

# Mycorrhizas

Manuel Moscariello

January 31, 2021

# Contents

Contents	2
<b>I. Introduction</b>	<b>4</b>
1. Our closest cousins	5
2. Ancient lovers	6
3. Orchids	8
3.1. Distribution and ecology of OMFs . . . . .	9
<b>II. Materials and methods</b>	<b>11</b>
4. The database	12
5. Phylogenetic analysis	12
6. Multivariate analysis	13
<b>III. Results</b>	<b>16</b>
7. Phylogenetic analysis	17
8. Multivariate analysis	17
<b>IV. Discussion</b>	<b>22</b>

## *CONTENTS*

<b>V. Appendices</b>	<b>24</b>
<b>Bibliography</b>	<b>25</b>

# Part I.

## Introduction

# 1. Our closest cousins

The kingdom Fungi is one of the most diverse groups of organisms on Earth, and they are integral ecosystem agents with a huge impact on biogeochemical cycles, plant and animal pathology, plant nutrition and soil properties. While historically clustered together with plants (Copeland, 1938, 1956), towards the middle of last century it started to become clear that this lumping failed to properly deal with the differences between the two groups. In 1969 R. H. Whittaker published a paper dividing the organisms into five kingdoms: Animalia, Plantae, Fungi, Protista and Monera (Whittaker, 1969). By the 70s this division became widely accepted, and the Kingdom Fungi was recognized. Acknowledgement is just the first step in knowledge though. The understanding of the taxonomy, evolution and phylogenesis of fungi was still a matter of ample debate, one of those that may never end for lack of evidence. All the analysis were based on morphological differences, with all of its downsides.

Fossilized fungi are very difficult to come by, as they do not biomineralize like animals do, and has proven not only inconclusive with regard to the origin of fungi, but also rather incomplete relative to the evolutionary history of the various fungal lineages. The earliest compendium of fossil fungi is from the late 19th century (Meschinelli, 1898), and the symbiotic relationship with plants in fossils was suggested around that period (Renault, 1896), but the difficulty in the interpretation of morphological data made it impossible to actually understand what happened.

Earliest fossil with the morphological features of a fungus is dated to around 1 billion years ago, and was found in the Arctic Canada (Loron et al., 2019), and there is evidence of fungus-like organisms in fossils of around 800 Mya (Bonneville et al., 2020). Those findings are rare though, we surely have a richer diversity of fossils from the lower Devonian (around 400 Mya).

It wasn't until the large scale advent of molecular phylogenetics techniques that some light

could be properly shed on the history of fungi (James et al., 2006). From molecular clock analysis seems like fungi are sister group to animals, that is, the two lineages are close, diverging around 1.5 Billion years ago (Wang et al., 1999). The two groups form one supergroup called Opisthokonta (Cavalier-Smith, 1987), from the Greek opísthios (rear, posterior) and kontós (“pole” i.e. “flagellum”), since the group is characterized by flagellate cells that propel themselves with a single, posterior flagellum (in many cases lost).

IMG OF TREE WITH ANIMALS AND FUNGI AND PLANTS

## 2. Ancient lovers

So: fungi are animals’ cousins, and the two lineage diverged around 1.5 billion years ago. What happened then?

The ancestors of fungi are believed to be simple aquatic forms with flagellated spores, similar to members of the extant phylum Chytridiomycota (chytrids), which are now considered one of the early-diverging clade in the kingdom (James et al., 2006). The first terrestrial fungi colonized land probably before plants did (Heckman et al., 2001), as saprobe (taking nutrition out of dead matter) and/or in symbiosis with organisms capable of photosynthesis. It is commonly accepted that in order to colonize the land, plants had to develop a symbiotic relationship with fungi (Selosse and Le Tacon, 1998; Heckman et al., 2001; Bonneville et al., 2020), but it is not entirely clear whether this relationship was lichen-like or mycorrhizal-like.

IMG LICHEN

Lichens are the symbiotic relationship between a singol or more fungi (*mycobiont*) and a cyanobacteria or algae (*photobyont*). Think about an algae floating in water: for many reasons, it is not really equipped to deal with the challenges of a terrestrial life style: mainly, it won’t be able to mine substrate resources, to protect itself against dehydration, constant direct UV radiations and strong temperature fluctuations (Selosse and Le Tacon, 1998;

## 2. Ancient lovers

Blackwell, 2000). In a lichen, the photobiont is protected by the fungal stroma, and it can tolerate drought, cold, heat, intense light and barren rocky substrates. They also seem to be the first pioneers in a barren environment today, so everything would point to them being the right candidate for a first out-of-water plant-fungi symbiosis.

Yet, while this relationship evolved several times (Gargas et al., 1995), the only phyla we know that are capable of such process (called *lichenization*) are Ascomycota and, secondarily and later in time, Basidiomycota, and we can date the origin of those clades to about 400 Mya in the Devonian (Berbee and Taylor, 1993). Similarly we have fossils for lichens dating at the oldest in the Early Devonian (400 Mya) (Taylor et al., 1997; Honegger et al., 2013), while the first fossil land plants and fungi appeared 480 to 460 Mya, and molecular clock estimates suggests about 600–700 Mya (Berbee and Taylor, 1993; Heckman et al., 2001).

Therefore, lichens were likely not what opened the way to plants for land colonization. Let's look now at a mycorrhizal-like relationship.

Mycorrhiza is the symbiotic association between plants and fungi happening in the rhizosphere, that is, the plant's root system. It consist in an exchange of resources between the fungus and the plant, ideally the plant providing sugar to the fungus and the fungus providing minerals and nutrients to the plant, even though it's hard to pinpoint who's benefiting who and it may fall on different scales of the parasithic-mutualistic spectrum

What we know is that fossils resembling mycorrhizal relationships have fossil dating back to the Ordovician (with an age of about 460 million years), and are Glomales-like Arbuscular Mycorrhizal (AM hereafter) fungi, in a moment where the land flora is supposed to only consist of plants on the bryophytic level (Redecker et al., 2000). Now, plants can photosynthesize; these fungi can extract minerals from the substrate with great efficiency, protect the root system, extend the range from which water can be taken and protect the plants from pathogens. It's easy to see how this is going to end up in a passionate love story.

You could say “*wait a minute: those plants did not have true roots, how can we have a mycorrhizal relationship?*”. Good point. Fossil records provides evidence that fungal organisms entered in such symbiosis before the appearance of true roots, and as long as there is a multicellular host AM fungi are fine (Wang and Qiu, 2006; Bonfante and Genre, 2008).

Whether as lichen or as mycorrhiza, the symbiosis between plants and fungi is one of the most important, most ancient relationship in the history of living beings and it surely played

a crucial role in the successful colonization of the land by plants (Pirozynski and Malloch, 1975; Malloch et al., 1980; Harley and Harley, 1987; Trappe and Safir, 1987; Selosse and Le Tacon, 1998; Brundrett, 2002). The relationship is so beneficial (for one or both parts) that today is the norm, and is well established in c. 85% of extant plants (Cairney, 2000; Strullu-Derrien et al., 2018), with a high degree of complexity (van der Heijden et al., 2015).

### 3. Orchids

Let's move the camera away from fungi for a second. Don't worry, we'll get back to them soon enough, but now we need to introduce the second protagonist of the present work: orchids. Orchids are a diverse and widespread family of flowering plants, counting over 28,000 species in about 736 genera (Christenhusz and Byng, 2016), second only to *asteraceae* in terms of number even though they got on the scene only around 80 Mya (Ramírez et al., 2007), not very long ago. They are cosmopolitan, with a distribution spanning all continents except Antarctica and including most major island groups (Givnish et al., 2016). By the end of 2017 the IUCN Global Red List included assessments for 948 orchid species, of which 56.5% are threatened (Fay, 2018). In Europe all wild orchids are protected, being included in their entirety on Appendices I and II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CIT) as are many of the habitats they live in, and are in many countries Red List. Nonetheless, this protection has not staved off a general decline in the orchid flora of Europe (Jacquemyn et al., 2005; Kull and Hutchings, 2006). Major threats include habitat destruction and unsustainable (often illegal) harvesting, and because of their complex life histories orchids are thought to be particularly vulnerable to the effects of global environmental change (Kull et al., 2016; Gale et al., 2018)

Orchids exhibit an astonishing diversity of habitat adaptations, morphologies and pollination strategies, but some characteristics are common to the whole family. One of the most important is the reliance on Orchid Mycorrhizal Fungi (OMF hereafter) for reproduction and



### 3. Orchids

often outright survival. I told you we would soon get back to them. Orchids seeds are devoid of nutritional resources, and they completely rely on fungi for nutrition including water, minerals and carbon supply (Leake, 1994; RASMUSSEN and WHIGHAM, 1998; Merckx, 2013) in a nutritional strategy called “mycoheterotrophy”. After germination, seedlings often become autotrophic and subsequently revert to usual mycorrhizal functioning (Rasmussen, 1995; Cameron et al., 2008). Some species, especially from forest environments, remain mycoheterotrophic at adulthood though, developing partial photosynthetic capacity but still relying on fungi for carbon resources, a nutritional strategy called “mixotrophy” or “partial mycoheterotrophy” (Gebauer and Meyer, 2003; Julou et al., 2005; Selosse and Roy, 2009). Others never develop photosynthetic capacity and therefore rely completely on fungi for nutrition. This nutritional mode, which has evolved over 30 times independently in orchids, is called “obligate mycoheterotrophy” (Merckx, 2013). So: no OMFs, no orchids.

While the relationship between orchids and OMFs is known since over a century (Bernard, 1899; Rayner, 1927; Rasmussen, 2002; Selosse et al., 2011) and the mechanisms of this symbiosis are beginning to be properly understood, the knowledge from a taxonomical standpoint is still in full evolution. For many years orchids were thought to interact almost entirely with the members of the *Rhizoctonia* complex. It was later discovered not only that orchids have way more interactions with different fungi also from the *Ascomycetes* phylum, but also that *rhizoctonia* is a polyphyletic group, and was disassembled in different taxa, all members of the *Agaricomycetes*, most notably ***Sebacinales***, ***Ceratobasidiaceae*** and ***Tulasnellaceae*** (Dearnaley et al., 2012). There is also evidence of fungi from the *Ascomycota* phylum, especially in the order *Pezizales* (Selosse et al., 2004; Ouanphanivanh, 2008; Waterman et al., 2011), but they are the exception rather than the rule: the most common and known families of OMFs are in the *Basidiomycota* phylum, especially *Inocybaceae*, *Tulasnellaceae*, *Ceratobasidiaceae*, *Russulaceae*, *Sebacinaceae*, *Serendipitaceae* and *Thelephoraceae* (Taylor et al., 2004; Roy et al., 2009; Duffy et al., 2019)

#### 3.1. Distribution and ecology of OMFs

Since as we’ve seen orchids depend on OMFs for the germination of the seeds and in many cases for the nutrients also in adulthood, it is safe to assume that OMFs are everywhere orchids are. This is not the whole tale though, as we know that many OMFs can also turn to a

### 3. Orchids

soil free-living saprotrophic ecological niche (Oberwinkler et al., 2017) and form mycorrhizal relationship with plants other than orchids (Selosse and Martos, 2014), allowing them to spread to a bigger area. *Tulasnellaceae*, *Ceratobasidiaceae*, and *Sebacinales* (*Serendipitaceae* and *Sebacinaceae*) are ubiquitous, varying in their contribution to the total amount of OMFs depending on the area and in the level of specialization for the orchids. Other families, even though less common, still have a presence in most of the world (Jacquemyn et al., 2017).

This is all good and well as long as we look at the matter a broad scale. Here, the abiotic variables such as annual rainfall, soil chemistry etc. explain the distribution the best, but when we zoom at a more local level biotic factors, community composition and interactions may affect the OMFs distribution just as much, especially considering they are symbiotic organisms (Jacquemyn et al., 2017). I used *may*, as there is a lack of evidence in this regard, and many parts of the world are dramatically undersampled (e.g. all the African continent and most of the tropical areas of the planet), so conclusions are often drawn from a limited amount of very specific data. Part of the “problem” is how complex the relationships between orchids and OMFs are. They both vary in their degree of specialization, from a highly specialized to a more generalist approach (McCormick et al., 2004; Girlanda et al., 2011; van der Heijden et al., 2015), and OMFs can also turn to other ecologies, as pathogenic fungi and saprophitic free-living organisms (Veldre et al., 2013). The community question it’s critical: do OMFs from different families occur in different habitats? How much do the biotic factors impact the OMFs distribution compared to abiotic factors? Do OMFs belonging to the same family occur in different habitat? How much does is the preference for an habitat a shared characteristic for a group? Giving a partial answer to those questions, in regard to Europe, is the main aim of the present work.

## Part II.

### Materials and methods

## 4. The database

Data from bibliography regarding the distribution of OMFs in Europe was collected into a starting database. The data had to be of fungi isolated from known orchid roots, and had to be georeferenced at the very least with the name of a close enough place; also, each sample had to have a genbank accession code in order to get the sequences and do the analysis. Only sequences from well-known OMFs were considered, that is: Ceratobasidiaceae, Tulasnellaceae, Inocybaceae, Serendipitaceae, Sebacinaceae, Russulaceae and Thelephoraceae (Dearnaley et al., 2012) For each point six variables were extracted by using the ESDAC database (esd) and the World Clim database (wor): Nitrogen, Potassium and Phosphorus soil content, soil pH, minimum temperature of the coldest quarter and maximum precipitation of the wettest month. Those variables were selected because there is evidence that mycorrhizal fungi are very sensitive to nutrients in the soil: Nitrogen, Phosphorus and Potassium in high quantities (such as in eutrophicated soils because of agricultural fertilizers) have been seen to cause decline in the belowground mycorrhizal fungi species richness and cause dramatic changes in the community composition and structure (Lilleskov et al., 2002; Baar et al., 2002; Grant et al., 2011). Mycorrhizal fungi growth and community composition also seem to be influenced by the soil pH (Aarle et al., 2002; Carrino-Kyker et al., 2016), temperature and precipitation (Rillig et al., 2003). That's not all though: those variables may serve as important proxy for other conditions. Biomes and vegetation are correlated with the environmental condition, both because they change said conditions (like soil pH) and because all species have a range of tolerance. Also, human impact can often be seen by the amount of chemicals in the soil, especially close to cultivated fields. Environmental values were extracted using GDAL's `Sample Raster Values` tool (Using QGIS v. 3.16 as a GUI) and appended to the dataset

## 5. Phylogenetic analysis

In order to understand the distribution and ecology of the OMFs we need to get a better insight of their phylogenesis. The hypothesis was for sequences of the same family to be clustered together, with some doubts with the Sebaciniales as Serendipitaceae and Sebacinaceae are very close and only recently named so (?). The phylogenetic analysis were performed on the sequences deposited by the papers included in the database. The **primers** used were mainly ITS1F, ITS4, ITS3 and ITS4OF, all targeting **regions** between the 18S rRNA subunit and the 28S rRNA subunit, including the Internal Transcribed Spacers (ITS hereafter) 1 and ITS 2. Those primers were usually universal for *Basidiomycota* or in some cases more specific for *Tulasnellaceae* (like ITS4tul) or other taxa. Sequence DQ520100 from *Tremiscus helvelloides* was used as outgroup.

- Sequences were aligned using the MUSCLE algorithm (Edgar, 2004) and manually trimmed to a visually satisfying overlapping
- Ugene was used as main GUI, v. 37.0 (Okonechnikov et al., 2012)
- The Maximum Parsimony analysis was performed using TNT, v. 1.1 (tnt), using the Tree Bisection and Reconnection algorithm and with ten replics. 1000 trees were kept and a strict consensus tree was calculated. A bootstrap was performed on the tree with 200 replications to test the validity of the tree. Bootstrap values are displayed as node labels in the appendix tree
- The Bayesian analysis (MCMC) was performed using MrBayes, v. 3.2.7a (Huelsenbeck and Ronquist, 2001), using the Hasegawa-Kishino-Yano with a gamma rate heterogeneity among sites (`lset nst=2 rates=gamma;`). One million trees were generated and sampled each thousand, with four chains running. A final consensus tree was then calculated (see appendix)
- Trees were then visually edited with FigTree v. 1.4.4
- All parameters are available in the supplemental data, along with the files to reproduce the analysis.

## 6. Multivariate analysis

Before proceeding with the multivariate analysis, sequences have been clustered into Operative Taxonomic Units (**OTU** hereafter), by using *cd-hit* v. 4.8.1 (Li et al., 2001). This process yielded 210 OTUs, with the extremes of *Serendipitaceae* having two OTUs only, and *Tulasnellaceae* 52 OTUs. The database was then pivoted in a presence-absence matrix, and for further analysis it was splitted by family, so that each matrix only had all the OTUs for that single family, yielding 7 different matrices. This was necessary to test what internal variability each family has. Another matrix was obtained by grouping together all the observations from the same family, to test what the variability between the different families is. Principal Component Analysis (PCA hereafter) and Non-metric Multi Dimensional Scaling (NMDS hereafter) were performed on all the matrices by using the R package *vegan* (Dixon, 2003), to understand both how do the OTUs from different families cluster together (if they do) and what environmental factors are most relevant. For the NMDS an Euclidean distance method was used. Another matrix was obtained by using the single Families/OTUs as rows and removing the qualitative orchid species variable. This was done to understand the impact of the environmental variables only on each OTU, therefore trying to understand how different the realized niche (ie variance in the environmental variables) is between the groups.

In the PCA and NMDS we have taken into account how each OTUs presence was influenced by environmental factors, such as climate and soil conditions. By using Species Distribution Models (SDM hereafter) we also take in account the presence of other OTUs, trying to disentangle the assembly processes that lead to the community as we can observe from the data we have.

A bayesian analysis based hierarchical model of species community (HMSC) (Ovaskainen et al., 2017) was performed by using the *Hmsc* package in R, v. 3.0.9 (Tikhonov et al., 2020; hmsc-r, 2021). Three parallel chains were run, sampling every 500 results. Regression was done with a probit model (probability + unit), a non-linear model where the dependent variable can only take two variables, which was particularly apt for this dataset because of the binary nature of the presence-absence matrix. Using this framework, a plot with the species responses to environmental covariates (beta parameters) was produced, with at

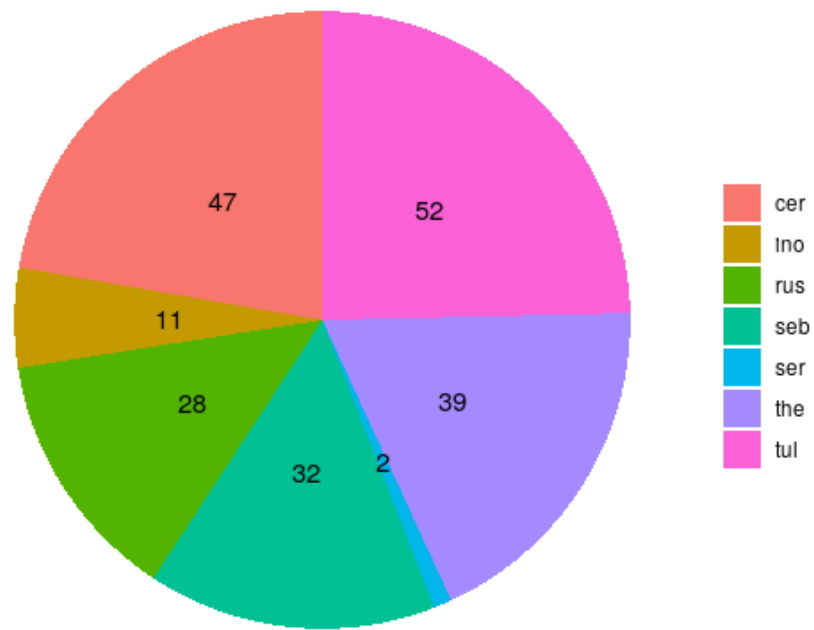


Figure 6.1.: Number of OTUs from each family

least a 85% posterior probability of being positive (red) or negative (blue). In addition, by using the presence-absence matrix, a correlation plot between the OTUs was established, looking at the positive associations with a statistical support of at least 85% shown in red and negative associations shown in blue.

## Part III.

### Results



## 7. Phylogenetic analysis

The **Bayesian analysis** yielded low probability branches. Nonetheless, it correctly put together the families, with the only notable exception of the *Serendipitaceae* and *Sebacinaceae* which were nested separately. This makes sense though, as they are both *Sebacinales* and the *serendipitaceae* were originally considered *sebacinaceae* B (Weiss et al., 2004) and were only recently given a new name and properly defined (Weiß et al., 2016) The **Maximum parsimony analysis** gave similar results, with very low bootstrap support The low quality of the trees is likely the result of the inconsistent overlap of the sequences. Many primers were used, and the resulting sequences were differently trimmed before depositing.

## 8. Multivariate analysis

The **PCA analysis** on the presence-absence matrix and the environmental variables combined showed how there is a substantial overlap of realized niche in the OTUs isolated from different orchids, with the notable exception of the Tulasnellaceae family; here we can see that the OTUs found in Ophrys are clustered separately from all the others, even though there is some overlap.

The first two components explained most of the variance, roughly 98%

The **NMDS analysis** on the single families seems to show that there is no highly relevant differentiation in the OTUs found in different orchid species, as the clustering wasn't really neat. Again, Tulasnellaceae seem to be the exception, with more distinct groups for

## 8. Multivariate analysis

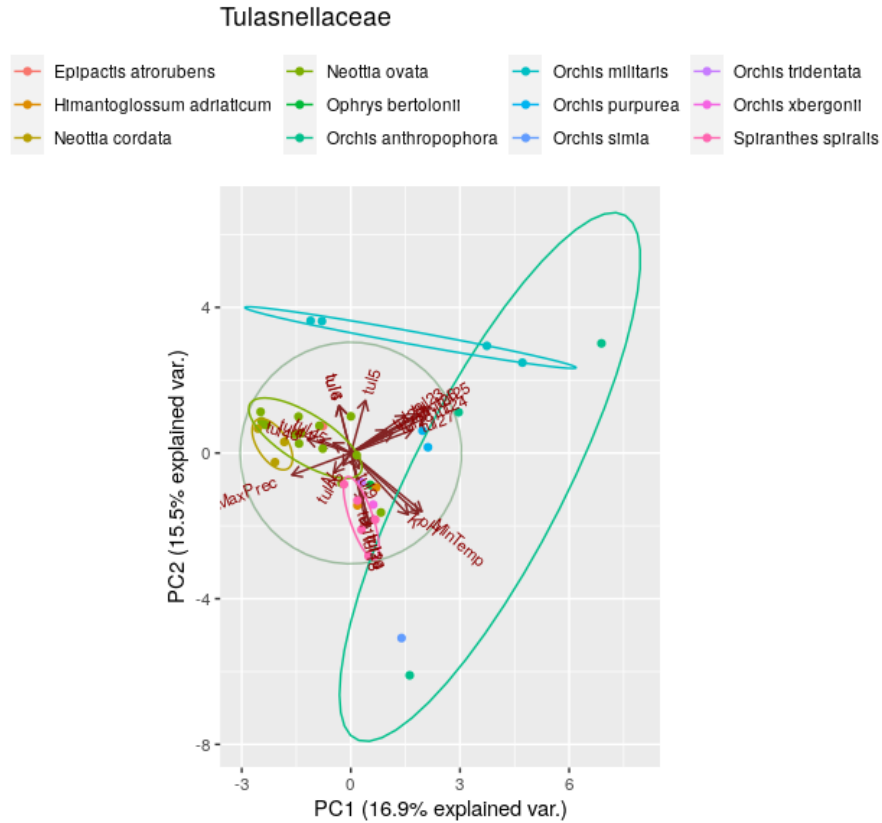
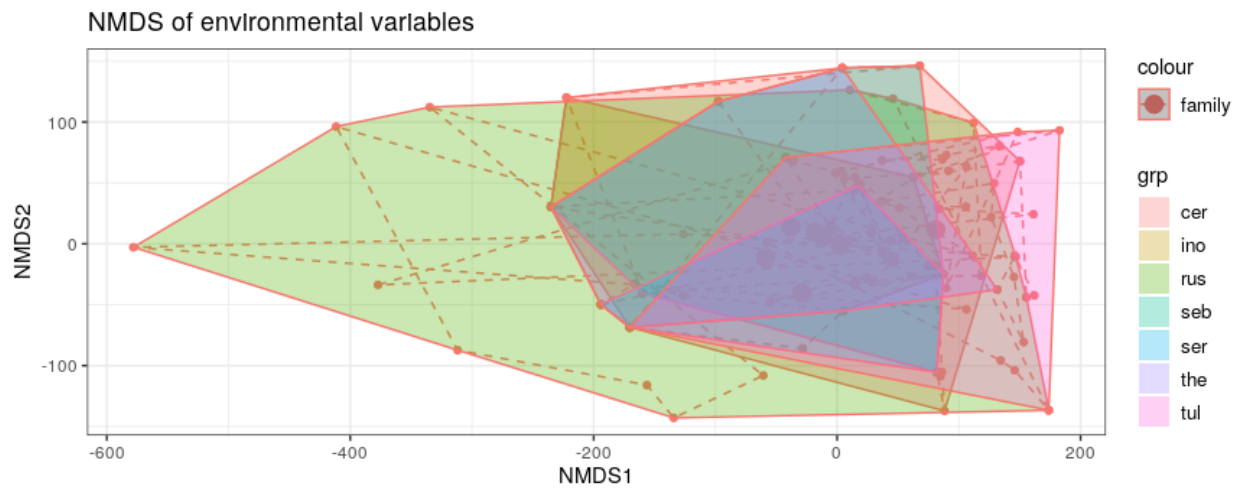


Figure 8.1.: PCA done on the Tulasnellaceae OTUs only

different orchid hosts; while this could be a bias caused by the higher number of samples, Ceratobasidiaceae and Thelephoraceae did not show this pattern even though the sample amount were roughly similar. This could point to a higher specialization of the Tulasnellaceae group, confirming previous observations (Dearnaley, 2007). The NMDS comparing the families yielded only a partial overlapping clustering, which could indicate that different orchids may have different degrees of specialization and realized niche; *Limodorum abortivum* seemed to exhibit the highest diversity, together with *Spiranthes spiralis*.

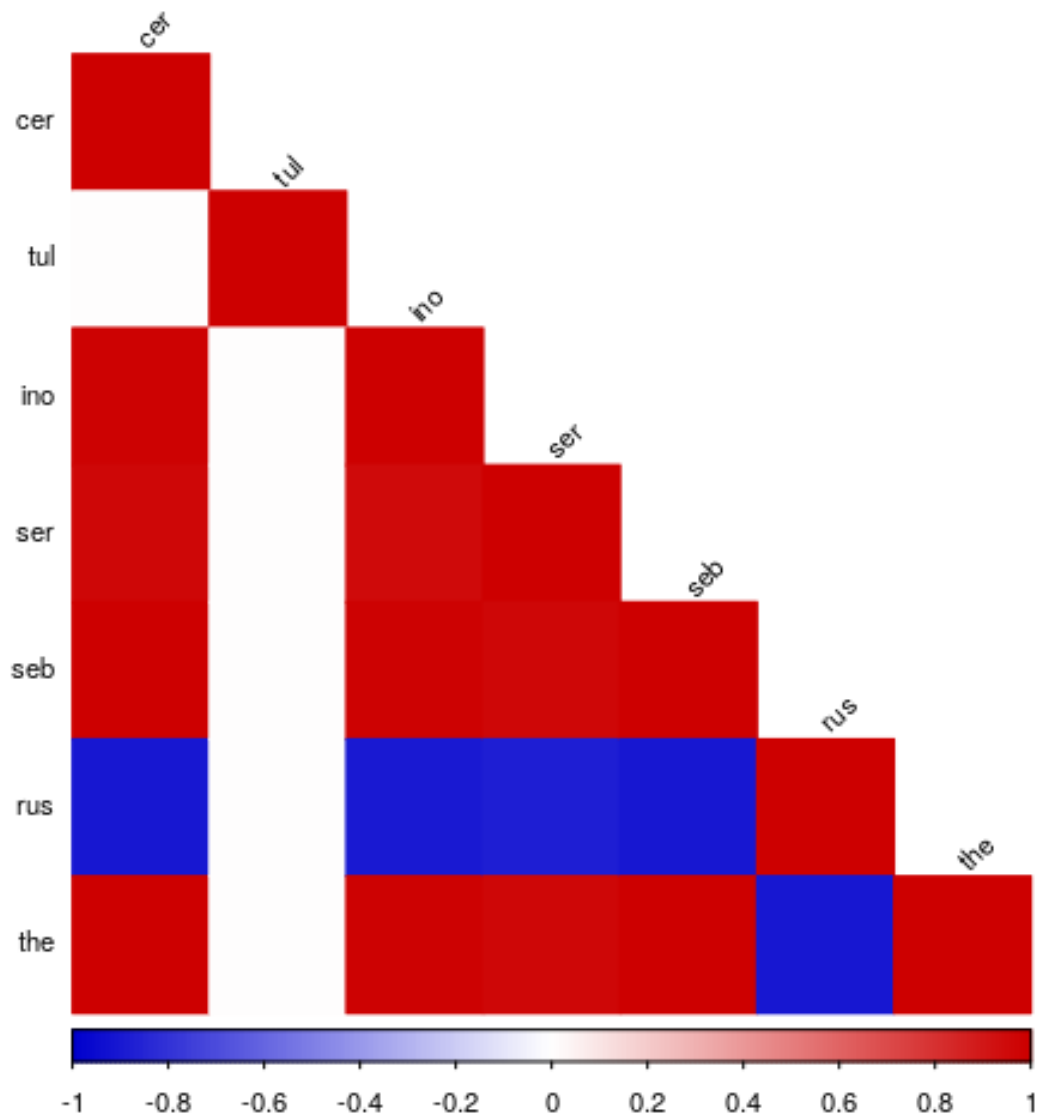
Taking the Orchid species out of the NMDS analysis and only looking at how different OMF families clustered based on the environmental conditions showed an unexpected pattern. Russulaceae seemed to have a way bigger variance, which points to a broader realized niche, compared to all other families; Tulasnellaceae, which is the most sampled and abundant OMF in the dataset, had less than half the variance and clustered in an area comparable to Sebaci-

## 8. Multivariate analysis



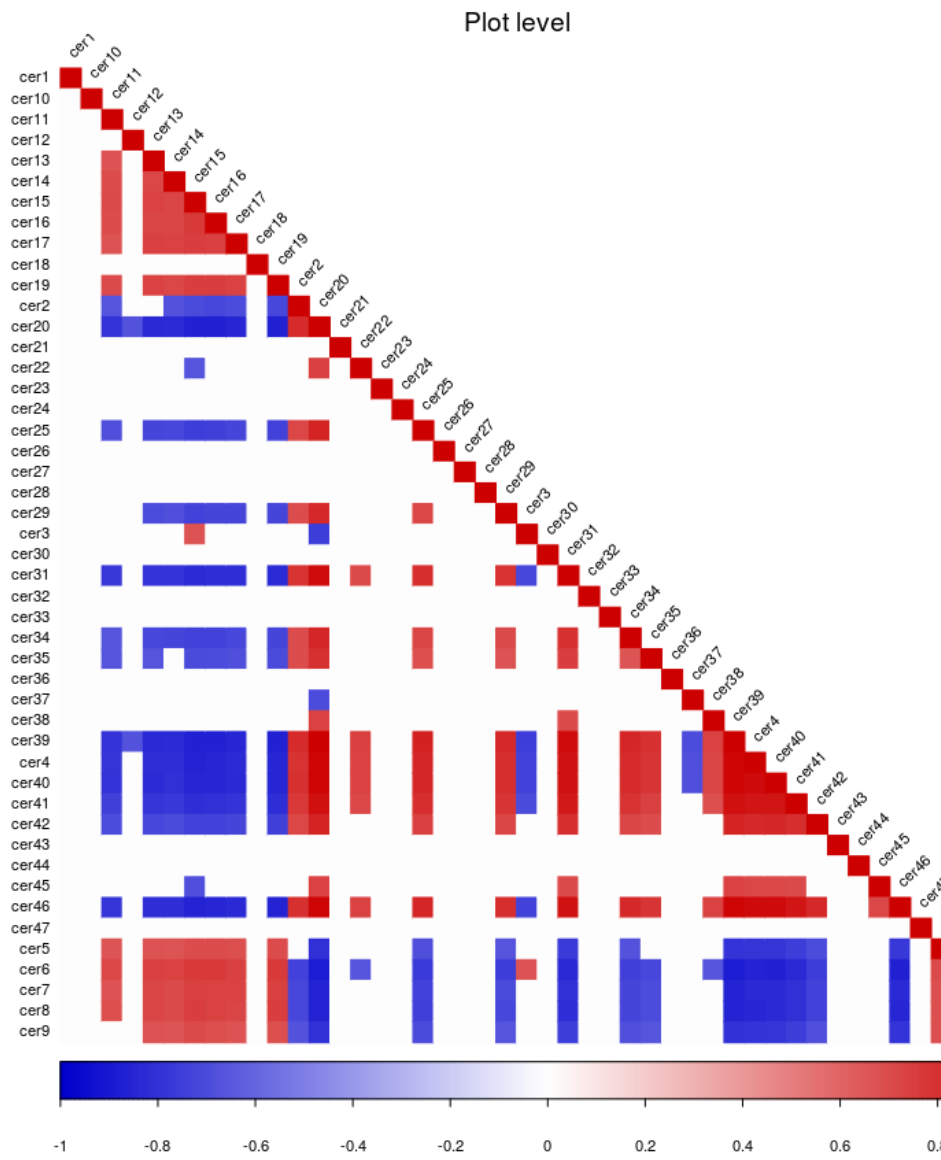
naceae.

The **HMSC** yielded basically two results. In the correlation between the families seemed like most families had a positive correlation, with two exceptions: Tulasnellaceae, who had no correlation (0) and Russulaceae, that had a negative correlation (-1). Apparently, Russulaceae not only have a bigger niche, they also don't share it. The mechanisms and reasons for such negative correlation could be interesting matter for future studies.

**All families**

OTUs from the same families seemed, on the other hand, to have no correlation with the others, positive or negative. This stands true for all families but Ceratobasidiaceae, which had more complex correlations, both positive and negative. Whether this is phylogenetically re-

## 8. Multivariate analysis



lated is to be understood.

## Part IV.

## Discussion

This could be a bias because of the higher number of samples in the dataset, repeating  
th

Part V.

Appendices



# Bibliography

Appendices — CITES. <https://cites.org/eng/app/appendices.php>.

ESDAC - European Commission. <https://esdac.jrc.ec.europa.eu/>.

TNT, a free program for phylogenetic analysis - Goloboff - 2008 - Cladistics - Wiley Online Library. <https://onlinelibrary.wiley.com/doi/full/10.1111/j.1096-0031.2008.00217.x>.

WorldClim. <https://www.worldclim.org/>.

I. M. V. Aarle, P. A. Olsson, and B. Söderström. Arbuscular mycorrhizal fungi respond to the substrate pH of their extraradical mycelium by altered growth and root colonization. *New Phytologist*, 155(1):173–182, 2002. ISSN 1469-8137. doi: 10.1046/j.1469-8137.2002.00439.x.

J. Baar, T. Bastiaans, M. van de Coevering, and J. Roelofs. Ectomycorrhizal root development in wet Alder carr forests in response to desiccation and eutrophication. *Mycorrhiza*, 12(3):147–151, 2002.

M. L. Berbee and J. W. Taylor. Dating the evolutionary radiations of the true fungi. <https://cdnsiencepub.com/doi/abs/10.1139/b93-131>, 1993.

N. Bernard. Sur la germination du *Neottia nidus-avis*. *Comptes Rendus Hebdomadaires Des Séances de l'Académie Des Sciences, Paris*, 128:1253–1255, 1899.

M. Blackwell. Terrestrial Life—Fungal from the Start? *Science*, 289(5486):1884–1885, Sept. 2000. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.289.5486.1884.

P. Bonfante and A. Genre. Plants and arbuscular mycorrhizal fungi: An evolutionary-developmental perspective. *Trends in Plant Science*, 13(9):492–498, Sept. 2008. ISSN 1360-1385. doi: 10.1016/j.tplants.2008.07.001.

S. Bonneville, F. Delpomdor, A. Préat, C. Chevalier, T. Araki, M. Kazemian, A. Steele,

# BIBLIOGRAPHY

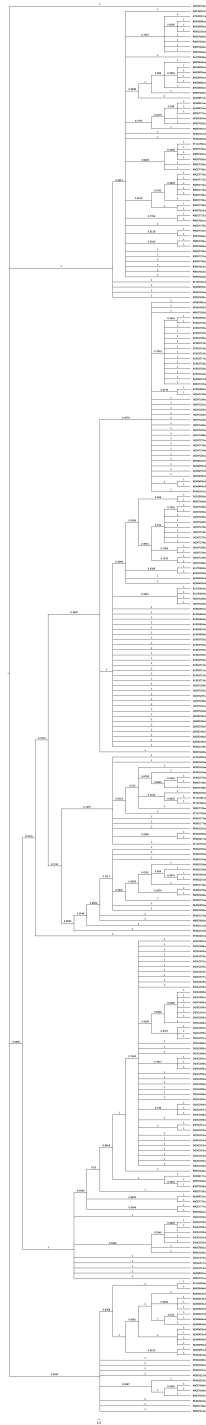


Figure 8.2.: Bayesian analysis tree

# BIBLIOGRAPHY

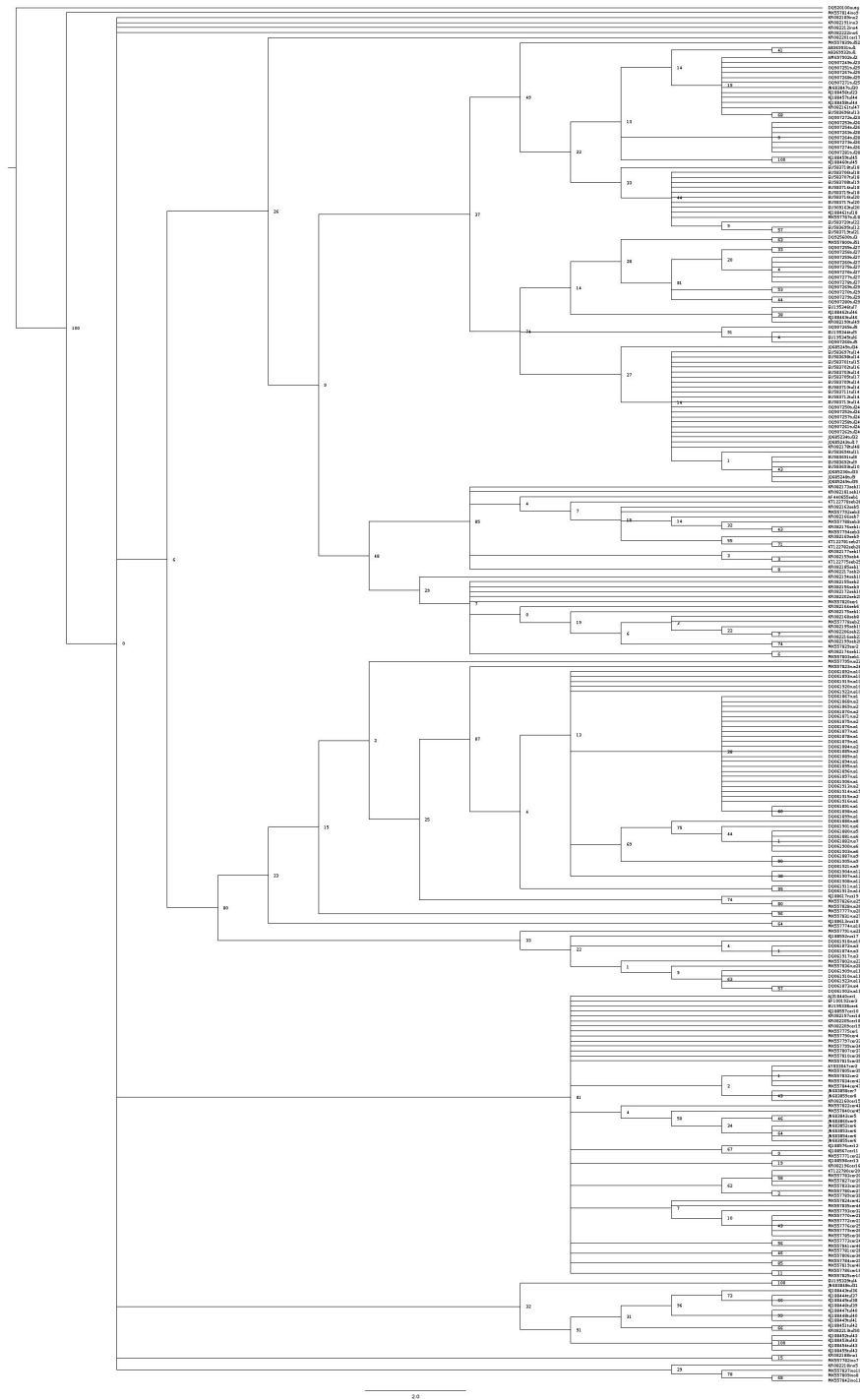


Figure 8.3.: Maximum Parsimony tree. Node labels are the bootstrap values

## BIBLIOGRAPHY

- A. Schreiber, R. Wirth, and L. G. Benning. Molecular identification of fungi microfossils in a Neoproterozoic shale rock. *Science Advances*, 6(4):eaax7599, Jan. 2020. ISSN 2375-2548. doi: 10.1126/sciadv.aax7599.
- M. C. Brundrett. Coevolution of roots and mycorrhizas of land plants. *New Phytologist*, 154(2):275–304, 2002. ISSN 1469-8137. doi: 10.1046/j.1469-8137.2002.00397.x.
- J. W. G. Cairney. Evolution of mycorrhiza systems. *Naturwissenschaften*, 87(11):467–475, 2000.
- D. D. Cameron, I. Johnson, D. J. Read, and J. R. Leake. Giving and receiving: Measuring the carbon cost of mycorrhizas in the green orchid, *Goodyera repens*. *New Phytologist*, 180(1):176–184, 2008. ISSN 1469-8137. doi: 10.1111/j.1469-8137.2008.02533.x.
- S. R. Carrino-Kyker, L. A. Kluber, S. M. Petersen, K. P. Coyle, C. R. Hewins, J. L. DeForest, K. A. Smemo, and D. J. Burke. Mycorrhizal fungal communities respond to experimental elevation of soil pH and P availability in temperate hardwood forests. *FEMS Microbiology Ecology*, 92(fiw024), Mar. 2016. ISSN 0168-6496. doi: 10.1093/femsec/fiw024.
- T. Cavalier-Smith. The origin of fungi and pseudofungi. In *Evolutionary Biology of the Fungi, British Mycological Society Symposium*, volume 12, pages 339–353. Cambridge Univ. Press, 1987.
- M. J. M. Christenhusz and J. W. Byng. The number of known plants species in the world and its annual increase. *Phytotaxa*, 261(3):201–217, May 2016. ISSN 1179-3163. doi: 10.11646/phytotaxa.261.3.1.
- H. F. Copeland. The Kingdoms of Organisms. *The Quarterly Review of Biology*, 13(4): 383–420, Dec. 1938. ISSN 0033-5770. doi: 10.1086/394568.
- H. F. Copeland. The classification of lower organisms. *The classification of lower organisms.*, 1956.
- J. D. W. Dearnaley. Further advances in orchid mycorrhizal research. *Mycorrhiza*, 17(6): 475–486, Sept. 2007. ISSN 1432-1890. doi: 10.1007/s00572-007-0138-1.
- J. D. W. Dearnaley, F. Martos, and M.-A. Selosse. 12 Orchid Mycorrhizas: Molecular Ecology, Physiology, Evolution and Conservation Aspects. In B. Hock, editor, *Fungal*

## BIBLIOGRAPHY

- Associations*, pages 207–230. Springer Berlin Heidelberg, Berlin, Heidelberg, 2012. ISBN 978-3-642-30825-3 978-3-642-30826-0. doi: 10.1007/978-3-642-30826-0\_12.
- P. Dixon. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, 14(6):927–930, 2003. ISSN 1654-1103. doi: 10.1111/j.1654-1103.2003.tb02228.x.
- K. J. Duffy, M. Waud, B. Schatz, T. Petanidou, and H. Jacquemyn. Latitudinal variation in mycorrhizal diversity associated with a European orchid. *Journal of Biogeography*, 46(5):968–980, 2019. ISSN 1365-2699. doi: 10.1111/jbi.13548.
- R. C. Edgar. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research*, 32(5):1792–1797, 2004.
- M. F. Fay. Orchid conservation: How can we meet the challenges in the twenty-first century? *Botanical Studies*, 59(1):16, June 2018. ISSN 1999-3110. doi: 10.1186/s40529-018-0232-z.
- S. W. Gale, G. A. Fischer, P. J. Cribb, and M. F. Fay. Orchid conservation: Bridging the gap between science and practice. *Botanical Journal of the Linnean Society*, 186(4):425–434, Mar. 2018. ISSN 0024-4074. doi: 10.1093/botlinnean/boy003.
- A. Gargas, P. T. DePriest, M. Grube, and A. Tehler. Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. *Science*, 268(5216):1492–1495, June 1995. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.7770775.
- G. Gebauer and M. Meyer.  $^{15}\text{N}$  and  $^{13}\text{C}$  natural abundance of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytologist*, 160(1):209–223, 2003. ISSN 1469-8137. doi: 10.1046/j.1469-8137.2003.00872.x.
- M. Girlanda, R. Segreto, D. Cafasso, H. T. Liebel, M. Rodda, E. Ercole, S. Cozzolino, G. Gebauer, and S. Perotto. Photosynthetic Mediterranean meadow orchids feature partial mycoheterotrophy and specific mycorrhizal associations. *American Journal of Botany*, 98(7):1148–1163, 2011. ISSN 1537-2197. doi: 10.3732/ajb.1000486.
- T. J. Givnish, D. Spalink, M. Ames, S. P. Lyon, S. J. Hunter, A. Zuluaga, A. Doucette, G. G. Caro, J. McDaniel, M. A. Clements, M. T. K. Arroyo, L. Endara, R. Kriebel, N. H. Williams, and K. M. Cameron. Orchid historical biogeography, diversification, Antarctica and the paradox of orchid dispersal. *Journal of Biogeography*, 43(10):1905–1916, 2016. ISSN 1365-2699. doi: 10.1111/jbi.12854.

## BIBLIOGRAPHY

- C. Grant, S. Bittman, M. Montreal, C. Plenchette, and C. Morel. Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal of Plant Science*, Mar. 2011. doi: 10.4141/P03-182.
- J. L. Harley and E. L. Harley. A Check-List of Mycorrhiza in the British Flora. *The New Phytologist*, 105(2):1–102, 1987. ISSN 0028-646X.
- D. S. Heckman, D. M. Geiser, B. R. Eidell, R. L. Stauffer, N. L. Kardos, and S. B. Hedges. Molecular Evidence for the Early Colonization of Land by Fungi and Plants. *Science*, 293(5532):1129–1133, Aug. 2001. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1061457.
- hmsc-r. Hmsc-r/HMSC, Jan. 2021.
- R. Honegger, D. Edwards, and L. Axe. The earliest records of internally stratified cyanobacterial and algal lichens from the Lower Devonian of the Welsh Borderland. *New Phytologist*, 197(1):264–275, 2013. ISSN 1469-8137. doi: 10.1111/nph.12009.
- J. P. Huelsenbeck and F. Ronquist. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17(8):754–755, 2001.
- H. Jacquemyn, R. Brys, M. Hermy, and J. H. Willems. Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and the Netherlands. *Biological Conservation*, 121(2):257–263, Jan. 2005. ISSN 0006-3207. doi: 10.1016/j.biocon.2004.05.002.
- H. Jacquemyn, K. J. Duffy, and M.-A. Selosse. Biogeography of Orchid Mycorrhizas. In L. Tedersoo, editor, *Biogeography of Mycorrhizal Symbiosis*, Ecological Studies, pages 159–177. Springer International Publishing, Cham, 2017. ISBN 978-3-319-56363-3. doi: 10.1007/978-3-319-56363-3-8.
- T. Y. James, F. Kauff, C. L. Schoch, P. B. Matheny, V. Hofstetter, C. J. Cox, G. Celio, C. Gueidan, E. Fraker, J. Miadlikowska, H. T. Lumbsch, A. Rauhut, V. Reeb, A. E. Arnold, A. Amtoft, J. E. Stajich, K. Hosaka, G.-H. Sung, D. Johnson, B. O’Rourke, M. Crockett, M. Binder, J. M. Curtis, J. C. Slot, Z. Wang, A. W. Wilson, A. Schüßler, J. E. Longcore, K. O’Donnell, S. Mozley-Standridge, D. Porter, P. M. Letcher, M. J. Powell, J. W. Taylor, M. M. White, G. W. Griffith, D. R. Davies, R. A. Humber, J. B. Morton, J. Sugiyama, A. Y. Rossman, J. D. Rogers, D. H. Pfister, D. Hewitt, K. Hansen, S. Hambleton, R. A. Shoemaker, J. Kohlmeyer, B. Volkmann-Kohlmeyer, R. A. Spotts, M. Serdani, P. W.

## BIBLIOGRAPHY

- Crous, K. W. Hughes, K. Matsuura, E. Langer, G. Langer, W. A. Untereiner, R. Lücking, B. Büdel, D. M. Geiser, A. Aptroot, P. Diederich, I. Schmitt, M. Schultz, R. Yahr, D. S. Hibbett, F. Lutzoni, D. J. McLaughlin, J. W. Spatafora, and R. Vilgalys. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature*, 443(7113):818–822, Oct. 2006. ISSN 1476-4687. doi: 10.1038/nature05110.
- T. Julou, B. Burghardt, G. Gebauer, D. Berveiller, C. Damesin, and M.-A. Selosse. Mixotrophy in orchids: Insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. *New Phytologist*, 166(2):639–653, 2005. ISSN 1469-8137. doi: 10.1111/j.1469-8137.2005.01364.x.
- T. Kull and M. J. Hutchings. A comparative analysis of decline in the distribution ranges of orchid species in Estonia and the United Kingdom. *Biological Conservation*, 129(1): 31–39, Apr. 2006. ISSN 0006-3207. doi: 10.1016/j.biocon.2005.09.046.
- T. Kull, U. Selgis, M. V. Peciña, M. Metsare, A. Ilves, K. Tali, K. Sepp, K. Kull, and R. P. Shefferson. Factors influencing IUCN threat levels to orchids across Europe on the basis of national red lists. *Ecology and Evolution*, 6(17):6245–6265, 2016. ISSN 2045-7758. doi: 10.1002/ece3.2363.
- J. R. Leake. The biology of myco-heterotrophic (‘saprophytic’) plants. *New Phytologist*, 127(2):171–216, 1994. ISSN 1469-8137. doi: 10.1111/j.1469-8137.1994.tb04272.x.
- W. Li, L. Jaroszewski, and A. Godzik. Clustering of highly homologous sequences to reduce the size of large protein databases. *Bioinformatics (Oxford, England)*, 17(3):282–283, Mar. 2001. ISSN 1367-4803. doi: 10.1093/bioinformatics/17.3.282.
- E. A. Lilleskov, T. J. Fahey, T. R. Horton, and G. M. Lovett. Belowground Ectomycorrhizal Fungal Community Change Over a Nitrogen Deposition Gradient in Alaska. *Ecology*, 83(1):104–115, 2002. ISSN 1939-9170. doi: 10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2.
- C. C. Loron, C. François, R. H. Rainbird, E. C. Turner, S. Borensztajn, and E. J. Javaux. Early fungi from the Proterozoic era in Arctic Canada. *Nature*, 570(7760):232–235, June 2019. ISSN 1476-4687. doi: 10.1038/s41586-019-1217-0.
- D. W. Malloch, K. A. Pirozynski, and P. H. Raven. Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants (A Review). *Proceedings of the Na-*

## BIBLIOGRAPHY

- tional Academy of Sciences*, 77(4):2113–2118, Apr. 1980. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.77.4.2113.
- M. K. McCormick, D. F. Whigham, and J. O’Neill. Mycorrhizal diversity in photosynthetic terrestrial orchids. *New Phytologist*, 163(2):425–438, 2004. ISSN 1469-8137. doi: 10.1111/j.1469-8137.2004.01114.x.
- V. S. Merckx. Mycoheterotrophy: An introduction. In *Mycoheterotrophy*, pages 1–17. Springer, 2013.
- L. Meschinelli. *Fungorum Fossilium Omnium: Hucusque Cognitorum Iconographia*. Sumptibus auctoris, typis Aloysii Fabris, 1898.
- F. Oberwinkler, D. Cruz, and J. P. Suárez. Biogeography and Ecology of Tulasnellaceae. In L. Tedersoo, editor, *Biogeography of Mycorrhizal Symbiosis*, Ecological Studies, pages 237–271. Springer International Publishing, Cham, 2017. ISBN 978-3-319-56363-3. doi: 10.1007/978-3-319-56363-3\_12.
- K. Okonechnikov, O. Golosova, M. Fursov, and the UGENE team. Unipro UGENE: A unified bioinformatics toolkit. *Bioinformatics*, 28(8):1166–1167, Apr. 2012. ISSN 1367-4803. doi: 10.1093/bioinformatics/bts091.
- N. Ouanphanivanh. Could orchids indicate truffle habitats? : Mycorrhizal association between orchids and truffles. *Acta Biologica Szegediensis*, 52(1):229–232, Jan. 2008. ISSN 1588-4082.
- O. Ovaskainen, G. Tikhonov, A. Norberg, F. G. Blanchet, L. Duan, D. Dunson, T. Roslin, and N. Abrego. How to make more out of community data? A conceptual framework and its implementation as models and software. *Ecology Letters*, 20(5):561–576, 2017. ISSN 1461-0248. doi: 10.1111/ele.12757.
- K. A. Pirozynski and D. W. Malloch. The origin of land plants: A matter of mycotrophism. *Biosystems*, 6(3):153–164, Mar. 1975. ISSN 0303-2647. doi: 10.1016/0303-2647(75)90023-4.
- S. R. Ramírez, B. Gravendeel, R. B. Singer, C. R. Marshall, and N. E. Pierce. Dating the origin of the Orchidaceae from a fossil orchid with its pollinator. *Nature*, 448(7157):1042–1045, Aug. 2007. ISSN 1476-4687. doi: 10.1038/nature06039.



## BIBLIOGRAPHY

- H. N. Rasmussen. *Terrestrial Orchids: From Seed to Mycotrophic Plant*. Cambridge University Press, Aug. 1995. ISBN 978-0-521-45165-9.
- H. N. Rasmussen. Recent developments in the study of orchid mycorrhiza. *Plant and Soil*, 244(1/2):149–163, 2002. ISSN 0032-079X.
- H. N. RASMUSSEN and D. F. WHIGHAM. The underground phase: A special challenge in studies of terrestrial orchid populations. *Botanical Journal of the Linnean Society*, 126(1-2):49–64, Jan. 1998. ISSN 0024-4074. doi: 10.1111/j.1095-8339.1998.tb02515.x.
- M. C. Rayner. Mycorrhiza. An account of non-pathogenic infection by fungi in vascular plants and Bryophytes. *Mycorrhiza. An account of non-pathogenic infection by fungi in vascular plants and Bryophytes.*, (No. 15), 1927.
- D. Redecker, R. Kodner, and L. E. Graham. Glomalean Fungi from the Ordovician. *Science*, 289(5486):1920–1921, Sept. 2000. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.289.5486.1920.
- B. Renault. *Bassin Houiller et Permien d’Autun et d’Épinac: Flore Fossile, 2. Partie. Fascicule 4*, volume 4. Imprimerie nationale, 1896.
- M. C. Rillig, K. K. Treseder, and M. F. Allen. Global Change and Mycorrhizal Fungi. In M. G. A. van der Heijden and I. R. Sanders, editors, *Mycorrhizal Ecology*, Ecological Studies, pages 135–160. Springer, Berlin, Heidelberg, 2003. ISBN 978-3-540-38364-2. doi: 10.1007/978-3-540-38364-2.6.
- M. Roy, T. Yagame, M. Yamato, K. Iwase, C. Heinz, A. Faccio, P. Bonfante, and M.-A. Selsosse. Ectomycorrhizal *Inocybe* species associate with the mycoheterotrophic orchid *Epipogium aphyllum* but not its asexual propagules. *Annals of Botany*, 104(3):595–610, Aug. 2009. ISSN 0305-7364. doi: 10.1093/aob/mcn269.
- M.-A. Selsosse and F. Le Tacon. The land flora: A phototroph-fungus partnership? *Trends in Ecology & Evolution*, 13(1):15–20, Jan. 1998. ISSN 0169-5347. doi: 10.1016/S0169-5347(97)01230-5.
- M.-A. Selsosse and F. Martos. Do chlorophyllous orchids heterotrophically use mycorrhizal fungal carbon? *Trends in Plant Science*, 19(11):683–685, Nov. 2014. ISSN 1360-1385. doi: 10.1016/j.tplants.2014.09.005.

## BIBLIOGRAPHY

- M.-A. Selosse and M. Roy. Green plants that feed on fungi: Facts and questions about mixotrophy. *Trends in Plant Science*, 14(2):64–70, Feb. 2009. ISSN 1360-1385. doi: 10.1016/j.tplants.2008.11.004.
- M.-A. Selosse, A. Faccio, G. Scappaticci, and P. Bonfante. Chlorophyllous and Achlorophyllous Specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) Are Associated with Ectomycorrhizal Septomycetes, including Truffles. *Microbial Ecology*, 47(4), Apr. 2004. ISSN 0095-3628, 1432-184X. doi: 10.1007/s00248-003-2034-3.
- M.-A. Selosse, B. Boullard, and D. Richardson. Noël Bernard (1874–1911): Orchids to symbiosis in a dozen years, one century ago. *Symbiosis*, 54(2):61–68, June 2011. ISSN 1878-7665. doi: 10.1007/s13199-011-0131-5.
- C. Strullu-Derrien, M.-A. Selosse, P. Kenrick, and F. M. Martin. The origin and evolution of mycorrhizal symbioses: From palaeomycology to phylogenomics. *New Phytologist*, 220(4):1012–1030, 2018. ISSN 1469-8137. doi: 10.1111/nph.15076.
- D. L. Taylor, T. D. Bruns, and S. A. Hodges. Evidence for mycorrhizal races in a cheating orchid. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1534):35–43, Jan. 2004. doi: 10.1098/rspb.2003.2557.
- T. N. Taylor, H. Hass, and H. Kerp. A cyanolichen from the Lower Devonian Rhynie chert. *American Journal of Botany*, 84(7):992–1004, 1997. ISSN 1537-2197. doi: 10.2307/2446290.
- G. Tikhonov, Ø. H. Opedal, N. Abrego, A. Lehtikainen, M. M. J. de Jonge, J. Oksanen, and O. Ovaskainen. Joint species distribution modelling with the r-package Hmsc. *Methods in Ecology and Evolution*, 11(3):442–447, 2020. ISSN 2041-210X. doi: 10.1111/2041-210X.13345.
- J. M. Trappe and G. R. Safir. Ecophysiology of VA mycorrhizal plants. 1987.
- M. G. A. van der Heijden, F. M. Martin, M.-A. Selosse, and I. R. Sanders. Mycorrhizal ecology and evolution: The past, the present, and the future. *New Phytologist*, 205(4):1406–1423, 2015. ISSN 1469-8137. doi: 10.1111/nph.13288.
- V. Veldre, K. Abarenkov, M. Bahram, F. Martos, M.-A. Selosse, H. Tamm, U. Kõljalg, and L. Tedersoo. Evolution of nutritional modes of Ceratobasidiaceae (Cantharellales,

## BIBLIOGRAPHY

- Basidiomycota) as revealed from publicly available ITS sequences. *Fungal Ecology*, 6(4): 256–268, Aug. 2013. ISSN 1754-5048. doi: 10.1016/j.funeco.2013.03.004.
- B. Wang and Y.-L. Qiu. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, 16(5):299–363, July 2006. ISSN 1432-1890. doi: 10.1007/s00572-005-0033-6.
- D. Y. Wang, S. Kumar, and S. B. Hedges. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proceedings of the Royal Society B: Biological Sciences*, 266(1415):163–171, Jan. 1999. ISSN 0962-8452.
- R. J. Waterman, M. I. Bidartondo, J. Stofberg, J. K. Combs, G. Gebauer, V. Savolainen, T. G. Barraclough, and A. Pauw. The Effects of Above- and Belowground Mutualisms on Orchid Speciation and Coexistence. *The American Naturalist*, 177(2):E54–E68, Feb. 2011. ISSN 0003-0147. doi: 10.1086/657955.
- M. Weiss, M.-A. Selosse, K.-H. Rexer, A. Urban, and F. Oberwinkler. Sebaciniales: A hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential\* \*Part 221 of the series Studies in Heterobasidiomycetes from the Botanical Institute, University of Tübingen, Tübingen. *Mycological Research*, 108(9):1003–1010, Sept. 2004. ISSN 0953-7562. doi: 10.1017/S0953756204000772.
- M. Weiß, F. Waller, A. Zuccaro, and M.-A. Selosse. Sebaciniales – one thousand and one interactions with land plants. *New Phytologist*, 211(1):20–40, 2016. ISSN 1469-8137. doi: 10.1111/nph.13977.
- R. H. Whittaker. New concepts of kingdoms of organisms. *Science*, 163(3863):150–160, 1969.