

Mycorrhizas in the tropical montane forest of South Ecuador

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Abstract: The tropical mountain rain forest area of the *Reserva Biológica San Francisco* in South Ecuador is exceptionally rich in indigenous tree species, orchids, ericads and in liverworts. Light microscopical studies revealed that all species are well colonized by structurally diverse fungi. Results from molecular analyses showed that the mycorrhizal fungal community of tropical forests in South Ecuador is highly diverse and includes Glomeromycota, Ascomycota and Basidiomycota. Beside of three ectomycorrhizal tree species all other tree species form arbuscular mycorrhizas with Glomeromycota; epiphytic and terrestrial orchids form orchid mycorrhizas with members of Basidiomycota (Tulasnellaceae, Sebacinaceae, Atractiellales). Sebacinaceae also occurred in mycorrhizas of hemiepiphytic ericads and formed cavendishioid mycorrhiza.

1. Introduction

Mycorrhizal fungi have an important ecological and economic role in tropical ecosystems. Investigations in the San Francisco Valley (South Ecuador, Loja-Chinchipe) showed that tropical mountain rain forests harbor a broad array of mycorrhizal associations and that mycorrhizal fungi are an integral part of these ecosystems. Even new mycorrhizal types and fungi were described from this area.

2. Materials and methods

The study site is located between 1800 and 2200 m above sea level on the slopes above the San Francisco River, Cordillera Real, South Ecuador, (3° 58' S, 79° 4' W). Mycorrhizas were sampled by tracing single roots from the stem down to the fine roots. Mycorrhizas were cleaned under tap water on the same day and one part of each sample was fixed in 70 % ethanol for light microscopy. The other part was dried in open tubes with an electric dryer at about 50°C for 24 hours, after which silica gel was added.

DNA was isolated from the dried root samples using the DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany).

From arbuscular mycorrhizas the 18S rDNA was amplified by using two rounds of PCR. The first PCR was performed with primers NS1/NS4 and for the nested PCR, primers AML1/ AML2 were used. For orchid and cavendishioid mycorrhizas the ITS and part of LSU was amplified. For details see SETARO et al. (2006), SUÁREZ et al. (2006) and KOTTKE et al. (2010).

Operational Taxonomic Units (OTUs) were defined as surrogates for species on the basis of sequence similarity with OPTSIL (GÖKER et al. 2009).

3. Results and discussion on ectomycorrhizal, arbuscular, orchid and cavendishioid mycorrhizae and their diversity

Three ectomycorrhizal species have been found in the tropical mountain rain forest of Reserva Biológica San Francisco so far, which are two *Neea* species and *Pisonia* sp., all belonging to the family Nyctaginaceae (HAUG et al. 2014). *Neea* sp. 1 forms typical ectomycorrhizal short roots. The fungal partners belong to three lineages: *Tomentella-Thelephora*, *Russula-Lactarius* and *Clavulina* (HAUG et al. 2005, TEDERSOO et al. 2010). In total six ectomycorrhizal fungi were found. Ectomycorrhizas of *Neea* aff. *floribunda* and *Pisonia* sp. show a particular morphology: there are no short roots and only parts of the long roots show ectomycorrhizal structures with hyphal mantle and Hartig net. Only one fungal partner from the *Tomentella-Thelephora* lineage is involved, respectively.

The fungal structures observed in the arbuscular mycorrhizas of the Andean trees were surprisingly diverse (BECK et al. 2007), but the gathered data are still insufficient to determine species. The only way to learn about AMF diversity is to extract and sequence fungal DNA directly from mycorrhizas. However, this method provides only sequences and therefore the morphological or biological species concept cannot be applied. Instead of species, these fungal sequences are assigned to Operational Taxonomic Units (OTUs, a surrogate for species). Results from these OTU analyses showed that the AMF community of tropical forests in South Ecuador is highly diverse. There are only few frequent OTUs, but a lot of rare ones (HAUG et al. 2013). Arbuscular mycorrhizal mycobionts of Ecuadorian forests belong to Glomerales, Diversisporales, Archaeosporales and Paraglomerales. The Glomeraceae are the most species-rich family followed by Acaulosporaceae and Archaeosporaceae. Each tree individual harbors several OTUs as mycobionts and different individuals from the same plant species show a great variety of fungal communities. All tree individuals are usually associated with one to three frequent OTUs and several rare OTUs. Frequent arbuscular mycorrhizal fungi are shared among different tree species of different families.

Orchid mycorrhizas are mutualistic interactions between orchids and a restricted group of Basidiomycota. Studies of terrestrial and epiphytic orchids in the tropical mountain rain forest of *Reserva Biológica San Francisco* revealed fungal communities with members of *Tulasnella*, *Sebacina* and even the discovery of a new group of mycobionts in orchids – the Atractiellomycetes (KOTTKE et al. 2010). This group of symbionts was recognized through TEM investigations which showed hyphae in the cortical cells with the characteristic simple septum and symplechosomes (BAUER & OBERWINKLER 1991, BAUER ET AL. 2006). Molecular investigations of the orchid mycorrhizas revealed sequences classified to Atractiellomycetes and completed the result.

This was an interesting finding because Atractiellomycetes belong to the Pucciniomycotina.

The cavendishoid mycorrhiza has been proposed by SETARO et al. 2006. Cavendishoid mycorrhiza is similar to arbutoid mycorrhiza in that it has a hyphal sheath, a Hartig net and intracellular hyphal coils. However, it is more similar to ericoid mycorrhiza in terms of fungal and plant affiliation. Cavendishoid mycorrhiza is mainly formed by hemiepiphytic Ericaceae belonging to the so-called Andean Clade of the subfamily Vaccinoideae. These plants occur only in the Neotropics and are frequent at *Reserva Biológica San Francisco*. Genera with cavendishoid mycorrhiza found at *Reserva Biológica San Francisco* are: *Cavendishia*, *Ceratostema*, *Diogenesia*, *Macleania*, *Orthaea*, *Psammisia*, *Sphyrospermum* and *Thibaudia*.

4. References

- BAUER, R., BEGEROW, D., SAMPAIO, J.P., WEISS, M. & OBERWINKLER, F. 2006: The simple-septate basidiomycetes: a synopsis. — *Mycol. Prog.* **5**: 41–66.
- BAUER, R. & OBERWINKLER, F. 1991: The symplechosome: a unique cell organelle of some basidiomycetes. — *Bot. Acta* **104**: 93–97.
- BECK, A., HAUG, I., OBERWINKLER, F. & KOTTKE, I. 2007: Structural characterization and molecular identification of arbuscular mycorrhiza morphotypes of *Alzatea verticillata* (Alzateaceae), a prominent tree in the tropical mountain rain forest of South Ecuador. — *Mycorrhiza* **17**: 607–625.
- GÖKER, M., GARCÍA-BLÁZQUEZ, G., VOGLMAYR, H., TELLERÍA, M.T. & MARTÍN, M.P. 2009: Molecular taxonomy of phytopathogenic fungi: a case study in *Peronospora*. — *PLOS ONE* **29**: e6319.
- HAUG, I., KOTTKE, I. & SUÁREZ, J.P. 2014: Ectomycorrhizas of three species of Nyctaginaceae in the tropical mountain rain forest of South Ecuador. — In BÂ, A.M., MCGUIRE, K.L. & DIÉDHIU, A.G. (eds.): *Ectomycorrhizal Symbioses in Tropical and Neotropical Forests*, pp. 19–28. — Boca Raton, London, New York: CRC Press.
- HAUG, I., SETARO, S. & SUÁREZ, J.P. 2013: Reforestation sites show similar and nested AMF communities to an adjacent pristine forest in a tropical mountain area of South Ecuador. — *PLOS ONE* **8**: e63524.
- HAUG, I., WEISS, M., HOMEIER, J., OBERWINKLER, F. & KOTTKE, I. 2005: Russulaceae and Thelephoraceae from ectomycorrhizas with members of the Nyctaginaceae (Caryophyllales) in the tropical mountain rain forest of southern Ecuador. — *New Phytol.* **165**: 923–936.
- KOTTKE, I., SUÁREZ, J.P., HERRERA, P., CRUZ, D., BAUER, R., HAUG, I. & GARNICA, S. 2010: Atractiellomycetes belonging to the “rust” lineage (Pucciniomycotina) form mycorrhizae with terrestrial and epiphytic neotropical orchids. — *Proc. Royal Soc. B* **277**: 1289–1298.

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- SETARO, S., WEISS, M., OBERWINKLER, F. & KOTTKE, I. 2006: Sebacinales form ectendomycorrhizas with *Cavendishia nobilis*, a member of the Andean clade of Ericaceae, in the mountain rain forest of southern Ecuador. — *New Phytol.* **169**: 355–365.
- 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.
- TEDERSOO, L., SADAM, A., ZAMBRANO, M., VALENCIA, R. & BAHAM, M. 2010: Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. — *ISME J.* **4**: 465–471.

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