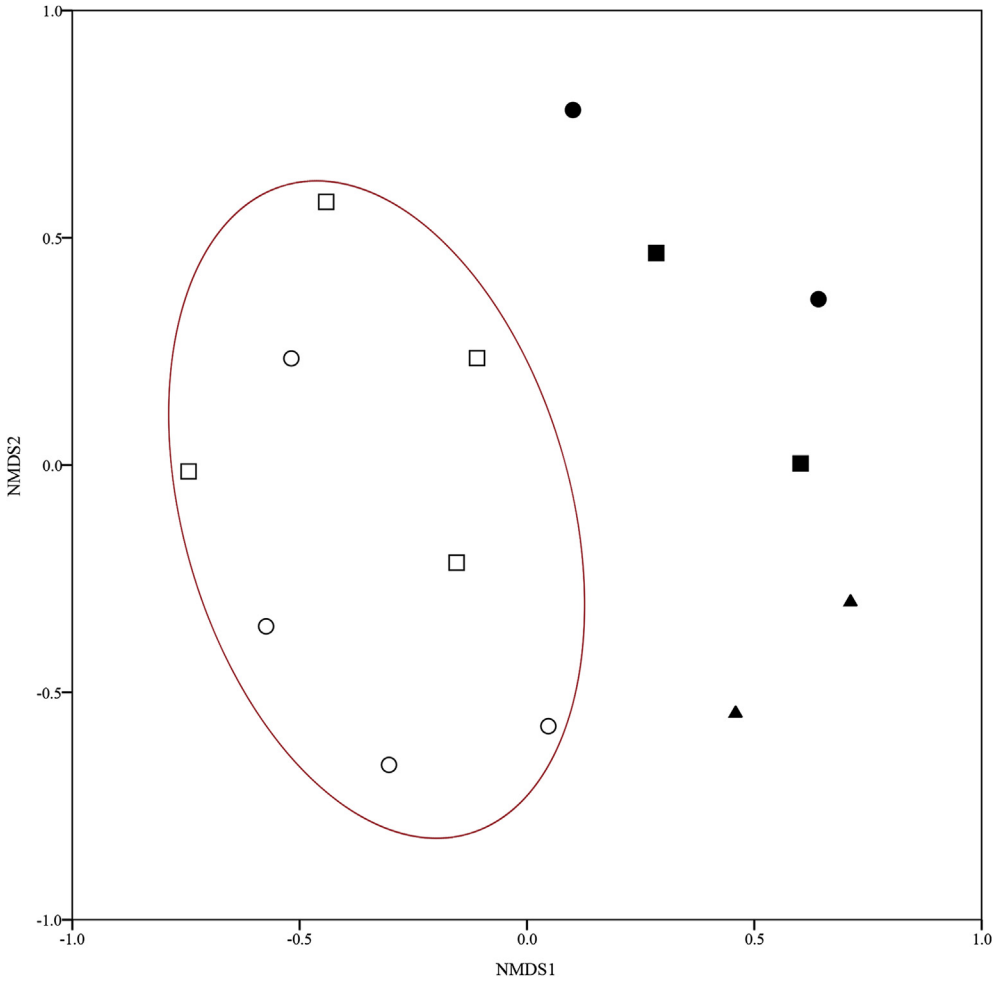

Fig. 4. Fungal endophyte communities detected in *Cyrtochilum flexuosum* (species 1, black dots), *Cyrtochilum myanthum* (species 2, black squares), *Maxillaria calantha* (species 3, black triangles), *Epidendrum marsupiale* (species 4, white dots) and *Cyrtochilum pardinum* (species 5, white squares), distributed in Podocarpus National Park (black figures) and Cajas National Park (white figures). Stress value 0.109211.

130 individuals), taxonomically assigned to *Hypoxylon griseobrunneum*, a member of Xylariales. Similar results were obtained in previous studies conducted in Southern Ecuador where Suárez et al. (2006) and Novotná et al. (2018) found that most of the isolates from the epiphytic orchids *Stelis* spp. belonged to the genus *Hypoxylon*. Moreover, in tropical latitudes, members of Xylariales have been reported as common, diverse and occasionally dominant root-associated endophytes (Yuan et al., 2009), present with different host plants, denoting preference and selectivity to some extent (Chen et al., 2013). In other tropical regions from Central America and La Réunion, Cantharellales was the order with the highest species richness (Martos et al., 2012; Kartzinel et al., 2013).

Fungal endophytes are probably part of a trade-off association that is still not fully understood (Kia et al., 2016). Undoubtedly, different fungal taxa impact differently orchid-fungi interactions through their contrasting set of traits (Kia et al., 2016). Consequently they may have an effect on plant productivity (Bever et al., 2012) and resistance to pathogen damages (e.g. Chen et al., 2013). According to their trophic mode, root-associated endophytes that inhabit plant roots could be symbiotroph, saprotroph, pathotroph-saprotroph, saprotroph-symbiotroph, pathotroph-symbiotroph or pathotroph (Ma et al., 2015; Kia et al., 2016). This was also clearly illustrated in the present study. Indeed, from the 1200 OTUs assigned to a putative life strategy, most belonged to saprotrophs (544 OTUs), followed by symbiotrophs (297 OTUs) and to a lesser extend to pathotrophs (152 OTUs). There were also 79, 50, 47, 24 and 7 fungal endophyte OTUs assigned as pathotroph-saprotroph, saprotroph-symbiotroph, pathotroph-saprotroph-symbiotroph, pathotroph-symbiotroph and pathotroph-saprotroph-symbiotroph, respectively. The ecological roles of endophytic fungi, assigned putatively in this study, and their effect on orchids, needs to be further evaluated in order to comprehend the root-fungal Orchidobiome, the diversity and community structure of fungi of the roots of orchids. Oliveira et al. (2014) suggested the necessity to clarify the effects of endophytic fungi on endangered orchids in Brazil, mainly because fungal communities potentially contribute to orchid adaptation to changing environmental conditions. Currently, endophytic fungi in orchids remain poorly characterized because the proportion of OTUs identified may be not assigned to a species in a public database (Oliveira et al., 2014; Ma et al., 2015). Thus, for fungal taxonomic work, it is of the highest priority to increase the DNA databases (Abarenkov et al., 2010a) that will allow a more comprehensive characterization of fungal communities associated with orchids but also with other plants and ecosystems. In addition, the use of transmission electron microscopy (e.g. Suárez et al., 2006, 2008) or the evaluation of nutrient flow (e.g. Cameron et al., 2006) could provide new evidence about functional roles of root-associated fungal endophytes.

4.2. Communities of fungal endophytes associated with co-existing orchid species

The few studies on root-associated fungal endophytes conducted so far were mostly focused on species identification (e.g. Bayman and Otero, 2006; Boddington and Dearnaley, 2008). However, the potential abiotic and biotic drivers determining fungal endophyte community assembly have usually not been considered. In the present study, the influence of the orchid species co-existence in root-associated fungal endophytes was evaluated as a potential driver for endophyte communities. In natural ecosystems co-existent species, in theory, do not compete for the same resources (Tilman, 1982) unless small-scale habitat heterogeneity is present and consequently a segregation of niches could be expected (Selosse, 2014). Studies on sympatric orchids reported distinctive mycorrhizal communities that could represent niche partitioning which may contribute to orchid co-existence (Jacquemyn et al., 2014; Cevallos et al., 2017). Our results at PNP corroborate these observations. Indeed, distinct endophyte communities were found between co-existing orchid species at both study sites. For instance, we found mycorrhizal fungi that promote nutrient uptake (Rasmussen, 2002) but also non-mycorrhizal fungi that are thought to be sources of bioactive compounds (Xing et al., 2015). Such diversity in fungal functional attributes could help orchids to adapt to the changing environmental conditions (Oliveira et al., 2014). Considering the mutualism-parasitism continuum hypothesis (Johnson et al., 1997), changes in ecological context could cause a transition from mutualism to parasitism or *vice versa*. In orchid communities, species form complex networks of orchid-fungi interactions (Martos et al., 2012) although such associations do not always have benefits for both partners. For instance, at the early developmental stages of the orchid, the associated fungi do not receive any reward from the orchid seeds (Smith and Read, 2008), and the relationship has been considered parasitism to some extent (Dearnaley, 2007). Meanwhile, the interaction between fungi and adult orchids, where both partners have benefits (or at least a transitory backflow), is recognized as mutualism (Cameron et al., 2006; Fochi et al., 2017). In some interactions, fungi that facilitate seed germination could also associate with orchids at the adult stage (Cameron et al., 2008). In this case, there is a transition in the relationship, from parasitism to mutualism (van der Heijden et al., 2015).

In addition, host phylogeny seems to drive fungal community assemblage. This was evidenced by Jacquemyn et al. (2011) who identified similar mycorrhizal fungi associated with different *Orchis* species. Likewise, in the Angraecoid orchid subtribe the reported mycorrhizal fungi were closely related species (Martos et al., 2012). Interestingly, in the present study we found (in site 1) that closely related *Cyrtochilum* species shared more similar endophytic fungal communities among each other than with *M. calantha*, in accordance with the results of Cevallos et al. (2017). This observation was, however, not repeated at site 2. Therefore, increasing the number of individuals, at both sites, should help to corroborate whether the host phylogeny is a driver of endophyte community composition.

In contrast to the findings at sites 1 and 2 (PNP), co-existing orchids *E. marsupiale* and *C. pardinum*, at CNP (sites 3, 4, 5 and 6), had similar fungal endophyte communities although both orchid species belong to distinctive tribes (Epidendreae and Cymbidieae, respectively). In this case host phylogeny is not a determining driver for endophyte community structure. Deep phylogenetic studies evaluating the evolutionary histories between orchids could contribute to deduce more precise ecological premises (e.g. Freudenstein and Chase, 2015).

Apart from host phylogeny, fungal endophyte community composition could be influenced by many abiotic and biotic factors (Barnes et al., 2016). For instance, altitude is widely recognized as an important factor that determines the fungal communities in soil, especially in the Andes (Geml et al., 2014). Although microorganisms are considered globally cosmopolitan, abiotic factors probably impact the fungal diversity, especially in habitats with harsh environmental conditions (Jumpponen et al., 2017). However, the 45 OTUs identified at both PNP and CNP, seem not to be restricted by abiotic factors. It is likely that the fungi that overlapped between all studied orchids, at both areas of montane rainforest, have exceptional characteristics and are prone to develop under a wider gradient of abiotic conditions (Cray et al., 2013).

4.3. Root-associated fungal endophytes across orchid populations

Factors that could drive fungal endophyte community composition across orchid populations are still not fully described or assessed. Environment and geography affect the diversity and composition of some root-associated fungal communities but the effect of each factor could be variable or specific across ecosystems (Barnes et al., 2016). Here we showed that root endophyte communities associated with *C. flexuosum, C. myanthum* and *M. calantha,* evaluated independently per orchid species, varied substantially across orchid population, suggesting that host phylogeny is not a determining factor for endophyte community composition. Our results corroborate previous observations of Jacquemyn et al. (2016) on mycorrhizal fungi, revealing that fungi can be characteristic of a specific site as a result of local conditions. This supported the theory that orchid endophytic fungi are subjected to local selection because of particular environmental conditions (Ma et al., 2015) at each sample site. At PNP, the study sites have similar forest structure and are classified as evergreen upper montane forests (Beck et al., 2008), but each site has particular temperature and rain conditions. Oliveira et al. (2014) reported similar results in an early study in a Neotropical region in Brazil, where *Hadrolaelia jongheana, Hoffmannseggella caulescens,* and *Hoffmannseggella cinnabarina* displayed different endophyte community composition with few overlapping fungal endophytes as a consequence of local factors (soil conditions, vegetation and climate).

Our results at CNP showed that populations of *E. marsupiale* and *C. pardinum*, evaluated independently per orchid species, had similar endophyte communities. This was probably because the sites at CNP are located in the same mountain foothill under similar temperature and rainfall conditions (ETAPA, 2005), corroborating the effect of the site on fungal endophyte communities. Moreover, it is likely that proximity between sites could facilitate fungal dispersal (Jumpponen et al., 2017). As a consequence, overlapping endophytic fungi are more probable. Notwithstanding that very little is known about the restrictions of fungal endophyte distribution (Queloz et al., 2011), the possibility that our results were somewhat the consequence of geographic proximity between sites or an effect of contrasting environmental conditions due to high altitudinal levels at mountain areas, cannot be ruled out. Moreover, it is not excluded that orchid species in CNP present an ancestral ecological conservatism in endophyte preferences. Because identified fungi were not specifically associated with a single orchid species, it is probable that orchids had preferences for several widely distributed fungal groups (Otero et al., 2007).

In conclusion, the fungal endophyte communities assessed on epiphytic orchids seem to follow different strategies of assembly. Fungal endophytes at PNP appear to be impacted by host phylogeny and sites, while the results at CNP suggest that neither host phylogeny nor the sites had an effect on fungal endophyte communities. Either way, the assessment of additional orchid species and

sites could help elucidate which factors determine root-associated fungal endophytes.

4.4. Comparison of endophyte communities between the two areas of montane rainforest (PNP vs CNP)

Sampling approaches at both areas of montane rainforest were not totally comparable because each one has specific environmental conditions that resulted in a particular ecosystem structure (Baquero et al., 2004) in addition to the specific orchid species sampled per area. Thus, a thorough analysis combining data from both areas of montane rainforest to make inferences about the effect of biotic or abiotic factors on OMF communities was beyond the scope of this study. However, the contrast of all the fungal endophytes associated with epiphytic orchids (independently of orchid species) between the two areas of montane rainforest could give some insights about the fungal community structure at a larger scale. We showed that orchid root-associated fungal endophyte communities were highly different between PNP and CNP. Our results corroborate the hypothesis of Baas Becking (1934) that local environmental conditions could configure fungal endophyte communities, assuming the hypothesis that microorganisms are widely distributed and the environment is shaping which ones are able to grow. In addition to the distinct fungal endophyte community composition at PNP and CNP, we also identified a set of endophytic fungi that were present in both areas of montane rainforest. Although the distance between both areas of montane rainforest is approximately 125 km, 45 fungal OTUs core-species (21 orders) were found in both situations. These fungi were mycorrhizal, saprobes or latent pathogens. Following the premise that endophytes are cosmopolitan, it is likely that overlapping endophytic fungi have a large population distribution (Fitter, 2005; Jumpponen et al., 2017) but may not be very specialized. Overlapping endophytes probably have ecological plasticity and are able to play different roles in the interaction (Pecoraro et al., 2017). Based on observations on orchid mycorrhizal fungi (Cevallos et al., 2017), we hypothesize that endophytic fungal core-species could represent an advantage because being frequently available they could fill some physiological demands (e.g. nutritional or pathogen defense) when other fungi are not available. Clarifying the potential roles of the fungal endophytes in orchids' life needs to be explored to better understand the ecological dynamics of plant-fungi interactions (Oliveira et al., 2014).

Acknowledgements

This work was supported by the Académie de Recherche et d'Enseignement Supérieur Wallonie-Bruxelles (ARES) within the frame of a PRD project entitled 'Reinforcement of the fungal expertise in Ecuador via case studies of fungal plants interactions in selected ecosystems and the development of biotechnology-oriented fungal resources' and the Secretaria de Educación Superior, Ciencia, Tecnología e Innovación of Ecuador [grant number PIC-13-ETAPA-003].

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.funeco.2018.05.002.

References

Abarenkov, K., Henrik Nilsson, R., Larsson, K.-H., Alexander, I.J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vrålstad, T., Liimatainen, K., Peintner, U., Kõljalg, U., 2010a. The UNITE database for molecular identification of fungi recent updates and future perspectives. New Phytol. 186, 281–285.

Abarenkov, K., Tedersoo, L., Nilsson, R.H., Vellak, K., Saar, I., Veldre, V., Parmasto, E., Prous, M., Aan, A., Ots, M., Kurina, O., Ostonen, I., Jõgeva, J., Halapuu, S., Põldmaa, K., Toots, M., Truu, J., Larsson, K.H., Kõljalg, U., 2010b. Plutof-a web based workbench for ecological and taxonomic research, with an online implementation for fungal its sequences. Evol. Bioinforma. 6, 189–196.

Baas Becking, L.G.M., 1934. Geobiologie of inleiding tot de milieukunde. W.P. Van Stockum & Zoon, Den Haag.

Baquero, F., Sierra, R., Ordóñez, L., Tipán, M., Espinosa, L., Rivera, MaB., Soria, P., 2004. La vegetación de los Andes del Ecuador. EcoCiencia. ESLA. EcoPar. Div. Geográfica– IGM, pp. 1–28.

Barnes, C.J., Van der Gast, C.J., Burns, C.A., McNamara, N.P., Bending, G.D., 2016. Temporally variable geographical distance effects contribute to the assembly of root-associated fungal communities. Front. Microbiol. 7, 195.

Bayman, P., Otero, J.T., 2006. Microbial endophytes of orchid roots. In: Schulz, B., Boyle, C., Sieber, T. (Eds.), Microbial Root Endophytes. Springer-Verlag, Berlin, pp. 153–178.

Beck, E., Makeschin, F., Haubrich, F., Richter, M., Bendix, J., Valarezo, C., 2008. The ecosystem (Reserva Biológica san Francisco). In: Beck, E., Bendix, J., Kottke, I., Makeschin, F., Mosandl, R. (Eds.), Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, No. 198. Springer, Heidelberg, pp. 1–14.

Bendix, J., Rollenbeck, R., Richter, M., Fabian, P., Emck, P., 2008. Climate. In: Beck, E., Bendix, J., Kottke, I., Makeschin, F., Mosandl, R. (Eds.), Gradients in a Tropical Mountain Ecosystem of Ecuador. Ecological Studies, No. 198. Springer, Heidelberg, pp. 63–73.

Bever, J.D., Platt, T.G., Morton, E.R., 2012. Microbial population and community dynamics on plant roots and their feedbacks in plant communities. Annu. Rev. Microbiol. 66, 265–283.

Bidartondo, M.I., Burghardt, B., Gebauer, G., Bruns, T.D., Read, D.J., 2004. Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. Proc. Biol. Sci. 271, 1799–1806.

Boddington, M., Dearnaley, J.D.W., 2008. Morphological and molecular identification of fungal endophytes from roots of *Dendrobium speciosum*. Proc. R. Soc. Queensl 114, 13–17.

Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. 154, 275–304.

Bunch, W.D., Cowden, C.C., Wurzburger, N., Shefferson, R.P., 2013. Geography and soil chemistry drive the distribution of fungal associations in lady's slipper orchid, *Cypripedium acaule*. Botany 91, 850–856.

Cameron, D.D., Johnson, I., Read, D.J., Leake, J.R., 2008. Giving and receiving: measuring the carbon cost of mycorrhizas in the green orchid. Goodyera Repens. New Phytol. 180, 176–184.

Cameron, D.D., Leake, J.R., Read, D.J., 2006. Mutualistic mycorrhiza in orchids: evidence from plant-fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid *Goodyera repens*. New Phytol. 171, 405–416.

Cevallos, S., Sánchez-Rodríguez, A., Decock, C., Declerck, S., Suárez, J.P., 2017. Are there keystone mycorrhizal fungi associated to tropical epiphytic orchids. Mycorrhiza 27, 225–232.

Chen, J., Zhang, L.C., Xing, Y.M., Wang, Y.Q., Xing, X.K., Zhang, D.W., Liang, H.Q., Guo, S.X., 2013. Diversity and taxonomy of endophytic Xylariaceous fungi from medicinal plants of *Dendrobium* (Orchidaceae). PLoS One 8 e58268.

Colwell, R.K., 2013. EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples. User's Guide and application published at:, Version 9. http://purl.oclc.org/estimates.

Cray, J.A., Bell, A.N.W., Bhaganna, P., Mswaka, A.Y., Timson, D.J., Hallsworth, J.E., 2013. The biology of habitat dominance; can microbes behave as weeds? Microb. Biotechnol 6, 453–492.

Dearnaley, J.D.W., 2007. Further advances in orchid mycorrhizal research. Mycorrhiza 17, 475–486.

Dearnaley, J.D.W., Martos, F., Selosse, M.A., 2012. Orchid mycorrhizas: molecular ecology, physiology, evolution and conservation aspects. In: Hock, B. (Ed.), The Mycota (A Comprenhensive Treatise on Fungi as Experimental Systems for Basic and Applied Research). Springer, Heidelberg, pp. 207–230. Berlin.

Dressler, R.L., 1990. The Orchids: Natural History and Classification. USA. Harvard University Press, Cambridge MA.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998.

ETAPA, 2005. Aprobado vía acuerdo ministerial el 1 de abril del Plan de manejo integral del Parque Nacional Cajas (Cuenca, Ecuador).

Fitter, A.H., 2005. Darkness visible: reflections on underground ecology. J. Ecol. 93, 231–243.

Fochi, V., Chitarra, W., Kohler, A., Voyron, S., Singan, V.R., Lindquist, E.A., Barry, K.W., Girlanda, M., Grigoriev, I.V., Martin, F., Balestrini, R., Perotto, S., 2017. Fungal and plant gene expression in the *Tulasnella calospora - Serapias vomeracea* symbiosis provides clues about nitrogen pathways in orchid mycorrhizas. New Phytol. 213, 365–379. https://doi.org/10.1111/nph.14279.

Freudenstein, J.V., Chase, M.W., 2015. Phylogenetic relationships in Epidendroideae (Orchidaceae), one of the great flowering plant radiations: progressive specialization and diversification. Ann. Bot. 115, 665–681.

Garnica, S., Riess, K., Bauer, R., Oberwinkler, F., Weiß, M., 2013. Phylogenetic diversity and structure of sebacinoid fungi associated with plant communities along an altitudinal gradient. FEMS Microbiol. Ecol. 83, 265–278.

Geml, J., Pastor, N., Fernandez, L., Pacheco, S., Semenova, T., Becerra, A., Wicaksono, C., Nouhra, E., 2014. Large-scale fungal diversity assessment in the

Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. Mol. Ecol. 23, 2452–2472.
Götzenberger, L., de Bello, F., Bråthen, K.A., Davison, J., Dubuis, A., Guisan, A., Lepš, J., Lindborg, R., Moora, M., Pärtel, M., Pellissier, L., Pottier, J., Vittoz, P., Zobel, K., Zobel, M., 2012. Ecological assembly rules in plant communities-approaches, patterns and prospects. Biol. Rev. 87, 111–127.
Gradstein, S.R., Homeier, J., Gansert, D., 2008. The Tropical Mountain Forest - Patterns and Processes in a Biodiversity Hotspot. University Press, Göttingen.
Herrera, P., Kottke, I., Molina, M.C., Méndez, M., Suárez, J.P., 2018. Generalism in the interaction of Tulasnellaceae mycobionts with orchids characterizes a biodiversity hotspot in the tropical Andes of Southern Ecuador. Mycoscience 59, 38–48.
Herrera, P., Suárez, J.P., Kottke, I., 2010. Orchids keep the ascomycetes outside: a highly diverse group of ascomycetes colonizing the velamen of epiphytic orchids from a tropical mountain rainforest in Southern Ecuador. Mycology 1, 262–268.
Jacquemyn, H., Brys, R., Merckx, V.S.F.T., Waud, M., Lievens, B., 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., Lievens, B., 2015. Mycorrhizal networks and coexistence in species-rich orchid communities. New Phytol. 206, 1127–1134.
Jacquemyn, H., Merckx, V.V., Brys, R., Tyteca, D., Cammue, B.P., Honnay, O.a., 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., Waud, M., Lievens, B., Brys, R., 2016. Differences in mycorrhizal communities between *Epipactis palustris, E. helleborine* and its presumed sister species *E. neerlandica*. Ann. Bot. 118 (1), 105–114.
Jiménez-Valverde, A., Hortal, J., 2003. Las curvas de acumulación de especies y la necesidad de evaluar la calidad de los inventarios biológicos. Rev. Ibérica Aracnol. 8, 151–161.
Johnson, N.C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol. 135, 575–585.
Jumpponen, A., Herrera, J., Porras-Alfaro, A., Rudgers, J., 2017. Biogeography of root-associated fungal endophytes. In: Tedersoo, L. (Ed.), Biogeography of Mycorrhizal Symbiosis. Springer, Cham, pp. 195–222.
Kageyama, S.A., Mandyam, K.G., Jumpponen, A., 2008. Diversity, function and potential Applications of the root-associated endophytes. In: Varma, A. (Ed.), Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-function, Biotechnology, Eco-physiology, Structure and Systematics. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 29–57.
Kartzinel, T.R., Trapnell, D.W., Shefferson, R.P., 2013. Highly diverse and spatially heterogeneous mycorrhizal symbiosis in a rare epiphyte is unrelated to broad biogeographic or environmental features. Mol. Ecol. 22, 5949–5961.
Kia, S.H., Glynou, K., Nau, T., Thines, M., Piepenbring, M., Maciá-vicente, J.G., 2016. Influence of phylogenetic conservatism and trait convergence on the interactions between fungal root endophytes and plants. ISME J. 1–14.
Kohout, P., Těšitelová, T., Roy, M., Vohník, M., Jersáková, J., 2013. A diverse fungal community associated with *Pseudorchis albida* (Orchidaceae) roots. Fungal Ecol. 6, 50–64.
Kottke, I., Setaro, S., Haug, I., Herrera, P., Cruz, D., Fries, A., Gawlik, J., Homeier, J., Werner, F.A., Gerique, A., Suárez, J.P., 2013. Mycorrhiza networks promote biodiversity and stabilize the tropical mountain rain forest ecosystem: perspectives for understanding complex communities. In: Bendix, J., Beck, E., Bräuning, A., Makeschin, F., Mosandl, R., Scheu, S., Wilcke, W. (Eds.), Ecosystem Services, Biodiversity and Environmental Change in a Tropical Mountain Ecosystem of South Ecuador. Springer, Berlin Heidelberg, 187e203.
Kristiansen, K.A., Taylor, D.L., Kjøller, R., Rasmussen, H.N., Rosendahl, S., 2001. Identification of mycorrhizal fungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single-strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences. Mol. Ecol. 10, 2089–2093.
Ma, X., Kang, J., Nontachaiyapoom, S., Wen, T., Hyde, K.D., 2015. Non-mycorrhizal endophytic fungi from orchids. Curr. Sci. 109, 36.
Mandl, N., Lehnert, M., Kessler, M., Gradstein, S.R., 2010. A comparison of alpha and beta diversity patterns of ferns, bryophytes and macrolichens in tropical montane forests of southern Ecuador. Biodivers. Conserv. 19, 2359–2369.
Martos, F., Dulormne, M., Pailler, T., Bonfante, P., Faccio, A., Fournel, J., Dubois, M.-P., Selosse, M.-A., 2009. Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. New Phytol. 184, 668–681.
Martos, F., Munoz, F., Pailler, T., Kottke, I., Gonneau, C., Selosse, M.A., 2012. The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. Mol. Ecol. 21, 5098–5109.
Minga, D., Ansaloni, R., Verdugo, A., Ulloa, C., 2016. Flora del Páramo del Cajas, Ecuador. Universidad del Azuay, Cuenca.
Montesinos, F., 1996. Árboles y arbustos del bosque Mazán. Tomo I. ETAPA. Empresa Munic. Teléfonos, Agua Potable Alcantarillado.
Novotná, A., Benítez, Á., Herrera, P., Cruz, D., Filipczyková, E., Suárez, J.P., 2018. High diversity of root-associated fungi isolated from three epiphytic orchids in southern Ecuador. Mycoscience 59 (1), 24–32. http://doi.org/10.1016/j.myc.2017.07.007.
Oksanen, A.J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., Hara, R.B.O., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2016. Package " Vegan": Community Ecology Package.
Oliveira, S.F., Bocayuva, M.F., Veloso, T.G.R., Bazzolli, D.M.S., da Silva, C.C., Pereira, O.L., Kasuya, M.C.M., 2014. Endophytic and mycorrhizal fungi associated with roots of endangered native orchids from the Atlantic Forest, Brazil. Mycorrhiza 24, 55–64.
Otero, J.T., Flanagan, N.S., Herre, E.A., Ackerman, J.D., Bayman, P., 2007. Widespread mycorrhizal specificity correlates to mycorrhizal function in the neotropical, epiphytic orchid *Ionopsis utricularioides* (Orchidaceae). Am. J. Bot. 94, 1944–1950.
Pecoraro, L., Huang, L., Caruso, T., Perotto, S., Girlanda, M., Cai, L., Liu, Z.-J., 2017. Fungal diversity and specificity in *Cephalanthera damasonium* and *C. longifolia* (Orchidaceae) mycorrhizas. J. Syst. Evol. 9999, 1–12.
Pridgeon, A., 1995. The Illustrated Encyclopaedia of Orchids. Timber Press, Portland.
Queloz, V., Sieber, T.N., Holdenrieder, O., McDonald, B.A., Grünig, C.R., 2011. No biogeographical pattern for a root-associated fungal species complex. Glob. Ecol. Biogeogr. 20, 160–169.
R Development Core Team, 2014. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Austria, Vienna.
Rasmussen, H.N., 2002. Recent developments in the study of orchid mycorrhiza. Plant Soil 245, 149–163.
Riofrío, L.R., Naranjo, C.N., Iriondo, J.M.I., Torres, E.T., 2007. Spatial structure of *Pleurothallis, Masdevallia, Lephanthes* and *Epidendrum* epiphytic orchids in a fragment of montane cloud forest in South Ecuador. Lankesteriana 7, 1–2. https://doi.org/https://doi.org/10.15517/lank.v7i1-2.18447.
Riofrío, M.L., Cruz, D., Torres, E., de la Cruz, M., Iriondo, J.M., Suárez, J.P., 2013. Mycorrhizal preferences and fine spatial structure of the epiphytic orchid *Epidendrum rhopalostele*. Am. J. Bot. 100, 1–10.
Selosse, M.A., 2014. The latest news from biological interactions in orchids: in love, head to toe. New Phytol. 202, 337–340.
Setaro, S., Kottke, I., Oberwinkler, F., 2006. Anatomy and ultrastructure of mycorrhizal associations of neotropical Ericaceae. Mycol. Prog. 5 (243). https://doi.org/10.1007/s11557-006-0516-7.
Sklenár, P., Dusková, E., Baslev, H., 2011. Tropical and temperate: evolutionary history of Páramo Flora. Botanical Rev. 77, 71–108.
Smith, D.P., Peay, K.G., 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. PLoS One 9 (2) e90234.
Smith, S.E., Read, D., 2008. Mycorrhizal Symbiosis. Academic Press, New York, USA.
Suárez, J.P., Eguiguren, S., Herrera, P., Jost, L., 2016. Do mycorrhizal fungi drive speciation in *Teagueia* (Orchidaceae) in the upper Pastaza watershed of Ecuador? Symbiosis 36, 135–136.
Suárez, J.P., Weiss, M., Abele, A., Garnica, S., Oberwinkler, F., Kottke, I., 2006. Diverse tulasnelloid fungi form mycorrhizas with epiphytic orchids in an Andean cloud forest. Mycol. Res. 110, 1257–1270.
Suárez, J.P., 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. Mycol. Prog. 7, 75–85. https://doi.org/10.1007/s11557-008-0554-4.
Sudheep, N.M., Sridhar, K.R., 2012. Non-mycorrhizal fungal endophytes in two orchids of Kaiga forest (Western Ghats), India. J. For. Res. 23, 453–460.
Tedersoo, L., Nilsson, R.H., Abarenkov, K., Jairus, T., Sadam, A., Saar, I., 2010. Methods 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. New Phytol. 188, 291–301.
Těšitelová, T., Kotílinek, M., Jersáková, J., Joly, F.X., Košnar, J., Tatarenko, I., Selosse, M.-A., 2015. Two widespread green *Neottia* species (Orchidaceae) show mycorrhizal preference for Sebacinales in various habitats and ontogenetic stages. Mol. Ecol. 24, 1122–1134.
Tilman, D., 1982. Resource Competition and Community Structure. Princeton University Press, New Jersey.
Turenne, C., Sanche, S., Hoban, D., Karlowsky, J., Kabani, A., 1999. Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis System. J. Clin. Cal. Microbiol. 37, 1846–1851.
van der Heijden, M.G.A., Martin, F.M., Selosse, M.-A., Sanders, I.R., 2015. Mycorrhizal ecology and evolution : the past, the present, and the future. New Phytol. 205, 1406–1423.
Waterman, R.J., Bidartondo, M.I., Stofberg, J., Combs, J.K., Gebauer, G., Savolainen, V., Barraclough, T.G., Pauw, A., 2011. The effects of above- and belowground mutualisms on orchid speciation and coexistence. Am. Nat. 177, E54–E68.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomial RNA genes for phyologenetics. In: Innis, M.A., Gelfand, D., Sninsky, J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, Inc., New York, pp. 315–322.
Xing, X., Gai, X., Liu, Q., Hart, M.M., Guo, S., 2015. Mycorrhizal fungal diversity and community composition in a lithophytic and epiphytic orchid. Mycorrhiza 25, 289–296.
Yuan, L., Yang, Z., Li, S., Hu, H., Huang, J.L., 2010. Mycorrhizal specificity, preference, and plasticity of six slipper orchids from South Western China. Mycorrhiza 20, 559–568.
Yuan, Z.L., Chen, Y.C., Yang, Y., 2009. Diverse non-mycorrhizal fungal endophytes inhabiting an epiphytic, medicinal orchid (*Dendrobium nobile*): estimation and characterization. World J. Microbiol. Biotechnol. 25, 295–303.
Zotz, G., Winkler, U., 2013. Aerial roots of epiphytic orchids: the velamen radicum and its role in water and nutrient uptake. Oecologia 171, 733–741. https://doi.org/10.1007/s00442-012-2575-6.