Molecular- and microscopy-based approaches for the study of ectomycorrhizal communities in Brazilian restinga

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Introduction

Although science is always advancing, we are still beginning to understand the different biotic and abiotic elements that shape biological communities and how they interact. As if the diversity of organisms in ecosystems was not enough, there are also interspecific interactions that perform a central role in community diversity maintenance, populations dynamic and community structure (Mougi & Kondoh 2012). While struggling to manage nutrient production and forest restoration above ground, we often ignore a whole level of the biodiversity: the intricate interrelationships among organisms that take place under the soil. An example of such interaction is the association between fungi and plant root tips, called mycorrhizae (Heijden et al. 2008). Today, about 80% of the surveyed land plant species are engaged in at least one kind of mycorrhizal association (Wang & Qiu 2006) and it is estimated that the fungus is responsible for a large part of the plant intake of nutrients such as nitrogen and phosphorus, critical for the development of plants. Copper, iron and zinc are also absorbed by the fungus (Heijden et al. 2008) and the mycelium that surrounds the root tips can also provide resistance to biotic and abiotic stresses (Gilbert & Johnson 2017). In return, the fungus receives carbon fixed by the plant through photosynthesis (Rhodes 2017).

Mycorrhizae are generally classified in seven different types, according to the symbiotic partners and some morphological features (Smith & Read 2008). Ectomycorrhizae (ECM) are the of the most common type and involve several families of Spermatophyte plants that interact mainly with macrofungi of the Basidiomycota phylum (Smith & Read 2008). ECM are characterized by the formation of a mantle, composed by hyphae that wrap around the root tip, and the Hartig net, composed by hyphae that typically penetrate into the intercellular space of the root cortical tissue (Smith & Read 2008), or sometimes only between the epidermal cells (Brundrett 2004). The remaining types of mycorrhizae refer to associations between Basidiomycota or Ascomycota fungi and specific plant families like Ericaceae and Orchidaceae (Brundrett 2002).

The current paradigm on ECM states that their diversity and distribution peak at boreal and temperate areas of the globe (Tedersoo et al. 2012), while in the tropics, ECM would be restricted to monotypic forests (Bâ et al. 2014). However, very recent new observations of ECM are popping up all around native species in South American forests (Roy et al. 2017; Alvarez-Manjarrez et al. 2018; Vanegas-Leon et al. 2019) leading to new insights on the ecology and evolution of Neotropical mycorrhizae and potential uses of Neotropical ECM fungi in areas such as agriculture and environmental restoration. A recent study conducted by our group found morphological and molecular evidence of ECMs in Atlantic Forest fragments, suggesting plant and fungal partners that have never been reported before as ECM (Vanegas-León 2017, Vanegas-León et al. 2019). This exciting finding indicates that the occurrence and importance of ECM in the neotropics is probably underestimated and we know nothing regarding the role of this relationship to the organisms and to the biological community.

The Atlantic Forest is one of the richest biomes in the world, but only around 12% of the original forest is left in Brazil. In Santa Catarina, less than 5% of the area have mature forests and the larger part is composed by secondary forest with an average of approximately 44 tree species per forest remnant. The area has been exploited for wood, cattle and real state that threat the maintenance of older trees and the growth of new healthy trees. Recent data indicated that 908 ha of woods had been reduced between 2017 and 2018 in Santa Catarina (SOS Mata Atlântica 2019). Specifically, 206 ha of coastal ecosystems called restingas also disappeared in the same period. The restingas work as a buffer on the coastal areas and shows sandy, acidic and nutrient-poor soils, often with high salinity levels (Falkenberg 1999). Even though this is a difficult environment to live in, it is estimated that restingas harbor around 225 plant species in Santa Catarina (Korte et al. 2012). These species probably present interesting adaptations to cope with the challenging conditions of their environment, but we know very little about it. Among the possible interactions, we believe that ECM could thrive in this type of environment, since the soil shows some similarities with boreal and temperate forests (i.e. sandy, with low contents of inorganic nutrients and high organic matter content). Based on this, we believe that restinga may be a very interesting site to look for tropical ECM-forming species. In fact, our group has already found some of them. So far, we had encouraging results with the plant species *Guapira* *opposita* (Veill.) Reitz (Nyctaginaceae Juss.), which was confirmed as capable of forming at least five different morphotypes of ECM with unknown fungi. All these tropical ECM morphotypes were very different from those of boreal ECM. This raises the possibility that they function differently as well.

The morphoanatomical description of ECM has an inherent value (Comandini et al. 2012). The combination of morphological characters of ECM, and their molecular characteristics, integrated with other data (for example, secondary metabolites), contributes to the definition of a more natural phylogenetic classification of the group (Comandini & Rinaldi 2006). The ectomycorrhizal morphology and anatomy, in addition to increasing the knowledge of ecological habits, both of the plant symbiont and of the fungus, can be used to improve the phylogenetic resolution within a taxon (Comandini & Rinaldi 2001).

Preliminary data collected by the proponent demonstrated that the neotropical ECM found in restinga environments have a distinct morphology when compared to the most widely known ECM in temperate regions. In some specimens collected, Hartig's mantle and network are established in such a way that the epidermal cells of the root do not seem to exist, or the hyphae that penetrate intercellularly form a distinct morphological structure, which has been called "paraepidermal" (Alvarez-Manjarrez et al. 2018). This morphological difference suggests the possibility that they work in a different way as well, and future studies should focus on understanding this process and checking if there is an exchange of nutrients between the organisms involved in this symbiosis. However, to start studying the physiology of the fungus-plant relationship, we need to know the identity of the partners through their morphological and molecular patterns (Ferlian et al. 2018).

Objectives

Aiming to understand the subterranean biodiversity existing in Brazilian restinga areas, the present project aims to:

- Characterize, based on morphological and molecular analyzes, the ectomycorrhizae collected in restinga areas;
- Identify, through molecular analysis, the plant components involved in this symbiosis;
- Propose a new morphological concept for neotropical ectomycorrhizae through a comparative analysis between the most representative taxa of restinga ECM and the traditional ECM from boreal and temperate zones.

Material & Methods

The methodology used for the development of the proposal considers techniques used for the study of ectomycorrhizae, to describe the morphology of the ECM and understand the relationships between the plant and the fungus (Rinaldi et al. 2008). We have little preliminary data pointing that the same plant individual can form different ECM morphotypes, suggesting that there are more than one fungal species in their roots. In order to try and assess overall fungal diversity in the restinga we will perform field trips to collect basidiomes and take soil samples to estimate the ECM richness.

Collection: — To start addressing some issues regarding tropical ECM we will focus our efforts in areas of the Atlantic Forest in Florianópolis, Santa Catarina, Brazil, which include several restinga areas. Sampling in these areas will be opportunistic. Samples of soil and the root system of plants that are below basidiomas of taxa recognized as ECM will be collected. Basidiomas and plants will be collected and identified according to traditional methods in taxonomy and by molecular data (Iotti & Zambonelli 2006). The soil samples will be washed and, under a stereomicroscope, the root tips with ECM (at least those with a mantle) will be selected to be analyzed morphologically and molecularly.

Morphological analysis:—The description of the morphotypes found will include macro and micromorphological aspects of the root tips with and without the fungus. The descriptions will be carried out under a stereoscope and light optical microscope to measure and describe the different hyphae present in the mantle. Analyzes in confocal microscopy will be done at the root tips to describe in detail the anatomy and morphology of the hyphae and the root cells (Comandini & Rinaldi 2006). This type of study could give us hints on how this symbiosis works, as well as a possible way to quickly recognize the participating organisms without destructive analyses.

Molecular analysis and phylogenetic inference:—The molecular study of the root tips will allow the identification of the associated plant and fungus. Specific ITS primers will be used as a barcoding region to identify plants and fungi. Leaves of the plants that grow around the collection points will be sampled to build a molecular library to be used to compare with the root sequences (Leonardi et al. 2018). Phylogenetic analyzes will be performed using the Maximum Likelihood and Bayesian Inference methods, with parameters to be defined, on the CIPRES platform, and the analyzes will be enriched with sequences obtained through public databases (GenBank - https://www.ncbi.nlm.nih.gov/nucleotide/ and UNITE - http://unite.ut.and is/).

Literature Cited

Alvarez-Manjarrez J, Garibay-Orijel R & Smith ME (2018) Caryophyllales are the main hosts of a unique set of ectomycorrhizal fungi in a Neotropical dry forest. Mycorrhiza 28: 103-115.

Bâ AM, McGuire KL & Diédhiou AG (2014) Ectomycorrhizal symbioses in Tropical and Neotropical forests. CRC Press, Boca Raton. 286p.

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytologist 154: 275-304.

Brundrett MC (2004) Diversity and classification of mycorrhizal associations. Biological Reviews 79: 473-495.

Comandini O & Rinaldi AC (2001) Together, but not for ever: ectomycorrhizal symbiosis is an unstable affair. Mycological Research 105:130–131.

Comandini O, Contu M & Rinaldi AC (2006) An overview of Cistus ectomycorrhizal fungi. Mycorrhiza 16:381–395.

Comandini O, Rinaldi AC & Kuyper TW (2012) Measuring and estimating ectomycorrhizal fungal diversity: a continuous challenge. Em: Pagano, M. eds. Mycorrhiza: occurrence in natural and restored environments, 1 ed., cap. 9, Nova Science Publishers, New York, pp. 165–200.

Falkenberg DB (1999) Aspectos da Flora e da vegetação secundária da restinga de Santa Catarina, sul do Brasil. Insula 28: 1-30.

Ferlian O, Biere A, Bonfante P et al. (2018) Growing research networks on mycorrhizae for mutual benefits. Trens in Plant Science 23(11): 975–984.

Gilbert L & Johnson D (2017) Plant-plant communication through common mycorrhizal networks. In: Advances in Botanical Research, v. 82, cap. 4: 83-97.

Heijden MGA, Bardgett RS & Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11: 296-310.

Iotti M & Zambonelli A (2006) A quick and precise technique for identifying ectomycorrhizae by PCR. Mycological Research 110: 60–65.

Korte A, Gasper AL, Kruger A & Sevegnani L (2012) Composição florística e estrutura das restingas de Santa Catarina. In: Vibrans AC, Sevegnani L, Gasper AL & Lingner DV (eds.). Inventário Florístico Florestal de Santa Catarina, Vol. IV, Floresta Ombrófla Densa. Blumenau. Edifurb.

Leonardi M, Neves MA, Comandini O & Rinaldi A (2018) Scleroderma meridionale ectomicorrhizae on Halimium halimifolium: expanding the Mediterranean symbiotic repertoire. Symbiosis. doi.org/10.1007/s13199-018-0548-1.

Mougi A & Kondoh M (2012) Diversity of interaction types and ecological community stability. Science 337: 349-351.

Rhodes CJ (2017) The whispering world of plants: 'The Wood Wide Web'. Science Progress 100: 331-337.

Rinaldi AC, Comandini O & Kuyper TW (2008) Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Diversity 33: 1−45.

Roy M, Vasco-Palacios A, Geml J et al (2017) The (re)discovery of ectomycorrhizal symbioses in Neotropical ecosystems sketched in Florianópolis. New Phytologist 214: 920-923.

Smith SE & Read DJ (2008) Mycorrhizal symbiosis. 3° ed. Academic Press, New York. 815p.

Tedersoo L, Bahram M, Toots M et al. (2012) Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. Molecular Ecology 21: 4160-4170.

Vanegas-León ML, Sulzbacher M, Rinaldi A et al (2019) Are Trechisporales ectomycorrhizal or non-mycorrhizal root endophytes? Mycological Progress 18: 1231-1240.

Vanegas-Léon ML (2017) Ectomicorrizas tropicais: estudos de casos na Mata Atlântica. 78 pp. Dissertação. Universidade Federal de Santa Catarina, Brasil. Florianópolis, CAPES.

Wang B & Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16: 299-363.