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# ROOT ASSOCIATED FUNGI OF PATAGONIAN ENDEMIC ORCHIDS

Master's Thesis

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# 1. Introduction

Orchidaceae is one of the largest plant families with 27,135 described species and 899 genera (The Plant List, 2010). The highest number of orchid species occurs in tropical and subtropical regions, where they mainly grow as epiphytes on trees and shrubs (Pereira *et al.,* 2014). Remaining orchids are terrestrial and some can grow on rocks or very rocky soil (Weston *et al.,* 2005). Orchids may have specific associations with their pollinators as well as with mycorrhizal fungi, thus they make an excellent models for investigating biological interactions (Selosse, 2014). For example, many orchid species present food deception by mimicking attractive flowers, but not rewarding pollinators with nectar and some orchids have the pollination strategy, where blossoms mimic some female insects to attract male insects (Singer, 2003). Orchids cheat their fungal symbionts by parasitizing a mycorrhizal partner of a nearby photosynthetic plant, which means they are parasitizing the plant as well (Bayman *et al.,* 2006). All orchids fully depend on orchid mycorrhizal (OrM) fungi during early stages of development, when fungi provide inorganic and organic nutrients to seeds and seedlings (Rasmussen, 1995). Eventually most orchids become autotrophic and only minority of them remain fully myco-heterotrophic throughout their life (Leake, 1993), meaning that the plant is heterotrophic during early stages of development, but gets its resources from mycorrhizal fungi. Nonetheless, fungal partner provide N, P and water flows to autotrophic orchids during their adult stage (Dearnaley, 2007). OrM forming fungi can form characteristic hyphal coils, called pelotons, within the plant root cells, which will increase the interfacial surface area between orchid and fungus (Bayman *et al.,* 2006). Plant receives the essential nutrients and carbon from both living and senescent pelotons (Selosse, 2014).

The most common OrM fungi (OrMF) associated with both terrestrial and epiphytic green orchids belong to Tulasnellaceae, Sebacinales and Ceratobasidiaceae (Dearnaley *et al.,* 2012; Martos *et al.,* 2012). When not associating with orchid roots, these fungal groups have diverse ecological strategies, like saprotrophic, pathogenic or in some cases might form ectomycorrhizal symbioses (EcM) with neighbouring trees (Dearnaley *et al.,* 2012). In addition, several species of EcM basidiomycetes as well as ascomycetes has been found to form OrM associations (Selosse *et al.,* 2004; Dearnaley *et al.,* 2012). Habitat type may have a significant impact on OrM fungal composition. Orchids in open grasslands are associating with Sebacinales and Cantharellales, whereas terrestrial orchids in ectomycorrhizal forests form mycorrhizas with Russulaceae, Sebacinales and Thelephoraceae (Dearnaley *et al.,* 2012). Nonphotosynthetic orchids tend to associate primarily with Sebacinales (Taylor *et al.,*

2003). Taylor *et al.* (1999) indicated that some orchids associate exclusively with ectomycorrhizal fungi belonging to the family of Russulaceae. In addition to OrMF, orchids may be colonized by fungal endophytes. Mycorrhizae forms mutualistic relations between plant roots and fungi, whereas endophytes are growing inside plant tissues without causing symptoms of disease (Bayman *et al.,* 2006). Endophytes have been shown to indicate fitness benefits to host plants, including tolerance to herbivory, heat, salt, disease and drought (Rodriguez *et al.,* 2008). Orchid endophytes are commonly found in soil and as endophytes of other plants (Bayman *et al.,* 2006). Stark *et al.* (2009) identified most orchid endophytes from ascomycetes, for example *Exophiala, Fusarium, Leptodontidium* or *Tetracladium*. It has been suggested that orchid roots are more commonly colonized by endophytes after flowering (Kohout *et al.,* 2013).

South America is one of the richest places on earth for orchid diversity (Dixon *et al.,* 2003). Most of the orchids there are epiphytic and growing in tropics, but in Patagonia is temperate climate and therefore different conditions. Unfortunately, there is very limited knowledge about Patagonian orchids in English, as most of the studies are written in Spanish. At least 23 species belonging to three genera (*Chloraea* 13 species, *Gavilea* 9, and *Codonorchis* 1) were cited from Patagonia (Fracchia *et al.,* 2014 and references therein). In this study, we investigated mycorrhizal associations in roots of genus's *Chloraea, Gavilea* and *Codonorchis.* The genus *Chloraea* comprises 52 species, including two varieties, of which *c.* 37 species are found in Chile and Argentina, 14 species in Bolivia, Peru and northern Argentina; and 1 species in eastern Argentina, Brazil and Uruguay. The genus *Gavilea* encompasses 16 species found in Chile and Argentina (including the Juan Fernandez and Falkland Islands) (Govaerts, 2014). *Codonorchis lessonii* grows in Argentina, Chile and Falkland Islands, inhabiting most commonly forests dominated by *Nothofagus pumilio* and *N. betuloides* (Vidal *et al.,* 2012 and references therein). In addition, the genus *Codonorchis* includes another accepted species (*C. canisioi*), which is found in the southernmost part of Brazil, where it is also endemic (Govaerts, 2014).

One approach to isolate OrMF is cultivation from orchid roots, but this method has poor ability to detect slow-growing or uncultivable fungi. More accurate approach is based on direct DNA extraction from roots (Dearnaley *et al.,* 2012). Few recent studies have isolated mycorrhizal fungi from some species in those genera and found different OrM symbionts. Specifically, some species of the genus *Chloraea* (*Ch. collicensis* and *Ch. gavilu*) and *C. lessonii* were associated with fungal species of *Tulasnella* (Fracchia *et al.,* 2014; Pereira *et al.,*

2014) whereas species of the genus *Gavilea* (*G. australis* and *G. lutea*) tended to associate with *Ceratobasidium* and *Tulasnella* (Fracchia *et al.*, 2014). Overall the species from Patagonia have been poorly studied, compared to orchids from other regions.

In this present study our main goals were to determine i) fungal taxa associated with these endemic orchids in Patagonia and ii) the effect of different environmental factors on the species richness and community composition of root associated fungi.

# 2. Materials and methods

## 2.1. Study sites and sampling

In November 2013, roots of Patagonian endemic orchids were collected from 26 study sites in western Argentina (Fig. 1). Study sites spanned from 336 to 1265 m.a.s.l. (meters above sea level), and included different habitats, like forests, dry areas and grasslands. At each study site, we collected one root from a single plant, i.e. 6 plants of *Codonorchis lessonii* (d'Urv.) Lindl., 5 of *Chloraea alpina* Poepp., 1 of *Ch. chica* Speg. & Kraenzl., 1 of *Ch. magellanica* Hook.f., 4 of *Gavilea odoratissima* (Poepp. & Endl.) Poepp. and 9 of *Gavilea sp*. (Supporting Information, Table S1). Collecting time corresponded to the time when orchids started to flower but some sampled orchids (N=10) were non-flowering and their identification was decided based on the molecular data. Sampling was done using a spade and a knife. All root samples were placed into plastic bags and processed on the same day. They were cleaned from adhering soil, surface sterilized using commercial house bleach for 30s, followed washing in sterilised water.

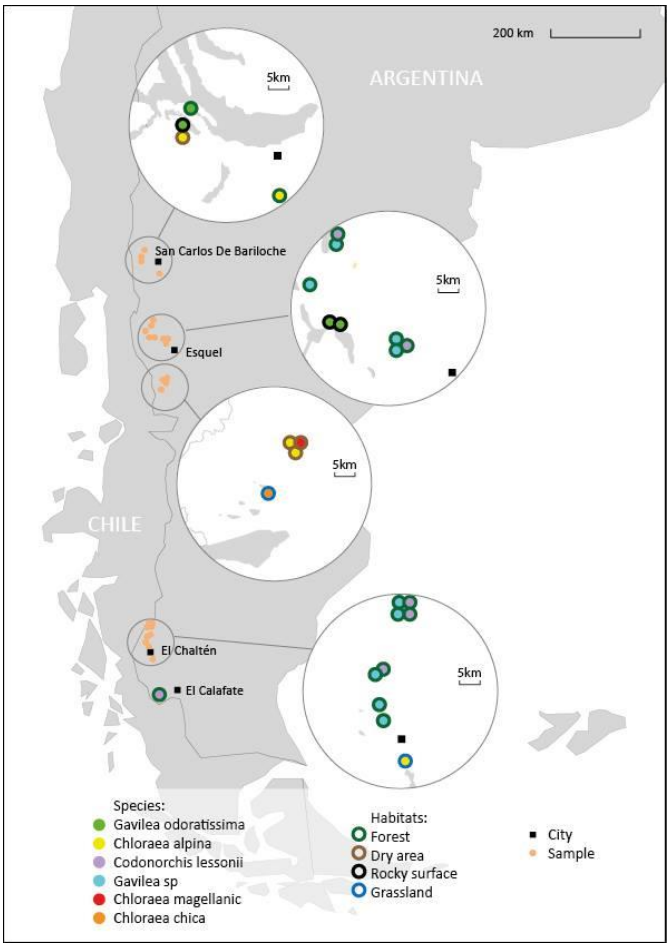


**Fig. 1.** The map of study sites in western Argentina.

## 2.2. Molecular analyses

Before DNA extraction, 0.05 g on randomly selected root fragments were powdered in 2-ml tubes using two 3-mm tungsten carbide beads in Mixer Mill MM400 (Retsch GmbH, Haan, Germany). DNA was extracted from root samples with PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. To ensure that a wide variety of fungal species including Tulasnellaceae were identified, we amplified Internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA) was amplified with primer pairs ITS1ngs/ITS4ngs and ITS1Fngs/ITS4ngs (Oja *et al.,* 2015; Tedersoo et al., 2014, 2015). Each of these primers was supplemented with a 10-12 bases multiplex identifier (MID) tag in the 5'end had at least four differences to each other (Tedersoo *et al.,* 2014). PCR amplification was performed in a 25 µl reaction volume containing 2 µl of DNA, 0.5 µl of each primer, 17 µl of dH2O and 5 µl of HOT FIREPol Blend Master Mix Ready to Load (Solid Biodyne, Tartu, Estonia). DNA samples were denatured for HotStart PCR before amplification at 95ºC for 15 min, followed by 35 cycles of 30s at 95ºC, 30s at 55ºC, 1 min at 72ºC and finally 72ºC for 10 min. Next, the PCR products were pooled and checked for the presence of a product on 1% agarose gel. In case of no visible band or strong band, we repeated the amplification by adjusting the number of cycles between 25 and 35. The PCR products were purified using Exo-Sap enzymes (Sigma, St. Louis, MO, USA) and 20 µl of the purified PCR product was normalized using a Sequalprep™ Normalization Plate (96) Kit (Invitrogen Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The pooled PCR products were finally precipitated with ethanol, which cleans and concentrates DNA. The PCR products were pyrosequenced using Roche GS FLX+ platform and Titanium chemistry.

## 2.3. Bioinformatics and statistical analyses

Using ACACIA 1.52 (Bragg *et al*., 2012), the pyrosequencing reads were cleaned based on the quality information. Too short sequences (<200bp in length) and sequences possessing any mismatch to the MID tags or primers were removed using MOTHUR 1.30.2 (Schloss *et al.,* 2009). Sequences were demultiplexed based on the MID tags and primers. Putative chimeras were identified and removed with UCHIME (Edgar *et al.,* 2011). The remaining sequences clustered into operational taxonomic units (OTUs) with a 97% similarity threshold using CROP 1.33 (Hao *et al.,* 2011). Next, we removed all global singletons for minimizing the effects of artifactual sequences (Tedersoo *et al.,* 2010). Remaining OTUs were

taxonomically identified based on their representative sequences using Biopython scripts to run BLASTn queries against the International Nucleotide Sequence Databases Consortium (INSDC) and UNITE (Abarenkov *et al.*, 2010). Based on the best BLASTn hits, OTUs were categorized into eukaryote kingdoms. We considered BLASn e-value $<e^{-50}$ reliable to assign OTUs into the fungal kingdom. Furthermore, fungal OTUs were identified to lower taxonomic levels following Index Fungorum (www.indexfungorum.org). OTUs, which were not identified were determined manually in UNITE database. We assigned OTUs to their corresponding Species Hypothesis (SH – Kõljalg *et al.*, 2013) in UNITE database.

The effects of host and habitat on the richness of non OrM fungal OTUs and potentially OrM fungal OTUs (members of Sebacinales, Tulasnellaceae and Ceratobasidiaceae) was analysed by one-way ANOVA as implemented in the Statistica package (version 7, 2004; StatSoft Inc., Tulsa, OK, USA).

Pairwise distances between samples were calculated based on GPS coordinates using Fields package or R (Furrer *et al.*, 2015; R Development Core Team, 2015). We used rdist.earth function to create distance matrix based on coordinates. We ran these analyses separately for the richness of non OrM fungal OTUs and potentially OrM fungal OTUs (members of Sebacinales, Tulasnellaceae and Ceratobasidiaceae). To identify the main predictors of fungal taxa richness and root colonization, we used the general least squares (GLS) model, based on environmental factors. For all tests, we calculated residuals of OTU richness in relation to the square root of the number of obtained sequences to account for differences in sequencing depth (Tedersoo *et al.*, 2014).

We also tested the effect of host, habitat and geographical variables on non-OrM fungal and potentially OrM fungal community composition with multivariate permutation analysis of variance as implemented in the Adonis routine of the Vegan package of R (Oksanen *et al.*, 2015). Considering that geographical distances cannot be analysed as locations, phylogenetic eigenvectors of principal components of neighbour matrices (PCNM) were derived from the distance matrix, forward selected in the Packfor package of R (Dray *et al.*, 2013) and used in statistical analyses. The final multivariate models were constructed based on forward selection criteria. We used Bray-Curtis index to calculate the community distance matrix from Hellinger-transformed community matrix. In addition, we ran separate analyses to test the effect of same factors on the non-OrM fungal community composition for each host genus. We visualized the differences in mycorrhizal community structure using a nonmetric multidimensional scaling (NMDS) ordination as implemented in the Ecodist

package of R (Goslee & Urban, 2007). Ellipses were added, which indicate 95% confidence intervals around centroids of each category.

We calculated indicator species (species that reflect environmental conditions) using the IndVal function implemented in the labdsb package of R (Roberts, 2015). This method assigns indicator value index between a species and each group, then identifies the group with the highest association value and uses randomization methods (permutation test) to test statistical significance of value. We used host and habitat as discreet variables and altitude (m.a.s.l.) and latitude for continuous variables. Altitude was divided into four groups: 1) <558 m.a.s.l. (336-558 m.); 2) ~690 m.a.s.l. (686-693 m.); 3) ~830 m.a.s.l. (829-845 m.); 4) >1000 m.a.s.l. (1047-1210 m.). Similarly, we divided latitude into following groups: 1) 41ºS; 2) ~42ºS (42.7ºS – 42.8ºS); 3) ~43ºS (43.7ºS – 43.8ºS); 4) ~50ºS (49.0ºS – 50.4ºS). Samples, which had less than four OTUs or fewer sequences than 10, were removed from analyse.

# 3. Results

## 3.1. Fungal identification

The quality-filtered data set comprised of 1844 OTUs (66, 473 sequences), of which 44.3% were singletons. After removing singletons, data set resulted in 839 fungal OTUs and 128 OTUs of nontarget eukaryotic organisms. The remaining 60 OTUs with low BLASTn hit (E-value > $e^{-50}$) or with no BLASTn hit were discarded. In addition, we excluded *Penicillium* (4 OTUs, 634 sequences) from the dataset, since it may have been contamination. With the forward primers ITS1Fngs and ITS1ngs, we detected more fungal OTUs and sequences, which clustered into 384 OTUs (24, 734 sequences) compared with reverse ITS4ngs primer, which recovered 325 OTUs (19, 945 sequences). In addition, we found more OrMF OTUs and sequences (27 OTUs, 8030 sequences). Therefore further analyses of fungal communities are focused on the data set obtained with the ITS1Fngs and ITS1ngs primers.

The most OTUs and sequences belonged to Ascomycota (60.8% of all OTUs, 12, 905 sequences), followed by Basidiomycota (34.0%, 11, 030 sequences), Mucoromycotina (4.1%, 389 sequences) and Glomeromycota (1.0%, 18 sequences). Less than 6% of OTUs (429 sequences) remained unidentified at the phylum level. The most OTU-rich orders from Ascomycota were Helotiales (13.1%, 2916 sequences) and Chaetothyriales (4.6%, 433 sequences). Most sequences of Helotiales belonged to the genus *Tetracladium* (1279 sequences) and in Chaetothyriales to the family *Herpotrichiellaceae* (345 sequences), which are considered to be a putative fungal endophytes. Of Basidiomycota, Agaricales (6.1%, 234 sequences) was the most OTU-rich order. In Agaricales, the majority of sequences belonged to family *Cortinariaceae* (92 sequences) and *Inocybaceae* (100 sequences).

## 3.2. Fungal richness and community composition

The richness of non-OrMF and OrMF were not significantly affected by any environmental factors. In addition, there was no spatial effect, which means the placement of the sampling did not have any effect to the fungal richness. Likewise, environmental and geographical variables had no effect on the OrMF community composition (Supporting Information, Fig. S1). On the other hand, non-OrMF community composition was affected by several environmental factors (Fig. 2). In particular, host species explained 27% ($F_{5,25}$=1.609, P=0.001) of non-OrMF community composition, followed by habitat ($R^2$=0.125, $F_{3,25}$=1.238, P=0.019), altitude ($R^2$=0.054, $F_{1,25}$=1.598, P=0.001) and latitude ($R^2$=0.048, $F_{1,25}$=1.422,

P=0.012). In a separate analysis with each orchid, variables did not have any effect to the community structure, except with *Chloraea*, where host explained 38.4% ($F_{2,5}$=1.244, P=0.047) and altitude 25% ($F_{1,5}$=1.666, P=0.01) of non-OrM fungal community composition.

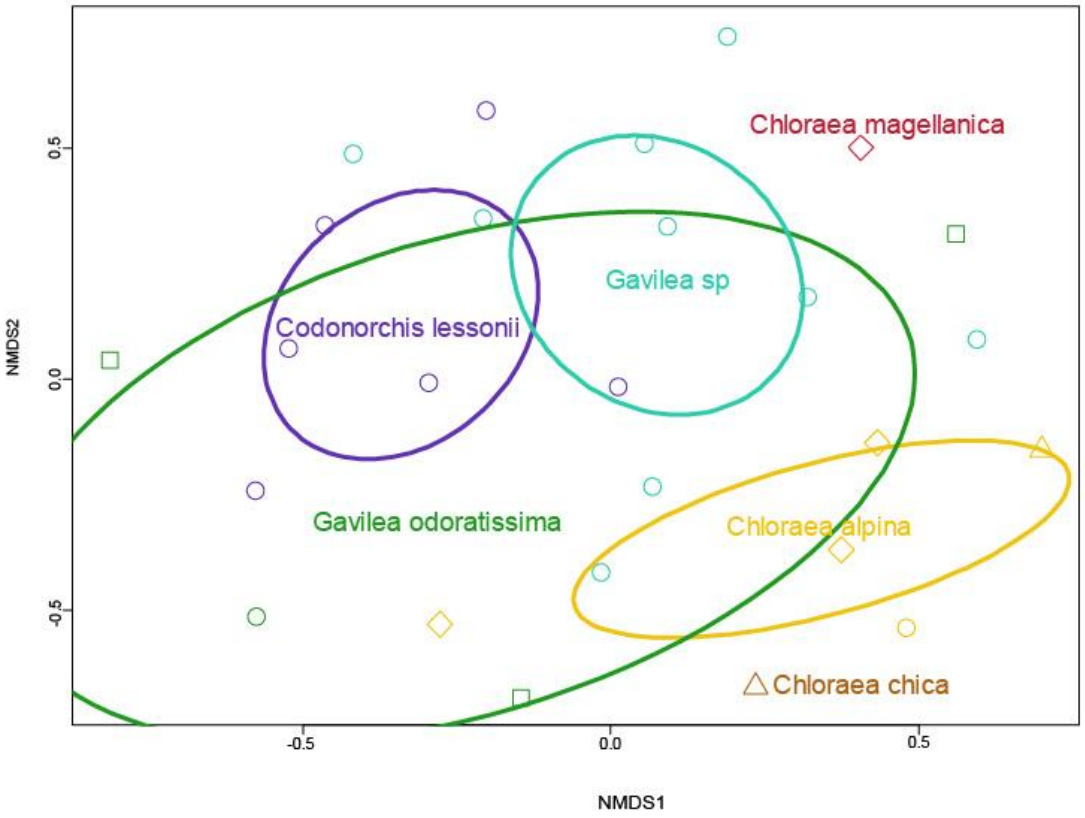


**Fig. 2** Nonmetric multidimensional scaling (NMDS) ordination plots demonstrating the effect of habitat (diamond - dry area; square - forest; circle - grasslands; triangle - rocky surface) and host (green – *G. odoratissima;* purple – *C. lessonii;* blue – *Gavilea sp;* red – *Ch. magellanica;* yellow – *Ch. alpina;* orange – *Ch. chica*) on the community composition of non-OrMF.

Even though there was no significant effect of orchid species on fungal communities, there were some differences between them. The roots of genus *Gavilea* were most commonly associated with Ceratobasidiaceae (10 OTUs, 2954 sequences) and Tulasnellaceae (5 OTUs, 1305 sequences), followed by Sebacinales (1 OTU, 4 sequences). Similar results occurred in roots of *C. lessonii*, which were dominantly associated with Ceratobasidiaceae (9 OTUs, 2281 sequences), followed by less abundant Tulasnellaceae (5 OTUs, 110 sequences) and Sebacinales (1 OTU, 76 sequences). Roots of *Ch. chica* and *Ch. alpina* were exclusively associated with Ceratobasidiaceae (98.4%, 9 OTUs, 1279 sequences). By contrast, we found

no OrMF associated with the roots of *Ch. magellanica*, only putative endophyte from genus Helotiales (4 OTUs, 226 sequences) were present.

Indicator species analyses revealed that there were more fungal OTUs, which had preferential associations with a particular host species, than habitat or altitude. Specifically 6 OTUs were preferentially associated with *Codonorchis* and 1 OTUs *Chloraea*, whereas 1 OTUs preferred rocky surface, 1 OTUs dry area and 3 OTUs >1000 meters above sea level (Table 1). We found no indicative species for any latitude or altitude lower than 1000 m.a.s.l. groups. Neither with forest or grassland habitats or for *Gavilea*.

**Table 1.** Relationships between environmental factors (host, habitat ant altitude of the host plant) and the occurrence of particular fungi in the roots as based on indicator species analyses. Preferential associations of OTUs with a particular host (C – *Codonorchis*, Ch – *Chloraea*), habitat (R – rocky surface, D – dry area) and altitude (x meters above sea level) are indicated.

| Fungal taxon | OTU | Species hypothesis (SH) code | No. of orchid individuals | Host | Habitat | Altitude |
|---|---|---|---|---|---|---|
| *Acremonium* | OTU0036 | SH025034.06FU | 5 | | | >1000** |
| *Amphisphaeriaceae* | OTU0305 | SH015348.06FU | 5 | | R* | |
| *Exophiala* | OTU0175 | SH029555.06FU | 15 | | D* | |
| *Leotiomycetes* | OTU0373 | SH013881.06FU | 7 | | | >1000* |
| *Mastigobasidium* | OTU0355 | SH016036.06FU | 7 | C** | | |
| *Mortierella* | OTU0295 | SH017856.06FU | 4 | C* | | |
| *Mortierella* | OTU0266 | SH016060.06FU | 4 | C* | | |
| *Myxotrichaceae* | OTU0292 | SH236509.06FU | 4 | C* | | |
| *Peziza* | OTU0002 | SH018963.06FU | 9 | Ch** | | |
| *Tetracladium* | OTU0503 | SH018983.06FU | 13 | C** | | |
| *Tetracladium* | OTU0603 | SH018983.06FU | 14 | C** | | |
| *Umbelopsis* | OTU0108 | SH144402.06FU | 4 | | | >1000* |

Significance levels: *P < 0.05; **P < 0.01.

# 4. Discussion

All studied orchid species were most commonly associated with Ceratobasidiaceae, followed by Tulasnellaceae and Sebacinales. We detected that the roots of genus *Gavilea* were associated with Ceratobasidiaceae (60%) and Tulasnellaceae (30%), which is consistent with the previous work (Fracchia *et al.,* 2014). Although Fracchia *et al.* (2014) identified Tulasnellaceae as a prominent OrMF taxa associating with *C. lessonii*, we found much broader spectrum of OrMF in *C. lessonii* roots, including members of Ceratobasidiaceae (as dominant) and Sebacinales. In the case of *Chloraea*, the roots of *Ch. chica* and *Ch. alpina* were exclusively associated with fungal partners from Ceratobasidiaceae, whereas Pereira *et al.* (2014) described only Tulasnellaceae in association with the roots of *Ch. collicensis* and *Ch. gavilu.* All of these contrasts with previous studies may be due to the methodological differences. In particular, both of these previous works used culture-based methods for identifying symbiotic partners, whereas we detect fungi from roots with the direct DNA extractions. The major disadvantage of culture-dependent technique is the poor ability to detect slow-growing or uncultivable fungi (Dearnaley *et al.,* 2012). However, based on our sampling method we were not able to determine the nature of interaction between orchids and non-OrMF.

The number of non-OrMF and OrMF in roots of orchid species was not significantly affected by any studied environmental factor. Although we did not detect any effect of the studied factors, some other characteristics could explain this result, for example soil pH (McCormick & Jacquemyn) or water availability (Illyes *et al.,* 2009). Orchids studied in this work, were all endemic to this region and in general, there is no relationship between orchid rarities and the range of their mycorrhizal fungi (McCormik & Jacquemyn., 2013).

Community composition of OrMF was not affected by any analysed environmental factors or geographical variables. It may be explained that OrMF are widely spread in this region and they are not dependent on any here studied factors. It has been suggested that many mycorrhizal fungi are widespread and occur in a wide variety of habitats (McCormick & Jacquemyn, 2013).We could assume that orchids associate with available fungi and have limited preferences towards their mycorrhizal fungi, which has been also found in previous studies (Otero *et al.,* 2004; Shefferson *et al.,* 2006). Bonnardeaux *et al.* (2007) evidenced that mycorrhizal generalist orchids appear to be capable of growing in a wider variety of habitats. Other results showed contradictory results, although it has been found that the type of habitat

has little influence on the composition (Tesitelova *et al.,* 2015), opposite has been also documented (Oja *et al.,* 2015). On the other hand, community composition of non-OrMF was significantly affected by host species. However the biological reasons remain unknown. Because of the great diversity of endophytic fungi in orchid roots and the variation in methods among previous works, it is difficult to determine preferences in the interaction (Bayman *et al.,* 2006).

In addition we found habitat effect on the non-OrMF community composition. Majority of our samples were collected from *N. pumilio* forests, where might be poor lighting availability and this may be the reason why we had significant effect of geographical factors and habitat type in non-OrMF. In Patagonia, the only native EcM host is *Nothofagus* (Tedersoo *et al.,* 2010), which ectomycorrhizal fungal communities are dominated by Basidiomycota (species of *Cortinarius, Inocybe, Tomentella, Thelephora, Clavulina, Tulasnella* and *Sebacina*) (Nouhra *et al.,* 2013). Selosse *et al.* (2004) suggested that the replacement of typical OrMF with other fungal taxa may be a strategy to secure access to fungal carbohydrates where typical OrMF are not available or where light availability is limited, for example in forests.

Indicative fungal species reflect the environmental conditions. Several species, which we identified, are also been found in orchid mycorrhiza in the Czech Republic, Japan, Germany and France (Malinova *et al.,* unpublished; Ogura-Tsujita *et al.,* 2008; Stark *et al.,* 2009; Julou *et al.,* 2005). Other fungal taxa have not been found in orchid mycorrhiza, but in soil and roots of other plants. *Exophiala* and *Tetracladium* have been previously identified as possible orchid endophytes (Stark *et al.,* 2009), but their exact role in orchids is still unclear. In this study, we found *Exophiala* to be significantly affecting composition in dry habitats, but *Tetracladium* had significance within host (*Codonorchis*). There is an evidence, that plants associated with *Exophiala* sp. associated plants can grow better under heat stress (Khan *et al.,* 2012), therefore it may improve orchids conditions in dry habitats. Ascomycetes are known to occur OrMF in orchid roots (Selosse *et al.,* 2004), but further studies are needed to clarify the function of the ascomycete associates towards orchids used in this work.

# Summary


## Root associated fungi of Patagonian endemic orchids

Eva Luukas

Orchidaceae is one of the largest plant families with 27,135 species. Orchids have specific associations with their mycorrhizal fungi, which form orchid mycorrhiza (OrM). Fungi forming OrM are mostly saprotrophic or pathogenic and belong to Ceratobasidiaceae, Tulasnellaceae or Sebacinales or in some case ectomycorrhizal species. In addition, orchids may be colonized by fungal endophytes.

In this study our main goals were to determine fungal taxa associated with these endemic orchids in Patagonia and the effect of different environmental factors on the species richness and community composition of fungi. We gathered root samples from South Argentina, in 26 study sites. Fungi were identified by using direct DNA extraction from orchid roots.

All of the host plants were mostly associated with Ceratobasidiaceae, followed by Tulasnellaceae and Sebacinaceae. Fungal richness was not affected by any of the studied factors. In community composition, we found significant effects of host, habitat and geographical factors to non-OrM forming fungi.

# Kokkuvõte

## Patagoonia endeemsete orhideede juurtega seotud seened

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Orhideelised ehk käpalised on üks suurimaid õistaimede sugukondi, kuhu kuulub 27 135 liiki ja ligikaudu 900 perekonda. Orhideedel on spetsiifilised suhted nii tolmeldajate kui ka seentega. Põhilisteks orhidoidset-mükoriisat moodustavateks seenteks peetakse seeni seltsist Sebacinales ning sugukondadest Ceratobasidiaceae ja Tulasnellaceae. Lisaks nendele seentele võivad orhidoidset-mükoriisat moodustada mitmed ektomükoriissed seened kand- ja kottseente rühmast ning peale selle võivad orhideede juured olla sama-aegselt koloniseeritud erinevate endofüütsete seente poolt.

Selle töö eesmärkideks oli identifitseerida orhidoidset-mükoriisat moodustavad taksonid Patagoonia endeemsetel orhideedel ning välja selgitada, millised keskkonna- ja geograafilised tingimused mõjutavad nende seente liigirikkust ja kooslust. Selleks koguti 26 juureproovi kuuelt orhideeliigilt Lõuna-Argentiinast. Seeneliikide identifitseerimiseks kasutati kõige kaasaegsemat nn uue põlvkonna DNA järjestusmeetodite abi.

Kõik töös uuritud Patagoonia endeemsed orhideed olid põhiliselt seotud seensümbiontidega sugukonnast Ceratobasidiaceae, ning vähesel määral leiti seensümbionte sugukonnast Tulasnellaceae ja seltsist Sebacinales. Üldiselt ei olnud nende seente liigirikkus ja kooslus mõjutatud ühegi uuritud faktori poolt. Olulised erinevused ilmnesid mitte orhidoidset-mükoriisat moodustavate seente koosluste puhul, mis oli mõjutatud nii peremeestaimest, kasvukohast kui ka geograafilistest faktoritest.

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# Supporting Information

**Table S1** Locations and description of study sites

| Study sites | Geographical coordinates | Elevation (m) | Vegetation | Sampled species |
|---|---|---|---|---|
| El Calafate | 49°22'S, 72°52'W | 398 | Dry grassland | *Chloraea alpina* |
| El Chaltén | 43°44'S, 71°24'W | 829 | Sandy, dry area covered with low vegetation | *Ch. alpina* |
| El Chaltén | 43°44'S, 71°24'W | 832 | Sandy, dry area covered with low vegetation | *Ch. alpina* |
| El Chaltén | 41° 5'S, 71°32'W | 1210 | Sandy, dry area | *Ch. alpina* |
| El Chaltén | 43°49'S, 71°28'W | 1199 | Grassy, shrubby slope | *Chloraea chica* |
| El Chaltén | 43°44'S, 71°24'W | 826 | Sandy, dry area covered with low vegetation | *Chloraea magellanica* |
| El Chaltén | 49°11'S, 72°57'W | 469 | *Nothofagus pumilio* forest | *Codonorchis lessonii* |
| El Chaltén | 49° 4'S, 72°52'W | 585 | *N. pumilio* forest | *C. lessonii* |
| El Chaltén | 50°26'S, 72°46'W | 336 | Under *N. pumilio* | *C. lessonii* |
| El Chaltén | 49° 4'S, 72°52'W | 703 | *N. pumilio* forest | *C. lessonii* |
| Esquel | 42°50'S, 71°29'W | 1231 | *N. pumilio* forest | *C. lessonii* |
| Esquel | 42°37'S, 71°39'W | 661 | *N. pumilio* forest | *C. lessonii* |
| Esquel | 42°48'S, 71°41'W | 693 | Rocky surface | *Gavilea odoratissima* |
| Esquel | 41° 3'S, 71°32'W | 838 | *N. pumilio* forest | *G. odoratissima* |
| Esquel | 42°48'S, 71°39'W | 558 | Rocky surface near lake shore | *G. odoratissima* |
| Esquel | 41° 5'S, 71°32'W | 845 | Rocky surface | *G. odoratissima* |
| Esquel | 42°50'S, 71°29'W | 1150 | *N. pumilio* forest | *Gavilea sp* |
| Esquel | 49° 4'S, 72°51'W | 751 | *N. pumilio* forest | *Gavilea sp* |
| Esquel | 49° 4'S, 72°52'W | 686 | *N. pumilio* forest | *Gavilea sp* |
| Esquel | 49°17'S, 72°56'W | 743 | *N. pumilio* forest | *Gavilea sp* |
| Esquel | 49°15'S, 72°56'W | 729 | *N. pumilio* forest | *Gavilea sp* |
| Esquel | 49°11'S, 72°57'W | 499 | *N. pumilio* forest | *Gavilea sp* |
| San Carlos de Bariloche | 41°13'S, 71°17'W | 1047 | *N. pumilio* forest | *Ch. alpina* |
| San Carlos de Bariloche | 42°37'S, 71°39'W | 662 | *N. pumilio* forest | *Gavilea sp* |

| | | | | |
|---|---|---|---|---|
| San Carlos de Bariloche | 42°50'S, 71°29'W | 1265 | *N. pumilio* forest | *Gavilea sp* |
| San Carlos de Bariloche | 42°43'S, 71°44'W | 541 | Mixed forest near lake shore | *Gavilea sp* |

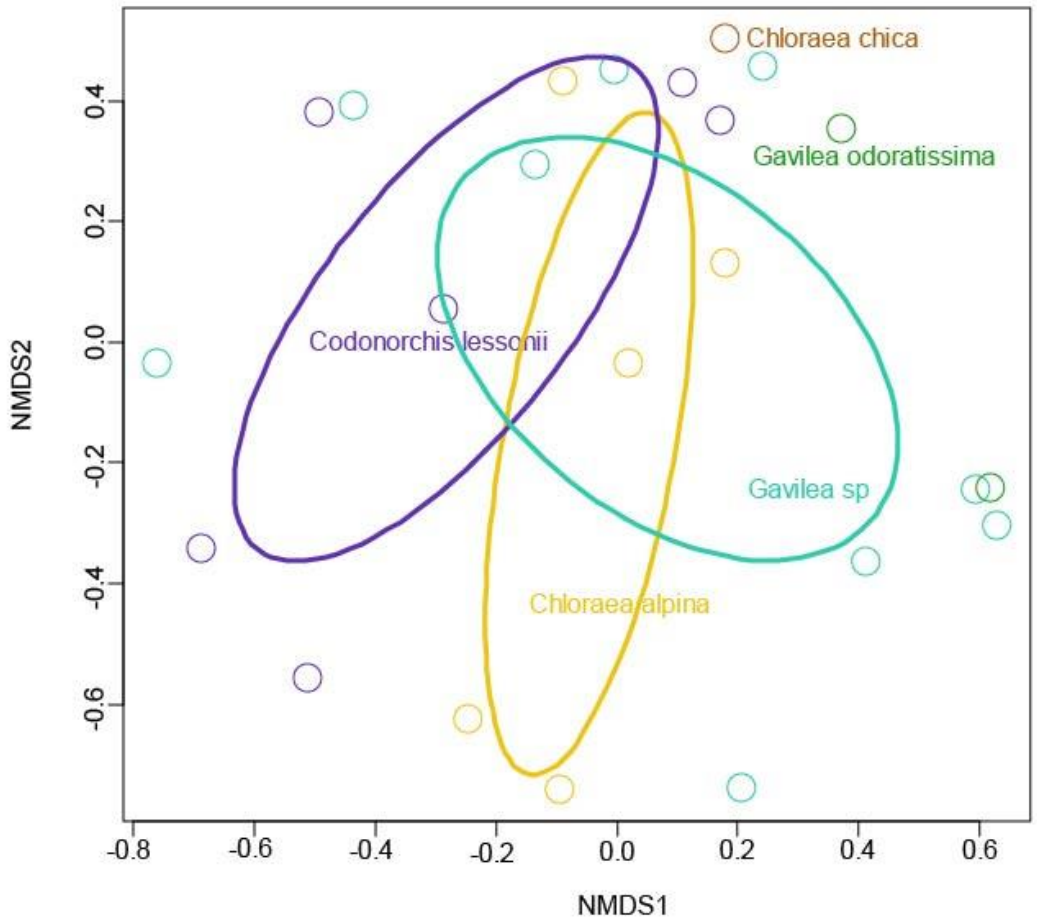


**Fig. S3** Nonmetric multidimensional scaling (NMDS) ordination plots demonstrating the effect of host (green – *G. odoratissima;* purple – *C. lessonii;* blue – *Gavilea sp;* yellow – *Ch. alpina;* orange – *Ch. chica*) on the community composition of OrMF.

# Lihtlitsents lõputöö reprodutseerimiseks ja lõputöö üldsusele kättesaadavaks tegemiseks

Mina, Eva Luukas,

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Petr Kohout ja Jane Oja,

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Tartus, 26. Mai 2015 Eva Luukas