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Leho Tedersoo *Editor*

Biogeography of Mycorrhizal Symbiosis

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Preface

The distribution of organisms has fascinated scientists and naturalists since the times of Karl Linné (1707–1778), Alexander von Humboldt (1769–1859), Charles Darwin (1809–1882) and Alfred Wallace (1823–1913), who can be regarded as the founding fathers of biogeography. These brilliant scientists and their followers published thousands of studies on the distribution patterns of macroscopic organisms such as plants and animals and, to some extent, macroscopic fungi. Until the late 1990s, information about the distribution of microscopic information was extremely scant and the understanding was often biased due to the inability to distinguish biological species. The advent of molecular DNA-based identification in 1990s and development of high-throughput methods in late 2000s enabled, for the first time, to shed light on the ecology and biogeography of microorganisms. A vast majority of these studies concerned bacteria and other free-living microorganisms, either providing support or conflicting counterevidence to the hypothesis of Lourens Baas Becking (1895–1963) that 'Everything is everywhere, but, the environment selects'). Because mycorrhizal symbiosis is essential for plant mineral nutrition and ecosystem nutrient cycling and the distribution of fungal symbionts is determined by the host plant, this fragmented knowledge motivated me to compile recent state-of-the-art information about the biogeographic aspects of fungi and their plant associations. This has been my topic of curriculum and field of research since 2001, which provided me a good position for such a challenge.

My initial idealistic view about the book was to cover all topics that are related to the biogeography of mycorrhiza, from definitions of the mycorrhizal groups and methods, to local processes such as assembly rules, dispersal mechanisms, means of reproduction and gene flow, to global patterns including climate effects, biogeography of representative groups, macroecology as well as global overviews and syntheses. Although a few renowned researchers declined to contribute, around 80% of the planned chapters were covered and a majority of these go beyond the state of the art in our current knowledge or provide completely novel insights into the biogeographic aspects of mycorrhizal plants and fungi. Hereby I also express my sincere gratitude to all expert reviewers (2–5 for each chapter), who readily

accepted the refereeing task and greatly helped to improve most chapters anonymously or non-anonymously.

The book is arranged so that it starts with overviews of methods and local processes, continues with regional and global-scale reviews and meta-analyses of specific groups and ends with more general syntheses in macroecology. Although all above-described chapters represent separate reviews, syntheses, extended case studies or a combination of these, many of the chapters synergistically add complementary or additive information to our overall knowledge. Chapter 1 provides an excellent overview of the most up-to-date methods in biogeography and phylogeography with several novel examples based on the poison-cap (*Amanita*) mushrooms. Chapters 2 and 3, respectively, review the current information about population ecology and dispersal mechanisms of ectomycorrhizal fungi and collectively indicate that the means of dispersal of fungi have a strong effect on the fungal population genetics, which has a major influence on population ecology, speciation and large-scale biogeographic patterns. Chapter 4 gives a novel synthesis about the mechanisms of coexistence among ectomycorrhizal symbionts on a fine scale, pointing to the particular importance of host plants. Chapter 5 reviews the mechanisms driving fungal diversity and composition of all mycorrhiza types from landscape to regional scale, indicating context-dependent latitudinal effects. These two chapters indicate that the main drivers of diversity and composition of EcM fungi differ greatly across the geographic scale, with increasing importance of host, edaphic, climatic and historical factors at larger scales. Chapter 6 is solely focused on ectomycorrhizal fungi, revealing several novel phylogenetic lineages and providing instructions for their high-throughput sequencing-based identification. Chapter 7 reviews the distribution of arbuscular mycorrhizal fungi and presents a novel global-scale niche analysis of the most common taxa in the context of species recognition. Chapters 8 and 9 provide timely reviews of the global distribution of orchid mycorrhiza and ericoid mycorrhiza, respectively, pointing to the gaps in knowledge and urgent research needs. Chapter 10 reviews the current state of knowledge about the ecology and biogeography of non-mycorrhizal root endophytes. The three latter chapters provide evidence that biogeographic patterns of ericoid mycorrhizal fungi, arbuscular mycorrhizal fungi and root endophytes have a lot in common in overall biogeographic patterns that, taken together, differ from ectomycorrhizal fungi as based on previous studies. Chapter 11 presents an overview of the global distribution of studies that determine mycorrhizal status and reviews recent knowledge about factors underlying the level of mycorrhizal colonization on a global scale. Chapter 12 strongly complements with Chap. 9 by providing an overview of the taxonomy, ecology and biogeography of the enigmatic basidiomycete genus *Tulasnella*, the main orchid root symbiont. Chapters 13, 14 and 15 focus on the biogeography, recognition of species and population ecology of the ectomycorrhizal fungi from the genus *Laccaria*, the asexual *Cenococcum geophilum* complex and the gourmet mushroom *Tricholoma matsutake*, respectively. Together with Chap. 1 and some recent overviews, these three chapters indicate that phylogenetic history and diversification patterns may strongly differ among ectomycorrhizal fungal groups that are potentially related to ecological

conditions, historical origin and reproductive biology. Chapter 16 describes the low mycobiont diversity and biogeography of a stress-tolerant tropical tree *Coccoloba uvifera* in the Caribbean basin and reveals several events of historical fungal host shifts from North American trees. Chapter 17 reviews the distribution of mycorrhizal types and alternative root nutritional strategies in Australia in a phylogenetic and historical perspective, pinpointing to multiple Australian plant groups that may exhibit hitherto overlooked mycorrhiza-like fungus–plant root associations. Chapter 18 presents a reanalysis of global diversity of ectomycorrhizal fungi from the perspective of dark diversity, species pool and community completeness, indicating the additional value of these alternative measures of diversity. Chapters 19 and 20 define the evolutionary lineages of ectomycorrhizal plant groups and illustrate their historical and present-day distribution patterns. Furthermore, Chap. 20 synthesizes the invasion potential of ectomycorrhizal plants considering probable global change scenarios. Finally, Chap. 21 provides a timely update about the principal definition and global distribution of mycorrhizal types and non-mycorrhizal plants in relation to habitat conditions and plant life form, with a strong additional focus on mycorrhiza misdiagnosis issues.

Taken together, this book provides a comprehensive overview of the distribution patterns of all mycorrhizal types, with individual contributions seeking to explain the underlying causes such as differences in dispersal mechanisms and phylogenetic and historical constraints. As major novelties, the book describes best practices and novel methods in biogeographic and phylogeographic studies (Chap. 1) and sets standards to the overall definition and interpretation of mycorrhizal symbiosis in plants (Chap. 21), including the novel treatment of ectomycorrhizal plant species in phylogenetically defined groups (Chap. 19), as well as revising this information for mycorrhizal fungi (Chap. 6).

The reviews and syntheses covered in this book open new perspectives in plant and fungal ecology and biogeography. Information about the mycorrhizal habit of plants enables construction of global distribution maps of mycorrhizal symbiosis when integrated with data about vegetation structure and density. Furthermore, knowledge about the distribution of mycorrhizal plants and fungi and their putative functional capacities allows modelling of soil processes in order to understand the role of mycorrhizal types in determining patterns of carbon and soil nutrient cycling from landscape to global scale and evaluating the shifts in these mycorrhiza-mediated processes under global change. The so far missing information about the driving forces of population dynamics, intraspecific and interspecific competition and community assembly rules would greatly improve our understanding of spatial and temporal turnover in fungal communities in the evolutionary perspective. Due to striking differences in ecophysiology and reproductive biology among fungi representing different mycorrhizal types, the evolutionary and biogeographic processes may differ greatly among these groups of fungi and urgently warrant further fundamental research.

Tartu, Estonia Leho Tedersoo

Contents

Chapter 1
Overview of Phylogenetic Approaches to Mycorrhizal Biogeography, Diversity and Evolution

Santiago Sánchez-Ramírez, Andrew W. Wilson, and Martin Ryberg

1.1 Introduction

For more than two centuries biologists have been interested in understanding the distribution of biodiversity. Following the work of Agustin Pyramus de Candolle and Alexander von Humboldt in the eighteenth century, biogeography has changed from being a merely descriptive discipline to a field rooted in ecological and evolutionary principles (Crisci et al. 2003). Biogeography has now diversified into many branches that specialize on different spatial, temporal, and taxonomic scales, but can be classified into two major categories known as *ecological* and *historical* biogeography (Wiens and Donoghue 2004). For historical biogeography (from here on just termed biogeography), the last decades of the twentieth century witnessed paradigm shifts between dispersal and vicariance schools (Zink et al. 2000). Nowadays, it is generally accepted that multiple evolutionary processes such as dispersal, speciation, extinction, and species interactions contribute to biodiversity build-up and distribution (Hubbell 2001; Ricklefs 2004; Wiens and Donoghue 2004; Mittelbach et al. 2007; Ree and Sanmartín 2009; Ronquist and Sanmartín 2011; Birand et al. 2012).

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Undoubtedly, the bulk biogeographic knowledge has garnered around the study of plant and animal distribution. In contrast, patterns in microorganisms (fungi included) have been more elusive. This has led to considerable debate on how microorganisms disperse and are structured geographically (Finlay 2002; Martiny et al. 2006; Peay et al. 2007, 2010a). For instance, a classic view in microbial biogeography is that "everything is everywhere, but the environment selects" (Baas-Becking 1934). This hypothesis is based on two major assumptions. One is that many microorganisms have dispersal capabilities (e.g. vegetative reproduction and massive spore production) that allow propagules to be present "virtually" everywhere (Stolp 1988). This perception may be confounded with the fact that most microorganisms have simple morphologies, suggesting they are "cosmopolitan", when, in fact, there are many different species (Finlay 2002; Peay et al. 2010a). The second is the role of the environment as a selective filter during colonization, which may limit the establishment of propagules in new regions. This last point can relate to geographical bonds that many microorganisms have with their hosts (Werren et al. 1995; Corby-Harris et al. 2007), in spite of their potential for global propagation (Brown and Hovmøller 2002). While this hypothesis would provide a simple test to assess the mechanisms behind microbial geographical structure, their cryptic nature is a complicating factor.

In the last three decades, the study of fungal ecology and evolution has experienced a revolution after the introduction and advancement of molecular tools (Horton and Bruns 2001; Bruns and Shefferson 2004; Peay et al. 2008). DNA-based analyses provide a means to overcome the "micro" dimension, making relevant biological units quantifiable. For instance, environmental meta-barcoding can reveal diversity that is unobservable to the naked eye. Similarly, molecular phylogenetics can help understand evolutionary relationships between observable and unobservable diversity, enabling the exploration of microbial diversity dynamics in both temporal and spatial scales.

Fungi are among the most diverse organisms on Earth. Not only accounting for the thousands of described species or the millions of missing ones, but also referring to the vast complexity of ecological interactions above- and below-ground (Hawksworth 2001; O'Brien et al. 2005; Mueller et al. 2007; Blackwell 2011; Tedersoo et al. 2014b). The mycorrhizal symbiosis is one of the most common forms of mutualistic relationships in nature. Plant, fungal, and bacterial partners interact in intricate ways in the rhizosphere contributing in large extent to nutrient recycling and carbon sequestration (Smith and Read 2010; Bonfante and Genre 2010). Mycorrhizal fungi are scattered across the fungal tree of life, where most can be found in four main fungal groups. The Glomeromycota is a fungal phylum exclusively composed of fungi forming arbuscular mycorrhizae (AM) (Schüßler et al. 2001; Redecker and Raab 2006). Fungi forming ectomycorrhizae (EcM) appeared more recently and are spread across the largest fungal phyla: the Basidiomycota, with about 50 known lineages; the Ascomycota, with about 40 known lineages; and the /endogone1, /endogone2 and /densospora lineages in the Mucoromycotina of Zygomycota (Tedersoo et al. 2010; Tedersoo and Smith 2013; Chap. 6). AM fungi interact with the vast majority of plant biota (ca. 80%

land plants), but are taxonomically species-poor (Bonfante and Genre 2010; Öpik et al. 2013; Pagano et al. 2016), whereas EcM fungi are more diverse, but only interact with a limited number of families of mostly woody plants, including Pinaceae, Fagaceae, Betulaceae, Salicaceae, Myrtaceae, Nothofagaceae, Dipterocarpaceae, and some members of the Rosaceae and Fabaceae, which dominate many tree communities of temperate, tropical, alpine, and boreal ecosystems of the Northern and Southern hemispheres (Malloch et al. 1980; Alexander 2006; Smith and Read 2010; Chaps. 19 and 20). Mycorrhizal fungi are key players in all terrestrial ecosystems except Antarctica. By tracing their evolutionary and ecological history, we can better understand the role of past environmental and biotic events in shaping distribution and diversity patterns that we observe today. In addition, host association data can provide interesting points of view for the emergence and conservation of mycorrhizal host communities over evolutionary time scales.

In this review, we seek to highlight phylogenetic approaches that may have valuable applications in current mycorrhizal phylo- and biogeographic research. Rather than enlisting different available methods (reviewed, for instance, in Ronquist and Sanmartín 2011), we conceptualize and discuss relevant methodological advancements, also recounting major methodological biases. We emphasize some examples from both EcM and AM fungi, and other organismal groups; particularly in the light of increasingly popular phylogenetic methods for species delimitation, divergence time estimation, and analyses involving the inference of historical distribution ranges, diversification rates, and trait evolution.

1.2 Barcoding, Species Delimitation, and the Need for Robust Phylogenies

Species are fundamental units for most biodiversity and evolutionary studies (Sites and Marshall 2004; de Queiroz 2007). Recognizing and defining species is a crucial task, not only for high-level species richness assessments and systematic studies, but also for population-level, intraspecific studies. For fungi, this task is particularly challenging given that more than 1.5 million fungal species are thought to exist (Hawksworth 2001; Blackwell 2011), yet less than 10% have a formal taxonomic description. Due to the fact that fungi live out most of their existence hidden from human eyes, the vast majority of undocumented species will likely remain that way. Before the rise of the molecular, PCR-based era in the 1990s (White et al. 1990), fungal taxonomy and systematics relied heavily on the morphological description of taxa. Many studies have shown this to be insufficient in describing fungal biodiversity (Taylor et al. 2000, 2006). More recently, fungal molecular phylogenies of related taxa commonly reveal the existence of species complexes composed of multiple cryptic lineages (Geml et al. 2006, 2008; Matute 2006; Jargeat et al. 2010; Leavitt et al. 2011a, b, 2015; Sánchez-Ramírez et al. 2015a, b). The term "cryptic species" is actually broadly applicable in fungi. Besides the common failure to

recognize species by morphological means alone, their hidden existence in the environment makes them generally difficult to study.

For more than twenty years, ribosomal DNA (rDNA) applications have been truly revolutionary in fungal research (White et al. 1990; Bruns et al. 1991; Horton and Bruns 2001; Schoch et al. 2012). In part, this is due to the efficiency of PCR primers that consistently amplify rDNA regions across many different fungal groups (Bruns and Gardes 1993; Schoch et al. 2012), and the variability and phylogenetic resolution found in different portions of the rDNA region (Bruns et al. 1991). For instance, the internal transcribed spacer region (ITS1-5.8S-ITS2 or simply ITS) is widely recognized as a species-level marker for fungi (Schoch et al. 2012). Other rDNA genes such as the 28S and 18S large and small subunits (LSU and SSU), usually provide resolution at higher taxonomic ranks due to being conserved, given their role as functional genes in the genome (Bruns et al. 1991; Bruns and Shefferson 2004). Early molecular studies were largely based on PCR and electrophoretic RFLP patterns, which were quickly replaced by DNA sequencing. With the availability of DNA sequence data, new advances were made in the fields of systematics and evolution through phylogenetics, and ecology through DNA barcoding. Early rDNA-based phylogenetics were a true turning point in fungal systematics, showing that many morphological characters did not reflect shared ancestry (e.g. homoplasy) (Hibbett et al. 1997a, 2000; Moncalvo et al. 2000, 2002; Hibbett and Binder 2002). In parallel, efforts on databasing initiatives (Bruns et al. 1998; Kõljalg et al. 2005; Abarenkov et al. 2010) and massive production of ITS sequences (Schoch et al. 2012; Hibbett 2016), have enhanced the accuracy and efficiency of fungal identification and classification (Peay et al. 2008).

In spite of their importance, transcendence, and widespread use in fungal biology, rDNA sequence data suffer from several deficiencies. In the case of ITS, which is probably the most popular, levels of intra- and inter-specific variation can be very different within and between species (Nilsson et al. 2008). Such inter-taxon differences may have an effect in sequence identity cut-off-based species delimitations, often used in environmental meta-barcoding studies, leading to an over- or under-estimation of diversity. ITS intra-genomic variability has also been reported, where multi-allelic copies have been found within the same genome (Simon and Weiß 2008; Lindner and Banik 2011). Base-calling errors and missing data in DNA chromatograms can arise in such cases, affecting downstream analyses such as multiple sequence alignments and phylogenetic analyses. While high levels of DNA variation is desirable in barcoding genes, too much variation, particularly at indel positions, can be problematic during alignments, causing misleading phylogenetic inference. In AM fungi, ITS is too variable and does not resolve species boundaries (Stockinger et al. 2010). Instead, the preferred barcoding rDNA gene is the small subunit (SSU), which has a resolution power at the family or order level in other groups (Stockinger et al. 2010; Bruns and Taylor 2016). Protein-coding genes, on the other hand, are generally easier to align because most positions along exons are subject to selection. They also have a wide range of phylogenetic scalability. For instance, amino acid alignments can be used for deep phylogenetics

(families, orders, classes), while synonymous codon positions and introns often have enough variation for more recent times-scales (species, populations). In principle, this has led initiatives, such as the Assembling the Fungal Tree of Life (AFTOL) project, to explore other genomic loci for resolving relationships among fungi (Blackwell et al. 2006). For some groups, such as *Cortinarius*, *Laccaria*, and *Amanita*, the ITS region has some utility in recognizing species, but protein coding genes such as *rpb2* and *tef1* are considered superior when defining intra- and inter-species boundaries (Frøslev et al. 2005; Sheedy et al. 2013; Sánchez-Ramírez et al. 2015a, b; Chap. 13). Nonetheless, protein-coding genes may be more challenging to work with at the production stage, given that primer pairs do not consistently amplify across taxa, or are too unspecific (Schoch et al. 2012). Moreover, in spite of their potential for environmental studies, protein-encoding loci are not widely accepted as barcoding markers among the community of fungal ecologists. In part, this might be due to the fact that protein-coding sequences, such as *rpb2*, are taxonomically not that well represented in nucleotide databases, and can be difficult to produce. However, protein-coding genes have promising advantages that might be worth exploring further for fungal environmental studies (Větrovský et al. 2016).

Simple barcoding for species identification usually involves the use of the Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990). BLAST is an algorithm that efficiently compares sequences to pre-existing databases, retrieving the best matching records. If the sequence is unknown, such as those from environmental samples, this method provides a way to define its taxonomic affinity, and potential geographic ties, depending on the availability of meta-data in the database used in the search. One way to determine if a sequence or a group of sequences belong to a molecular operational taxonomic unit (MOTU) is to establish a sequence identity cut-off (Nilsson et al. 2008; Fig. 1.1). Empirical studies looking at fungal intraspecific ITS variation have shown that a conservative threshold typically averages around 2–3% pairwise differences, with substantial variation between species (Nilsson et al. 2008; Hughes et al. 2009; Schoch et al. 2012). The process of clustering MOTUs can be fully automated given a set of aligned sequences using the barcode gap discovery method (ABGD; Puillandre et al. 2011; Fig. 1.1), or with sequence clustering algorithms such as UCLUST (Edgar 2010; Fig. 1.1). For instance, Tedersoo et al. (2014b) used ABGD to search for similarity thresholds to distinguish MOTUs in a data set of 757 sequences of Sebacinales. Moreover, considering the rate of molecular substitution in ITS and the rate of speciation, MOTUs may be over or underestimated depending on species-specific population histories (Ryberg 2015). A more sensitive approach would be, of course, to use data directly from phylogenetic trees to delimit species. This is, in fact, the purpose of models such as General Mixed Yule Coalescent (GMYC, Pons et al. 2006; bGMYC, Reid and Carstens 2012) and the Poisson Tree Processes (PTP/bPTP/mPTP, Zhang et al. 2013) that use branching patterns in a phylogenetic tree to determine, which branching events correspond to coalescence events (intraspecific) or speciation (interspecific) (Fig. 1.1). These models, however, rely heavily on the topology of the tree and assume that species are reciprocally monophyletic (Fujisawa and Barraclough 2013; Ryberg 2015). For

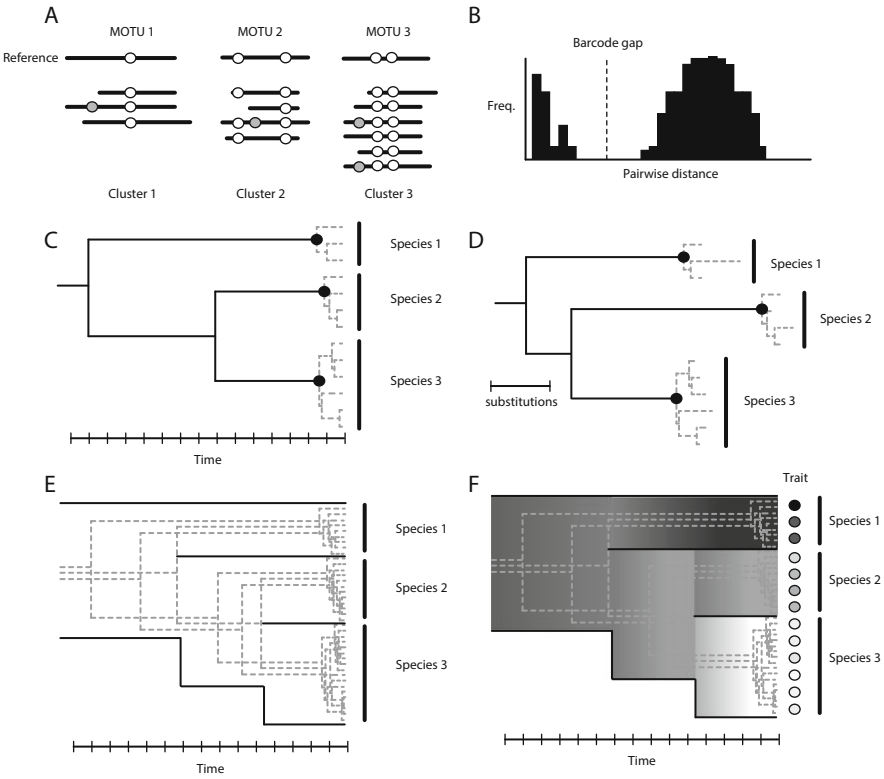

Fig. 1.1 Schematic presentation of species delimitation approaches. (**a**) Environmental sequence clustering based on a predefined similarity threshold. *White circles* represent species-specific barcodes. *Grey circles* represent intraspecific variation. (**b**) Similarity threshold estimation based on the ABGD method. (**c**, **d**) identification of population-level coalescent (*grey dotted lines*) and speciation (*black lines left*) branching events is the basis for GMYC-type species delimitations. Nodes representing the most recent common ancestor of each species are marked by a *black circle*; (**c**) represents the GMYC model, where trees are ultrametric, while (**d**) represents the PTP model, where branches represent substitutions. (**e**, **f**) Multi-locus species delimitation based on the multi-species coalescent model. Gene trees (*grey dotted lines*) from unlinked loci are used to infer the speciation history (species tree) and determine the most likely species delimitation scheme; (**f**) is an extension that allows incorporating information from continuous trait data

example, species with high population sizes will generally have longer coalescence times, leading to incomplete lineage sorting (Sánchez-Ramírez et al. 2015b). The accuracy of the GMYC model has been shown to drop in these situations, based on simulation data, leading to cases where species are not monophyletic (Fujisawa and Barraclough 2013). For these and other reasons it is generally recommended to use multiple approaches and data sources for species delimitation (Camargo et al. 2012; Carstens et al. 2013). Several studies with lichens (Leavitt et al. 2011a, b, 2015) and

the basidiomycete *Tulasnella* (Linde et al. 2014) have shown the discriminatory power of multiple multi-locus approaches for fungal species delimitation.

Most of the approaches mentioned above were developed specifically for single-locus data. However, there have been efforts to introduce the application of multi-locus approaches for the recognition of fungal species [e.g. the Genealogical Concordance Phylogenetic Species Concept (GCPSC); Taylor et al. (2000, 2006)]. Moreover, with the drop in sequencing costs and the availability of technology for massive sequencing, whole-genome approaches will be more common for phylogenetic reconstruction (Philippe et al. 2005; Cutter 2013). Biogeographic and phylogeographic analyses can benefit from large amounts of data in the sense that more robust phylogenies typically will lead to more solid evidence when testing hypotheses. Multi-locus data sets not only increase the number of molecular characters; they can also be used to delimit species more robustly using coalescent methods. Rannala and Yang (2003) introduced a model in which independent gene genealogies are fitted within the speciation history of a group of related species, into what it is now called a *species tree*. Species tree models (e.g. the multi-species coalescent) can take into account sources of gene tree incongruence (e.g. incomplete lineage sorting), while inferring species divergences and demographic histories (Rannala and Yang 2003; Liu et al. 2009; Heled and Drummond 2010). Different implementations of this model are now used to delimit species: Bayesian Phylogenetics and Phylogeography, or BP&P (Fig. 1.1e; Yang and Rannala 2010, 2014; Rannala and Yang 2013; Yang 2015); *BEAST model testing (Grummer et al. 2014); DISSECT and STACEY (Jones 2014) (for a recent review on coalescent-based species delimitation methods, see Mallo and Posada 2016). Novel extensions of BP&P are able to integrate phenotypic or geographic data together with genetic data to delimit species (Fig. 1.1f; iBPP, Solís-Lemus et al. 2015). Such advancements will probably bring systematists closer to the much-desired integrative taxonomy (Dayrat 2005; Will et al. 2005). Up to now, this approach has been used to delimit species in arthropods (Huang and Knowles 2016), reptiles (Pyron et al. 2016), and fish (Dornburg et al. 2016). However, we can envision environmental and geographic data, such as pH, humidity, elevation, latitude and longitude, being used as characters, in addition to genetic data, to delimit fungal species. Initiatives like UNITE that make these kinds of meta-data more easily accessible are therefore very valuable (Tedersoo et al. 2011).

1.3 Reconstructing the Geographic Past: Phylo- and Biogeography

Phylogeography and biogeography are two deeply connected disciplines focusing on the spatial dimension of biodiversity at different temporal scales. As a more recent field in evolutionary biology, phylogeography is concerned with explaining the geographic distribution of genetic diversity within a species (Avise et al. 1987; Avise 2000). This is accomplished by integrating approaches from phylogenetics and population genetics to tackle problems that lie between macro- and micro-

evolutionary scales (Avise 2009; Knowles 2009; Hickerson et al. 2010). Biogeography, on the other hand, is largely phylogeny-based and it is primarily concerned with distribution patterns of species or higher taxonomic ranks (Ronquist 1997; Ree and Sanmartín 2009). Both disciplines have phylogenetic roots, and as such, share many methodological approaches to infer geographic patterns.

Ancestral-state reconstruction (ASR) methods are widely used in phylo- and biogeorgaphic research (Ree and Sanmartín 2009; Ronquist and Sanmartín 2011). The basic concept behind ASR involves the projection of character states, that can be discrete or continuous (e.g. a saprotrophic vs. mycorrhizal ecology, latitude, elevation, fruiting morphology, etc.), backwards in time. Character states are usually assigned to sampled biological units (i.e. species or individuals) that occupy the tips of a phylogeny. These character states are then traced back from the tips down through the branches of the tree (for a recent review see Joy et al. 2016). In a geographic context, characters states can be either discrete and spatially defined areas (Maddison et al. 1992; Pagel 1994, 1999) or numeric geographical coordinates represented as continuous characters (Lemmon and Lemmon 2008; Lemey et al. 2010; Bloomquist et al. 2010).

ASR of discrete character states can be evaluated in a number of ways. Maximum parsimony optimizes the reconstruction to the minimum number of state transitions (e.g. Swofford and Maddison 1987). On the other hand, statistical methods apply maximum likelihood or Bayesian inference to optimize a stochastic continuous-time Markov-chain (CTMC) matrix (e.g. Pagel 1994, 1999; Pagel et al. 2004), which is used to describe transition probabilities between states or areas (O'Meara 2012; Sanmartín et al. 2008; Fig. 1.2). Ancestral area reconstruction methods often use a parsimony-based approach, such as the dispersal-vicariance analysis (DIVA) (Ronquist 1997). Others employ CTMC models, which are usually more parameter rich, such as the dispersal-extinction-cladogenesis (DEC) analysis (LAGRANGE, Ree and Smith 2008). Other CTMC models have been optimized to situations when the number of areas is large (BayArea, Landis et al. 2013) or include parameters that account for "jump" dispersal (e.g. founder-events) (BioGeoBears, Matzke 2013). At least two different programs, BioGeoBears and RASP (Yu et al. 2015), allow running different models within the same computing framework. Other packages allow the co-estimation of discrete CTMC phylogeographic models together with phylogenetic inference and divergence times (BEAST, Lemey et al. 2009; Drummond et al. 2012). These ancestral area reconstruction analyses differ in what processes they model. For example, if they allow for species to be distributed over more than one area (e.g. LAGRANGE) or not (e.g. Sanmartín et al. 2008). Perhaps they include separate processes for inheritance of ancestral areas at speciation events (e.g. LAGRANGE), or just include changes in ancestral areas along branches (e.g. BayArea). It is therefore important to consider what processes may be most important in any particular group to effectively formulate a hypothesis that is testable with these methods. Continuous geographic characters (e.g. geographic coordinates) have been more often used to infer phylogeographic patterns at a shallower temporal scale (Lemmon and Lemmon 2008; Lemey et al. 2010), where dispersal is more closely linked to the

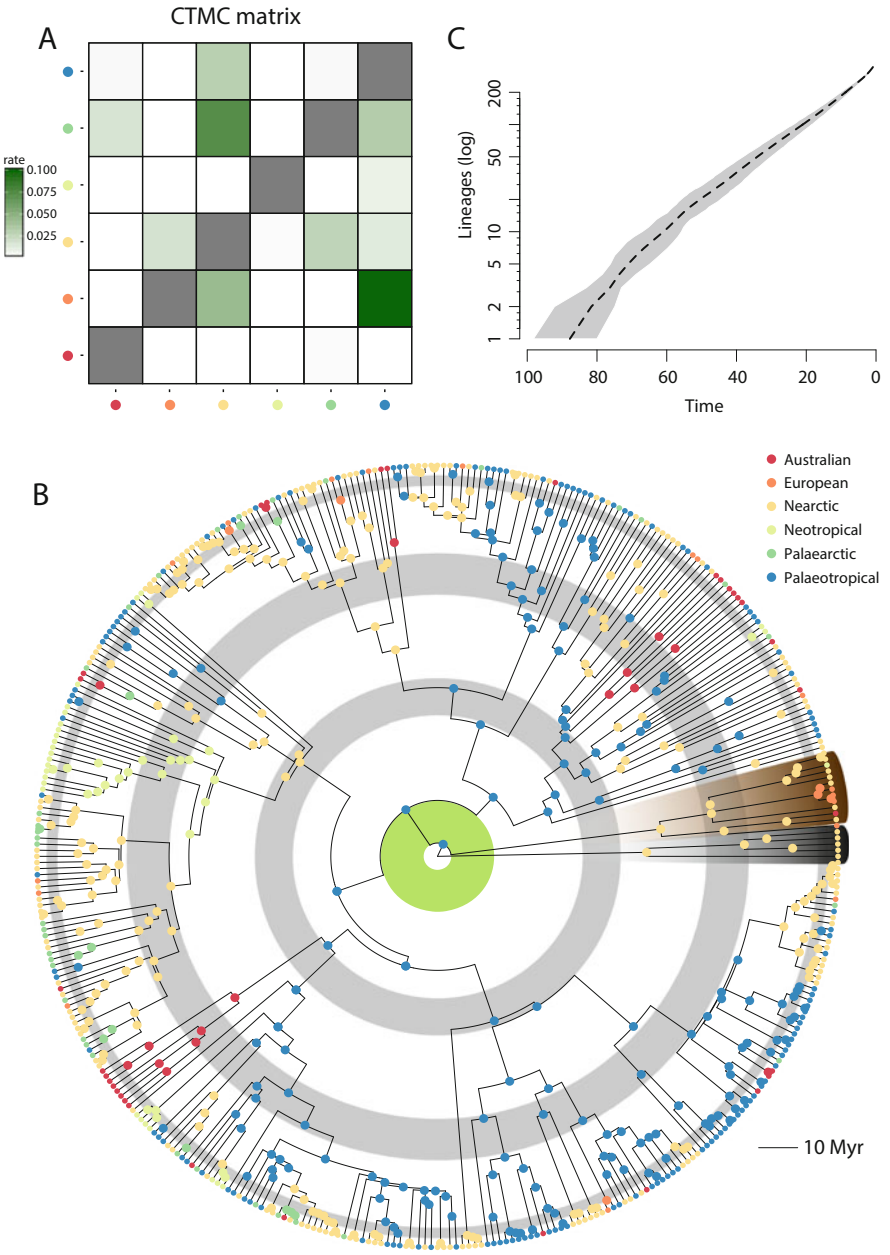

Fig. 1.2 Molecular dating and biogeographic reconstruction of the Amanitaceae. (**a**) Continuous-Time Markov Chain (CTMC) matrix depicting the rate of transition/dispersal between six biogeographic states; (**b**) time-calibrated molecular phylogeny of the Amanitaceae showing reconstructed and extant areas; and (**c**) lineage-through-time (LTT) plot of the phylogeny, excluding non-mycorrhizal taxa (clade highlighted in *brown* in the phylogeny) and the saprotrophic outgroup *Limacella* (highlighted in *black*). *Grey* concentric rings in A mark the Pliocene, Oligocene, and Palaeocene; *white rings* mark the Pleistocene, Miocene, Eocene, and the late Cretaceous; the *green ring* marks the time of the potential transition from the saprotrophic to mycorrhizal habit in Amanita

movement of individuals rather than rare discrete long-distance events. Empirical studies, for instance, have applied diffusion models to track the evolutionary dynamics of epidemic outbreaks (Lemey et al. 2010), human language (Bouckaert et al. 2012), and Pleistocene refugia (Gavin et al. 2014; Bryson et al. 2014). Some of these trait-evolution models are largely based on Brownian motion (BM), where traits evolve by small random changes that are controlled by a diffusion rate parameter (Felsenstein 1988). Extensions of BM allow traits to evolve constrained by a selection rate termed *alpha*, and are known as Ornstein–Uhlenbeck (OU) models (Hansen 1997; Butler and King 2004). OU models allow for the identification "preferred" trait optima, but they have been poorly explored in a geographic context.

Both discrete and continuous biogeographic and phylogeographic inference can also be achieved with standalone programs for ASR such as BayesTraits (Pagel et al. 2004), or through an integrated interphase such as R (R Core Team 2015), where packages like *ape* (Paradis et al. 2004) and *diversitree* (FitzJohn 2012) include built-in functions for ASR. R implementations are practical because they facilitate the direct manipulation and visualization of phylogenetic data. In addition, other visualization tools such as SPREAD (Bielejec et al. 2011) and Phylowood (Landis and Bedford 2014) are also important contributions that bring ease to the interpretation of complex historical phylo-/biogeographic processes.

Compared to plants and animals, fungal phylogeography and biogeography are considered to be in their early stages (Lumbsch et al. 2008; Beheregaray 2008; Peay and Matheny 2016). Some of the earliest phylogeny-based biogeographic analyses of fungi have concisely pointed out the importance of geography and molecular data to explain patterns of divergence and speciation—e.g. between intersterile groups in *Pleurotus* (Vilgalys and Sun 1994) and plant pathogens from the genus *Gibberella* (O'Donnell et al. 1998). Because many fungi interact with other organisms such as plants and animals, their distribution patterns have often been associated to those of their hosts (Bisby 1943; Horak 1983; Lichtwardt 1995). Nonetheless, mixed results have led to considerable debate on whether fungi exhibit biogeographic structure. Global and regional-scale studies have shown extensive cryptic lineages in EcM groups, some of which exhibit geographic structure, and associations with endemic

Fig. 1.2 (continued) (ca. 88–99 Myr). Altogether 789 LSU sequences of Amanitaceae with geographic distribution data, available (Sept. 2016) in NCBI were downloaded and aligned in Mafft (Katoh and Standley 2013). A maximum-likelihood tree was built with RAxML (Stamatakis 2014) and terminal species were delimited with mPTP (Zhang et al. 2013), keeping those with different species names in each cluster to compensate the lack of species-level resolution in LSU. A single sequence was randomly selected to construct a time-calibrated tree in BEAST v1.82 (Drummond et al. 2012), using a relaxed clock model with log-normal distribution, and calibrating with a normal distribution the nodes of the section *Caesareae* and the subgenus *Amanita*, based on Sánchez-Ramírez et al. (2015a). Terminal biogeographic states were recoded based on meta-data from the sequences and maximum likelihood reconstructions were performed using the functions *make.mkn*, *find.mle*, and *asr.marginal* in R package diversitree (FitzJohn 2012). The LTT plot is based on 1000 trees from the posterior distribution (in *grey*) and their mean (*dotted line*)

hosts (e.g. Sato et al. 2012 or specifically in *Amanita*, Geml et al. 2006, 2008; Cai et al. 2014; Sánchez-Ramírez et al. 2015a, b; *Boletus*, Feng et al. 2012; *Inocybaceae*, Matheny et al. 2009; *Laccaria*, Wilson et al. 2016a; Chap. 13; *Pisolithus*, Martin et al. 2002; *Strobilomyces*, Sato et al. 2007; *Tuberaceae*, Bonito et al. 2013). In contrast, other fungal biogeographic studies have shown more recent distribution patterns, typically explained by episodes of long-distance dispersal (Moyersoen et al. 2003; Kauserud et al. 2006; Moncalvo and Buchanan 2008; Geml et al. 2011) or cosmopolitan distribution (Pringle et al. 2005; Queloz et al. 2011). For EcM fungi, this notion implies that while some species have limited dispersal due to environmental constraints (e.g. Peay et al. 2007, 2010b, 2012; Sato et al. 2012), others are able to successfully establish propagules carried over transoceanic distances to exotic regions, where they might outcompete native fungi (Moyersoen et al. 2003; Vellinga et al. 2009; Pringle et al. 2009; Geml et al. 2011; Wolfe and Pringle 2012; Sato et al. 2012). Furthermore, a seemingly common observation has been a consistent association between continentally disjunct groups of fungi (e.g. between Asia and North America) (Wu et al. 2000; Mueller et al. 2001; Shen et al. 2002; Chapela and Garbelotto 2004; Oda et al. 2004; Geml et al. 2006, 2008; Halling et al. 2008; Cai et al. 2014; Sánchez-Ramírez et al. 2015a; Wilson et al. 2016a; Chap. 13; Fig. 1.2), similar to patterns found in temperate plants from the same regions (Wen 1999; Xiang et al. 2000; Qian and Ricklefs 2000). In several cases, there are hints of Palaeotropical origins and recent temperate diversification in different EcM groups (Matheny et al. 2009; Wilson et al. 2012, 2016a; Feng et al. 2012; Cai et al. 2014; Sánchez-Ramírez et al. 2015a; Fig. 1.2). This observation contrasts with higher species diversity seen in the Northern Hemisphere, both for EcM hosts and symbionts (Malloch et al. 1980; Halling 2001; Matheny et al. 2009). Alexander (2006) argues that the EcM habit is likely to have evolved in a Palaeotropical environment given that a putative EcM host ancestor in the Dipterocarpaceae is likely to have originated in Gondwana about 135 Ma (Moyersoen 2006), predating the Cretaceous radiation of other EcM Angiosperms (Lidgard and Crane 1988; Berendse and Scheffer 2009, but see Chap. 19). In the case of EcM fungi, this kind of evidence would support long-term host co-migration (e.g. Halling 2001; Põlme et al. 2013), followed by allopatric speciation/divergence and/or regional adaptation. If fact, studies in EcM groups commonly suggest patterns consistent with trans-continental dispersal over land masses (Halling et al. 2008; Geml et al. 2006, 2008; Matheny et al. 2009; Wilson et al. 2012; Bonito et al. 2013; Sánchez-Ramírez et al. 2015a; Fig. 1.2a, b), and at least two different studies have explicitly tested biogeographic models which support historical world-wide co-dispersal scenarios with plants (e.g. the Boreotropical hypothesis sensu Wolfe 1978; also see Lavin and Luckow 1993) in the Sclerodermatinae (Wilson et al. 2012) and *Amanita* sect. *Caesareae* (Sánchez-Ramírez et al. 2015a). Compared to EcM fungi, it is unfortunate that far less biogeographic studies have been conducted in AM fungi given the need to understand their evolutionary history (Chaudhary et al. 2008). While strict historical biogeographical studies are still scarce in AM fungi, macroecological studies have suggested that while geography and local environment explain some of the variance in global community structures, many operational taxa are globally distributed

(Chap. 7; Kivlin et al. 2011; Öpik et al. 2013; Davison et al. 2015; see Bruns and Taylor 2016 for a counter-argument). Moreover, phylogeographic analyses based on coalescent approaches have also been applied to test hypotheses about the cosmopolitan distribution of the AM species *Glomus mosseae*, indicating a recent expansion within the last few hundred years (Rosendahl et al. 2009).

1.4 Molecular Dating and the Fossil Record

Molecular phylogenies are necessary to study patterns and processes at macro- and micro-evolutionary scales (Avise and Wollenberg 1997; Barraclough and Nee 2001). The phylogeny takes the form of a topology or a graph depicting relationships between biological units, which includes basic information such as: (1) branch lengths indicating the amount of evolutionary change, (2) internal nodes or branching points, and (3) terminal nodes or tips, which represent sampled biological units. An important property of phylogenetic trees is that branch lengths can be represented as evolutionary time (Fig. 1.2). This notion comes from the molecular clock concept, introduced by Zuckerkandl and Pauling (1965), which states that the amount of molecular substitutions between taxa are proportional to the amount of time elapsed since their last common ancestor (Kumar 2005). Given this principle, branches and nodes in the tree can be scaled to time units and become "ultrametric" (i.e. every tip is equidistant to the root). In ultrametric trees, nodes represent divergence times in species-level trees, and coalescent times in population-level genealogies (Drummond and Bouckaert 2015). There are only a limited number of ways to time-calibrate ultrametric trees: (1) by calibrating terminal nodes (tip-calibration) based on known sampling dates; (2) by applying and assuming a known molecular clock (e.g. a molecular substitution rate, usually in the scale of number of substitutions per site per time unit—e.g. Myr, yr, generations); or (3) by calibrating internal nodes based on evidence from either the fossil record or geotectonic events.

Tip-calibration is practical for time-stamped samples of rapidly evolving organisms such as viruses, and some cases where ancient DNA is available (Drummond et al. 2003). Based on prior knowledge of substitution rates, a molecular clock model can be used to scale phylogenetic branches. In fact, the rate of substitution/mutation of some genes such as animal mitochondrial (Brown et al. 1979) and plant chloroplast genes (Clegg et al. 1994) have been well characterized across taxa and within populations. In contrast, substitution rates for rDNA genes, which are commonly used for fungal phylogenies, are quite variable between and within lineages (Bruns and Szaro 1992; Moncalvo et al. 2000; Berbee and Taylor 2001), deeming their use for time-calibrating fungal phylogenies impractical on their own, unless rates are specifically calculated for particular groups. However, rates need to be estimated from independent evidence in the first place, such as the fossil record or biogeographic events. In this case, internal node calibrations can be used as reference points to infer both molecular clock rates and divergence times (Kumar 2005; Ho and Phillips 2009).

Clock models for divergence time estimation have progressed over the last couple of decades (Welch and Bromham 2005; Ho 2014). The first clock model was conceptualized and implemented as a strict molecular clock (i.e. an evenly ticking clock), where every substitution happened at a constant rate within any given lineage. A new generation of "relaxed" clock models were later introduced allowing substitution rates to vary between lineages, accommodating for more biologically realistic evolutionary scenarios (Drummond et al. 2006; Drummond and Suchard 2010; Ho and Duchêne 2014). One of the most popular phylogenetics programs (with over 10,000 citations in last 10 years), and probably the *de facto* standard for time-tree analysis is the BEAST package (Drummond and Rambaut 2007; Drummond et al. 2012). Some of the advantages of BEAST over other software are that (1) phylogenies are co-estimated with divergence times, (2) the uncertainty in divergence time estimation can be measured, and (3) it offers flexibility and extensibility for model specification (Drummond and Rambaut 2007; Drummond et al. 2012; Bouckaert et al. 2014). Since the introduction of BEAST, together with the steady growth of DNA sequence data, time-calibration has regained much attention in phylogenetic research (Robinson 2006).

The fossil record can be a valuable source for studying ancestral distributions (Meseguer et al. 2015). Besides helping track the distribution of taxa and their extinct relatives in space and time (Lieberman 2003), ages of fossils can be used as priors for time-calibrating molecular phylogenies (Ho and Phillips 2009). In addition, well-sampled records can also provide information about extinction rates (Jablonski 2008) and data that can be used in newer models for divergence-time estimation. For example, the *fossilized birth-death* process uses "total evidence" from the fossil record, integrating information from rates of speciation, extinction, and fossilization (Heath et al. 2014; Zhang et al. 2016).

Compared to many plant and animal records, the fungal fossil record is depauperate (Berbee and Taylor 2010). One of the reasons is because most fungal structures are made of soft tissues that decay rather quickly, making fossilization difficult (Pirozynski 1976; Taylor et al. 2014). Another challenge is their correct classification and taxonomic assignment, which is largely based on reproductive structures that rarely fossilize. Given these difficulties, mycologists have often relied on secondary calibrations (e.g. using age constraints based on a previous time-calibration), where they either estimate a "taxonomically broad" phylogenetic tree with external fossil calibrations to generate prior calibration distributions (e.g. Skrede et al. 2011; Wilson et al. 2012, 2016a; Tedersoo et al. 2014b; Cai et al. 2014; Sánchez-Ramírez et al. 2015a; Zhao et al. 2016), use node ages from other studies based on fossil records or molecular clocks (Jeandroz et al. 2008; Matheny et al. 2009; Ryberg and Matheny 2011, 2012), or fix the global substitution/mutation rate of a particular gene (Rosendahl et al. 2009; Bonito et al. 2013; Sánchez-Ramírez et al. 2015b). Using a diverse array of approaches can lead to inconsistent results and lack of reproducibility. In order to aid mycologists in their quest to time-calibrate molecular phylogenies we provide a condensed overview of potentially useful fossils (e.g. well-identified fossils representing the minimum age of certain groups; Table 1.1).

Table 1.1 List of fungal fossils with potential for phylogenetic time-calibration (based on Taylor et al. 2014)

Genus/species/material	Age (Ma)	Period	Source	Taxonomic group	References
Scutellosporites devonicus	400	Lower Devonian	Rhynie chert	Gigasporaceae	Dotzler et al. (2006)
Hyphae with phialides (*Ornatifilum*)	440–400	Silurian-Devonian	Sweden, rocks	Ascomycota	Burgess and Edwards (1991)
Paleopyrenomycites devonicus	400	Lower Devonian	Rhynie chert	Sordariomycetes	Taylor et al. (1999, 2005)
Eomelanomyces cenococcoides	56	Lower Eocene	EM in amber from western India	Gloniaceae (e.g. *Cenococcum*)	Beimforde et al. (2011)
Hyphae with clamp connections	330	Lower Carboniferous	France	Basidiomycota	Krings et al. (2011)
Palaeancistrus martinii	305	Upper Carboniferous	North America	Basidiomycota	Dennis (1969, 1970)
Quatsinoporites cranhamii	113	Lower Cretaceous	Vancouver Island, British Columbia, Canada	Hymenochetaceae	Smith et al. (2004)
Palaeoagaricites antiquus	100	Upper Cretaceous	Burmese amber	Agaricales	Poinar and Buckley (2007)
Archaeomarasmius leggetti	94	Upper Cretaceous	New Jersey amber	Tricholomatoid/ Marasmioid clades	Hibbett et al. (1997b)
Nidula baltica	40–50	Middle Eocene	Kaliningrad region of Russia	Nidulariaceae	Poinar (2014)
Scleroderma echinosporites (spores)	41	Middle Eocene	British Columbia, Canada	Sclerodermataceae	Rouse (1962)
Suilloid EM	50	Middle Eocene	EM in Princeton chert, British Columbia, Canada	Suillineae	LePage et al. (1997)

Time-calibrated phylogenies can be used for testing hypothesis about the evolutionary history of organisms, in particular those with poor or no fossil record. For instance, some of the oldest putatively Glomeromycota fossils from the Ordovician (ca. 460 Ma, Redecker et al. 2000) and Devonian (Dotzler et al. 2006) suggest that AM fungi where already associated with plants during the transition from an aquatic to a terrestrial environment (Malloch et al. 1980; Brundrett 2002). Molecular dating studies endorse this hypothesis, placing the origin of AM fungi between 400 and 600 Ma (Simon et al. 1993; Berbee and Taylor 2001; Lucking et al. 2009). On the other hand, EcM symbiosis has evolved more recently. Based on molecular clock estimates using SSU branch lengths of several EcM lineages and evidence from the fossil record (e.g. permineralized EcM from the Eocene; LePage et al. 1997), Bruns et al. (1998) suggested that EcM symbioses could have radiated independently and simultaneously during the Tertiary (e.g. Eocene-Oligocene). This was when the climate initiated its cooling trend leading to a more temperate environment dominated by members of the Pinaceae and Fagales (Wolfe 1978; Prothero and Berggren 1992; Zachos et al. 2001). In contrast, Halling (2001) proposed that EcM symbiosis evolved together with the Pinaceae—most of which are able to form EcM associations—during the Jurassic (ca. 180 Ma; Gernandt et al. 2008), and subsequently diversified further as a result of angiosperm radiation in the Cretaceous (125–65 Ma; Berendse and Scheffer 2009). Using time calibrated phylogenies of nine EcM lineages of Agaricales, Ryberg and Matheny (2012) rejected both hypotheses on the basis of discordant clade ages, most of which occurred after the Jurassic, during the Cretaceous and Palaeogene periods (from ca. 100–40 Ma). However, other groups, such as the truffles (Tuberaceae) might have had an older evolutionary history, spanning from the late Jurassic (ca. 156 Ma) and later diversifying during the Cretaceous and Palaeogene (Jeandroz et al. 2008; Bonito et al. 2013). Supporting the findings of Ryberg and Matheny (2012), our case analysis indicates that the EcM habit in *Amanita* could have evolved during the late Cretaceous (ca. 90 Ma; Fig. 1.2b). The genus *Amanita* is a particularly interesting system to study the evolution of EcM symbiosis. First, its close saprotrophic sister group is known (Wolfe et al. 2012b); second, several *Amanita* genomes have been sequenced to date, which may facilitate comparative assessments of genomic machineries between mycorrhizal and non-mycorrhizal species (Nagendran et al. 2009; Wolfe et al. 2012a; Hess and Pringle 2014; Hess et al. 2014; van der Nest et al. 2014); and third, the growing number of biogeographic and phylogeographic studies (Oda et al. 2004; Geml et al. 2006, 2008; Cai et al. 2014; Sánchez-Ramírez et al. 2015a, b, c; Zhang et al. 2015) providing ample resources for phylogenetic inference.

1.5 Tracking Species Richness Over Time and Space: Diversification Rates

Speciation and extinction are the ultimate processes responsible for biodiversity build-up (Hubbell 2001; Ricklefs 2004, 2007). One way to look at patterns of variation in species diversity throughout evolutionary time is to measure the amount of fossil species (Jablonski 2008; Liow 2010). However, not all taxonomic groups have reliable fossil record. Alternatively, branching patterns in (well-sampled) molecular phylogenies can be interpreted or modeled as diversification processes (Barraclough and Nee 2001; Nee 2006; Ricklefs 2007; Purvis 2008). Yule (1925) developed one of the first models of phylogenetic bifurcation. This model described a process of pure birth (speciation) where lineages split independently from one another at a constant rate—usually termed λ. Later, a model that allowed both birth and death of lineages (the birth-death model) was introduced (Raup 1985; Nee et al. 1992). This incorporated an additional parameter controlling the rate at which lineages went extinct—usually termed μ. From then on, several different macro-evolutionary models have been developed with the intention of better describing plausible diversification scenarios (Moen and Morlon 2014).

Another way to assess how nodes in the phylogenetic tree are distributed relative to the root or the tips is to plot the cumulative number of lineages as a function of time (Nee 2006). This is known in the literature as a lineage-through-time (LTT) plot (Fig. 1.2c). A different method is the γ-statistic, which measures the branching patterns in molecular phylogenies numerically by quantifying the degree of deviation from a constant-rate expectation ($\gamma = 0$) (Pybus and Harvey 2000). Positive values ($\gamma > 0$; significant if >1.96 at 95% confidence) indicate that nodes are closer to the tips ("exponential" LTT plot), which reflect recent diversification bursts or background extinction, whereas negative γ values ($\gamma < 0$; significant <-1.64 at 95% confidence) indicate that nodes in the tree are closer to the root ("logistic" LTT plot), suggesting a rapid burst of diversification followed by a slowdown (Pybus et al. 2002; Crisp and Cook 2009). In fact, the latter signature is a common pattern observed in phylogenies from different plants and animals (McPeek 2008; Morlon et al. 2010). These slowdown patterns can be attributed to many different scenarios, including diversity-dependence due to niche saturation (Rabosky and Lovette 2008; Phillimore and Price 2008; Etienne et al. 2012), time-dependency (Stadler 2011), and protracted speciation (Etienne and Rosindell 2012).

Other models measure diversification rates as a function of character states, and are particularly useful for biogeographic and trait-evolution analyses. They have 'blossomed' into a family of trait-dependent models that range from a basic binary (two discrete states) model (BiSSE, Maddison et al. 2007), to multi-states (MuSSE, FitzJohn 2012), to geographic states (GeoSSE, Goldberg et al. 2011), to continuous traits (QuaSSE, FitzJohn 2010), all of which are implemented in likelihood and Bayesian frameworks in the R package *diversitree* (FitzJohn 2012). The most recent addition is the hidden-state speciation and extinction (HiSSE) model, which attempts to correct for potentially unaccounted states that could also influence rates of diversification (Beaulieu and O'Meara 2016). Furthermore, complex

mixtures of diversification rate-variation can also be detected using reversible-jump MCMC algorithms, such as BAMM (Rabosky 2014).

Unsurprisingly, most empirical analyses have focused on patterns in plants and animals (McPeek 2008; Butlin et al. 2009), leaving microorganisms understudied. Nevertheless, diversification analyses can prove to be powerful approaches to understand diversity dynamics though evolutionary time in groups with a poor fossil record, such as fungi. Likewise, these approaches can help mycologists test evolutionary hypotheses regarding the role of hosts, soil chemistry, geography, and other underlying mechanisms driving fungal diversification.

A long-standing question in EcM evolution has been the high degree of functional convergence and the high relative diversity of different EcM groups (Malloch et al. 1980; Bruns et al. 1998; Hibbett et al. 2000; Halling 2001; Brundrett 2002). Although most EcM fungi converge into a similar ecological niche, they are scattered across the fungal tree of life occurring independently in at least 80 phylogenetic lineages (Tedersoo et al. 2010; Chap. 6). Substantial variation in species diversity can be found among EcM lineages/clades; for instance, the /cortinarius lineage comprises >2000 species, while only 1–4 species are found in the /meliniomyces lineage and other helotialean groups (Tedersoo et al. 2010). If we hypothesize that all EcM lineages/clades originated around the same time (i.e. have the same clade age), and assume that they diversify at a constant rate, then we would expect clades to have similar number of species (same clade size). In contrast, observations of EcM richness pattern suggest otherwise; either that (a) EcM clades originated at different times and have diversified under a constant rate, or that (b) EcM clades originated within a similar time-frame but their diversification rate is variable within and/or among clades, or that (c) both times and rates vary. The relationship between clade age and clade size has been studied and discussed broadly for plant and animal clades, with a more or less generalized conclusion that both variables are decoupled, supporting variable diversification rates among clades (Ricklefs 2006; Rabosky et al. 2012; Scholl and Wiens 2016). Ryberg and Matheny (2012) showed that both ages and rates of diversification vary among several EcM clades of Agaricales. They also tested the hypothesis of a potential initial rapid radiation followed by a diversification slowdown tentatively caused by rapid niche occupation, as shown to occur in other taxa (Rabosky and Lovette 2008; Etienne et al. 2012). However, models of rate constancy could not be rejected. If the degree of statistical power was adequate, this observation could imply that diversification in these fungi is not driven primarily by niche specialization, which can happen where there is competition (Ackermann and Doebeli 2004), probably depending on other sources of speciation, such as allopatry or parapatry (Ryberg and Matheny 2012). Compared to EcM fungi, AM fungi appear to have much lower rates of diversification. While formal diversification rate analyses are still lacking in AM fungi, it is possible to estimate the net diversification rate based on an approximation by Magallón and Sanderson (2001). Based on a clade size of 200–300 spp. (Öpik et al. 2013), a crown age of 460 Ma (Redecker et al. 2000), and the assumption of a constant diversification rate, the Glomeromycota would have a net diversification rate of about 0.01 speciation

events per million years. Notably, this in 3–14 times lower than speciation rates in some EcM agarics (Ryberg and Matheny 2012).

Other diversification studies in fungi focusing on trait or character state evolution have found support for different trends. For instance, a study in the saprotrophic agaric *Coprinellus* found a correlation between higher rates of lineage accumulation and trait diversification as evidence of an adaptive radiation linked to the appearance of auto-digestion as a key innovation trait (Nagy et al. 2012). Another study on gasteriod fungi showed that net diversification rates (e.g. speciation–extinction) in several gasteroid lineages are elevated in comparison to non-gasteroid lineages across the Agaricomyces (Wilson et al. 2011). While this result was not significant, equilibrium frequency calculations that incorporated the one-way (irreversible) transition of gasteromycetation suggested a trend toward increased gasteroid diversity. Furthermore, after finding evidence of multiple independent dispersal events from the New World to the Old World in the Caesar's mushrooms (*Amanita* sect. *Caesareae*), Sánchez-Ramírez et al. (2015a) tested the hypothesis of increased diversification after the colonization of a new environment, finding evidence that supports both higher speciation and extinction in New World compared to Old World lineages. This suggests higher species turnover in the New World, which is probably coupled with recent drivers of diversification such as glacial cycles (Sánchez-Ramírez et al. 2015a, b).

Most of these studies have focused on isolated clades, making broader comparisons difficult. Nevertheless, a recent initiative known as the Agaricales Diversification (aDiv; https://sites.google.com/site/agaridiv2013/home) project seeks to generate a LSU and *rpb2* data set for about 3000 species of Agaricales. A primary goal of the project is to explore diversification drivers within key ecological groups in the Agaricales (Szarkándi et al. 2013). An order-level time-calibrated phylogeny can offer a unique opportunity for testing broader hypotheses on EcM evolutionary ecology.

1.6 Evolutionary Ecology

The field of evolutionary ecology is concerned with studying the evolution of species interactions, specifically targeting biological or environmental processes that influence changes in diversity over evolutionary time scales. An obvious step towards understanding the evolution of modern ecological roles is to integrate phylogenetic information with geographic and environmental data (Ricklefs 2004; Wiens and Donoghue 2004; Pinto-Sánchez et al. 2014), as well as community assembly data (Webb et al. 2002; Cavender-Bares et al. 2009; Cadotte and Davies 2016). Having a historical view about biodiversity is crucial to advance our understating of past and present-day patterns.

A well-recognized spatial pattern across the tree of life is the general latitudinal diversity gradient (LDG), which shows that species richness is highest at tropical latitudes and decreases towards the poles (Hillebrand 2004; Brown 2014). While

this latitude-diversity relationship has been observed for many groups of plants and animals over past decades, these patterns in soil fungi have only recently been recognized. Studies have shown that the general LDG holds for soil fungi as a whole (Tedersoo et al. 2014a), but for EcM fungi the diversity peaks at temperate latitudes (Tedersoo and Nara 2010; Tedersoo et al. 2012, 2014a; Chap. 18). This means that EcM species richness is higher in temperate regions, compared for instance to tropical or boreal regions. From a macro-evolutionary perspective, processes such as speciation, extinction, and dispersal are the ultimate contributing factors to the LDG (Mittelbach et al. 2007). Recent studies based on phylogenetic and ecological data have linked higher species richness in the temperate region to higher rates of diversification. For example, Kennedy et al. (2012) found that a single temperate clade in the genus *Clavulina* had 2.6 times higher speciation rate that the rest of the group, which was inferred to be mainly tropical. Sánchez-Ramírez et al. (2015c) used the time-calibrated phylogeny of *Amanita* sect. *Caesareae* and continuous geographic data to test for the role of latitude as a driver of diversification. Model testing, together with continuous trait evolution, suggest that lineages diversify at a faster rate at temperate latitudes compared to tropical climate, supporting the findings of Kennedy et al. (2012). Further support has come from a study in the genus *Russula* that reported overall higher net diversification rates in extra-tropical lineages with continual transitions between temperate and tropical environments (Looney et al. 2016). In the light of the growing evidence in favor of higher rates of diversification in the temperate region, it would be interesting to test if these bouts of temperate diversification occurred simultaneously during the Miocene cooling trend that coincided with orogenic activity around the globe and an increase in dominance of EcM plants (Askin and Spicer 1995; Potter and Szatmari 2009; Chap. 20). Until now, these studies have focused on geographic traits, either discrete or continuous, but studies in other groups (e.g. amphibians) have shown how climatic data can be coupled with comparative phylogenetic methods to look at how ecological niches evolve in relation to diversification processes (Pyron and Wiens 2013).

Macro-ecological studies also indicate that other groups of fungi have particular patterns of diversity that vary, not only with respect to latitude, but also with respect to other environmental factors such as temperature or precipitation (Arnold and Lutzoni 2007; Öpik et al. 2013; Tedersoo et al. 2014a; Treseder et al. 2014; Davison et al. 2015). For instance, compared to EcM fungi, AM and endophytic fungi appear to be more diverse in tropical and subtropical regions, and their communities seem to be more differentiated (Arnold 2007; Arnold and Lutzoni 2007; Öpik et al. 2013). A similar pattern rises for fungal pathogens and saprotrophs (Tedersoo et al. 2014a). Both differences in diversity patterns across fungal taxa, as well as differences in their ecological modes, might reflect a historical relationship with their ancestral ecological niche. In spite of heavy criticism about sampling, Treseder et al. (2014) found support to the hypothesis that tropical environments tend to harbor older taxa, compared to younger taxa that tend to reside at more temperate ones.

Another topic of interest regarding evolutionary ecology of mycorrhizal fungi is the co-evolution of host associations. While AM fungi are obligate mutualists with a wide range of hosts (Giovannetti and Sbrana 1998; Bonfante and Genre 2010), EcM fungi can be either generalists or specialists (Molina et al. 1992; Bruns et al. 2002), some of which may be potentially facultative (Baldrian 2009). Examples of high specificity in EcM associations are interactions between certain fungi and myco-heterotrophic plants (Bidartondo and Bruns 2005; Bidartondo 2005), the bolete genus *Suillus* and members of the Pinaceae (Kretzer et al. 1996; Bidartondo and Bruns 2005; Nguyen et al. 2016), and alder-associated mycobiota (Tedersoo et al. 2009; Kennedy et al. 2011, 2015; Põlme et al. 2013). Studies in some of these systems can provide insights into the co-evolution of plant-fungal interactions. High degree of symbiont affinity in the Monotropoideae (Ericaceae) has been evidenced by unique congeneric associations among different myco-heterotrophic plant lineages (Bidartondo and Bruns 2002). Waterman et al. (2011) studied how pollinators and symbionts affected speciation, coexistence, and distribution in orchid species. They show that shifts in symbiont partners are important for plant coexistence, but not for speciation, as most closely related species tend to have the same EcM partners (Waterman et al. 2011, 2012). Given that specific EcM and bacterial communities can be found in *Alnus*-dominated forests, several studies have focused on how the natural history of the host has affected the distribution of the symbionts. Kennedy et al. (2011) compared community assemblages in different *Alnus*-dominated locations in Mexico and other locations in the Americas. They found a striking similarity in the composition of MOTUs between the different locations, giving support to the hypothesis of host-fungal co-dispersal. Similarly, Põlme et al. (2013, 2014) found that the evolutionary history of *Alnus* species had a strong impact on EcM and bacterial (*Frankia*) community structure.

Historical biogeographic analyses have also evidenced host co-dispersal based on phylogenetic and ASR data (Matheny et al. 2009; Wilson et al. 2012; Sánchez-Ramírez et al. 2015a). A number of studies have focused on evolutionary transitions between gymnosperm and angiosperm hosts, with the aim of investigating ancestral host preferences in EcM fungi. A period of rapid speciation in *Leccinum* has been associated to different host changes from an Angiosperm ancestor (den Bakker et al. 2004). Also, Matheny et al. (2009) found that members of the EcM family Inocybaceae were ancestrally associated to Angiosperms and later switched to members of the Pinaceae. Similar patterns have been observed in the Hysterangiales (Hosaka et al. 2008), the truffle family Tuberaceae (Bonito et al. 2013), as well as in gasteroid boletes (Sclerodermatineae) (Wilson et al. 2012). Ryberg and Matheny (2012) showed some support for older EcM agaric clades (e.g. *Hygrophorus*) being ancestrally associated with Pinaceae hosts, whereas younger clades (e.g. Inocybaceae and *Cortinarius*) were ancestrally associated with Angiosperms.

1.7 Methodological Biases and Caveats

As a word of caution, we point out a number of biases and caveats, some that can arise through the application of specific methodology, and others that are inherent of mycological fieldwork and fungal biology in general. We emphasize that some of these points should be considered when making phylo-/biogeographic inferences or interpretations of observed patterns.

Mycorrhizal fungi spend most of their life cycle dwelling in the rhizosphere underground. EcM fungi, for instance, only produce fruiting bodies (on which morpho-taxonomy is based on) during a narrow time-frame (e.g. one or two months). Also, fruiting bodies decay rather quickly, which can further narrow the observational window. Other EcM groups such as members of the Sebacinales or Thelephorales are rarely collected in the field, but have been found to be quite abundant underground (Gardes and Bruns 1996; Dahlberg 2001; Tedersoo et al. 2006; Porter et al. 2008). AM fungi are only known to reproduce asexually, which can complicate morphological species delimitations and sampling strategies (Helgason and Fitter 2009). These limitations can have implications for fungal diversity assessments in general, but specially in a geographic context. Probably due to logistic reasons and bureaucracy in certain regions, fungal taxa from different geographic locations have been studied disproportionately. Given that significantly more biodiversity research is conducted in North America and Europe (Wilson et al. 2016b), mycorrhizal fungi from these regions (most of the times in temperate ecosystems) have been sampled and studied more often than others (Dahlberg 2001; Dickie and Moyersoen 2008). Historically, many more fungal biodiversity surveys (Mueller et al. 2007) and genetic analyses (Douhan et al. 2011) have been conducted in temperate regions than in tropical ones. This systematic sampling bias can thus generate gaps in our understanding of the distribution of fungal taxa, which can have profound effects in the proposition and assessment of biogeographic hypotheses.

Human-mediated dispersal is also well documented in fungi. In particular, AM and EcM fungi can easily travel with soil or roots of trees that have been translocated for reforestation practices, food production, or as ornamental plants (Dunstan et al. 1998; Vellinga et al. 2009). Many of them are able to invade and spread in non-native habitat (Pringle et al. 2009). These events can also introduce noise in biogeographic inference. Nevertheless, long-distance dispersal is also a natural process by which spores travel long distances and establish in distant locations (e.g. Moyersoen et al. 2003; Bonito et al. 2013; Geml et al. 2011).

Reliable data on host association is often unavailable for many mycorrhizal species, which can directly affect studies on host coevolution. Accurately identifying hosts can be tedious if done through inoculation studies, or misleading if done in the field. While the most straight-forward way to identify a host is by molecular means (Muir and Schlötterer 1999; Sato et al. 2007; Wilson et al. 2007), this step is not done routinely. This also concerns the correct and detailed annotation of sequences deposited in GenBank, which often lacks isolation source data, including

geographic location and host (Vilgalys 2003; Bidartondo et al. 2008; Tedersoo et al. 2011). Establishing such connections is critical to effectively investigate how photobionts shape biogeographic patterns in fungi.

Phylogenetic analyses are known to be subject to sampling issues. For instance, the accuracy in dated molecular phylogenies strongly depends on taxonomic sampling (Heath et al. 2008), in particular for clades used for fossil-calibration (Linder et al. 2005). The shape of a phylogenetic tree can change significantly if the sampling is non-random or incomplete, which is often the case in fungal phylogenies (Hibbett et al. 2011; Ryberg and Matheny 2011; Hinchliff et al. 2015), affecting the interpretation of diversification processes (Pybus and Harvey 2000; Pybus et al. 2002; Ryberg and Matheny 2011). Most models for ASR are also susceptible to sampling, as state or location transition probabilities will tend to be more accurate in better sampled phylogenies. It is also unclear how robust models including cladogenetic processes are to missing branching events in the tree. BiSSE-type analyses have also undergone scientific scrutiny for their high false-positive rates due to phylogenetic pseudo-replication (Maddison and FitzJohn 2015; Rabosky and Goldberg 2015), and issues with the size of phylogenetic trees (Davis et al. 2013). Many of these issues can be controlled for by doing simulations (e.g. Rabosky and Goldberg 2015), or by applying models that directly account for the issues (e.g. HiSSE, Beaulieu and O'Meara 2016). Similarly, implementations of other models, such as BAMM have also been critiqued (Moore et al. 2016). Finally, although phylo-community methods are appealing approaches to answer many questions about mycorrhizal (and fungal/microbial) biogeography, most of the species-level data comes from ITS sequences, which are often problematic to align over distantly related taxonomic groups.

1.8 Conclusions and Future Directions

For about a decade, fungal (and microbial) biogeography has been regarded as a young, emerging field (Martiny et al. 2006; Lumbsch et al. 2008; Douhan et al. 2011). Nonetheless, it is clear that a slow but steady body of knowledge is amassing around our understanding of the dimensions of fungal diversity. This includes the notion that the 'everything-is-everywhere' paradigm does not hold generally true, and that an historical perspective is necessary to understand the diversity of any given area (Peay et al. 2010a, 2016; Peay and Matheny 2016).

The steady stream of sequence data promises to supply us with information to solve many of the questions on fungal biogeography. However, most sequences come from the ITS region, which is difficult to use in wider taxonomic contexts, and the necessary meta-data for studies of biogeography and host associations are often lacking. Some of the major challenges relate to accurate and biologically meaningful species delimitations, as well as the generation of robust phylogenies for molecular dating and testing biogeographic hypothesis. Genomic initiatives (e.g. Kohler et al. 2015) and cheaper sequencing (i.e. next-generation sequencing)

will undoubtedly provide unprecedented molecular resources for phylogenomics, that together with better models, promise to solve many of the current downfalls.

Although there are only a handful of studies about diversification and evolutionary ecology of fungi (many of which are focused on EcM symbioses), results seem to be consistent with biogeographic scenarios that point to recent high diversification rates in temperate regions, compared to more ancient and historically conserved tropical patterns (Kennedy et al. 2012; Treseder et al. 2014; Sánchez-Ramírez et al. 2015c; Looney et al. 2016). We envision future phylogeny-based studies incorporating more ecological data (e.g. physiological, climatic, environmental, and geographic traits) and future meta-barcoding-based studies incorporating more phylogenetic data. The first point could be achieved, in part, by making use of geographic information system resources, such as WorldClim (http://www.worldclim.org), while the second could be achieved by implementing supertree approaches (e.g. Beaulieu et al. 2012; Qian and Jin 2016). With regards to EcM phylogeography, there is virtually no study to date (Google searched on Oct. 18, 2016) that has used geographic-coordinate-based diffusion models to infer ancestral distribution ranges in fungi. Similarly, there are very few studies that have applied palaeo-distribution modeling to infer refugial areas during the last glacial maximum (Sánchez-Ramírez et al. 2015b; Feng et al. 2016), in spite of its great potential to understand EcM population dynamics during the last tens of thousands of years.

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Chapter 2
Population Biology and Ecology of Ectomycorrhizal Fungi

Lucie Vincenot and Marc-André Selosse

2.1 Introduction

Despite their wide diversity, physiological peculiarities and inconspicuousness of fungi have long hampered their study and recognition of their prevalence in ecosystems (Webster and Weber 2007). In forest environments, understanding of biology and diversity of ectomycorrhizal fungi (EcMF) has been challenging due to the limited availability of morphological and ecological characters to delineate species. Most Ascomycota and Basidiomycota produce conspicuous fruitbodies, but many others fruit hypogeously (e.g. *Rhizopogon*, *Tuber* spp.), inconspicuously (e.g. corticioid and resupinate fungi) or never (e.g. *Cenococcum geophilum*). Although several early mycologists had rather advanced thinking about fungal ecology, the development of molecular tools and phylogenetic analyses in the last decades has enabled a giant step to assess species diversity (Dettman et al. 2003). At infraspecific level, there are more serious obstacles. As for other 'non-model' organisms, progress in EcMF population ecology is held back by the difficulty to distinguish between individuals. Fruitbodies are not representative of individuals since a mycelial genet (=genetic individual) can produce several fruitbodies, or even no fruitbody over the observation period, because of either environmental variation or sampling effort (Todd and Rayner 1980; Selosse et al. 2001; Moore et al. 2008; Halme and Kotiaho 2012).

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The scarcity of phenotypic criteria to characterise individuals first led to the use of somatic incompatibility (SI) to distinguish among genets (e.g. Fries and Mueller 1984; Dahlberg and Stenlid 1994). Even though SI brought first insights into EcMF population ecology by enabling description of genotypes' numbers and sizes, this technique was barely sufficient to precisely differentiate among kin and fully describe genetic diversity of fungal populations (Jacobson et al. 1993; Anderson and Kohn 1998). Soon, a wide range of molecular markers (e.g. AFLP, RAPD, RFLP, SSR, SNP amongst many) were developed to identify EcMF genotypes. Population genetics studies first described patterns in local populations and explored their ecological drivers. Further technical progress in developing molecular markers and power of associated analyses broadened the scope of EcMF population studies (Anderson and Kohn 1998; Horton and Bruns 2001). Subsequent studies integrated biological features deciphered at local scale, such as mating systems and dispersal, to regional scale and up to distribution ranges. That broadening of scales helped documenting the role of wider environmental, biotic interactions and biogeographic drivers in shaping EcMF diversity.

2.2 Fine-Scale Population Genetic Structure

2.2.1 Mating System and Colonisation Following Forest Stage

Early population-level research aimed at tracking the persistence of inoculated ectomycorrhizal genotypes and evaluating their competitive interaction with indigenous populations (*Amanita muscaria*, Sawyer et al. 2001; *Laccaria bicolor*, Selosse et al. 1998, 1999; *Lactarius deliciosus*, Hortal et al. 2009; *Suillus collinitus*, El Karkouri et al. 2005; *Tuber melanosporum*, Guérin-Laguette et al. 2013) or assessing the genetic diversity and origin of commercial mushrooms (*Tricholoma matsutake*, Murata et al. 2005; *Tuber magnatum*, Rubini et al. 2005). These studies also revealed the mating system and life history strategies of fungi during forest ecological succession.

The pioneer studies by Dahlberg and Stenlid (1990, 1994) investigated genet distribution of *Suillus* spp. at various forest stages with SI. Young stands were dominated by numerous small genets, whereas older stands harboured less numerous, larger genets. During the forest chronosequence, colonisation by spores would have established the first, small genets, part of which would have extended belowground by mycelium growth, eliminating others by competition. In mature stands, large and competitive genets would dominate, potentially favoured by a greater ability to spread in soil by mycelial growth (Dahlberg and Stenlid 1994).

This paradigm was soon applied to various EcMF through mapping and genotyping of fruitbodies. These surveys were further used to distinguish EcMF species falling into 'early-stage' and 'late-stage' categories, i.e. displaying either

pioneer traits (*R*-strategists) or traits associated with later successional traits (*C* and *S*-strategists; strategies sensu Grime 1977), respectively. A ruderal strategy was observed for populations of *Russula vinosa* (Liang et al. 2004), *L. bicolor* (Selosse et al. 1999), *Russula brevipes* (Bergemann et al. 2006) and *Tricholoma terreum* (Huai et al. 2003) that formed many densely fruiting small genets (<4 m) with a prevalence of sexual reproduction and spread by sexual spores. As expected, these *R*-strategists ('early-stage') were observed in young forests, e.g. *Laccaria amethystina* in primary successional *Larix kaempferi* stands (Wadud et al. 2014), *Suillus granulatus* in a young *Pinus strobus* stand (Lee and Koo 2016) and *Rhizopogon vinicolor* in recently disturbed *Pseudotsuga menziesii* stands (Kretzer et al. 2005; Dunham et al. 2013). Typical *C*/*S*-strategists ('late-stage') were characterised by habitat in old stands and predominance of a few large, perennial and potentially competitive genets that suppress the establishment of conspecifics from meiospores. A population of *Russula* species from subsect. *Foetentinae* harboured dominant genets extending up to 70 m, competing with small genets producing a single fruitbody in a primary dipterocarp forest (Riviere et al. 2006). Fiore-Donno and Martin (2001) detected a single, large genet of *Xerocomus chrysenteron* (110 m-extent) and of *X. pruinatus* in a mature stand. Rubini et al. (2011) recovered a completely clonal *T. melanosporum* population, with a single strain dominating a truffle ground and likely impairing the establishment of other genets. Another noteworthy spontaneous genet, reaching a 40 m width and estimated 300 m^2, was described in a natural population of *Suillus pungens* in a mature *Pinus muricata* forest (Bonello et al. 1998; Table 2.1 *in* Douhan et al. 2011).

2.2.2 Species' Ecological Strategy and Environmental Constraints

However, using genet distribution as a proxy of species' ecological strategy and mating system soon revealed shortfalls. First, multiple discrepancies arose in the expected balance between sexual reproduction and mycelial expansion considering forest stage. Redecker et al. (2001) observed small genets of *Lactarius xanthogalactus*, *Russula cremoricolor* and *Amanita francheti* in a late successional forest, suggesting that basidiospore recruitment was more important than previously recognised. Small genets (mean size <10 m) were also displayed by *Amanita alboverrucosa* in native mature forest stands (Sawyer et al. 2003), *Cantharellus formosus* in old-growth Douglas fir stands (Dunham et al. 2003) and *Tricholoma scalpturatum* in mature stands (Carriconde et al. 2008a). Thus, EcMF colonisation strategy might be also explained by species' intrinsic features. Furthermore, various species showing a mixed pattern of mid-sized genets, occasional large individuals and numerous very small genets could not be categorised as 'early-' or 'late-stage' strategists; as a result, the

respective prevalence of sexual recombination (i.e. arrival of new spores) and vegetative growth (i.e. genet persistence and extension) could not be inferred. For instance, *Tricholoma matsutake* associated with *Pinus densiflora* formed a mosaic of numerous small mycelial genotypes intermingled with a dominant genet (Murata et al. 2005). Various EcMF species display such a mixed strategy, like *Amanita* spp. (Sawyer et al. 2003), *A. muscaria* (Bagley and Orlovich 2004), *Cortinarius rotundisporus* (Sawyer et al. 1999), *Cenococcum geophilum* (Wu et al. 2005), *Pisolithus* spp. (Anderson et al. 2001), *Russula brevipes* (Bergemann and Miller 2002), *Suillus spraguei* (Burchardt et al. 2011) and *Tuber aestivum* (Molinier et al. 2016).

Local environmental conditions and intraspecific competition are therefore a key to the local population structure. Fine-scale genet distribution patterns, that were contradicting the strategies theory, were observed in unexpected habitats. For example, Selosse (2003) described old populations (>70 years) of *Leccinum duriusculum* composed of small genets, and a young population (<20 years) with large genets, suggesting that genet size results from the way how neighbours' density limits genet expansion and that small genets are not necessarily recently established. In mature stands, small genets were observed for *Suillus grevillei* (Zhou et al. 1999) and *Laccaria amethystina* (Gherbi et al. 1999; Fiore-Donno and Martin 2001). Genotyping of ectomycorrhizae also revealed a single large dominant *Rhizopogon vesiculosus* genet in a recently disturbed site (Kretzer et al. 2005; Dunham et al. 2013). Moreover, for some species, characterisation of conspecific populations associated to contrasted habitat characteristics further confirmed the crucial influence of forest maturity and level of disturbance on individual colonisation strategy and genetic diversity in local populations, e.g. for *Suillus* spp. (Dahlberg and Stenlid 1990, 1994) and *Hebeloma cylindrosporum* (Gryta et al. 1997, 2000; Guidot et al. 2001, 2002). Those studies do not fully invalidate Dahlberg and Stenlid's paradigm, but highlight the balance between specific biological features, infraspecific variation, site history and environmental parameters in shaping population structure.

2.2.3 Above- and Belowground Patterns of Genet Distribution

Another challenge of the use of fruitbodies to identify genets is that spatiotemporal distribution of fruiting may inaccurately reflect dynamics and abundance of genets belowground. For several species, though, comparisons of above- and belowground distribution showed strong spatial and temporal correspondence, suggesting that fruitbodies are indeed a good proxy (*H. cylindrosporum*, Guidot et al. 2001; *Suillus pictus*, Hirose et al. 2004; *L. laccata*, Wadud et al. 2014; *T. magnatum*, Murat et al. 2013). Awareness of intraspecific variability in fruiting behaviour (phenology, abundance) related to individual and microhabitat variation (*H. cylindrosporum*,

Guidot et al. 2001; *Laccaria* spp., Selosse et al. 2001; *S. pictus*, Hirose et al. 2004) motivated population surveys over several fruiting seasons. Analysis of spatiotemporal persistence confirmed rapid turnover of genets in some populations (*Pisolithus* spp., Anderson et al. 2001; *H. cylindrosporum*, Guidot et al. 2001, 2003; *L. laccata*, Wadud et al. 2008, 2014; *R. brevipes*, Bergemann et al. 2006), but also revealed some erratic fructification patterns, with often-small, 'dormant' (non-fruiting) genets actually persisting in soils without fruiting, e.g. for *Laccaria* (Gherbi et al. 1999; Selosse et al. 2001; Hortal et al. 2012) or *T. melanosporum* (Taschen et al. 2016). Besides validating species ecological strategies theory, the studies investigating temporal persistence confirmed the role of habitat variation, such as microdisturbance combined with infraspecific variation, in shaping population structure. In terms of infraspecific variability, not all genets persist equally as mycelium, nor expand or produce fruitbodies in similar amounts and frequencies. This also suggests infraspecific variation in competitiveness of each genet and points to the trade-off in resource allocation between sexual reproduction (fruiting) and clonal expansion (Johnson et al. 2012).

2.2.4 Local Dispersal Patterns

Beyond mating system and ecological strategy, genet mapping has contributed to basic knowledge about fungal biology, such as spore dispersal patterns (Fig. 2.1; Chap. 3). Although spore dispersal from fruitbodies is difficult to study, spatial autocorrelation analyses of kinship document the size of genetic neighbourhood and effective dispersal range (Peakall et al. 2003). Strong genetic autocorrelation among fruitbodies demonstrate the prevalence of short-distance (<20 m) dispersal of basidiospores, validating a decreasing spore deposition with increasing distance from the fruitbody (Morkkynen et al. 1997; Galante et al. 2011), which has been found in populations of *Laccaria* spp. (Wadud et al. 2014), *Tricholoma scalpturatum* (Carriconde et al. 2008a) and *Suillus grevillei* (Zhou et al. 1999, 2001). In non-agaricoid species, fine-scale spatial autocorrelation analyses revealed similar to even closer dispersal range, for example positive autocorrelation in *T. melanosporum* extends up to 6–8 m (Murat et al. 2013; Taschen et al. 2016) or up to 400 m in *Cantharellus formosus* (Dunham et al. 2006). Longer-range dispersal, probably relayed by mycophages, has been suggested for hypogeously-fruiting species such as *Rhizopogon* spp. (no spatial autocorrelation over 5.5 km; Kretzer et al. 2005) or *Tuber* spp. (very little spatial autocorrelation over 100 km; Taschen et al. 2016; Fig. 2.1).

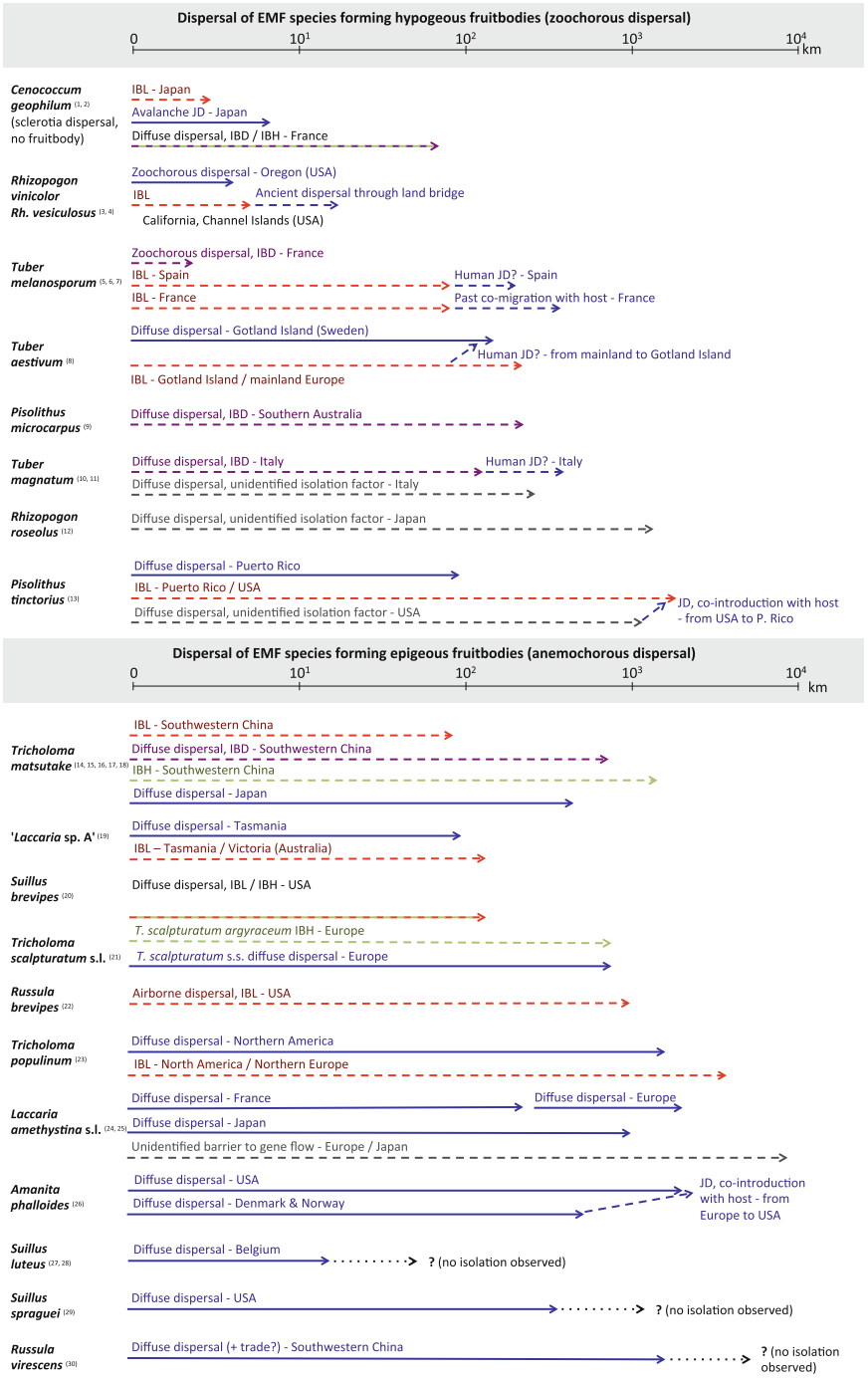

Fig. 2.1 Inferred dispersal mechanisms operating at diverse scales for several EcMF species. *Plain arrows*: uninterrupted gene flow; *dotted arrows*: discontinued gene flow. *JD* Jump Dispersal,

2.2.5 *Cryptic Sexuality*

Understanding of fine-scale genetic structure has enabled to shed light or re-evaluate life cycles and potential occurrence of cryptic sexual reproduction or parasexuality. The common generalist EcMF species *Cenococcum geophilum* is considered asexual (LoBuglio 1999), but its populations show unexpectedly high levels of genetic diversity and recombinant genotypes that are conflicting with the idea of clonality (e.g. Panaccione et al. 2001; Gonçalves et al. 2007; but see Douhan et al. 2007; Chap. 14). A solid cue for cryptic sexual or parasexual cycle in *C. geophilum* was recently found in Portuguese populations displaying variation in genome size and ploidy level (Bourne et al. 2014). Life cycles have been re-evaluated in *Tuber* spp. as well. Rubini et al. (2005) suggested recombination in *T. magnatum*, contradicting the so-far assumed strict selfing (Paolocci et al. 2006). Similarly, Riccioni et al. (2008) demonstrated outcrossing in populations of *T. melanosporum*, although a high inbreeding occurs in this species, due to the recruitment of sexual partners from the immediate vicinity (Taschen et al. 2016). Fine-scale studies on genetic structure have clarified autecology of EcMF species and paved the way for investigating how environmental parameters can shape population structure and genetic diversity.

2.3 Response to Environment and Biotic Interactions

2.3.1 *Environmental Constraints Shape EcMF Populations*

Limiting spore dispersal can lead to divergence between populations, while local standing genetic variation may allow ecological specialisation. Variation within local subpopulations can be stronger than among populations, as evidenced by analyses of molecular variance (AMOVA—*T. magnatum*, Rubini et al. 2005; *T. melanosporum*, Murat et al. 2004; *R. brevipes*, Bergemann et al. 2006). To distinguish between the effects of environmental parameters and the effects of geography, studies compared EcMF populations Spatial Genetic Structure (SGS, related to geography) to genetic structure explained by contrasted habitats.

Fig. 2.1 (continued) IBD Isolation By Distance, *IBH* Isolation By specialisation to Host/Habitat, *IBL* Isolation By Landscape. [1]Jany et al. (2002), [2]Wu et al. (2005), [3]Grubisha et al. (2007), [4] Kretzer et al. (2005), [5]García-Cunchillos et al. (2014), [6]Murat et al. (2004), [7]Taschen et al. (2016), [8] Wedén et al. (2004), [9]Hitchcock et al. (2011), [10]Mello et al. (2005), [11]Rubini et al. (2005), [12]Okuda et al. (2013), [13]Rivera et al. (2014), [14]Amend et al. (2009), [15]Amend et al. (2010), [16]Lian et al. (2006), [17]Xu et al. (2008), [18]Zeng and Chen (2015), [19]Sheedy et al. (2015), [20]Branco et al. (2015), [21]Carriconde et al. (2008b), [22]Bergemann et al. (2006), [23]Grubisha et al. (2012), [24]Roy et al. (2008), [25]Vincenot et al. (2012), [26]Pringle et al. (2009), [27]Muller et al. (2004), [28]Muller et al. (2007), [29]Rivera et al. (2014) and [30]Cao et al. (2013)

Gryta et al. (2006) compared the structure of populations within two *Tricholoma* species from similar-stage black poplar forests with contrasting disturbance levels (i.e. recurrent river flooding versus undisturbed). For both species, genet size, population genetic diversity and life-history strategy differed between the two habitats, pointing towards a possible specialisation to flooding. In *S. brevipes*, Branco et al. (2015) characterised genome-wide variation of two Californian populations from coastal and mountainous environments, separated by a 300 km-wide gap without host. Diversity analyses revealed robust delineation between populations from the two regions despite a low genetic divergence. Furthermore, genome-wide selection footprint analyses (F_{ST} outlier detection) detected several genomic regions diverging between populations, including the *Nha-1-like* locus that is involved in salt tolerance. These results point towards adaptive response of coastal populations to saline stress. This is a pioneering example of population genomics to characterise EcMF adaptation to local environment.

Effects of soil parameters on EcMF population diversity have been investigated more deeply. For instance, *C. geophilum* populations showed some clustering in relation to soil pH, with haplotypes specific to either calcareous or acid soil stands, suggesting adaptive response to soil acidity (Jany et al. 2002). Most studies have focused on heavy metals. *Suillus luteus* ecotypes displayed an adaptive tolerance to zinc and cadmium, mediated by metal efflux (Colpaert et al. 2000, 2011). Further research in physiological response to Zn along a gradient of soil contamination showed a correlation between Zn-tolerance and Zn level in soil, validating the hypothesis of an adaptive response to Zn pollution (Colpaert et al. 2004). However, genetic diversity in Zn-contaminated versus non-contaminated sites showed similarly high genetic diversity for neutral markers, with no genetic structure related to contamination nor geographic distance (Muller et al. 2004, 2007). Combination of sexual reproduction and effective gene flow by spore immigration may have compensated local selection of the Zn-tolerance trait and allowed local adaptation without genetic drift in contaminated sites.

Other studies focused on soils intrinsically rich in heavy metals, such as serpentine soils, which usually shape EcMF community structure (e.g. Urban et al. 2008). *Pisolithus albus* sampled in a mosaic of Ni-contaminated sites within a non-contaminated continuum revealed two genetic clusters that were related to soil type but not to geographic distance (Jourand et al. 2010). All *P. albus* isolates from non-contaminated soils were sensitive to Ni in vitro, whereas isolates from high-Ni soils were ranged from sensible to tolerant to Ni. This exemplifies the selection of ecotypes, although partly counterbalanced by gene flow from external, non-adapted populations. Similarly, isolates of *Cenococcum geophilum* from contrasting Maryland soils revealed ecotypes specific to serpentine or to non-serpentine soils, with higher genetic diversity in non-serpentine soils (Panaccione et al. 2001). Isolates from serpentine soils (Gonçalves et al. 2007, 2009) showed variable but always higher Ni tolerance in vitro compared to isolates from non-serpentine soils.

2.3.2 EcMF Specialisation Towards Hosts

Beyond environmental constraints, infraspecific variation in EcMF populations can be ascribed to biotic interactions due to the obligate symbiosis with their hosts. Most EcMF species are considered generalists, i.e. establishing symbiotic relationships with a variety of host tree species, genera or even families (Smith and Read 2008; Smith et al. 2009). At community level, numerous EcMF species are shared between species, forming multidimensional common ectomycorrhizal networks (Selosse et al. 2006; Bahram et al. 2014). At infraspecific level, single host trees have been shown to simultaneously associate with several genetic individuals, for example in *C. geophilum* (LoBuglio and Taylor 2002; Jany et al. 2002), *L. deliciosus* (Hortal et al. 2009), *T. terreum* (Huai et al. 2003), *R. vesiculosus* and *R. vinicolor* (Beiler et al. 2010), *T. matsutake* (Lian et al. 2006) or *T. melanosporum* (Bertault et al. 2001; Rubini et al. 2011; Taschen et al. 2016). Coexistence of several genets with different ecophysiological abilities (Hortal et al. 2012), as well as colonisation by different EcMF, can be beneficial for the host tree. This could be a key for the dominance of generalism, because it would allow the selection of the best partners by each host tree, corresponding to their ecophysiology (Douglas 1998). However, selection by host trees, if not diluted by recurrent recombination with exogenous genotypes, can lead to population specialisation and perhaps to enhanced efficiency (Bruns et al. 2002; Rochet et al. 2011). Hence, high genetic differentiation among EcMF populations could be related to host specialisation, although it cannot be related to phenotype differences (cryptic species; Taylor et al. 2006; Tedersoo et al. 2008).

To test host generalism, several studies compared genetic structure of EcMF populations associated with distinct hosts. *Laccaria amethystina* has been observed under a very broad range of temperate hosts from several deciduous families as well as Pinaceae (Fries and Mueller 1984). Comparing populations from monospecific stands, Roy et al. (2008) showed that host identity or geography each explained less than 0.90% of total variance, while diversity within populations accounted for more than 91.0% of variance (residual variance being distributed between populations within host pool or within region). Furthermore, no correspondence was observed between genotype clustering and host identity, supporting overall host generalism. Multilocus comparison of *Tricholoma populinum* populations associated with multiple poplar species from North America and Scandinavia showed no structure related to hosts (Grubisha et al. 2012). This further suggested geographic divergence of *T. populinum* after host divergence (*P. balsamifera*/*P. trichocarpa*), indicating host generalism for *Populus* spp.

Conversely, several studies revealed specialisation to a narrower range of hosts than expected. For instance, in an inoculated population of *Suillus collinitus*, cluster analysis distinguished two clades, one associated to *Pinus halepensis* only and the other to *P. sylvestris* and *P. pinea*, suggesting host specialisation (El Karkouri et al. 2005). Two sympatric genetic clusters detected in populations *Cortinarius arcuatorum* were associated with Fagaceae or conifers (Garnica et al. 2011). In

Tricholoma matsutake, significant genetic differentiation was observed among distant populations that could not be explained completely neither by climate, altitude nor geographic distance. Their distinct association with *Pinus densiflora*, *P. yunnanensis* or *Quercus monimotricha* suggested that local specialisation towards tree species was driving the genetic structure (Zeng and Chen 2015).

Hoeksema and Thompson (2007) experimentally tested host adaptation in *Rhizopogon occidentalis* by cross-inoculating multiple populations of coastal pine species (*P. contorta*, *P. radiata*) and multiple populations of the fungus. Relative performance of host and fungi were assessed based on ecophysiological traits. The host × EcMF population interactions revealed a decline in mean fungal colonisation correlated with increasing geographic distance between plant and fungal origin, suggesting specialisation to local host populations. Such an elegant transplant experiment approach could be coupled with genomics studies to look for adaptation footprints.

Specialisation to host, if subjected to strong local selection and the lack of exogenous gene flow, may lead to (sometimes cryptic) sympatric speciation. Phylogenetic and nucleotide diversity analyses of *A. muscaria s.lat.* revealed the co-occurrence of three sympatric cryptic species, whose divergence could have been driven by host specialisation (Geml et al. 2006). Comparative phylogenies of hosts and EcMF partners also supported coevolution to be a speciation factor in fungi associated with alders, particularly in the genera *Alnicola*, *Alpova* and *Lactarius* (Rochet et al. 2011). Similarly, four cryptic, partially or totally sympatric species were detected within the *P. involutus* complex, with partly overlapping host ranges, showing a speciation driven collectively by the environment and hosts (Jargeat et al. 2013).

2.3.3 Impact of Dispersal on Population Structure

EcMF population structure can be influenced by means of dispersal of sexual and asexual propagules (Fig. 2.1) that can be mediated by wind, soil mesofauna (Lilleskov and Bruns 2005; Roets et al. 2011) and/or mammals (Johnson 1996). Then, effective dispersal of spores can be restricted by dispersers' own home range. Description of EcMF populations' SGS has allowed some inference about how means of dispersal shape EcMF populations diversity. For instance, the absence of positive spatial autocorrelation in *Rhizopogon vinicolor* and *R. vesiculosus* over 5.5 km suggests effective kilometre-scale dispersal of spores, rather than a continuous diffusion with decreasing abundance from their immediate vicinity, a pattern concordant with a dispersal by small forest mammals (Kretzer et al. 2005). Dependence of hypogeous species on such dispersal agents could shape the strong local SGS among islands by interruption of zoochory, e.g. in *Rhizopogon* spp. (Grubisha et al. 2007; Okuda et al. 2013).

In *Cantharellus formosus*, highly similar genotypes were retrieved several kilometres apart, raising the possible explanation of human or animal

mycophagous dispersal (Dunham et al. 2003). Human activities can indeed disperse EcMF propagules (Selosse et al. 1999), even unconsciously, as documented for pathogenic fungi (Fisher et al. 2012). For instance, European populations of *Tuber aestivum* revealed four genetic clusters inconsistent with geography; the absence of SGS over up to 2400 km could be explained by human dispersal via inoculated plant material (Molinier et al. 2016). Regional-scale substructure of *T. melanosporum* populations may also be related to active human inoculation and trade of plants (García-Cunchillos et al. 2014). Interestingly, Taschen et al. (2016) revealed no loss of natural regional SGS in inoculated plantations. In Puerto Rico, the founder effect (strong local SGS and reduced allelic richness) of exotic *Pisolithus tinctorius* in pine plantations points towards co-introduction with host trees (Rivera et al. 2014). The toxic and invasive *Amanita phalloides* rapidly colonised (several km/year) the west coast of North America since the nineteenth century (Pringle and Vellinga 2006). European and North American populations showed genetic differentiation but no isolation, confirming recent divergence (Pringle et al. 2009). While the European populations showed SGS, with high genetic diversity and effective population size, the North American population displayed no SGS but signs of a genetic bottleneck (low polymorphism, no private alleles), confirming the hypothesis of a recent introduction of *A. phalloides*.

A growing body of studies thus hint towards local specialisation to environment, either driven by local physical variation or by interactions with symbiotic partners and dispersers. However, some EcMF display evidence of generalism to environmental conditions and hosts. For these species, population genetic structure could be driven by dispersal efficiency over greater distances and population divergence at a broader scale.

2.4 Landscape and Habitat Distribution Shape Modern and Past Populations

2.4.1 Isolation By Distance Among Populations

Although generalist EcMF species can establish in various range of environments, their distribution range may encompass barriers to gene flow, shaping SGS at regional or continental scales. Gene flow over such distances can be mediated by long-distance dispersal (LDD), or by diffuse, continuous dispersal of propagules within the distribution area (Lomolino et al. 2010). The effects on demography of these two mechanisms entail different signatures in populations' SGS. LDD leaves a founder effect signature in small dispersed populations, including a sharp drop in neutral diversity, and posterior genetic drift. Such cues were observed in *Pisolithus* spp. populations in New Zealand, probably resulting from multiple LDD events from Australia (Moyersoen et al. 2003). A possible

ancient LDD event founded Scandinavian *T. populinum* populations, showing complete reproductive isolation from North America and strongly impoverished genetic diversity (Grubisha et al. 2012).

By contrast, diffuse dispersal represents a continuous gene flow of propagules over limited distance, where resistance to dispersal entails correlation between geographic distance and genetic distance, i.e. the characteristic Isolation By Distance (IBD) pattern. Various EcMF display IBD at variable scales. IBD was observed in *Rhizopogon* spp. over 50 km distance in California Chanel Islands (Grubisha et al. 2007), in *C. geophilum* over 250 km in France (Jany et al. 2002), in *T. magnatum* over 450 km in Italy (Rubini et al. 2005), in *Pisolithus microcarpus* over 700 km in Southeastern Australia (Hitchcock et al. 2011) and in *T. scalpturatum* over 2500 km in Western Europe (Carriconde et al. 2008b). The increased reproductive isolation with longer distance can lead to lineage divergence, as revealed by combined population genetics analyses and phylogeography of wide-ranged EcMF species previously described as trans- or multicontinental (*A. muscaria*, Geml et al. 2006; *T. populinum*, Grubisha et al. 2012). Range disruption between populations can even hide cryptic speciation, as detected in *L. amethystina* across Eurasia (Vincenot et al. 2012).

2.4.2 Landscape Genetics

Beyond Euclidian geographic distance, geographic features such as mountains ranges, water bodies and watersheds and dominant airstreams can hamper dispersal of propagules (Manel et al. 2003; Zeller et al. 2012). On Mount Fuji, landscape features appear to strongly impact population structure of *Cenococcum geophilum*, whose sclerotia are not dispersed by wind. Its populations situated <10 km apart but separated by a valley were genetically differentiated (Wu et al. 2005).

Landscape effects were tested in topographically peculiar southwestern China. *Tricholoma matsutake* populations from Yunnan and Sichuan provinces displayed high genetic diversity and low but significant differentiation among populations (with significant $F_{ST} = 0.10$; Amend et al. 2010). Genetic distance did not correlate with the elevation gradient, but a significant IBD pattern appeared over 1100 km. At finer scale, strong differentiation was detected at over 65 km, and populations were significantly less diverged within than between watersheds. Landscape distance, calculated as the shortest route between populations below treeline, i.e. along suitable habitat, significantly correlated with genetic distance (Cushman et al. 2006).

In China, three geographic clusters within *Tuber indicum* are shaped by hydrographic network; one cluster corresponds to Mekong river paleoregion, whereas two other clusters are separated by the contemporary Yangtze River (Feng et al. 2016). In *T. himalayense*, by contrast, genetic structure was inconsistent with contemporary landscape. However, its populations from different watersheds were isolated due to the southward postglacial displacement of suitable habitats

that progressively decreased their connectivity (Feng et al. 2016). Thus, rivers are barriers to EcMF gene flow by spore dispersal, at least for hypogeous animal-dispersed EcMF.

2.4.3 Extensive Gene Flow

Populations of various EcMF species may display extensive gene flow in areas devoid of barriers. This was documented at regional scale in *T. aestivum* over 180 km (Wedén et al. 2004), *L. amethystina* over 450 km in France (Roy et al. 2008) and over 950 km in Japan (Vincenot et al. 2012), *T. matsutake* in China over 70 km (Amend et al. 2009) and in Japan over 450 km (Murata et al. 2005) and *S. spraguei* over 600 km (Rivera et al. 2014). Broadening of geographical scale further revealed unexpected, very extensive gene flow over thousands of kilometres. *Russula virescens* populations showed no SGS pattern over 2700 km (Cao et al. 2013). Unexpectedly, mountains and valleys of Yunnan did not act as dispersal barriers for *R. virescens* that contrast with *T. matsutake* populations (see Sect. 2.3.2). In western Europe, highly outbreeding *L. amethystina* populations have a low global F_{ST} (0.04) from Spain to Estonia, with a marginally significant signal of IBD over 2900 km (Vincenot et al. 2012), probably due to the absence of physical barriers and host generalism (Roy et al. 2008). Similarly, host generalism would favour extensive gene flow of *P. microcarpus*, associated with various acacias and eucalypts, over southeastern Australia (Hitchcock et al. 2011).

EcMF dependence on host partners requires a habitat continuum for diffuse dispersal. Even over short distances, corridors of vegetation can assist gene flow, as for *S. grevillei* (Zhou et al. 2001) or *P. microcarpus* (Hitchcock et al. 2011). While gene flow between populations of host specific EcMF species is restricted by host distribution (e.g. '*Laccaria* sp. A', Sheedy et al. 2015; *Rhizopogon roseolus*, Okuda et al. 2013; *Suillus brevipes*, Branco et al. 2015), host generalism favours efficient gene flow and establishment in wide areas (Vellinga et al. 2009).

2.4.4 Co-migration with Hosts

Biogeographic analyses of EcMF population history have revealed demographic fluctuations following host populations, still reflected in modern population structure. In southern Australia, populations of '*Laccaria* sp. A', specifically associated with *Nothofagus cunninghamii*, follow their host's SGS. In Tasmania, higher genetic diversity, richness, effective population size and admixture as compared to populations from Victoria would correspond to a Tasmanian refugium for '*Laccaria* sp. A' during the last glaciation, followed by postglacial co-expansion towards mainland Australia with their host (Sheedy et al. 2015). Southwestern Mediterranean truffle populations could have retreated to Italian

and Iberian glacial refugia, as present populations reflect postglacial co-expansion with their hosts. Recent gene flow was detected among *Tuber magnatum* populations from central Italy (autocorrelation up to 450 km), while those from southern and northwestern Italy differed significantly (Mello et al. 2005; Rubini et al. 2005), suggesting a glacial refugium in central Italy and a postglacial co-expansion with hosts southward and northward. *Tuber melanosporum* shows moderate differentiation between populations from central Italy, France and Spain, with highest diversities in southernmost populations, providing support to Italian and Iberian glacial refugia (Riccioni et al. 2008). Furthermore, an Italian glacial refugium for *T. melanosporum* is supported by the genetic bottleneck signature in Italian populations (Murat et al. 2004). As the distribution of *T. melanosporum* haplotypes is consistent with that of oak trees, this species may have followed the two postglacial re-colonisation routes of oaks, through the Rhone valley and through southern France to the Atlantic coast (Murat et al. 2004; Bertault et al. 2001; Payen et al. 2015). Host-associated glacial refugia for *T. melanosporum* were also found in Iberian Peninsula (García-Cunchillos et al. 2014).

These studies highlight how host demographic history shaped modern populations of EcMF species. Co-migration patterns and parallel EcMF and host biogeography enable to reconstruct the history of ectomycorrhizal forests. Populations of *T. matsutake* show unexpected isolation across the Gibraltar Strait between Europe and Morocco, perhaps due to co-migration with *Cedrus atlantica* along southern Mediterreanean coast, since coalescence analysis points towards a common ancestor in Anatolia (Chapela and Garbelotto 2004). Between North America and Europe, a similar isolation was found, with significant regression with landscape distances across the Bering Strait. *Tricholoma matsutake* populations would thus have co-migrated with their hosts from North America, their centre of diversification, towards Eurasia through Beringia rather than through the Atlantic land bridge (Chapela and Garbelotto 2004). Co-migration through Beringian land bridges was also suggested for *C. arcuatorum* and *C. elegantior* (Garnica et al. 2011). Phylogeographic and coalescence analyses of American *A. muscaria* populations identified two endemic groups in Alaska, without evidence of recent gene flow from southern regions (Geml et al. 2006, 2010), again supporting a Beringian glacial refugia. Beyond documenting the history of EcMF populations, such large-scale population genetic studies contribute to understanding of EcMF biogeography.

2.5 Conclusions and Perspectives

The effective range of dispersal is an indicator of fruitbody and propagule types (Douhan et al. 2011). Fine-scale population genetic analyses clarify EcMF autecology and ecophysiology such as mycelium growth rate, persistence, reproductive biology, mating system (especially in ascomycetous EcMF whose study is now

starting) and dispersal mechanisms (Fig. 2.1). The role of microenvironmental parameters and individual plasticity on mycelium development and fructification patterns remains to be investigated, potentially with gene expression patterns at very-fine scale to re-explore the population dynamics studies of the 1990s. Currently, molecular tools can be applied to ectomycorrhizae and improve our ability to study the mycelial stage.

Nevertheless, EcMF population studies contribute to recognition of these organisms as a crucial part of ecosystem functioning and history. For instance, successful combination of demographic reconstruction, phylogenetics and paleoecology (e.g. Geml et al. 2010; Murat et al. 2004) have shown that EcMF are relevant contributors to the understanding of historical biogeography of host and associated vegetation.

Douhan et al. (2011) stated that we are reaching the era of population genomics for EcMF, giving access to adaptive traits beyond neutral traits deciphering only historical trends. Detection of selective footprints by comparing genomic structure of contrasted populations are now emerging to detect local specialisation and adaptation signatures (Bourne et al. 2014; Branco et al. 2015). This promising approach for understanding EcMF response to environmental constraints could be successfully coupled with transcriptomic analyses to identify genes actively involved in adaptation (e.g. Zampieri et al. 2011 for *T. melanosporum* cold adaptation). Another powerful combination of EcMF population genomics would associate host population genomics in order to look for genetic co-adaptation on both sides and its functional outcomes (e.g. Hoeksema and Thompson 2007). With the development of genomic and transcriptomic technologies, associating genomics and traits variation analyses in contrasted environments and hosts would open the understanding of ecology and evolution of EcMF populations.

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Chapter 3
Spore Dispersal in Ectomycorrhizal Fungi at Fine and Regional Scales

Thomas R. Horton

3.1 Introduction

Like fungal pathogens, the distribution of EcM fungi is directly influenced by patterns of host specificity and the distribution of their host plants. Readers interested in host specificity are referred to Molina et al. (1992) and Molina and Horton (2015). In this chapter, I review how the life cycles of ectomycorrhizal (EcM) fungi impact their fine and regional scale dispersal (short- and medium-distance dispersal, respectively). I also consider life history traits that impact establishment following dispersal. The majority of case studies highlighted here are from coniferous ecosystems. My hope is that the material included has broad applicability with respect to the biogeography of EcM fungi, and while the species may change, the families and genera of the fungi considered are represented in EcM tree systems across the globe. I present nuances unique to wind and animal vectors with respect to spore dispersal and establishment. Finally, I close by reviewing three life history traits that help fungi in Basidiomycota overcome problems inherent to establishment after dispersal to uncolonized areas: secondary homothallism, dispersal via mycophagy, and the production of resistant propagules.

It is important to keep in mind that studying EcM fungi under controlled conditions continues to be difficult, because most EcM fungi are difficult to isolate and grow in the absence of a host plant. Most species are not easy to grow even with a host plant, and only a handful have been observed to fruit under laboratory, growth chamber, or greenhouse conditions (Nara 2008). Furthermore, germination cues of spores of most species are still largely unknown making it difficult to assess spore viability, grow single spore isolates, or perform mating studies. Knowledge of the life history traits of EcM fungi comes from genera that grow in culture including

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Amanita, *Boletus*, *Cenococcum*, *Hebeloma*, *Laccaria*, *Pisolithus*, *Rhizopogon*, *Suillus*, *Tuber*, among others (see Cairney and Chambers 1999). Absent from this list are genera that are often important in EcM plant communities such as *Amphinema*, *Cortinarius*, *Inocybe*, *Lactarius*, *Piloderma*, *Ramaria*, *Russula*, and *Tricholoma*. The inferences about the ecology of most EcM fungi are necessarily based on lessons from the first set of taxa and from field studies.

Relying on field studies is limited by our ability to detect the presence of a species in an area, let alone its relative abundance. Firstly, although the fungi may be active on roots, they may not fruit when investigators are on site in any given year. Secondly, mycorrhiza and especially active mycelia are difficult to observe in belowground samples. Fortunately, there have been great advances with the development of molecular tools that allow us to detect EcM fungi from vegetative structures such as EcM root tips, hyphae, and propagules in soil (Gardes and Bruns 1993; Horton and Bruns 2001; Peay et al. 2008), and much of the work reviewed below was derived from such approaches. However, even if below ground in its vegetative state, the chance of a soil sample containing hyphae, root tips, or propagules from a particular EcM fungal species is very low (Horton and Bruns 2001; Taylor 2002). Although some may see these issues as a source of frustration, I see them as a fascinating opportunity to tinker with methods to work with EcM fungi and develop new strategies to target outstanding questions.

There are other inherent difficulties when researching the role of spore dispersal in the establishment of a new individual. To emphasize this point, consider the following: there are good data for the release of a basidiospore via ballistosporic discharge and Buller's drop (Buller 1924; Pringle et al. 2005) but little data on the fate of a spore following its release. Spores can be trapped under field conditions following dispersal, but how far a spore has traveled, and especially which sporocarp released it, is very difficult to determine unless captured in close proximity to the sporocarp (Galante et al. 2011; Li 2005). Further, there are data on how many spores some species produce (Buller 1924), but there are only limited data on how spores actually become established as new individuals, or even how many become established at all. Indeed, experiments to isolate environmental conditions impacting spore release and germination have only been conducted on a handful of EcM fungal species (Halbwachs and Bässler 2015). As a result, conditions that affect dispersal and establishment are still largely speculative for most species.

3.2 Spore Liberation

EcM fungi that are hypogeous (fruit below ground) or produce puffballs have lost the ability to forcibly discharge their spores and instead release them passively (Table 3.1). However, most EcM fungi have two mechanisms to forcibly eject spores: ballistosporic discharge in Basidiomycota and the bursting of the asci in operculate Ascomycota. Many pathogenic fungi fruit on plant parts of their host positioned above the ground, contributing to increased dispersal distances and the

Table 3.1 Examples of genera of ectomycorrhizal fungi grouped by their fruiting habit

Fruiting habit	Release of spores	Primary dispersal mechanisms	Basidiomycota	Ascomycota	Zygomycota *s.lat.*
Epigeous	Forcible[a]	Wind	*Amanita*, *Boletus*, *Cantharellus*, *Cortinarius*, *Inocybe*, *Lactarius*, *Leccinum*, *Paxillus* *Russula*, ***Suillus***[b], *Tricholoma*, *Tylopilus*	*Geopyxis*, *Peziza*, ***Wilcoxina***[c]	
Hypogeous	Passive	Mammal mycophagy	***Rhizopogon****(Suillus)*[d], *Truncocolumella*, *Alpova*, *Martelliela (Russula)*, *Gymnomyces (Russula)*, *Hydnagium (Laccaria)*, *Thaxterogaster (Cortinarius)*, *Hymenogaster (Cortinarius)*, *Amanita*, *Archangeliella (Lactarius)*, *Hysterangium (Gomphales)*, *Gautieria (Ramaria)*	*Genea*, ***Tuber***, *Balsamia*, *Terfezia*, *Elaphomyces*, *Choiromyces*	*Endogone*
Puffball	Passive	Wind	*Scleroderma*, *Pisolithus*		
Resupinate[e]/ Thelephoroid	Forcible	Wind	*Thelephora*, *Tomentella*, *Tylospora*		
Secotioid[f]	Passive	Wind/animal?	*Gastroboletus*, *Gastrosuillus*		

(continued)

Table 3.1 (continued)

Fruiting habit	Release of spores	Primary dispersal mechanisms	Basidiomycota	Ascomycota	Zygomycota *s.lat.*
Sclerotia[g]	Passive	Belowground	*Austropaxillus*, *Boletus*, *Cortinarius*, *Gyrodon*, *Hebeloma*, *Leccinum*, *Paxillus*, *Pisolithus*, *Scleroderma*	*Acephala*, ***Cenococcum***, *Phialocephala*	
Asexual spores	Passive	Belowground		***Wilcoxina***	*Glomus*, *Gigaspora*, *Sclerocystis*

Epigeous = above ground; hypogeous = below ground; puffball = spores enclosed in aboveground or erumpent sporocarp; resupinate = spores produced in a flat layer adhering to a surface such as a stick or log, but can also be loosely incorporated in the litter, may be epigeous

[a]Forcible discharge refers to ballistospores that are forcibly ejected from basidia (Basidiomycota) or ascospores that are forcibly ejected from asci (Ascomycetes); Passive release refers to spores that remain associated with the sporocarp but may be dispersed through mechanical means such as wind, rain, or animal traffic

[b]Bold type indicates there are species in the genus known to produce resistant propagules

[c]Wilcoxina produces sexual ascospores and asexual chlamydospores. Chlamydospores can build up in soils as a resistant propagule bank

[d]Names in parentheses are epigeous taxa thought to be most closely related to the hypogeous forms

[e]Resupinate sporocarps lie flat on the substrate without a cap or stem

[f]Like hypogeous sporocarps, secotioid sporocarps release spores passively within an enclosed hymenium. In contrast to hypogeous forms, this form includes remnant tissue derived from the stem and cap with varying levels of modification. Although they fruit above ground, they do not forcibly eject spores

[g]Dormant fungal tissue that resists decay and can survive deleterious conditions. See Smith et al. for a thorough treatment on fungi that produce sclerotia

chance to encounter a suitable host. In contrast, most EcM fungi fruit close to the ground, and even in those Basidiomycota with relatively long stems, the top of the cap is still positioned only about 15–20 cm above the ground. EcM fungi in Ascomycota that fruit above ground (epigeous habit) typically do not produce stems, and while they release spores into the air, they also do so close to the ground as exemplified by the small cup-shaped sporocarps produced by *Wilcoxina mikolae* (Fig. 3.1). Fungi that fruit close to the ground disperse spores at a fine scale, a fruiting habit that serves to maintain close proximity to suitable hosts and substrates.

Basidiospores produced by epigeous Basidiomycota are asymmetrical, a morphology related to ballistosporic discharge and the role of Buller's drop (Buller 1924; Pringle et al. 2005). The ballistosporic discharge mechanism in Basidiomycota is impressive in terms of the forces generated to release spores from the hymenium (Money 1998). However, the energy released quickly dissipates and likely does not greatly affect dispersal beyond the hymenium within the cap of the mushroom (Fig. 3.2; see also Stolze-Rybczynski et al. 2009). Those interested in spore liberation and aerial dispersal are directed to Buller (1922), Ingold (1971), Stolze-Rybczynski et al. (2009), and a recent review by Halbwachs and Bässler (2015) on Basidiomycota.

The caps of Basidiomycota are positioned such that spores fall down from the hymenium layer via gravity and most spores do not impact other gills as they fall. Some fungi such as *Amanita* spp. will reposition the caps through gravitropism to allow the spores to fall to the ground should the mushroom land on its side. Once the spores fall away from the boundary layer of the mushroom, they will be carried in wind currents. Many spores will still fall to the ground if wind currents are minimal (Nazaroff 2014) or when the caps are positioned very close to the ground or when spore bearing tissue is incorporated in the litter (e.g., in resupinate spp. such as *Tomentella* spp.). Many spores released from caps positioned 15–20 cm above the ground can still be captured close to the originating sporocarp with sticky

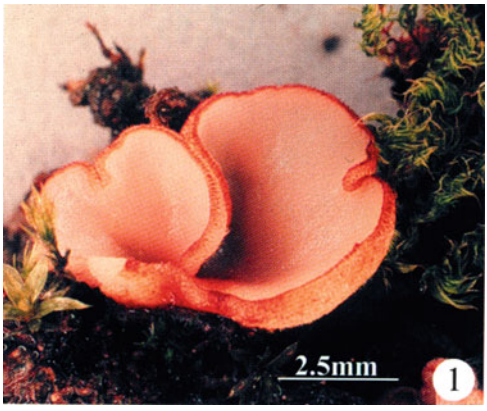

Fig. 3.1 A sporocarp of the diminutive *Wilcoxina mikolae* (note the scale bar). Modified from Trevor et al. (2001)

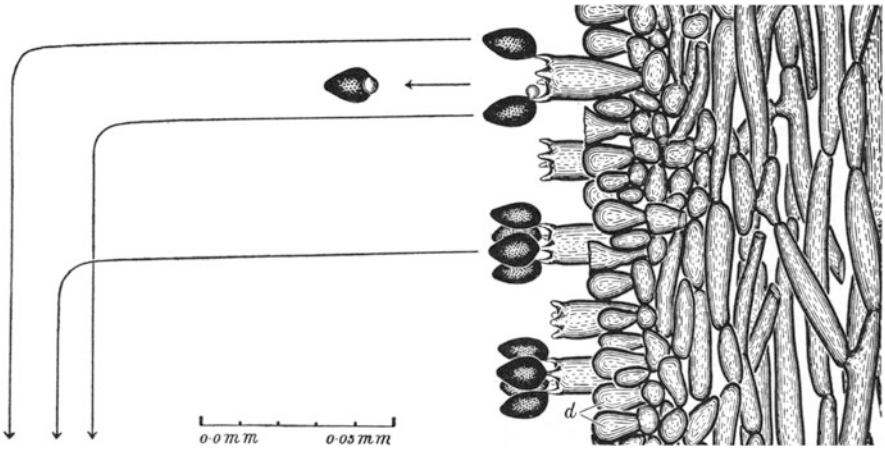

Fig. 3.2 A vertical section taken transversely to the long axis of a *Paneolus campanulatus* gill showing the probable trajectories of spores discharged from the hymenium. Three trajectories of spores, discharged in still air, are indicated by the *arrows*. Magnification, ×465. Note that only one side of a gill face is shown and the spores are not traveling far enough to impact the opposing gill face before gravity takes over. Modified from Fig. 97 in Buller (1922)

slides placed on the ground near the mushroom (Galante et al. 2011; Horton et al. 2013).

EcM fungal fruit bodies produced by Ascomycota primarily in *Helotiales* and *Pezizales* are also produced very close to the soil surface (Fig. 3.1). These spores are shot upward a few centimeters from the cup-shaped ascocarps. I am not aware of any reports documenting how far ascospores travel after being released from epigeous EcM Ascomycota such as *Peziza* or *Wilcoxina*. Interestingly, it appears that many EcM ascomycetes produce asexual propagules below ground (*Cenococcum*, *Wilcoxina*, etc.) or sexual spores in hypogeous sporocarps (*Tuber*, *Elaphomyces*, *Genea*, etc.).

3.3 Into the Air

Spores of EcM fungi are microscopic (measured in μm) and visible only in mass as a spore print from a basidiocarp or a cloud of spores from an ascocarp. While spores of many species are dispersed through the air by wind, it is very difficult to determine the distances the spores travel. Several years after Mount St. Helens erupted, Allen (1987) captured an average of one EcM spore (*Thelephoraceae* spp.) per 24 trap hours in wind traps positioned at various locations within the blast zone. Li (2005) observed less than 2% of spores released from *Amanita* sporocarps dispersed beyond a radius of 5.2 m from the source. We used microscope slides covered with a mixture of paraffin and petroleum jelly placed at various heights

above the ground along transects out hundreds of meters into the open dunes from a forest edge only to capture so much debris that it was impossible to know if spores were also captured (Galante and Horton; unpublished data).

Galante et al. (2011) recovered spores that fell to the ground along 60 cm radii emanating out from sporocarps of six EcM fungus species chosen in part to reflect a diversity of sporocarp stature and spore ornamentation: *Inocybe lacera*, short stature and ridged spores; *Laccaria laccata*, short stature and spiny spores; *Lactarius rufus*, medium stature and spores with reticulate ornamentation; *Suillus brevipes*, medium stature and smooth spores; *Suillus tomentosus*, medium stature and smooth spores; and *Thelephora terrestris*, medium stature and knobby spores. Ninety-five percent of the spores observed along the horizontal transects for all species fell to the ground in the first 60 cm, with the data following a negative exponential decay curve. Dam (2013) reanalyzed the data from Galante et al. (2011) and found a variety of other models fit the data as well, and all predicted a large number of spores would be found on the ground close to the source caps. Dam (2013) suggested that basidiospores are of a size that they are influenced by the viscous drag of air and that most will be transported until they return to earth by rain (Gregory 1945). However, Nazaroff (2014) reviewed the deposition on surfaces of indoor bioaerosols 3–10 μm in diameter, the size range of fungal spores. He concluded that deposition attributable to gravitational settling onto surfaces is an important fate for such particles, even when fans generating wind up to 20 cm s^{-1} (about 0.7 km h^{-1}) were in the room. While it is likely that outdoor particle dynamics will follow the dynamics seen indoors to some extent, spores will also become entrained in faster air currents and settle away from the source sporocarp.

How many spores are released from a sporocarp, and then how many of those are carried away from the cap versus land near the cap remains speculative. Buller (1909) estimated a single sporocarp might release 1×10^9 spores, and using this figure, Galante et al. (2011) gave a conservative estimate that 1×10^7 spores could disperse in a vertical direction above the cap even if many spores fell to the ground near the source cap. In a follow-up study, vertical spore dispersal was investigated by placing sticky slides at increasing heights up to 65 cm above the caps of *Suillus luteus*. Like horizontal deposition around a sporocarp, vertical dispersal of spores fit a negative exponential decay, with 95% of the observed spores on slides within 60 cm above the caps (Sørensen et al. unpublished data).

One way to increase spore dispersal distance is to increase the height from which the spores are released. Galante et al. (2011) found that stem length was one of two characters that impacted local dispersal distance (the other being spore size). This increases the number of spores that can enter the air stream. The spores themselves have various ornamentations that may influence dispersal (Halbwachs and Bässler 2015), but the role of spore ornamentation on dispersal has not been fully explored yet.

Quantifying the airborne spores from EcM fungi is extremely difficult. Saprotrophic and pathogenic fungi can be "trapped" by placing petri dishes with homokaryotic cultures on various surfaces; the formation of a dikaryon indicates a spore of a compatible mating type landed on the homokaryotic mycelium. But EcM

Basidiomycota do not grow fast, and if homokaryotic cultures of EcM species that can be maintained in culture (e.g., *Laccaria*; Wong et al. 1989 and *Hebeloma*; Debaud et al. 1988) are placed into a field setting, they will be quickly overrun by fast-growing Ascomycota.

Using uncolonized seedlings as bait and qPCR of fungal barcodes from rainwater, Peay et al. (2012) provided important data for dispersal from 0.5 m to 5.4 km from a forest edge. They showed that there was a dramatic decline in spores captured between the first 10 and 100 m from the source of spores for the three most abundant EcM fungi: *Suillus pungens*, *Thelephora terrestris*, and *Tomentella sublilacina*. The fact that the spores were collected in rainwater is interesting as this lends support to the idea that airborne spores can be washed out by precipitation (Gregory 1945; Ingold 1971) and can thus be carried over considerable distances in wind currents until a rain event. The observation of *Tomentella sublilacina* spores in the study is also interesting as this fungus has a resupinate habit. This leaves open the question of how the spores became airborne. Perhaps spores were carried by invertebrates (Lilleskov and Bruns 2005).

3.4 Short-Distance Wind Dispersal: Home Is Where the Roots Are

A new genet establishing following short-distance dispersal benefits from being in the area where conditions supported the growth and fruiting of the species (the thallus that yielded the mushroom). The location is already occupied by compatible host trees, and the edaphic conditions are suitable for that species of EcM fungus. However, the genet is also attempting to establish in a location with other EcM fungi, making it difficult to find uncolonized roots, particularly in the face of competition with previously established thalli.

While the maximum size of EcM individuals (strain, clone, genet) can be on the order of meters to tens of meters, the mean size is typically less than 3 m (Douhan et al. 2011; Lilleskov et al. 2004; Chap. 2). An EcM fungus genet does not completely fill the soil volume occupied—anyone who has sorted and identified EcM fungi from root tips knows that multiple species will occur on adjacent root clusters at the scale of mm. It is likely that a genet is distributed patchily throughout the volume of soil with clusters of roots connected by sparse networks of hyphae (Agerer 2001) and that most of the soil is not occupied by the strain even within the boundaries of the thallus. A new genet may very well find uncolonized root tips to establish, even in the same general location as that occupied by the mother thallus. Shiros (or castles; fairy rings produced by *Tricholoma matsutake* and related species) provide good evidence of single strains occupying large areas, at least in occupied rings (Chap. 15). However, some shiros are composed of multiple genets (Lian et al. 2006), possibly the result of the establishment of new genets following

spore release from the original or mother thallus. The local population structure of other EcM fungi (those that do not necessarily produce classic fairy rings) are also typically a patchwork of individuals (Beiler et al. 2010; Dunham et al. 2003; Kretzer et al. 2004, 2005) with multiple genets and species that compete for root tips and other resources at a fine scale.

The ability to differentiate between parental, sibling, and other genotypes in EcM fungi remains difficult. One reason being, as pointed out by Fries (1978), it is notoriously difficult to induce spore germination of most EcM fungi, a necessary step when sorting out the sexual compatibility system of a species. Somatic compatibility tests are also difficult because many of the fungi do not grow well in culture even as dikaryons (Bonello et al. 1998). Further, highly variable microsatellite markers are not abundant in EcM fungi and microsatellite markers have been developed in only a handful of EcM taxa (Douhan et al. 2011). As Fries suggested over 30 years ago, understanding the genetic system of these fungi will lead to a greater understanding of their biology (Fries 1987), and we are still working toward that goal for many EcM species.

3.5 Medium-Distance Wind Dispersal: How Far Is Too Far?

Spores that disperse via wind face a major limitation to establishing a new individual if they are transported to uncolonized areas. The vast majority of EcM fungi cannot be maintained in culture without living host roots. This is good evidence that a propagule (spore, sclerotium, chlamydospore) from most EcM fungi may begin to form a new thallus, but the individual will not survive without a host. EcM fungi in Basidiomycota face an additional critical impediment to establishing a new individual from a single spore. Although some Basidiomycota can form functioning mycorrhizal roots as monokaryons (Gardes et al. 1990; Kropp et al. 1987; Kropp and Fortin 1988), the majority of species are thought to form vigorous mycorrhizae only as dikaryons. This means that a single spore germinant is not likely to survive long if the species is not already established in the area even if compatible hosts are present. Hyphae from a Basidiomycota spore must encounter haploid hypha from a compatible strain in order to form a dikaryon (functionally equivalent to a diploid but the two compatible nuclei remain independent). A major limitation to successful establishment following medium-distance dispersal is the low probability of encountering germinants of compatible strains. This is not a problem with local dispersal because so many spores are present from the source sporocarp and other sporocarps in the area. Even if encountering spore germinants only from the source genet, 25% of those spores will be mating-type compatible in fungi with a tetrapolar mating system and 50% of the spores will be compatible for fungi with a bipolar mating system. Three species of *Laccaria* are known to have a tetrapolar mating system (Doudrick and Anderson 1989; Kropp and Fortin 1988), while *Rhizopogon*

rubescens has a bipolar mating system (Kawai et al. 2008). Because of the difficulties with inducing germination and identifying successful matings (dikaryons) in EcM Basidiomycota, the mating system of most EcM fungi has yet to be elucidated (Kawai et al. 2008).

The chances of encountering spores of the same species after dispersal by wind may be low irrespective of the mating system. If the new location lacks other individuals of the same species, the source of spores may be from the area that yielded the dispersed spore. But when spores are dispersed in wind, the spore rain becomes increasingly diffuse with distance (Galante et al. 2011; Peay et al. 2012). Very few spores will be encountered in the new location unless there has been time for a resistant propagule bank to develop in the soil.

Peay et al. (2012) reported less than one spore per cm^2 per day was captured 1 km from the source location. It is reasonable to assume that as the distance increases from the source of the spores, the chance of encountering spores from the same source in the new location becomes more remote. This diffuse spore rain likely played a large role in the reduction of colonization on seedlings placed at increasing distances from the source location in the study by Peay et al. (2012). It is unclear where the break point is for dispersal distance at which the probability is too low for enough spore rain to support establishment, but very few spores appear to disperse at the scale of km (Peay et al. 2012) even for the most prolific producers of spores such as species of *Suillus* (e.g., spores per mushroom and number of mushrooms fruiting in an area). However, dispersal limitation does not tell the whole story. Species of *Clavulina*, *Cortinarius*, and *Tricholoma* were relatively abundant in spore traps in Peay et al. (2012) but were not observed on mycorrhizal roots of the bait seedlings. It appears some unknown factor or factors limit most EcM fungi from establishing new genets after dispersal to new areas, perhaps related to spore behavior, interactions with more competitive species under the conditions at the site, or simply the physiological interaction between these species and their hosts as seedlings (Last et al. 1987).

3.6 Ascomycota

EM fungi in Pezizales (Ascomycota) occur in the vegetative state as haploids, and a single spore can lead to a functioning individual following dispersal. Indeed, EcM roots formed by species of Ascomycota are colonized by haploid mycelia. Ultimately, the interaction of two compatible mating types is needed to complete the life cycle with plasmogamy and karyogamy occurring in the ascocarp followed by meiosis and a post-meiotic mitosis to form eight haploid spores in each ascus. In contrast to Basidiomycota, in Ascomycota the two sexually compatible haploid mycelia remain independent ecological entities during the majority of the life cycle, reducing the negative effect of a diffuse spore rain. This may in part explain why

EcM Ascomycota are commonly encountered on plants in disturbed habitats and primary successional settings where mycelial networks are lacking and EcM fungi must establish through spore inoculum.

3.7 Secondary Homothallism

About 1% of spores in some *Suillus* spp. are binucleate (Bonello et al. 1998; Horton 2006), which is the same percentage of spores that germinate and are dikaryotic based on the presence of clamps in the germinants (Bonello et al. 1998). The production of dikaryotic spores is achieved through secondary homothallism, essentially a mechanism for self-fertilization. In Basidiomycota, a post-meiotic mitosis occurs during spore production resulting in eight nuclei, a feature of the life cycle not shown in most textbooks (Malik and Vilgalys 1999). The mitotic division may occur in the spore, sterigma, or basidium. If mitosis occurs in the basidium, eight nuclei can migrate into the developing spores with the possibility that two nuclei of compatible mating types may be packed together, resulting in a dikaryotic spore. Jain (1976) put forth the reproductive assurance hypothesis for plants suggesting that there is a selective advantage to selfing if a single propagule can establish a new viable population after dispersal to an uncolonized location even though selfing may lead to inbreeding depression. Evidence for selfing in Basidiomycota is scant, but has been shown in *Suillus*, *Laccaria*, and *Hydnagium* (Bonello et al. 1998; Jacobson and Miller 1994; Mueller et al. 1993; Treu and Miller 1993). Although many species produce binucleate spores, most nuclei in such spores are derived from a mitotic division following migration of a single nucleus into the spore, which can only form a haploid mycelium. Interestingly, a low percentage of binucleate spores are produced even in fungi that produce uninucleate spores, raising the possibility that dikaryotic spores may result from nucleus packaging errors during spore development (see Horton 2006 for more on EcM fungi that produce binucleate spores). It is unclear whether the production of dikaryotic spores is as beneficial to EcM fungi as selfing is to pioneer plant species, but this may contribute to the success of *Suillus* and *Laccaria* spp. in early successional settings.

3.8 Mycophagy

Another way EcM fungi increase chances for establishment following spore dispersal is through mycophagy. Spores of many EcM fungi are dispersed by animals that consume the sporocarps and deposit the spores in their feces. There are many records of mammals eating epigeous and hypogeous fungi or the spores being found in stomach contents or fecal samples (Cazares and Trappe 1994; Colgan and Claridge 2002; Fogel and Trappe 1978; Izzo et al. 2005; Luoma et al. 2003; Maser

et al. 1978). Alsheikh and Trappe (1983) reported a bird species eating desert truffles. Ashkannejhad and Horton (2006) found spores of both epigeous and hypogeous fungi (*Suillus* and *Rhizopogon* spp., respectively) in deer fecal pellets, and, importantly, pine seedlings inoculated with slurries made from the deer pellets yielded seedlings colonized by *Suillus* and *Rhizopogon* spp. Large mammals such as deer and wild boar can disperse spores up to several kilometers. Deer and boar fecal pellets can contain millions of spores (Ashkannejhad and Horton 2006; Nuñez et al. 2013) many of which are mating-type compatible, suggesting that dispersal of spores via mycophagy by large mammals has important advantages over the diffuse spore rain dispersed via wind over similar distances.

On Isla Victoria, Argentina, conifers were not establishing outside the perimeter of plantations despite the fruiting of compatible EcM fungi introduced with the conifers in the plantations (Nuñez et al. 2009; Simberloff et al. 2002). This pattern revealed two things. Firstly, the conifers were not associating with native EcM fungi associated with *Nothofagus*, at least not to the extent that supported establishment (Hayward et al. 2015a). Secondly, wind dispersal of spores from fungi fruiting in the plantations was not leading to conifer invasion into native *Nothofagus* stands. However, European wild boar and deer were recently introduced on the island and have established growing populations. Now the introduced mammals are eating the conifer-specific EcM fungi in the plantations. Seedlings inoculated with boar or deer fecal pellets collected during the fruiting season outside the plantations yielded conifer-specific EcM fungi on the seedlings (Nuñez et al. 2013). It appears as if the introduction of the conifers and their specific EcM fungi was not enough to lead to an invasion, probably because of dispersal limitations of the fungi (Nuñez et al. 2009). However, the conifers are now spreading into the native *Nothofagus* stands, suggesting that mammalian dispersal vectors of the EcM fungi were necessary for the invasion to proceed (Nuñez et al. 2013). Although the Northern Hemisphere conifers were able to associate with a few EcM fungi associated with in the Southern Hemisphere *Nothofagus*, they still could not establish in those stands. Like the primary successional system investigated by Ashkannejhad and Horton (2006) in Oregon, USA, the fungi supporting the spread of the conifers were primarily suilloid species in the genera *Suillus* and *Rhizopogon* that were introduced with the pines to Isla Victoria and dispersed by large mammals.

Spores dispersed by mycophagist mammals must survive passage through the digestive track of the animal to remain viable as inoculum. This same feature of resistance may allow the spores to lie dormant for an undetermined amount of time in soils. Ashkannejhad and Horton (2006) found spores of *Suillus* and *Rhizopogon* remained viable as inoculant in dry fecal pellets stored at room temperature for 1 year. Bruns et al. (2008) found that spore inoculum potential of four *Rhizopogon* spp. increased with time over a 4-year period.

3.9 Resistant Spores

The production of resistant spores that can remain dormant in soils may be another important life history trait that increases the chance for establishment of EcM fungi following dispersal. Horton (2006) used DAPI stain to observe the number of nuclei in spores collected from spore prints for a large number of EcM fungus species across multiple genera. While nuclei were observed in fresh spores of all fungi, spores from the same spore prints did not show nuclei after storage for 1 year at room temperature except *Rhizopogon* and *Suillus*. These data support other evidence suggesting that the spores of most EcM fungi are relatively short-lived but that *Rhizopogon* and *Suillus* produce spores are resistant and may form dormant spore banks in soils (Baar et al. 1999; Bruns et al. 2008; Horton and Bruns 1998).

EcM fungi that generate a resistant spore bank benefit from dormancy in an analogous way that plants benefit from soil seed banks (Simpson et al. 1989). Both *Suillus* and *Rhizopogon* appear to produce resistant spores, but *Rhizopogon* spp. may be unique in their dormancy mechanisms (Nara 2008). Horton et al. (1998) and Baar et al. (1999) sampled pine seedlings establishing after a stand replacing fire. *Rhizopogon* and to a lesser extent *Suillus* were the primary EcM fungi found colonizing postfire seedlings. This was in contrast to belowground studies from the area prior to the fire showing a variety of EcM fungi in the genera *Russula*, *Lactarius*, *Amanita*, *Laccaria*, and *Boletus* on mature trees and a near absence of *Rhizopogon* and *Suillus* (Bruns et al. 2005; Gardes and Bruns 1996; Horton and Bruns 1998; Taylor and Bruns 1999). These results supported results in Taylor and Bruns (1999) who showed a shift from a complex assemblage of fungi on in situ Bishop pine roots harvested in soil cores from the mature forest to a community dominated by *Rhizopogon* and *Suillus* on Bishop pine seedlings grown in soils from the same cores in a bioassay experiment. Mycorrhizal infection was likely from mycelial networks in the mature forest while the soil bioassay method selected fungi with resistant propagules in the soils, mostly spores but also sclerotia (e.g., *Cenococcum*) or chlamydospores (e.g., *Wilcoxina*). Removal of soils from the forest disrupts the mycelial networks and kills them by severing the mycelia from the host roots (carbon source). The hyphae may be infective initially (Horton et al. 1998) but appear to lose efficacy as soils dry out. However, EcM fungi that produce resistant propagules are able to survive the death of the host and drying of the soils and can colonize seedlings in bioassay experiments (see Table 3.1). This same feature enables these EcM fungi to survive lengthy dispersal events, facilitating establishment in new locations.

3.10 Conclusions and Future Perspectives

The understanding of spore dispersal in EcM fungi remains somewhat speculative for a number of reasons. Spores are difficult to track in field settings, and spores of most EcM species do not germinate readily if at all under laboratory conditions.

The fungi cannot be easily grown in lab even with a compatible host. The fungi are difficult to observe from samples of mycelia or mycorrhizal root tips collected from the field. Further, the sporocarps of many species may not be easily detected because of their cryptic nature and sporadic and ephemeral fruiting habits. However, applications of various PCR-based methods are providing insights about the distribution of the fungi, their population structure, and knowledge about establishment in new areas.

Spores of epigeous taxa are primarily dispersed via wind. Airborne spores may be deposited in close proximity to the sporocarp or be carried over considerable distances by wind. Spores that become airborne may be deposited with gravity on surfaces or remain airborne until a rain event washes them out of the air, but more empirical evidence is needed for airborne or water-borne spore deposition of EcM fungi.

Once spores are airborne, they likely become increasingly diffuse with dispersal distance. This may impact fungi in Basidiomycota more than Ascomycota because Ascomycota are haploid in the vegetative state and only need a single spore to establish a new thallus. Spores of Basidiomycota may not be able to successfully establish in uncolonized locations, because the hyphae of a haploid germinant are believed to be short-lived (but some species can form inferior mycorrhiza as haploids). A diffuse spore rain reduces the chance for hyphae from a germinating Basidiomycota spore from encountering hyphae from a compatible spore to form a dikaryon in uncolonized locations.

While many species of EcM fungi in Basidiomycota form binucleate spores, only a few are known to produce dikaryotic spores through secondary homothallism. The fitness cost of selfing for those that undergo selfing through secondary homothallism may be outweighed by gains from establishment in new areas. Species that produce dikaryotic spores may be able to establish easier after dispersal to uncolonized areas, because the germinant is a dikaryon and can form functioning EcM associations as long as compatible hosts are available.

Animal dispersal is relatively common in EcM fungi. The animals eat the sporocarps and pass viable spores through the digestive tract. Large mammals such as deer and boar can disperse spores over hundreds and sometimes thousands of meters. The fecal material can contain millions of spores from a single species but also contain a mix of spores from multiple species. Because there are so many spores of a species in the feces, the chance of a germinant encountering mating-type compatible spores is high and so fungi dispersed via mycophagy have an excellent chance of establishment in new locations, again, as long as roots of a compatible host plant are available.

Some EcM fungi produce resistant propagules in the form of sexual and asexual spores, as well as sclerotia. These propagules can lie dormant in soils until conditions or a suitable host becomes available. How long such propagules can survive in soils is not well documented. Spores from some species of *Rhizopogon* appear to become more viable over time in the dormant state.

Finally, it is interesting that the life history of *Suillus* and *Rhizopogon* spp. includes features that appear to contribute to successful establishment in new

locations, particularly resistant propagules and secondary homothallism. These same life history features may help explain why these fungi are often coinvasive with Pinaceae (Chu-Chou and Grace 1983a, b; Hayward et al. 2015a, b; Nuñez et al. 2009). The fungi were initially transported by humans to the Southern Hemisphere in nonsterile soil or litter from host stands or plantations in the Northern Hemisphere. Understanding the invasion biology of this model system may lead to insights into invasions of other mycorrhizal plants (Nuñez et al. 2008).

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Chapter 4
Processes Maintaining the Coexistence of Ectomycorrhizal Fungi at a Fine Spatial Scale

Laura M. Bogar and Kabir G. Peay

4.1 Introduction

Ectomycorrhizal fungi present a paradox when it comes to questions of scale. On the one hand, the bulk of their environmental influence—from decomposition to interactions with plant roots—is mediated at a micron scale, with much of the important chemistry occurring in a thin layer of mucilage ensheathing the hypha. On the other hand, these organisms defy their traditional categorization as microbes, living as genets ranging in size from a few centimeters to more than a dozen meters in diameter (Bonello et al. 1998; Gherbi et al. 1999; Kretzer et al. 2004; Chap. 2). Their dispersal is markedly limited by distance and host availability (Peay et al. 2010; Peay and Bruns 2014), and the influence they exert on global carbon and nitrogen cycles is decidedly macroscopic (Näsholm et al. 2013; Averill et al. 2014). But the interactions that determine what these communities do in the ecosystem happen when a hypha encounters a soil particle, another hypha, or a root. How do these fine-scale interactions, taken together, contribute to the levels of diversity we observe in ectomycorrhizal fungal communities?

The species-level diversity of ectomycorrhizal fungi has been well documented at the scale of forest stands (DeBellis et al. 2006), individual plants (Bahram et al. 2011), and soil samples (Anderson et al. 2014) and tends to be highest in the temperate zones (Tedersoo et al. 2012a). Although sampling strategies have changed enormously since the advent of next-generation sequencing techniques, measures of ectomycorrhizal fungal diversity have remained fairly consistent. It seems reasonable to say that the early direct sequencing studies represent a lower bound on the number of coexisting ectomycorrhizal species, while the next-generation data represent a closer estimate of true richness. In a given sampling

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plot, typically encompassing 100–400 m^2, observed richness can vary from as few as 16 (Saari et al. 2005) to more than 100 (DeBellis et al. 2006) ectomycorrhizal fungal species, and estimated richness is often much higher than observed. An individual tree may host 15 (Saari et al. 2005) to 122 (Bahram et al. 2011) ectomycorrhizal fungal species on its root system. Although these large-bodied fungi can extend for meters, the root system of a single tree could hypothetically host as many ectomycorrhizal fungal species as it has fine roots for colonization, with each fine root typically supporting just one ectomycorrhizal fungus (Smith and Read 2008). Even a single gram of soil can accommodate hundreds of meters of hyphae (Ekblad et al. 2013), potentially of many different fungal species, but ectomycorrhizal fungal species richness remains fairly limited.

This is particularly true when considering species richness at a centimeter scale, such as in particular soil samples, although spatial turnover in ectomycorrhizal fungal community composition remains fairly high. It is not uncommon to find several ectomycorrhizal fungal species within a few centimeters of each other (Gardes and Bruns 1996; Genney et al. 2006), but core-scale diversity is rarely greater than 10–20 species-level taxa per sample (Anderson et al. 2014). With such low species richness at a small scale, the fact that that these large-bodied microbes maintain relatively high diversity at larger scales suggests substantial variation in community composition from one soil core to the next. Beta diversity is high in many temperate habitats, with autocorrelation in ectomycorrhizal fungal communities dropping off after 3–4 m (Lilleskov et al. 2004; Pickles et al. 2012). This scale increases dramatically at lower latitudes, however, to ~150 m (Bahram et al. 2013). Here, we review the current understanding of how ectomycorrhizal fungi coexist at this fine scale and the ecological processes that generate core-to-core variability in fine-scale composition.

To explore possible drivers of fine-scale ectomycorrhizal community structure, we will apply the framework proposed by Vellend (2010) to consider mechanisms contained within each of four main processes: selection, dispersal, drift, and speciation. We define "fine-scale processes" as those occurring within a few centimeters of soil, although micron- and meter-scale processes can also have important fine-scale effects (Fig. 4.1). At the centimeter scale, only selective process can typically act directly, but all of the first three processes may be critically important in determining which fungi can coexist. The fourth process, speciation, may seem less immediately relevant to fine-scale ectomycorrhizal richness, but likely plays an important role in determining the composition of the regional species pool, from which local communities are drawn. We will address major factors influencing each of these processes for ectomycorrhizal fungi on a fine scale, with the goal of identifying which processes may be most important, clarifying the current state of research on these mechanisms, and suggesting promising areas for future investigations. In particular, we will investigate the extent to which spatial scale—of resource heterogeneity, fungal foraging, host carbon allocation, and even the size of fungal individuals—influences ectomycorrhizal fungal coexistence, and review the literature surrounding partitioning of both soil resources and host-derived carbon among ectomycorrhizal fungi at a fine scale.

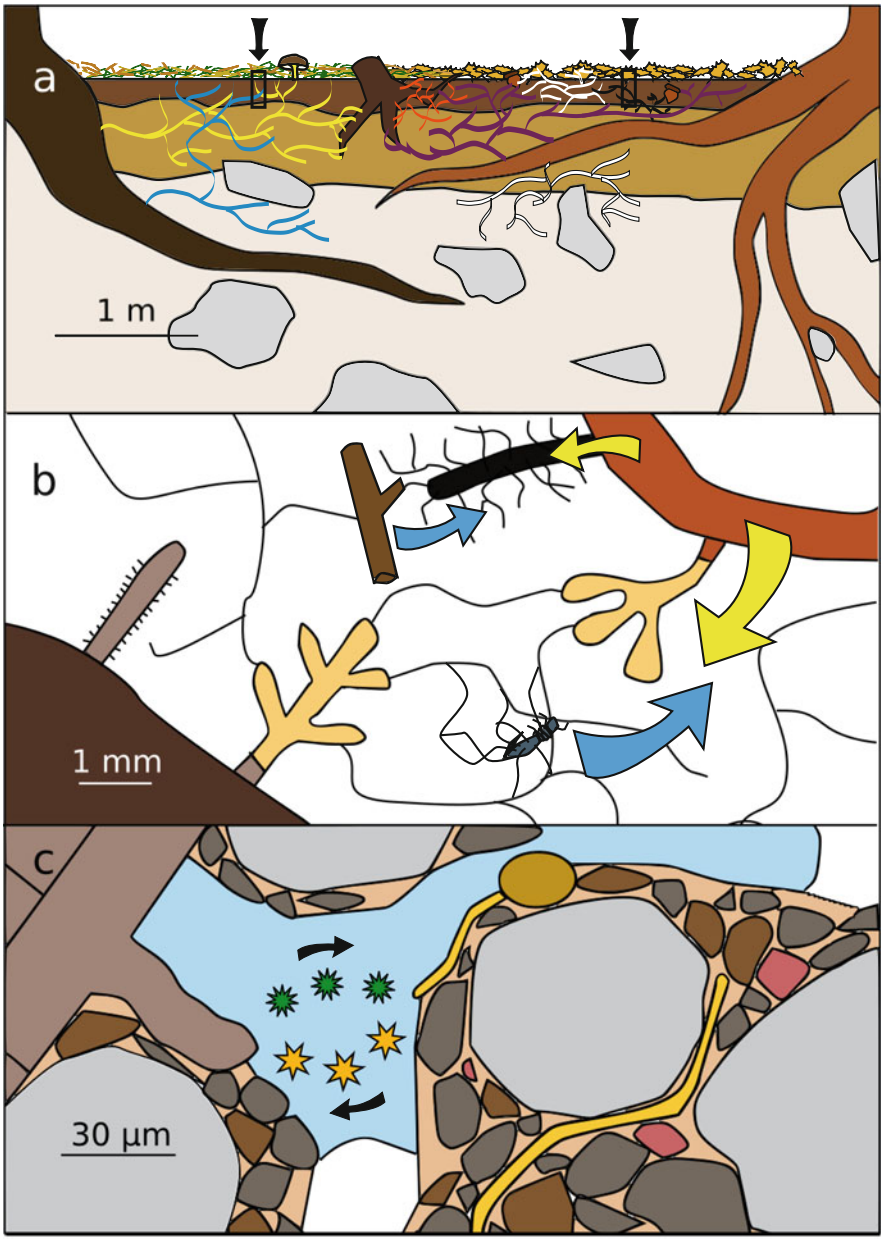

Fig. 4.1 The centimeter-scale distribution of ectomycorrhizal fungi is affected in important ways by processes that operate at several scales. At the meter scale (**a**), ectomycorrhizal fungi may compete with each other and saprotropic fungi for space, water, and patchily distributed resources (here, illustrated with seeds and a buried stump). Sampling cores (*arrows*) intersect with fungal individuals to capture a subset of the community but always offer an incomplete picture. Dynamics at individual root tips (**b**) are also important, as fungi compete for resource patches (here, a decaying invertebrate and twig) and trade soil resources (*blue arrows*) for carbon from the host plant (*yellow arrows*). *Arrow* size illustrates a hypothetical quantity of resource traded, with some

4.2 Ecological Selection

Just as natural selection changes the frequency of a phenotype within a population, ecological selection changes the frequency of a species within a community (Vellend 2016). Many traditional ecological theories pertain to selective processes: competition and fundamental niche differences are the primary mechanisms of ecological selection, while realized niches and their resulting community patterns can be seen largely as outcomes of these two selective processes. An ectomycorrhizal fungal hypha's responses to fine-scale physical, chemical, and biological heterogeneity will influence the fitness of the genet to which it belongs and the frequency of its species in the community and will alter how the forest system functions with respect to decomposition rates (Talbot et al. 2013), carbon storage (Averill et al. 2014), and resilience after disturbance events (Jones et al. 2003). Probably the most important selective pressures are related to soil environment and substrate—the ability of a fungus to acquire nitrogen, phosphorus, and host-derived carbon, and its tolerance of seasonal and environmental heterogeneity can vary substantially between individuals, populations, and species.

Selective processes are closely tied to the idea of the ecological niche. Any time two species of ectomycorrhizal fungi respond differently to their environment—that is, differ in their niche dimensions—changes in the environment may create ecological selection pressure that will lead to predictable changes in the composition and function of the community. These selective processes are among the dominant forces shaping ectomycorrhizal fungal community structure and observed niche occupancy at a centimeter scale. The context-dependent fitness differences between species play an important role in determining which taxa can coexist in a local patch by mediating competition and niche partitioning and influence the degree to which neighboring soil patches support similar communities.

4.2.1 Selective Processes: Fundamental Niche Differences Among Ectomycorrhizal Fungi

Fundamental niche differences are a key target of ecological selection that play an important role in determining the form and function of ectomycorrhizal fungal communities at a fine scale. Fungi may differ in their abilities to take up certain

Fig. 4.1 (continued) partners receiving more (*larger arrows*) than others. At a micron scale (**c**), hyphae traverse a heterogeneous system of soil particles and pores to find resources and roots. Here, we have illustrated a germinating spore communicating via diffusible signals (seven-point stars) with a plant root (signals represented by eleven-point stars) to determine whether they may initiate a compatible mycorrhizal interaction. Resources are distributed patchily at each of these scales, while competition is ubiquitous, presenting unique challenges for these organisms and likely determining coexistence at a centimeter scale

forms of nitrogen or phosphorus and may also differ in their tolerances for different quantities of host carbon. Over evolutionary time, other habits that may arise as the outcomes of inter- and intraspecific interactions—fruiting phenology, preferred soil habitat, and specificity for a particular host plant—can also become a part of the fundamental niche as organisms specialize on particular habits and lose the ability to behave otherwise. In this section, we will focus on how ectomycorrhizal fungi differ in their fundamental resource uptake niches with respect to nitrogen, phosphorus, and carbon.

Soil resource uptake: Nitrogen and phosphorus are, in many systems, the most important soil resources provided by ectomycorrhizal fungi to their host plants (Smith and Read 2008). Both of these resources exist in diverse forms in the soil, with organic forms often originating in decaying organic matter, and inorganic forms commonly adsorbed to mineral particles or dissolved in the soil solution (Finlay et al. 1992; Talbot and Treseder 2010; Cairney 2011). To exploit these resources fully, fungi must employ a broad range of chemical strategies, from oxidative degradation to break down recalcitrant tissues to direct enzymatic uptake of nitrogen and phosphorus when the resources are available (Lindahl and Tunlid 2015). Most nitrogen in the soil is in organic forms, encompassing a broad array of chemistries that vary in their availability to ectomycorrhizal fungi (Talbot and Treseder 2010), although anthropogenic inputs of inorganic nitrogen can also be important for ectomycorrhizal fungal communities (Lilleskov et al. 2001). Phosphorus supplies vary by soil type and age and exist in inorganic (phosphates) and organic (phosphate mono- and diesters and inositol phosphates) forms (Turner 2008; Cairney 2011). While it has long been suspected that these resources are important, recent studies have provided a more complete picture of how substrate partitioning contributes to observed patterns of fine-scale diversity and composition (Tedersoo et al. 2003; Courty et al. 2010; Taylor 2014).

Evidence for fundamental niche partitioning with respect to soil resources is strongest in culture-based axenic and co-xenic studies, in which a single fungus is offered a range of nutrient substrates for uptake with either exogenous hexoses or an otherwise non-mycorrhized host plant as a carbon source. These studies eliminate the influence of co-occurring fungal competitors and, although the setting is highly artificial, make it possible to estimate the fundamental niche of a given fungus along a resource axis of interest by providing "optimal conditions" for fungal performance (Crowther et al. 2014). Ectomycorrhizal fungi have long been known to exhibit interspecific variation in their use of particular nitrogen sources in these experimental settings, such as nitrate (Nygren et al. 2008), amino acids (Abuzinadah and Read 1988), and protein (Finlay et al. 1992). For phosphorus, in vitro work has also confirmed that different ectomycorrhizal fungal species can exploit mineral and organic sources to different extents (Lapeyrie et al. 1991) and that these various phosphorus acquisition strategies can correspond to differences in plant nutrition (Baxter and Dighton 2005). Ectomycorrhizal fungi embody myriad resource uptake niches that help to support the diversity observable in the field.

Importantly, the resource use profiles of these fungi should be generally nested: a fungus that can use a complex or recalcitrant substrate, such as protein or inositol phosphate, would be expected to be able to use a simpler resource as well, such as

ammonium or inorganic phosphate, but the reverse may not be true (e.g., Finlay et al. 1992). This asymmetry may be associated with trade-offs. For instance, perhaps fungi with narrower substrate niches make up for their specialization by being better competitors for space on the root system. In ectomycorrhizal fungi, trade-offs are known to exist between spore-based colonization and competitive abilities (Nara 2009; Kennedy et al. 2011), and a recent study showed a trade-off between enzyme production and root tip colonization (Moeller and Peay 2016). Further experiments will be required, however, to determine if substrate specialization per se corresponds to reduced competitive ability. More experimental tests of these potential trade-offs would be helpful to clarify how this nested functional diversity contributes to fine-scale coexistence.

Carbon resources: Host-derived carbon may be one of the most significant common resources in ectomycorrhizal fungal communities. Since these fungi are, in ecological settings, obligate biotrophs, this resource is a necessity for all members of the guild. Unlike soil-derived resources, which can take many forms and exist in diverse substrates, host-derived carbon is available to ectomycorrhizal fungi only as hexoses at the growing tips of fine host roots (Nehls et al. 2007). The lack of variability means that this critically important niche axis is also probably the most difficult to partition and may be the site of the most intense competition in ectomycorrhizal fungal assemblages. The fundamental carbon niche of ectomycorrhizal fungi is likely to be almost identical across taxa. Despite this, there may be opportunities for ectomycorrhizal fungi to reduce competition for this resource by varying their carbon demands.

Carbon resource partitioning by differential demand may be important in ectomycorrhizal fungal communities at a fine spatial scale. The carbon demand of ectomycorrhizal fungi is context dependent, increasing in the presence of resource patches (Bidartondo et al. 2001) or competitors (Leake et al. 2001), but the extent to which it might differ systematically between ectomycorrhizal taxa remains unclear. Ectomycorrhizal fungi have traditionally been divided into two principal categories: early-stage fungi with limited carbon demands that are stronger dispersers than they are competitors, and late-stage fungi that need more carbon and are strong competitors but poor dispersers (Deacon and Fleming 1992; Bruns 1995). Early-stage, low-carbon fungi tend to end up on the distal root tips of mycorrhizal seedlings, while the late-stage fungi tend to establish themselves closer to the stem, perhaps moving outward with successional time (Gibson and Deacon 1988; Peay et al. 2011). If the root can be modeled as a "leaky hose" of carbon resources leading from the stem to more distal roots (Bruns 1995), the observed spatial partitioning of these early- and late-stage fungi may in fact represent a partitioning of the carbon resources available from the host plant. Substantial discrepancies have also been observed between the number of root tips colonized by a particular fungal species and the quantities of hyphae and fruit bodies observable in the surrounding soil (Gardes and Bruns 1996; Kjøller 2006), suggesting that there likely are important differences among fungi in the amount of carbon extracted per infected root tip. These differences in carbon use efficiency may also be reflected in the exploration types of particular fungi (Agerer 2001), with those

that produce a lot of extramatrical mycelium potentially being much stronger sinks than other types (Weigt et al. 2011). If ectomycorrhizal fungi demand different quantities of carbon that may be spatially partitioned, it is possible that the carbon niche space for these fungi is larger than it initially appears.

4.2.2 *Selective Processes: Competition Among Fungi and Between Fungi and Plant Roots*

Centimeter-scale competition, both among ectomycorrhizal fungi and between these fungi and plant roots, is a primary mechanism by which ecological selection can act on ectomycorrhizal fungal communities. Studies examining ectomycorrhizal fungal co-occurrence data at a centimeter scale often find evidence for both competitive and, occasionally, facilitative interactions (Agerer et al. 2002; Koide et al. 2005; Pickles et al. 2012). These processes can change resource use dynamics in significant ways, with important implications for resource availability and community composition in the systems where these fungi are active.

In studies involving multiple ectomycorrhizal fungi competing for host carbon, with niche space more densely filled by competing fungi, the available soil resources tend to be more completely exploited than when fewer fungi are involved. For example, Baxter and Dighton (2005) tested the effect of ectomycorrhizal fungal species richness on host plant phosphorus nutrition. In any given microcosm, there was only one chemical form of phosphorus, so there was no opportunity for niche partitioning to improve host nutrition under high fungal diversity. Despite this, the presence of multiple fungi improved plant phosphorus nutrition in the organic phosphorus treatment, indicating that competition among the fungi may have led to more complete utilization of the resource than occurred in the lower-richness fungal communities. Fungi may also try to provide more benefits to their hosts when fungal competitors are present, possibly contributing to the resource drawdown observed by Baxter and Dighton (2005). For example, the ECM fungus *Thelephora terrestris* was shown to increase acid phosphatase activity on seedlings when it was co-inoculated with the competitor *Rhizopogon occidentalis* (Moeller and Peay 2016). Increased symbiotic function in competitive settings has been documented in arbuscular mycorrhizal associations (van der Heijden et al. 1998) and is likely also an important competitive outcome structuring ectomycorrhizal fungal communities.

Most work on competition in ectomycorrhizal fungi has focused on intra-guild interactions, since the ecologies of ectomycorrhizal fungi are so similar that competition should be intense, but these fungi also rely on many of the same resources that saprotrophic and endophytic fungi do. Interactions between ectomycorrhizal and these other fungi are probably important in shaping the distributions of all of them. Endophytic fungi associated with ectomycorrhizas, for example, are diverse (Tedersoo et al. 2009), and their co-occurrence patterns with ectomycorrhizal fungi suggest a complex set of competitive and facilitative

interactions among these plant symbionts (Toju et al. 2016). It has long been hypothesized that competition between ectomycorrhizal and saprotrophic fungi may be important in determining rates of organic matter decomposition (Gadgil and Gadgil 1975), and it's possible that competition of this type could explain the substrate partitioning between these two groups observed by Lindahl et al. (2007).

Among ectomycorrhizal fungi at a local site, competition may explain centimeter-scale habitat specialization better than fundamental niche differences. Mujic and colleagues demonstrated that vertical partitioning of the soil column by two *Rhizopogon* species was likely driven by interspecific competition, not soil chemistry, despite substantial differences in soil characteristics between the upper and lower horizons used in the experiment (Mujic et al. 2015). At a community level, co-occurrence data also suggest that competitive interactions are important in structuring ectomycorrhizal fungal communities (Koide et al. 2005; Pickles et al. 2012), although there are exceptions (Kennedy et al. 2014). Nonrandom distributions of ectomycorrhizal fungi, both horizontally and vertically, are probably the result of both abiotic heterogeneity and competition with other decomposers and plant roots.

Unlike competition for soil resources, the outcome of which is probably controlled by how efficiently each competitor can exploit the resource, competition for carbon is mostly likely based on fungi interfering with each other's access to host roots. Many studies have demonstrated competition among ectomycorrhizal fungi for space on a host plant's root system, which likely corresponds to access to host-derived carbon (Hortal et al. 2008; Kennedy et al. 2009). Competitive interactions are likely the most significant force encountered by ectomycorrhizal fungi as they exploit host-derived carbon resources. Research into the extent to which ectomycorrhizal fungi vary in their carbon demand, the extent to which plants can reward particular fungi for high-quality symbiotic performance, and the extent to which carbon may be partitioned despite its limited diversity will be enormously useful in clarifying how ectomycorrhizal fungi negotiate their coexistence on shared host roots.

Competition for carbon and soil resources is not just important between different ectomycorrhizal fungal species. Intraspecific diversity, which should correspond to greater niche overlap and more intense competition among the fungi than interspecific diversity, may be even more important than species richness for improving host nutrition (Hazard et al. 2016). This may be partly the result of having a broader array of potential symbionts available, improving the chances of associating with a very high-quality partner, and may also correspond to more efficient utilization of available resources in diverse communities. Resource drawdown is important when considering fungal competition with plant roots: although ectomycorrhizal fungi have a reputation for improving plant nutrition in nitrogen-limited systems, evidence is accumulating to suggest that the fungi themselves induce the nitrogen limitation in the first place (Näsholm et al. 2013; Corrales et al. 2016) by immobilizing soil resource pools that might otherwise have been plant available. Since hyphae in the soil can have equivalent or greater surface area than plant roots (Rousseau et al. 1994), and often have more extensive and kinetically efficient

enzymatic repertoires for nitrogen uptake (Chalot and Brun 1998; Leake et al. 2004; Talbot and Treseder 2010), they may be in a position to immobilize the resource before plant roots have a chance to access it.

This direct competition between plant roots and ectomycorrhizal fungi may indirectly influence plant–plant competition. Those plants capable of trading photosynthate for nitrogen from ectomycorrhizal fungi are, effectively, drawing down the nitrogen more efficiently than plants associated with less effective nitrogen scavengers (e.g., arbuscular mycorrhizal plants). In the framework of Tilman's resource competition theory, ectomycorrhizal plants may have a lower R* for nitrogen than their competitors, meaning that they can draw down the resource and persist with lower levels of nitrogen than other plants (Tilman 1990). This may allow them to locally exclude plants that require nitrogen from non-ectomycorrhizal sources (Tilman 1990; Peay 2016). This may explain the frequency of monodominant stands of ectomycorrhizal plants in otherwise mixed forests (Torti et al. 2001), as locally induced nitrogen limitation may render it difficult for non-ectomycorrhizal plants to grow (Peay 2016).

The extent to which a fungus can draw down the available resources will, in many cases, determine its ability to compete with other fungi and plant roots in the vicinity, which in turn can affect host plant nutrition and aboveground competition between host and nonhost plants. Future work examining the scale and extent of resource drawdown, as well as the impact of inter- and intraspecific fungal competition on resource use, will add valuable nuance to our understanding of nitrogen and phosphorus dynamics in ectomycorrhizal fungal communities at a fine scale.

4.2.3 Outcomes of Selection: Realized Niche Partitioning

The realized niche of an ectomycorrhizal fungus represents the combined outcome of its fundamental niche, environmental factors, and interactions such as competition. Essentially, the realized niche serves as the ecological phenotype on which selective processes may act, shifting community composition over time. This realized ecology is the level at which competitive niche partitioning can occur, leading to complementarity in communities, convergent function across different ectomycorrhizal fungal assemblages, and structured patterns of association with particular host plants, including host specificity.

Soil resources: Studies of nitrogen and phosphorus uptake at the community level suggest that ectomycorrhizal fungal communities in similar environments tend to converge upon similar resource use profiles, despite differences in taxonomic composition and clear partitioning of resources among different fungal taxa. Co-occurring ectomycorrhizal fungi often have substantially different enzymatic capabilities for taking up nitrogen and phosphorus resources (Buée et al. 2007; Jones et al. 2010). Some of these differences may correspond to exploration type: low-biomass, short-distance exploration types tend to respond more positively to inorganic nitrogen inputs than long-distance exploration type fungi (Suz et al. 2014), and the fruiting

bodies produced by long-distance ectomycorrhizal fungi may be substantially more enriched in nitrogen-15 than those from short-distance fungi (Hobbie and Agerer 2010), suggesting they exploit different resource pools. It is worth noting that, in some systems, stable isotope measurements do not correspond well to potential enzyme activity measurements of root tips (Tedersoo et al. 2012b), suggesting that these measurements capture different aspects of fungal resource partitioning.

At the community level, different sites may have similar enzymatic profiles despite variation in the species composition and activity of particular taxa (Jones et al. 2010). The consistent community-level enzyme profiles suggest that, in many settings, ectomycorrhizal fungi compete for resources from a conserved set of substrates that selects for taxa with similar suites of enzymes. This result echoes findings from plant community ecology, where taxonomically dissimilar communities may converge to support a similar suite of functional traits over successional time (Fukami et al. 2005). Experiments manipulating community composition will be required to determine if this functional convergence principle holds true in ectomycorrhizal fungi, paying particular attention to spatial scale.

It will be essential to examine several lines of evidence to determine the true resource acquisition activities occurring in a given soil sample. For example, Bödeker and colleagues combined DNA and RNA sequencing with soil chemistry and enzyme activity measurements to implicate *Cortinarius* species in oxidative degradation of humus (Bödeker et al. 2014). Future work should capitalize on modern large-scale genomic, transcriptomic, and metabolomic tools to more fully characterize the function of ectomycorrhizal fungi in soils (see Shah et al. 2016 for a compelling in vitro example; similar experiments in natural settings would be especially illuminating). It would also be useful to determine the extent to which overlapping enzyme profiles may competitively exclude fungi that would otherwise coexist. Despite the need for more research, the available evidence strongly suggests that competitive niche reduction and resource partitioning processes act as powerful selective forces determining the composition and spatial turnover in ectomycorrhizal fungal communities at a centimeter scale and may push communities with different taxonomies into similar functions.

Host-derived carbon: Just as soil resource partitioning can be seen as an outcome of selective processes on the fundamental resource niches of ectomycorrhizal fungi, host-derived carbon can be partitioned among fungi based on which plants they can interact with and how much carbon those plant hosts are willing to allocate to them. Some of the variation in realized host compatibility and carbon allocation will be context dependent, affecting the realized niche, while other parts of it may be fundamental to the chemical and physiological cross talk between a particular plant and a particular ectomycorrhizal fungus. This essential variation in the dimensions of a fungus' carbon niche, mediated by its obligate relationship with a plant host, may help allow for the coexistence of diverse communities despite an essentially one-dimensional carbon resource.

Although ectomycorrhizal fungi generally are capable of interacting with a broad range of host plants, specificity may represent a unique case of realized niche partitioning in mixed forest environments. Studies of multiple-host forests tend to

find many ectomycorrhizal fungal generalists and a small handful of specialists (Kennedy et al. 2003; Ishida et al. 2007; Tedersoo et al. 2008). The breadth of the realized host range of a fungus can depend strongly upon the environment, from soil characteristics (Roy et al. 2013; Peay et al. 2015) to the community composition of potential host plants in the vicinity (Wolfe and Pringle 2012; Bogar and Kennedy 2013). The variation in and mutability of the host range of ectomycorrhizal fungi suggest that the phenomenon is underlain partly by physiological necessity on the part of the fungi—that is, differences in their fundamental niches that are acted upon by ecological selection—and partly by competitive dynamics with other fungi.

The chemistry and physiology driving symbiotic compatibility in the ectomycorrhizal association are, so far, poorly characterized. The mutualism as a whole likely relies on signaling between the plant and the fungus, mediated in part by fungal effector molecules that can interact with the plant's immune system to support symbiosis (Garcia et al. 2015). Ectomycorrhizal fungal genomes are rife with putative effector molecules (Kohler et al. 2015; Peter et al. 2016), but only one of these (MiSSP7 from *Laccaria bicolor*) has been functionally characterized to date (Plett et al. 2014). To understand why some fungi seem to prefer particular hosts (or, just as likely, why particular hosts prefer certain fungi), it is essential that we clarify the functions of plant and fungal signaling molecules, discerning the biochemical pathways with which they interact and the stages of symbiotic development at which each of them is most important.

Adding complexity, host range observed in the field is often context dependent. In laboratory microcosms, the fundamental host range of a fungus tends to greatly exceed the realized host range observable in field settings, a phenomenon known as ecological specificity (Smith and Read 2008; Molina and Horton 2015). It seems reasonable, then, to think that the host range observed in the field reflects context-specific niche partitioning among these obligately biotrophic fungi and may support their coexistence at a fine spatial scale.

Even once a compatible association is established, the plant likely plays an important role in determining which ectomycorrhizal fungi get the most carbon. At a fine scale, the balance between fungal carbon demand and plant carbon allocation may be a key determinant of ectomycorrhizal fungal community composition and probably has a major influence on competitive outcomes belowground. Plants can reward cooperation in the arbuscular mycorrhizal symbiosis by providing more carbon to effective partners (Bever et al. 2009; Kiers et al. 2011) and can punish defection of nitrogen-fixing bacterial symbionts by withholding oxygen (Kiers et al. 2003). It seems very likely that similar mechanisms operate in the ectomycorrhizal symbiosis. In a split-root system, ectomycorrhizal pine seedlings can direct more carbon resources to fungal partners with greater nitrogen supplies (L. Bogar, in prep.), but much work remains to determine the scale at which ectomycorrhizal plants can distinguish between different symbionts and the extent to which carbon allocation is truly coupled to the symbiotic performance of the fungi with respect to nitrogen, phosphorus, moisture, and other potentially important services.

The reverse process, in which fungi may give more resources to plant roots providing more carbon, has yet to be demonstrated in the ectomycorrhizal association, although it can happen in the arbuscular mycorrhizal symbiosis (Kiers et al. 2011) and may be an important mechanism reinforcing mutual cooperation. If the plant can specifically reward the most effective ectomycorrhizal fungal symbionts with carbon, the community that assembles on its roots could, potentially, be driven more strongly by the plant's nutritional needs than by other environmental variables. The influence of preferential carbon allocation on ectomycorrhizal fungal communities will be a fruitful area for future research.

The realized carbon niche of an ectomycorrhizal fungus depends in large part upon its interactions with host plants, particularly with respect to symbiotic compatibility and the amount of carbon the fungus can demand from a given host. The outcomes of these interactions, including phenomena such as ecological specificity, can be seen as the result of ecologically selective processes acting on fungal carbon acquisition. We hope that future work will improve our understanding of the degree to which ectomycorrhizal fungi can vary in their carbon demands and the extent to which symbiotic compatibility may be altered by environmental conditions.

Temporal niche partitioning and storage effects: Temporal niche partitioning can come about as the outcome of ecological selection on fundamental niche differences and competitive interactions and may play an important role in mediating ectomycorrhizal fungal coexistence. The theory of the storage effect suggests that species should be able to coexist under fluctuating conditions if they have unique responses to the environment, there is covariance between the environment and competition, and population growth is "buffered," allowing the species to survive temporarily adverse conditions (Chesson et al. 2001). Ectomycorrhizal fungi, as we have reviewed in the preceding sections, do respond differently to environmental variables, which have implications for competitive outcomes, and their populations are buffered by their persistence in spore banks (Bruns et al. 2009) and, potentially, by their simultaneous colonization of multiple host plants at any given time [see Moeller and Neubert (2016) for a discussion of the reciprocal hypothesis from the perspective of a host plant].

It has long been acknowledged that ectomycorrhizal fungal species fruit in different seasons, and this pattern is mirrored by the striking seasonal variation in the abundance of belowground fungal structures. Koide and colleagues, for instance, detected three distinctive temporal strategies employed by ectomycorrhizal fungal hyphae in a pine stand: one group of fungi maximized its activity in the spring, another in the fall, and the third appeared to exist at consistent abundances throughout the year (Koide et al. 2007). Distinct, taxon-specific seasonal strategies have also been observed in oak ectomycorrhizal root tips (Courty et al. 2008) and ectomycorrhizal hyphae (Voříšková et al. 2014).

The mechanisms driving seasonality in ectomycorrhizal fungi likely encompass a wide range of drivers, many related to the selective processes discussed in the preceding sections. Soil moisture, temperature, and litter quality may all vary seasonally and have substantial bearing on how well particular ectomycorrhizal fungi can grow (Ekblad et al. 2013). Resource allocation and fine root production by the host plant also varies seasonally. In general, mycelial extension of

ectomycorrhizal fungi appears to peak at approximately the same time as fine root production by the host plant, reflecting the tight interconnectedness of these carbon-reliant processes (Wallander et al. 2001). The extent to which carbon allocation changes from season to season has been observed to vary between plant species in a mixed forest (Epron et al. 2011)—this interspecific variation is probably in part attributable to differences in carbon fixation among the plants, but may also reflect differences in their symbiotic strategies.

Temporal changes in belowground carbon allocation, not clearly coupled to photosynthate availability, may reflect a bet-hedging strategy on the part of the plant. Moeller and Neubert found that, given an environment that varies at a moderate frequency (such as seasonal shifts), a plant may maximize its long-term fitness by investing carbon resources nonspecifically in their ectomycorrhizal fungal symbionts, supporting fungi that are not optimal partners at a given time (Moeller and Neubert 2016). In their model, this process allows the plant to maximize its exploitation of favorable conditions when they occur by accepting some carbon losses to the suboptimal fungi during less favorable seasons. Such seasonal bet-hedging may explain the persistence of a core ectomycorrhizal fungal community from season to season—the group that Koide and colleagues observed having no seasonal dynamics—despite major environmental fluctuations that otherwise should select for high temporal turnover in most ectomycorrhizal dominated forests.

Both theory and empirical data suggest that storage effects may help to explain the coexistence of the 10–20 ectomycorrhizal fungi typically present in a single forest soil core. At any given time, some proportion of the fungi identified in a given study are likely dormant, awaiting more favorable conditions or less intense competition before resuming activity. This has been demonstrated to be an important process in bacterial communities (Jones and Lennon 2010), and dormancy as spores is known to be an important mechanism for ectomycorrhizal persistence in soils (Bruns et al. 2009), though vegetative dormancy has not been thoroughly explored in this group. Ectomycorrhizal fungi may coexist at a fine spatial scale by timing their activities to avoid competition while maximizing the availability of host-derived carbon and soil resources.

Spatial niche partitioning: Ectomycorrhizal fungi experience tremendous heterogeneity in the soil environment, but it is unclear at what scale this variation impacts their realized niche dimensions with respect to hyphal foraging, competitive outcomes, and the fitness of fungal individuals. Certainly, ecological selection acting on fundamental niche differences and competitive outcomes should influence ectomycorrhizal fungal responses to environmental heterogeneity. The simultaneous macro- and microbial lifestyle of these fungi may allow them to take advantage of microscopic resources across a large area, reaping the benefits of long-distance resource translocation and micron-scale resource access available to few other organisms. If ectomycorrhizal fungi do integrate fine-scale heterogeneity across their bodies, however, it remains an open question whether patchiness at a scale smaller than a ramet should matter for the fitness of the fungus and at what scale these patchy resources might be partitioned.

There is some evidence that ectomycorrhizal fungi can occupy spatially distinct soil environments, which could create spatially variable selection and contribute to the coexistence of fungi that, in a homogenous environment, would be eliminated from the community by competition (Kim et al. 2008). Vertical niche partitioning by ectomycorrhizal fungi, for example, is well documented in a number of systems (Dickie and Xu 2002; Tedersoo et al. 2003; Bahram et al. 2015), with the most striking transitions in ectomycorrhizal fungal communities usually associated with the transition from the organic to the mineral horizon in a forest soil column (Taylor 2014). Ectomycorrhizal fungi also appear to occupy different habitats from saprotrophic fungi, preferentially occupying old litter and humus while leaving younger litter resources for saprotrophic fungi (Lindahl et al. 2007). It is unclear, however, what components of observed spatial niche partitioning are related to fundamental niche differences in fungi and which of them are the outcomes of species interactions constraining or expanding the soil environments a fungus can occupy. Observed patterns of spatial partitioning likely integrate fundamental niche differences, competitive outcomes, and niche construction in the soil environment.

Heterogeneity in the soil environment is thought to generally increase species richness in ectomycorrhizal fungal communities, allowing for higher root tip colonization and extrametrical mycelium production (Erland and Taylor 2002). The scale of this heterogeneity will determine the scale at which coexistence and spatial turnover occur in ectomycorrhizal fungal communities. At a large spatial scale, variation in factors such as moisture and resource availability certainly has important impacts on ectomycorrhizal fungal community structure (Lilleskov et al. 2002; Erlandson et al. 2016). In soils, however, much of the heterogeneity exists at such a fine spatial scale that it remains mostly unexplored. The environments traversed by a single hypha may span enormous variation in abiotic conditions, but this micron-scale detail is impossible to capture in a typical environmental sequencing project of homogenized soil cores (Vos et al. 2013). In particular, factors such as pH, soil moisture, nitrogen availability, and the prevalence of competitors can vary substantially at a fine spatial scale and have important implications for ectomycorrhizal fungal coexistence.

The fine-scale heterogeneity of soils is just beginning to be explored and probably has important implications for the structure of ectomycorrhizal fungal communities. It seems likely that ectomycorrhizal fungi are sensitive to centimeter-scale environmental heterogeneity, especially along vertical soil profiles, but that their relatively large body sizes may buffer them somewhat from the effects of soil variability at a finer scale. Fundamental niche differences may be responsible for the habitat specialization of some ectomycorrhizal fungi, but at a fine scale, competition appears to be an important force shaping community composition and spatial turnover. We hope that future experiments will explicitly account for the scale of patchiness in the environment to discern the extent to which environmental heterogeneity affects niche partitioning and competitive outcomes for ectomycorrhizal fungi.

4.3 Drift

Ecological drift is an elusive but essential process contributing to community assembly and coexistence across spatial scales. Regrettably, it has received much less attention by ectomycorrhizal researchers than selective processes. Vellend (2016) presents ecological drift as a corollary to genetic drift, encompassing any process acting on individual organisms whose outcome is random with respect to species identity. This concept ties neutral theory into a broader theoretical framework, providing a mechanism by which ecologically neutral, random events can produce distinctive patterns in community composition (Hubbell 2001). Drift typically acts on a community in the form of demographic stochasticity, randomly altering birth and death rates to shift species composition in the community as a whole. Although drift per se can be difficult to detect—the absence of evidence for selective processes is not the same as evidence for drift—there are particular conditions in which drift likely plays an important role. As in population genetics, communities with fewer individuals should be more subject to drift than larger ones. In situations where ecological drift is important, this process is predicted to lead to low local diversity, high spatial turnover, and weak relationships between community composition and environmental factors (Vellend 2016).

Although the abundances of ectomycorrhizal fungi are hard to measure, the available evidence suggests that only a small handful of fungal individuals typically coexist at a small scale. This may explain why the first two predictions—low local diversity and high spatial turnover—are common features of ectomycorrhizal fungal communities. Although few studies have explicitly examined drift in this group of organisms, its importance may become clear in studies designed to examine other forces. Isolated trees in Point Reyes, California, for example, hosted communities of ectomycorrhizal fungi whose composition could not be explained by soil chemistry, distance to intact forest, or the community composition of neighboring tree islands (Peay et al. 2010). The apparent randomness of ectomycorrhizal fungal community structure on these isolated trees suggests that drift may be important at the scale examined. In southeast China, too, Gao et al. (2015) could explain only a modest amount of the variation they observed in ectomycorrhizal fungal community structure among intermediate-aged and old forest plots, despite accounting for a comprehensive set of possible selective factors. Situations like this—with exhaustively measured selective factors explaining only modest amounts of observable variation—are among the most plausible illustrations of drift in the ectomycorrhizal fungal literature.

Like neutral theory, ecological drift can provide a specific null hypothesis that will be a useful tool for interpreting community patterns of ectomycorrhizal fungi, especially in small assemblages and at small spatial scales. Unfortunately, research into fine-scale ectomycorrhizal community structure often refrains from reporting the information required to implicate drift in community structure. Samples from similar locations (e.g., root samples from a particular species of host plant) tend to be pooled in reporting, so the rate of community turnover from plant to plant is

difficult to infer. Modeling of microbial communities has suggested that rates of dispersal and strength of selection will also impact the extent to which drift can shape a community (Evans et al. 2016)—feedbacks among these processes in ectomycorrhizal fungal assemblages would be a fruitful area for future research. Understanding how drift acts on ectomycorrhizal fungal communities could greatly improve our understanding of their dynamics in situations that, with our current emphasis on selective processes, may otherwise seem inexplicable.

4.4 Dispersal

Vellend's framework (2016) includes dispersal as another central mechanism shaping community ecology, akin to gene flow in an evolutionary context. This process typically combines elements of both selection and drift—selection determining the relative dispersal abilities of particular species, and drift determining which of those species in particular establish in any given patch. The rate of arrival of species to a community will influence both the species composition within a few centimeters of soil and the rate at which that community shifts through space and time.

Although there has been significant debate over the importance of dispersal limitation in organisms with microscopic propagules (Finlay 2002; Green and Bohannan 2006), several lines of evidence suggest that dispersal limitation is important for ectomycorrhizal fungi. Some of the earliest work supporting this idea comes from the plant invasion literature: many early attempts to cultivate pines outside their native ranges failed, likely due to ectomycorrhizal symbiont limitation (Richardson et al. 1994; Pringle et al. 2009). Since these initial attempts, however, pines have become important invasive plants in many of these same regions, probably thanks to the subsequent introduction and spread of suitable fungal partners (Vellinga et al. 2009). The initial limitation of pine establishment outside its native range strongly suggests that ectomycorrhizal fungi are dispersal limited at the global scale, although chance long-distance dispersal can certainly happen (Geml et al. 2012). At the landscape scale, measurements of spore dispersal (Peay et al. 2012) and fungal richness on isolated hosts (Peay et al. 2010) suggest that ectomycorrhizal fungal dispersal is markedly limited by distance and strongly affected by environmental variables such as wind speed and solar radiation (Peay and Bruns 2014). This landscape-scale dispersal limitation appears to slow Pinaceae invasions, even in areas where the plants are well established (Nuñez et al. 2009). Fine-scale dispersal by spores is also severely distance limited (Chap. 3). Although a single mushroom can produce local spore densities in the tens of thousands per cubic meter, the vast majority of these propagules fall within 3–53 cm of the sporocarp, depending on its morphology (Li 2005; Galante et al. 2011). Even when a spore lands near a suitable host, germination efficiencies vary substantially and are often low (Nara 2009), reducing effective dispersal rates and

generating predictable patterns of community assembly on new host plants (Chap. 3).

Hyphal extension may be just as important in mediating dispersal success as spore movement, particularly when considering which spores will establish after arriving in a new environment. It is well known that seedlings planted under mature ectomycorrhizal host plants will often acquire communities resembling those of the canopy trees (Dickie and Reich 2005; Cline et al. 2005), while seedlings planted in disturbed habitats, or many meters away from established ectomycorrhizal inoculum sources, will host a predictable and distinct set of ruderal fungi on their roots (Taylor and Bruns 1999; Peay et al. 2012). These fungi typically are thought to colonize plants by way of airborne spores, as considered earlier, or by resistant propagules in the soil. Fungi in the spore bank may persist for years (Bruns et al. 2009) and play an important role in colonizing newly emerged host roots, especially in disturbed habitats with few active mycelia. It has been hypothesized that early-stage ruderal fungi may be excellent at infecting available hosts from spore, but not be strong competitors relative to the late-stage fungi characteristic of mature forests (Taylor and Bruns 1999). Trade-offs between spore-based colonization ability and hyphal function have been suggested with respect to competition (Kennedy et al. 2011) and enzyme activity (Moeller and Peay 2016), but much work remains to establish the extent and consistency of these trade-offs for ectomycorrhizal fungi. At a fine spatial scale, the ectomycorrhizal community is likely primarily driven by root colonization from active mycelia drawing carbon from nearby roots, supplemented by species inputs from the surrounding pool of resistant propagules.

The relative dispersal abilities of ectomycorrhizal fungi are very likely the product of selective forces, while realized spore dispersal events are often stochastic. Spore establishment and hyphal extension, which are key components of successful dispersal, are probably controlled mainly by selective processes at a fine scale, principally competition with other soil fungi. Dispersal processes integrate ecological selection and drift to allow for the movement of species, contributing in important ways to the fine-scale diversity observable in ectomycorrhizal fungal communities.

4.5 Synthesizing Dispersal, Drift, and Selection: Priority Effects

Fine-scale community patterns in ectomycorrhizal fungi depend, to a large extent, on what the community has looked like in the past. This historical contingency integrates the effects of dispersal, drift, and frequency-dependent selection (via competition and facilitation) to produce familiar patterns of ectomycorrhizal community assembly. Arrival order can have an enormous influence on the community of ectomycorrhizal fungi that develops on an individual seedling, whether we consider spore arrival or the presence of established mycelium colonizing a bait

plant. In general, this order will be determined by the composition of the regional species pool, the dispersal abilities of the fungi in the pool, and stochastic events influencing dispersal probabilities (Peay and Bruns 2014). The influence of arrival order on community outcomes, known as priority effects, can themselves be mediated by niche preemption, in which the early-arriving species blocks the establishment of a later arriver by occupying the niche it would have needed, or niche modification, in which the early-arriving species prevents establishment of a later arriver by altering the environment (Fukami 2015). Both of these processes are likely important in ectomycorrhizal fungal communities. Priority effects have been documented many times in mycorrhizal fungi and likely play a large role in determining community structure in many systems (Kennedy et al. 2009; Werner and Kiers 2015). Niche preemption is perhaps best documented, with early-arriving taxa occupying space on the root system and thus having both a territorial and a carbon supply advantage over later-arriving individuals (Kennedy and Bruns 2005; Kennedy et al. 2009). Niche modification may also be important, particularly when considering the influence of preexisting fungi on soil chemistry and available resources. Ectomycorrhizal fungi may differ significantly in their abilities to leverage particular soil resources for their own growth or trade with the host plant, as reviewed earlier in this chapter, and may change features such as local pH (Rosling et al. 2004) and water potential (Koide and Wu 2003). These trait differences probably have important effects on later-arriving fungi, but the extent to which this occurs is not well documented. Future research should specifically investigate the effects of early-arriving fungi on later ones, with an eye to the mechanisms involved. Understanding how selection, drift, and dispersal interact to produce priority effects and historical contingency will greatly improve our ability to predict the ecological trajectories of ectomycorrhizal fungal communities, from individual sampling cores to landscape scales.

4.6 Speciation

At a fine spatial scale, most community dynamics of ectomycorrhizal fungi are probably driven by selective processes, drift, and local dispersal. Vellend (2016) is right, however, to include speciation in his ecological framework: community ecology spans many scales, and, if the organisms of interest have short generation times, or if the scale of the study includes an evolutionarily relevant time frame, speciation may be an important source of new diversity in an ecological community. Past speciation, especially at the regional scale, can also have important influences on the composition of the local species pool (Ricklefs 1987). In the metaphor to population genetics, speciation in a community is analogous to mutation in a population. Like mutation, speciation is most influential in large communities and across long timescales.

When considering the influence of speciation on the fine-scale structure of ectomycorrhizal fungal communities, it may be most useful to examine the

interplay between the fine-scale selective processes we have reviewed and larger-scale diversification events occurring in ectomycorrhizal fungal lineages. In particular, speciation processes are probably important in generating ectomycorrhizal fungi with narrow host ranges, a phenomenon that may help promote fine-scale coexistence in mixed forests. Although the coevolution of hosts and symbionts does not typically increase species diversity (Hembry et al. 2014), host shifts appear to be important opportunities for diversification for ectomycorrhizal fungi (Den Bakker et al. 2004; Garnica et al. 2011; Rochet et al. 2011). This may be because host shift events often open up novel ecological opportunities for the fungi that undergo them. In the case of *Amanita phalloides*, host shifts coinciding with range expansion have led to substantial increases in the resources invested in above-ground reproduction (Wolfe and Pringle 2012), an important ecological adjustment which could eventually contribute to a speciation event. If there is a trade-off wherein increased fitness on a new host requires diminished fitness on alternative hosts, we might expect to see a narrow host range develop in a host-shifted clade of ectomycorrhizal fungi (see Poisot et al. 2011 for a discussion of how trade-offs may lead to ecological specialization). Research investigating the extent to which such trade-offs exist, and the frequency with which host shifts might accompany reductions in fundamental host range, will be essential in explaining the variation in host ranges observable in modern ectomycorrhizal fungi.

This variation in host range is the outcome of speciation processes but has important implications for ecological selection and ectomycorrhizal fungal community functioning at a centimeter scale. As reviewed earlier in this chapter, differences in host range may contribute to the fine-scale coexistence of ectomycorrhizal fungi by reducing the competition for space on the roots of any given host plant. At a fine spatial scale, the effects of speciation will nearly always be mediated by selective processes, but the speciation itself is critical to generating the diversity present in a given soil sample. Future research tying together these ecological and evolutionary processes will improve our understanding of both in structuring ectomycorrhizal fungal communities.

4.7 Conclusions

The paradox of the ectomycorrhizal fungal lifestyle is essentially a problem of scale. Although it is clear that fine-scale patchiness in soil resource distribution, competitor density, and host carbon availability can be important for ectomycorrhizal fungi, the structure of a community within a few centimeters of soil is determined by processes that operate across many scales. The distribution of resources in the soil likely varies at a micron scale, while fungal individuals can extend across meters in the soil. Host-derived carbon is likely the subject of intense competition, and the scale at which its availability is regulated remains unclear. These selective mechanisms have been a major research focus for decades, but it is increasingly clear that drift and dispersal play important roles in structuring

communities and promoting coexistence of ectomycorrhizal fungi. Experimental tests of dispersal dynamics, especially at a fine spatial scale, are sorely needed to determine the primary mechanisms involved and the relative influences of drift and selective processes on community assembly and turnover across small spatial scales. Drift, in particular, has probably haunted many studies designed to test selective forces on small communities. Acknowledging its influence, and designing ways to quantify drift explicitly, should be a focus for community ecology as a whole. Speciation, though rarely witnessed in community-level studies of ectomycorrhizal fungi, likely plays an important role in determining the regional species pool, from which communities are drawn to occupy small patches of soil. Taken together, the balance of influence among these four key processes—selection, drift, dispersal, and speciation—will vary depending upon the spatial and temporal scale under consideration. Being explicit about the processes of interest, and testing the scales at which these processes are important, will help us understand how ectomycorrhizal fungi coexist at the margins of microbial and macroscopic life.

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Chapter 5
Altitudinal Gradients in Mycorrhizal Symbioses

The Current State of Knowledge on How Richness and Community Structure Change with Elevation

József Geml

5.1 Introduction

Montane habitats generally are recognized as biodiversity hotspots as well as areas of high endemism (Lomolino 2001). Despite representing about one-eighth of the world's land area outside Antarctica, mountains harbor about one-third of all terrestrial species (Spehn et al. 2012; Antonelli 2015). Ever since the first scientific studies of Darwin, Wallace and von Humboldt on mountain biota, documenting changes in species richness and community composition has been at the center of ecological and biogeographic studies (Lomolino 2001; McCain and Grytnes 2010). Mountains provide unique opportunities to test various ecological theories and, to some extent, to study possible effects of climate change as they are characterized by gradients of abiotic factors, such as temperature, available moisture, etc. (Guo et al. 2013). However, in most organismal groups we still lack answers to fundamental questions regarding patterns of taxonomic richness and community composition (Lomolino 2001; Guo et al. 2013).

5.2 Environmental Factors

Numerous abiotic factors that shape biological communities change more or less predictably with increasing elevation. Among these, temperature is the most predictable with an average decrease of ca. 0.6 °C per 100 m elevation increase (Barry 2008). Changes in precipitation, another environmental factor crucially important

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for living organisms, along altitudinal gradients is much less predictable in general terms due to its complex relationship with regional climate and topography (Barry 2008). In mid- and high latitudes, precipitation tends to increase with elevation, while tropical mountains often show little variation in rainfall along an altitudinal gradient or exhibit a moderate mid-elevation peak (McCain and Grytnes 2010). There are several other environmental factors that interplay with temperature and precipitation to determine biological productivity. Among these, solar radiation, cloud cover, soil type, and nutrient content also vary substantially with elevation, as does surface area due to geometric constraints. For example, cloud forests, perhaps the most characteristic vegetation type of tropical montane habitats, are created by largely persistent cloud cover at certain elevations. Living organisms occupy different habitats along altitudinal gradients according to their physiological requirements for abiotic factors (temperature, water availability, etc.) and based on their interaction dynamics with other species. The resulting, largely predictable, changes in habitat and community structure with increasing elevation have been a focal point for ecological and evolutionary research and have contributed to the understanding of spatial patterns of biodiversity and their underlying mechanisms.

5.3 General Altitudinal Patterns in Terrestrial Ecosystems

Most studies focusing on biological communities along altitudinal gradients have focused on changes in richness (i.e., the number of taxa) of various taxonomic groups of vascular plants, insects, and vertebrates. Most studied organismal groups have been reported to display either a monotonal decline in richness with increasing elevation, a mid-elevation peak, or some combinations of the two, e.g., low-elevation richness plateau followed by a mid-elevation peak or by a monotonal decline (Colwell et al. 2004; Cardelús et al. 2006; McCain 2009).

Patterns of monotonal decline have generally been attributed to the decrease in environmental energy (e.g., temperature) and the decrease in suitable habitat area (Stevens 1992; Rosenzweig 1995). Possible explanations for mid-elevation peak in richness include increased rainfall and relative humidity and the mostly geometric effect of overlapping distributions of species with broad elevation range as observed in various organismal groups (Colwell and Lees 2000; Grytnes and Vetaas 2002; Sanders 2002; Colwell et al. 2004; McCain 2004; Cardelús et al. 2006; Grytnes et al. 2008). In some instances, in groups specialized in habitats with sparse vegetation (e.g., lichens), species richness can increase with elevation (Grytnes et al. 2006; Geml et al. 2014).

It has been noted repeatedly that certain altitudinal patterns of richness correlate with functional groups and, because ecological function often is evolutionarily conserved, with taxonomic groups. In other words, various ecological groups of plants and animals have been shown to exhibit diversity peaks at different elevations (Cardelús et al. 2006; McCain 2009; McCain and Grytnes 2010; Guo et al. 2013). Considering their particular importance to mycorrhizal fungi, it is noteworthy that,

among plants on a global level, trees generally have higher richness in lower elevations, while shrubs and herbs tend to be most diverse in mid-elevations (Guo et al. 2013).

5.4 The Distribution of Fungi Along Altitudinal Gradients

5.4.1 General Aspects

The vast majority of studies on the effect of elevation on richness have focused on vascular plants and animals, while information on changes in richness and community composition of fungi along elevation gradients remains scarce. The limited number of relevant fungal studies used various techniques and often targeted specific groups, such as morphological identification of macrofungi (Kernaghan and Harper 2001; Gómez-Hernández et al. 2012), freshwater ascomycetes (Shearer et al. 2015), culturable soil ascomycetes (Devi et al. 2012), and root-colonizing arbuscular mycorrhizal fungi (Gai et al. 2012); DNA sequencing of leaf and root endophytic fungi (Coince et al. 2014), bryophyte-associated fungi (Davey et al. 2013), wood-inhabiting fungi (Meier et al. 2010), and ectomycorrhizal root tips (Bahram et al. 2012; Nouhra et al. 2012; Coince et al. 2014; Miyamoto et al. 2014; Jarvis et al. 2015; Rincón et al. 2015); and deep DNA sequencing of soil samples (Geml et al. 2014; Merckx et al. 2015; Rincón et al. 2015). For non-mycorrhizal fungi, the first results show either nonsignificant effect of elevation on richness (Meier et al. 2010; Davey et al. 2013; Geml et al. 2014; Rincón et al. 2015) or a more or less monotonic decrease in species richness with increasing elevation (Devi et al. 2012; Gómez-Hernández et al. 2012; Geml et al. 2014; Shearer et al. 2015), often depending on the taxonomic or functional groups in question. Moreover, the vast majority of these studies from different biomes detected strong compositional shifts with increasing elevation, regardless of richness (e.g., Meier et al. 2010; Gómez-Hernández et al. 2012; Davey et al. 2013; Geml et al. 2014; Merckx et al. 2015; Rincón et al. 2015).

5.4.2 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (phylum Glomeromycota) are obligately symbiotic and form mycorrhizal associations with ca. 80% of land plants, including ca. 200,000 species of herbs, grasses, trees, hornworts, and liverworts (Davison et al. 2015). Despite their Paleozoic origin that coincided with the colonization of land by plants, extant taxa of Glomeromycota mostly appeared and achieved global distribution after the major continental shifts of the Mesozoic (Davison et al. 2015). The number of taxa is estimated to be between 340 and 1600 based on molecular

studies, and the vast majority of them occur in more than one continent and in multiple climatic zones (Davison et al. 2015; Van der Heijden et al. 2015).

The accumulating data suggest that, despite their large spores being more suited for short-distance dispersal, arbuscular mycorrhizal fungi are surprisingly effective dispersers even across considerable geographic distances over long timespans and that the regional species pool at any given locality represents a relatively large portion of the total global diversity (Davison et al. 2015; Van der Heijden et al. 2015). At the global level, the primary factors that are expected to determine the composition and richness of arbuscular mycorrhizal fungal communities are geographic distance, climate, and edaphic factors, in particular precipitation and soil pH (Davison et al. 2015; Öpik and Davison 2016). For example, arbuscular mycorrhizal fungal richness has been shown to correlate negatively with latitude (Davison et al. 2015) and positively with soil pH (Porter et al. 1987; Coughlan et al. 2000). Overall, the relative importance of dispersal to environmental filtering seems to depend on geographic scale and shows substantial variation (Vályi et al. 2016).

Given their obligately symbiotic lifestyle, vegetation is expected to have a strong influence on the distribution of arbuscular mycorrhizal fungi. Even though plant species richness does not correlate with the taxonomic richness of Glomeromycota on a global scale (Tedersoo et al. 2014), vegetation type does influence both richness and community composition of arbuscular mycorrhizal fungi at smaller spatial scales (Davison et al. 2015; Vályi et al. 2016). In addition to marked microclimatic and edaphic differences among distinct habitats on a landscape scale, the identity and distribution of host plants can influence the spatial distribution of arbuscular mycorrhizal fungi even within relatively homogenous habitats. For example, despite the apparent lack of species-level specificity in arbuscular mycorrhizal symbioses, different plant species often associate with different sets of glomeromycete species from the species pool of a given site (Sýkorová et al. 2007; Gosling et al. 2013; Vályi et al. 2016). Functional differences among arbuscular mycorrhizal fungi likely explain at least partly such preferential associations among certain host–symbiont pairs (Davison et al. 2011). For example, it has been shown that host plants and arbuscular mycorrhizal fungi with similar life strategies, e.g., competitiveness and tolerance for stress or disturbance, preferentially associate with each other (Chagnon et al. 2013).

There have been only a handful of studies documenting changes in root colonization, community composition, and/or richness of arbuscular mycorrhizal fungi along altitudinal gradients. Ruotsalainen et al. (2004) did not find statistically significant shift in root colonization of several herb species along an elevation gradient ranging from sea level to 1400 m a.s.l. in subarctic Norway based on morphological assessments. Similarly, Fisher and Fulé (2004) found no correlation of root colonization of corn seedlings by arbuscular mycorrhizal fungi across soil samples taken from various forest types between 2595 and 3308 m a.s.l. in Arizona, USA. Lugo et al. (2008) morphologically identified arbuscular mycorrhizal fungi in rhizospheric soil samples taken from underneath various grass species in Puna vegetation in the Andes between 3320 and 3870 m a.s.l. Despite the relatively short gradient (550 m) that featured a single vegetation type, the authors observed

that species richness of arbuscular mycorrhizal fungi decreased significantly with increasing elevation (Fig. 5.1). A follow-up study from Lugo et al. (2012) based on an extended set of field sites ranging from 3220 to 4314 m a.s.l. in the same region documented significant decrease in root colonization in the sampled grass species. This trend was evident both in total colonization rates in all samples and within host plant species. Gai et al. (2012) compared species richness and root colonization rate of arbuscular mycorrhizal fungi along a Tibetan elevation gradient between 1990 and 4648 m a.s.l. The vegetation types ranged from subtropical broad-leaved forest, through various temperate conifer forests to alpine scrubland and meadow. Both species richness and root colonization rate decreased significantly with increasing elevation regardless of host plant identity, particularly above 3000 m a.s.l. (Gai et al. 2012) (Fig. 5.1). Geml et al. (2014) used DNA metabarcoding of soil samples to characterize soil fungal communities in the subtropical Yungas forests of the Andes. Their sampling sites ranged from 405 to 2160 m a.s.l., representing the three major forest types: piedmont, montane, and montane cloud forest. The results showed strong compositional turnover with increasing elevation and revealed that although total fungal richness did not change with increasing elevation, richness estimates changed markedly in several fungal groups. In particular, richness of operational taxonomic units (OTUs) of arbuscular mycorrhizal fungi was negatively correlated with elevation (Geml et al. 2014) (Fig. 5.1). As part of a multitaxon study on the evolution of endemism on Mt. Kinabalu in Malaysia, Merckx et al. (2015) analyzed DNA metabarcoding data generated from soil samples taken along an altitudinal gradient from 425 to 4000 m a.s.l. and showed that the majority of phylogenetic lineages in the genus *Glomus* were restricted to low elevations. Finally, Bonfim et al. (2016) found strong altitudinal turnover of arbuscular mycorrhizal fungi in a Brazilian Atlantic Forest gradient from 80 to 1000 m a.s.l., but, in contrast to other studies, the authors found greatest richness at the highest sampling site. Given the low number of sites along their single gradient, site-specific confounding factors may at least in part explain these results (Bonfim et al. 2016).

The emerging picture from the above studies is that both richness and abundance of arbuscular mycorrhizal fungi show a more or less monotonous decrease with increasing elevation, although regional deviation from this general pattern may exist. As noted above, there are various environmental factors that correlate with elevation at differing spatial scales. While the decrease in temperature with increasing elevation is predictable globally, biotic and edaphic factors can change along an altitudinal gradient on a more local scale and could strongly influence the distribution of arbuscular mycorrhizal fungi. For example, soil pH often is negatively correlated with elevation, particularly in areas with an increase in precipitation and resulting changes in vegetation and soil organic matter (Gai et al. 2012; Geml et al. 2014), and several of these factors likely contribute to the decrease in richness. On the other hand, the dataset of Lugo et al. (2008) suggests that even temperature per se may influence richness, when vegetation and edaphic factors remain comparable along the gradient. The positive correlation between arbuscular fungal richness and temperature is also supported by their higher richness in low latitudes on a global scale, as noted above.

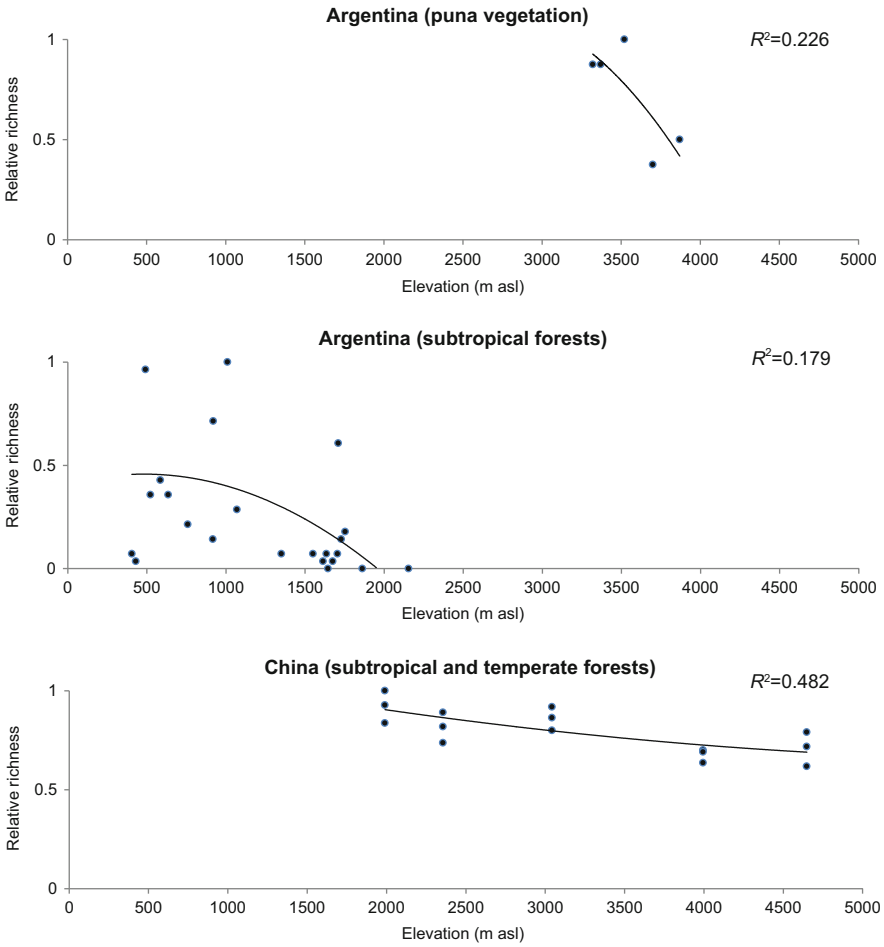

Fig. 5.1 Relative richness of arbuscular mycorrhizal fungi as a function of elevation based on data from previous studies: Puna vegetation in Argentina (Lugo et al. 2008), subtropical forests in Argentina (Geml et al. 2014), and subtropical and temperate forests in China (Gai et al. 2012). Relative richness was calculated in relation to the highest per-site richness value in each study. Correlation coefficients were inferred using quadratic regression

5.4.3 Ectomycorrhizal Fungi

Ectomycorrhizal symbioses worldwide involve ca. 6000 plant species and more than 20,000 fungal species (Rinaldi et al. 2008; Brundrett 2009). Although only about 2% of the estimated number of plant species form ectomycorrhizal associations (Brundrett 2009), the vast majority of them are woody plants and include the ecologically and economically most important trees in most of the forested areas of the world, with possible exception of Neotropical lowland forests (Chap. 20). Plant

families involved in ectomycorrhizal symbioses include Betulaceae, Dipterocarpaceae, Fagaceae, Nothofagaceae, Pinaceae, and certain lineages in Cistaceae, Fabaceae, Juglandaceae, Myrtaceae, Nyctaginaceae, Polygonaceae, Rosaceae, Salicaceae, Tiliaceae, etc. (Chap. 19). According to the latest syntheses (Tedersoo and Smith 2013; Chap. 6), the ectomycorrhizal habit has evolved independently in ca. 80 fungal lineages that comprise more than 250 genera, mostly in the phyla Basidiomycota and Ascomycota. Most ectomycorrhiza-forming taxa likely radiated in the Cretaceous and Paleogene, as orders of Agaricomycetes and Pezizales probably originated around 200 and 150 million years ago, respectively, based on molecular clock estimates (Berbee and Taylor 2001; Chap. 1). These estimates, however, postdate the evolution of Pinaceae, the oldest extant plant family that form ectomycorrhizal symbiosis, whose oldest fossils are dated to 156 million years ago (LePage 2003; Tedersoo and Smith 2013).

On a global scale, ectomycorrhizal fungal richness is primarily influenced by the relative proportion and species richness of host plants, soil pH, mean annual temperature, and mean annual precipitation (Tedersoo et al. 2012, 2014). Specifically, richness of ectomycorrhizal fungi has repeatedly been shown to peak at intermediate annual temperatures (between 5 and 20 °C) and at mid-latitudes, particularly in northern temperate forests. In terms of soil pH, ectomycorrhizal fungi are known to prefer slightly acidic to neutral pH. The majority of ectomycorrhizal fungi have broad host range and associate with hosts representing a wide range of taxonomic groups, while several others are more specific to plant families or even genera (Molina et al. 1992). Richness and density of host plants correlate positively with ectomycorrhizal fungal richness both on global and more regional scales (Tedersoo et al. 2014). In fact, Pinaceae is most dominant in northern mid-latitudes where the richness peak of ectomycorrhizal fungi is observed (Tedersoo et al. 2014; Chap. 18).

Many ectomycorrhizal fungi in low- to mid-latitudes show dispersal limitation and pronounced phylogeographic patterns (Geml et al. 2008; Peay et al. 2012; Branco et al. 2015), while arctic-alpine species generally exhibit high level of intercontinental gene flow (Geml 2011; Geml et al. 2012). The global study of Bahram et al. (2013) on ectomycorrhizal fungal communities showed a strong impact of latitude, but not longitude, on phylogenetic community turnover, confirming the abovementioned differences in the dispersal capabilities among fungi inhabiting different latitudes. These studies suggest that ectomycorrhizal fungal species tend to have much more restricted geographic distribution than arbuscular mycorrhizal fungi and that differences in the size and composition of the regional species pools of ectomycorrhizal fungi likely influence strongly their altitudinal patterns in a given region.

Similarly to arbuscular mycorrhizal fungi, the number of studies on changes in richness and community composition of ectomycorrhizal fungi along altitudinal gradients is low. The few relevant studies largely investigated different geographic regions with distinct vegetation and climatic conditions and reported several types of observed patterns, which makes comparisons difficult (Fig. 5.2). Kernaghan and Harper (2001) collected sporocarps of ectomycorrhizal fungi along multiple

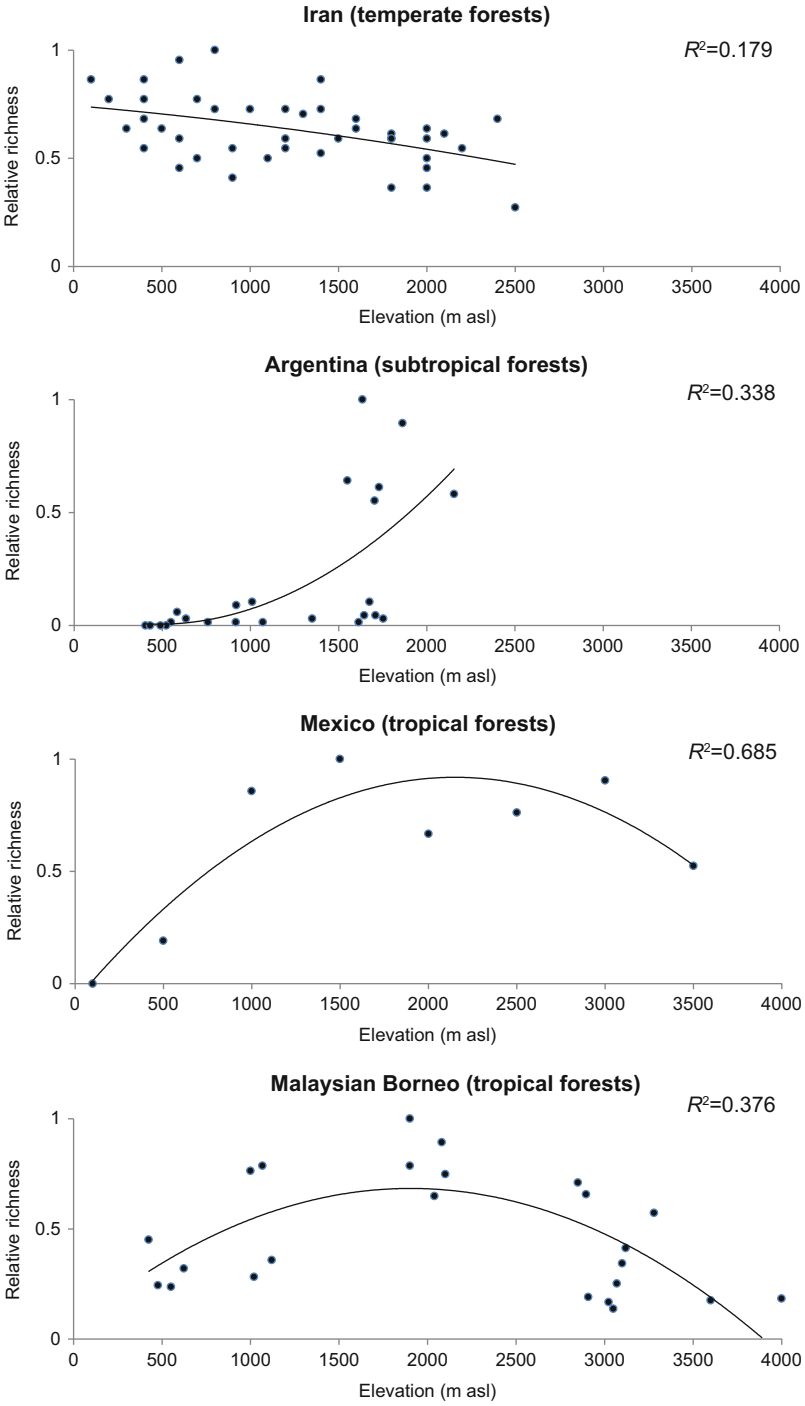

Fig. 5.2 Relative richness of ectomycorrhizal fungi as a function of elevation based on data from previous studies: temperate forests in Iran (Bahram et al. 2012), subtropical forests in Argentina

transects spanning the subalpine/alpine treeline ecotone (between 2000 and 2500 m a.s.l.) at two sites in Alberta, Canada. They found that richness and diversity of ectomycorrhizal fungi decreased with elevation despite the fact that host plant diversity was highest at the ecotone. Bahram et al. (2012) analyzed DNA sequence data generated from root tips of 367 ectomycorrhizal fungal species in mixed deciduous forests of Iran, ranging from sea level to ca. 2700 m a.s.l. The authors found that richness of ectomycorrhizal fungi declined with increasing elevation, partly explained by the decrease of rare species at high elevations due to their reduced competitive abilities under increased environmental stress (Bahram et al. 2012). In temperate forests in Argentina, Nouhra et al. (2012) compared sporocarp production of hypogeous fungi associated with *Nothofagus* species and reported higher richness in lower (800–900 m a.s.l.) than in higher elevations (1700–1800 m a.s.l.). Unlike the previous papers, Miyamoto et al. (2014) found highest richness of ectomycorrhizal fungi at mid-elevations on Mt. Fuji in Japan based on DNA sequences generated from root tips representing 73 ectomycorrhizal fungal species sampled between 1100 and 2250 m a.s.l. The authors contributed the observed patterns to the geometrical effect of overlapping elevation ranges (Miyamoto et al. 2014). The above studies spanned multiple vegetation types including different hosts that covaried with elevation, although ectomycorrhizal hosts were dominant throughout the sampled altitudinal gradients. In order to identify the role of abiotic drivers while keeping the host identity constant, Coince et al. (2014) analyzed pyrosequencing data of ectomycorrhizal root samples of *Fagus sylvatica* along multiple altitudinal gradients in France and Spain and found no statistically significant change in ectomycorrhizal fungal richness. Using the same methodology, similar results were obtained by Jarvis et al. (2015) and Rincón et al. (2015) regarding ectomycorrhizal fungi associated with *Pinus sylvestris* in various western European mountains. It is important to note, however, that all of the above studies showed compositional differences in ectomycorrhizal fungal communities along the altitudinal gradients as well as among the different regions regardless of the observed patterns of richness. In addition to the influence of host plant identity and abundance, temperature, precipitation, and edaphic factors, particularly soil moisture, pH, and C/N ratio, appear to be most influential in shaping ectomycorrhizal fungal communities in temperate mountains (Coince et al. 2014; Jarvis et al. 2015; Miyamoto et al. 2015; Rincón et al. 2015).

Even though substantially fewer studies have been published on the distribution of ectomycorrhizal fungi in subtropical and tropical mountains, studies from Mexico, Costa Rica, and northwestern Argentina concordantly suggest that richness of ectomycorrhizal fungi is by far the highest in montane cloud forests that generally occur between 1500 and 3000 m a.s.l. (Mueller et al. 2006; Gómez-Hernández et al.

Fig. 5.2 (continued) (Geml et al. 2014), tropical forests in Mexico (Gómez-Hernández et al. 2012), and tropical forests in Malaysian Borneo (Geml et al. 2017). Relative richness was calculated in relation to the highest per-site richness value in each study. Correlation coefficients were inferred using quadratic regression

2012; Geml et al. 2014; Wicaksono et al. 2016). Furthermore, a high percentage of ectomycorrhizal fungi appear to be restricted to montane cloud forests (Mueller et al. 2006; Wicaksono et al. 2016). The distribution of ectomycorrhizal hosts has been thought to shape the observed patterns strongly, because in these Neotropical regions the diversity and abundance of ectomycorrhizal host plants are by far the greatest in montane habitats, while low-elevation forests tend to harbor very few hosts (Mueller et al. 2006; Geml et al. 2014). The single published study on the distribution of ectomycorrhizal fungi in Palaeotropical mountains is that of Geml et al. (2017) on Mt. Kinabalu in Malaysian Borneo. Similar to the Neotropical studies, the data from Kinabalu indicated highest richness in most ectomycorrhizal fungal lineages in the mid-elevation montane forests, with the exception of tomentelloid fungi that showed a monotonal decrease in richness with increasing elevation. The high richness and restricted distribution of many ectomycorrhizal fungi in the montane forests suggest that mid-elevation peak richness is primarily driven by environmental characteristics of this habitat and not by the mid-domain effect (Geml et al. 2017). On Mt. Kinabalu, despite the decrease in host richness, the total relative basal area of ectomycorrhizal hosts is relatively constant (37–47%) along the sampled elevation gradient (Aiba and Kitayama 1999). This suggests that, in addition to host availability, ectomycorrhizal richness in subtropical and tropical mountains appears to peak at intermediate temperatures and high levels of available moisture, similar to the abovementioned latitudinal trends on a global scale. Tropical mountain environments are characterized by mid-elevation condensation zones where available moisture is usually the highest (Whitmore 1984; Rahbek 2005). As a result, many organismal groups that rely on high humidity show highest richness and abundance in montane cloud forests, e.g., orchids (Wood et al. 1993), ferns (Parris et al. 1992; Grytnes and Beaman 2006), epiphytic plants (Cardelús et al. 2006; Grytnes and Beaman 2006), bryophytes (Ah-Peng et al. 2012), and snails (Liew et al. 2010). Considering the distribution of ectomycorrhizal fungi, it is important to point out that tropical montane cloud forests are among the most vulnerable terrestrial ecosystems to climate change (Foster 2001; Pacheco et al. 2010; Wicaksono et al. 2016), as rising temperatures are resulting in a shifting cloud base that threatens their long-term survival (Still et al. 1999).

5.4.4 Orchid Mycorrhizal Fungi

While the knowledge on the altitudinal distribution of arbuscular mycorrhizal and ectomycorrhizal fungi is still rudimentary, much less attention has been paid to elevation patterns in other mycorrhizal fungi, such as those that form orchid and ericoid mycorrhizae. Most fungi that form mycorrhizas with orchids are facultative symbionts of orchids. Generally, fungi associated with green-leaved (i.e., only partially mycoheterotrophic) orchids mostly are saprotrophic, while nonphotosynthetic (fully mycoheterotrophic) orchids tend to associate with ectomycorrhizal fungi (Dearnaley et al. 2013). Furthermore, different species of orchids, as well as various life stages of the same orchid species, often represent different intermediate positions between the fully

autotrophic and fully heterotrophic spectrum of trophic mode (Dearnaley et al. 2013). Sebacinales, Ceratobasidiaceae, and Tulasnellaceae include most fungi that form mycorrhizas with orchids, but representatives of a wide range of other fungal taxonomic groups, particularly those that form ectomycorrhizas with trees, such as Pezizales, Russulales, Thelephorales, etc., can be associated with orchids as well, mostly in forests (Selosse et al. 2002; McCormick et al. 2004; Taylor et al. 2004; Waterman and Bidartondo 2008; Illyés et al. 2010; Dearnaley et al. 2013).

Despite the fact that substantial work has been done on the ecology of orchid mycorrhizal associations, there are very few published studies on their distribution at different elevations (Chap. 8). Taylor and Bruns (1999) investigated mycorrhizal specialization in nonphotosynthetic orchids *Corallorhiza maculata* and *C. mertensiana* in California. All symbiotic fungi found in the samples belonged to the ectomycorrhizal Russulaceae, and the authors found no shared fungal symbionts between the two orchid species. Moreover, there was a strong correlation with habitat and orchid mycorrhizal community composition, as populations of *C. maculata* above 2000 m a.s.l., corresponding to *Abies* forest, had no fungi in common with populations below 2000 m a.s.l. in forests dominated by *Pinus* and *Pseudotsuga* (Taylor and Bruns 1999).

Autotrophic orchids tend to be less specific with respect to mycorrhizal symbionts, ranging from "weedy" orchid species that associate with a broad range of fungi to locally endemic orchid species with more specialized symbionts (Suárez et al. 2006, 2008; Bonnardeaux et al. 2007; Rasmussen and Rasmussen 2009). The family Orchidaceae is most diverse in the tropics, and within tropical regions, their richness and abundance peak in mid-elevation montane forests (Küper et al. 2004; Cardelús et al. 2006; Acharya et al. 2011). Therefore, it is reasonable to hypothesize that orchid mycorrhizal fungal diversity and abundance may be highest in these montane forests. In addition, Kartzinel et al. (2013) found high spatial turnover of mycorrhizal symbionts of *Epidendrum firmum* in montane forests of Costa Rica, although neither biogeographic nor large-scale environmental factors were significantly correlated with community composition of orchid mycorrhizal fungi. Alternatively, differences in land use and fine-scale environmental factors may better explain the high spatial heterogeneity.

5.4.5 *Ericoid Mycorrhizal Fungi*

Most known ericoid mycorrhizal fungi can also grow as soil saprotrophs, while some can simultaneously colonize roots of other plants as well to form endophytic or ectomycorrhizal associations (Villarreal-Ruiz et al. 2004; Horn et al. 2013; Van der Heijden et al. 2015). Most fungi that are known to form ericoid mycorrhizas belong to the ascomycete order Helotiales and the basidiomycete order Sebacinales (Selosse et al. 2007; Walker et al. 2011; Geml et al. 2015; Van der Heijden et al. 2015). Even though members of Ericaceae, mostly evergreen or deciduous shrubs, can be found in all continents except Antarctica, they generally prefer cool and

relatively moist climates and acidic, nutrient-poor soils (Walker et al. 1994; Ojeda et al. 1998). The only published study specifically devoted to comparing community structure of ericoid mycorrhizas along an elevation gradient focused on fungal symbionts of *Vaccinium membranaceum* in Canada (Gorzelak et al. 2012). The study featured *V. membranaceum* root samples taken from various vegetation types, such as low- and mid-elevation spruce and hemlock forests, subalpine spruce and fir community, and alpine tundra at ca. 875, 1225, 1800, and 1925 m a.s.l., respectively. Gorzelak et al. (2012) isolated a total of ten fungal species from the root samples. Although all of them were found in multiple elevation sites and per-site richness values differed a little, there was substantial turnover in community composition: high-elevation fungal communities, characterized by *Rhizoscyphus ericae* and *Meliniomyces* sp., differed from lower elevation communities, where *Phialocephala fortinii*, *Cryptosporiopsis* sp., and *Neonectria radicicola* were dominant.

In the tropics, ericaceous plants tend to be restricted to montane habitats, particularly between 1000 and 3000 m a.s.l. (Luteyn 1989, 2002; Beaman and Beaman 1990; Kreft et al. 2004; Giriraj et al. 2008). Geml et al. (2014) found that richness in Helotiales and Sebacinales in the subtropical Andean forests was highest in the montane cloud forest zone (1500–3000 m a.s.l.). This pattern was particularly strong in Helotiales that included several indicator taxa with significant specificity and fidelity to the montane cloud forest. In addition to comprising numerous fungal species capable of forming ericoid mycorrhizas, both orders include many taxa with saprotrophic, root endophytic, or ectomycorrhizal trophic modes (Tedersoo et al. 2010). Therefore, studies specifically targeting ericaceous plants and their symbionts are needed to test whether or not the above trend holds (Chap. 9).

5.5 Conclusions

Despite significant advances in our knowledge on the diversity, distribution, and ecology of mycorrhizal fungi in the last decades, there is still very little known on how abiotic and biotic factors that correlate with elevation influence mycorrhizal communities. The first studies reviewed above suggest that altitudinal patterns of richness of mycorrhizal fungi are somewhat similar to those observed with respect to latitudinal gradients. For example, when considering the full range of biomes from tropical lowland forests to cold-dominated ecosystems, richness of arbuscular mycorrhizal fungi decreases with increasing altitude (and latitude), while ectomycorrhizal fungal richness tends to peak in mid-elevation (and mid-latitude) forests with temperate climates. Therefore, climate appears to be important in shaping the distribution of mycorrhizal fungi along altitudinal gradients in a variety of ways, e.g., by affecting microbial processes (e.g., decomposition) and edaphic factors, by altering species interaction dynamics among fungi and other members of the soil biota, and by influencing the abundance and diversity of hosts, etc. Distribution of hosts, which is also influenced by their biogeographic history, has

substantial influence on the above patterns, particularly in ectomycorrhizal fungi that associate with only ca. 2% of plant species and tend to have higher host specificity than arbuscular mycorrhizal fungi. Orchid and ericoid mycorrhizal symbioses, by definition, are also spatially limited by the distribution of Orchidaceae and Ericaceae, respectively. However, unlike most ectomycorrhizal fungi, both orchid and ericoid mycorrhizal fungi can grow as saptrotrophs and/or root symbionts of a wide range of plants, and, therefore, the distribution of these fungal taxa is not restricted to areas where orchids and ericaceous plants occur. Carefully planned and executed studies, ideally featuring altitudinal gradients across multiple geographic regions, are needed to statistically evaluate the contributions of globally relevant (e.g., temperature) and regionally (e.g., precipitation, host biogeography) or locally (e.g., edaphic factors) idiosyncratic variables to explain altitudinal patterns in mycorrhizal fungal richness as well as community composition.

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Chapter 6
Ectomycorrhizal Fungal Lineages: Detection of Four New Groups and Notes on Consistent Recognition of Ectomycorrhizal Taxa in High-Throughput Sequencing Studies

Leho Tedersoo and Matthew E. Smith

6.1 Introduction

Ectomycorrhizal (EcM) fungi represent a diverse group that forms mutualistic associations with plant roots. Due to different opinions and methods, there has been significant controversy in "separating the wheat from the chaff" when assigning mycorrhizal status to fungal species or Operational Taxonomic Units (OTUs) that are recovered from molecular identification studies (Rinaldi et al. 2008; Tedersoo et al. 2010; Tedersoo and Smith 2013). Based on phylogenetic information, the EcM fungal species and genera have been grouped into monophyletic "lineages" to reflect their independent evolution from non-mycorrhizal ancestors (Tedersoo et al. 2010). Accumulating evidence suggests that this is a unidirectional process by which mostly saprobic ancestors transition into a symbiotic lifestyle. These ectomycorrhizal biotrophic fungi subsequently lose the genes responsible for plant cell wall degradation (e.g., Kohler et al. 2015), and thus reversals to saprotrophy or other trophic lifestyles are rare, nonexistent, or transient.

Using sequence metadata as well as phylogenetic and statistical analyses, Tedersoo and Smith (2013) added additional lineages of previously unrecognized EcM fungi that were only known from sequence data obtained from plant roots and/or soil. These reports increased the number of EcM lineages to 78–82. Since 2013, a number of revealing molecular identification and phylogenetic studies have

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been published that motivated us to revise the EcM fungal lineages in order to match the most recent knowledge.

Molecular identification studies of fungi from soil typically rely on the best BLASTn matches or Naïve Bayesian Classifier (Porras-Alfaro et al. 2014) to assign representative sequences of OTUs to species, genera, families, and higher taxonomic ranks based on subjective similarity thresholds (Tedersoo and Nilsson 2016). Both traditional Sanger sequencing and high-throughput sequencing (HTS) studies usually fail to account for the fact that the ITS regions (as well as other molecular markers) differ in their rate of evolution and therefore in the level of separation between species and across lineages. For example, it is likely that an OTU with 95% full-length ITS sequence match to the taxon *Russula vinosa* represents an ectomycorrhizal species in the genus *Russula*, but the same is not necessarily true for *Cenococcum geophilum* or *Meliniomyces bicolor*. What can we conclude about the trophic mode of OTUs from soil or roots that match *R. vinosa* or any other EcM fungal taxon at 80%, 85%, or 90% similarity? Inclusion or exclusion of these taxa may strongly bias the view of the EcM to saprotroph ratio and the environmental effects on fungal guilds if these OTUs are highly abundant.

Although macroscopic EcM fungi are relatively well studied compared to some other fungal groups, molecular ecology studies in tropical ecosystems or in the Southern Hemisphere commonly encounter problems in identification due to a dearth of well-annotated reference sequences from identified specimens, axenic cultures, or EcM roots. If the studies are to compare overall fungal diversity, this is not a significant problem. However, trophic groups of fungi respond to different predictors and display different biogeographic patterns. Therefore, most studies attempt to separate EcM fungi, arbuscular mycorrhizal (AM) fungi, and putative plant pathogens from potential saprotrophs. So far, the assignment of a trophic status has been typically performed based on taxonomic assignments either manually or in a semiautomatic fashion (Nguyen et al. 2016). Although ecological traits should be clearly related to collections or at least reference OTUs or species hypotheses (SHs; Kõljalg et al. 2016) rather than genus or family names, there is currently no annotated system for rigorously incorporating additional information on important ecological, morphological, and physiological traits (e.g., EcM exploration type, fruit body type, enzymatic capacities, etc.). This means that most data sets require time-consuming manual trophic assignments based on expert knowledge in order to extract critical ecological details. The current system also renders the correctness of taxonomic labeling and specimen identification of great analytical importance. Hence, we aim to assign information about EcM fungal lineages to individual isolates (accessions) and SHs in UNITE and to establish group-specific ITS sequence similarity thresholds for delimiting EcM fungal lineages based on our previous experience with high-throughput sequencing.

6.2 Approaches

We critically evaluated recent studies about the phylogeny and molecular identification of EcM fungi published since 2013. We also rechecked the sequences and metadata accumulated in the International Nucleotide Sequence Databases consortium (INSDc) and UNITE over the same time period. Lastly, we ran simple maximum likelihood phylogenetic analyses as described in Tedersoo and Smith (2013) to establish the monophyly of putative EcM groups.

To reproducibly separate EcM fungi from non-mycorrhizal fungi in HTS studies, we compiled information about the BLASTn identification of soil fungi based on the ITS2 subregion in the 454 pyrosequencing (Tedersoo et al. 2014a, 2016a) and Illumina MiSeq (Tedersoo et al. 2015a, b) HTS data sets. We also added unpublished data targeting the full ITS region that was obtained by combining primers ITS9MUNngs and ITS4ngsUni (Tedersoo and Lindahl 2016) and Pacific Biosciences RS II platform for a subset of soil samples collected from Estonia and Australia. This approach is built on the inherent assumption that EcM fungi are monophyletic groups that are separated from non-mycorrhizal relatives and that EcM lineages display a "phylogenetic gap" compared with non-mycorrhizal sister taxa. There is ample evidence for this phenomenon in phylogenetic studies, where EcM lineages are usually separated from other non-EcM taxa with relatively strong statistical support and great phylogenetic distances (e.g., a long stem). The first author has used this approach in multiple studies published since 2014. Elaborating on this further and releasing this information was motivated by the urge to make interpretation of high-throughput sequencing data more reliable.

To be able to recognize these phylogenetic gaps and separate EcM groups from non-EcM taxa, we used both accumulated ITS Sanger sequence data and HTS data. Briefly, we compiled publicly available Sanger sequences from all EcM lineages and their putative sister groups (Tedersoo et al. 2011a; Tedersoo and Smith 2013) as references. Using the above HTS data sets, we established multiple statistical indices based on sequence length, sequence coverage, and BLASTn score. We studied the distribution of these metrics in different lineages and also in certain related groups *that matched best* to particular EcM lineages. Among multiple candidates, we selected "BLASTn score to query sequence length ratio (S/L ratio)" and "sequence identity (%)" as the most promising indices that display the most pronounced gap between EcM and non-EcM groups. We verified the results using additional BLASTn searches, retrieving the 100 best matches and/or via phylogenetic analyses (cf. Tedersoo and Smith 2013).

6.3 Additional EcM Fungal Lineages

The **/leotia lineage** is erected to accommodate the genus *Leotia* of Helotiales (Fig. 6.1). Kühdorf et al. (2015) demonstrated that certain species in the genus *Leotia* are common root symbionts that form arbutoid EcM of short-distance exploration type with *Comarostaphylis* in Costa Rica. Their description of a plectenchymatous mantle of narrow clampless hyphae and thick-walled emanating hyphae roughly matches the descriptions of EcM of various groups of Helotiales. The authors also showed that a disproportionate amount of environmental sequences affiliated with the EcM group originate from Sanger-sequenced ectomycorrhizal root tips. In our previous studies, we had not noticed this sequence grouping in *Leotia* and thus considered this group as non-EcM based on unconfirmed root tip data from Zambia and Australia (cf. Tedersoo et al. 2010). However, earlier assessments based on isotopic evidence had previously provided suggestive evidence for an EcM habit in *Leotia* (Zeller et al. 2007). So far, EcM associations have only been convincingly shown for a single clade that comprises the *L. lubrica* and *L. viscosa* species complexes (Kühdorf et al. 2015). We ran a maximum likelihood phylogeny by including all Sanger sequences from fruit bodies, EcM root tips, and soil affiliated to *Leotia* spp. and demonstrate that most species of *Leotia* are likely ectomycorrhizal (Fig. 6.1). This analysis indicates that the /leotia lineage is widely distributed in all continents except perhaps lowland South America. *Leotia* spp. associate with Pinaceae, Fagales, Arbutoideae, *Uapaca*, and putatively with the *Berlinia* group (Fabales) and Dipterocarpaceae in Africa and India (Tedersoo et al. 2014a).

The **/porpoloma lineage** is separated from /tricholoma based on the results of a multigene phylogenetic analysis by Sánchez-García et al. (2014). The /porpoloma lineage is comprised of a core group of *Porpoloma*, but excludes several species that have been transferred to other segregate genera: *P. umbrosum* and *P. metapodium* to *Pseudotricholoma*, *P. spinulosum* and *P. macrocephalum* to *Pogonoloma*, *P. bambusarium* to *Corneriella*, and *P. pes-caprae* to *Pseudoporpoloma* (Sánchez-García et al. 2014; Vizzini et al. 2016). As currently circumscribed, /porpoloma is a Southern Hemisphere lineage that is found in southern South America, Australia, and New Zealand. The root tip sequences originally assigned to /tricholoma (UDB002748 from *Pomaderris apetala*: Tedersoo et al. 2008; UDB007061 from *Nothofagus dombeyi* and UDB007096 and UDB007123 from *N. obliqua*: Nouhra et al. 2013) actually represent /porpoloma. Sequences belonging to /porpoloma have been also recovered from *Nothofagus nervosa* seedlings by Fernández et al. (2013) in Argentina (KJ701291). Microscopic studies of the Tasmanian and Patagonian material suggest that EcM of *Porpoloma* are similar to that of /tricholoma with a plectanchymatous mantle and hairy rhizomorphs that place it to the medium-distance fringe exploration type. *Pseudoporpoloma pes-caprae* represents a European grassland-inhabiting species that forms a sister group to the genus *Tricholoma* of the /tricholoma lineage (Sánchez-García et al. 2014; Vizzini et al. 2016). Following separation of *Porpoloma*, we treat the /tricholoma lineage as consisting only of *Tricholoma* species. The /tricholoma

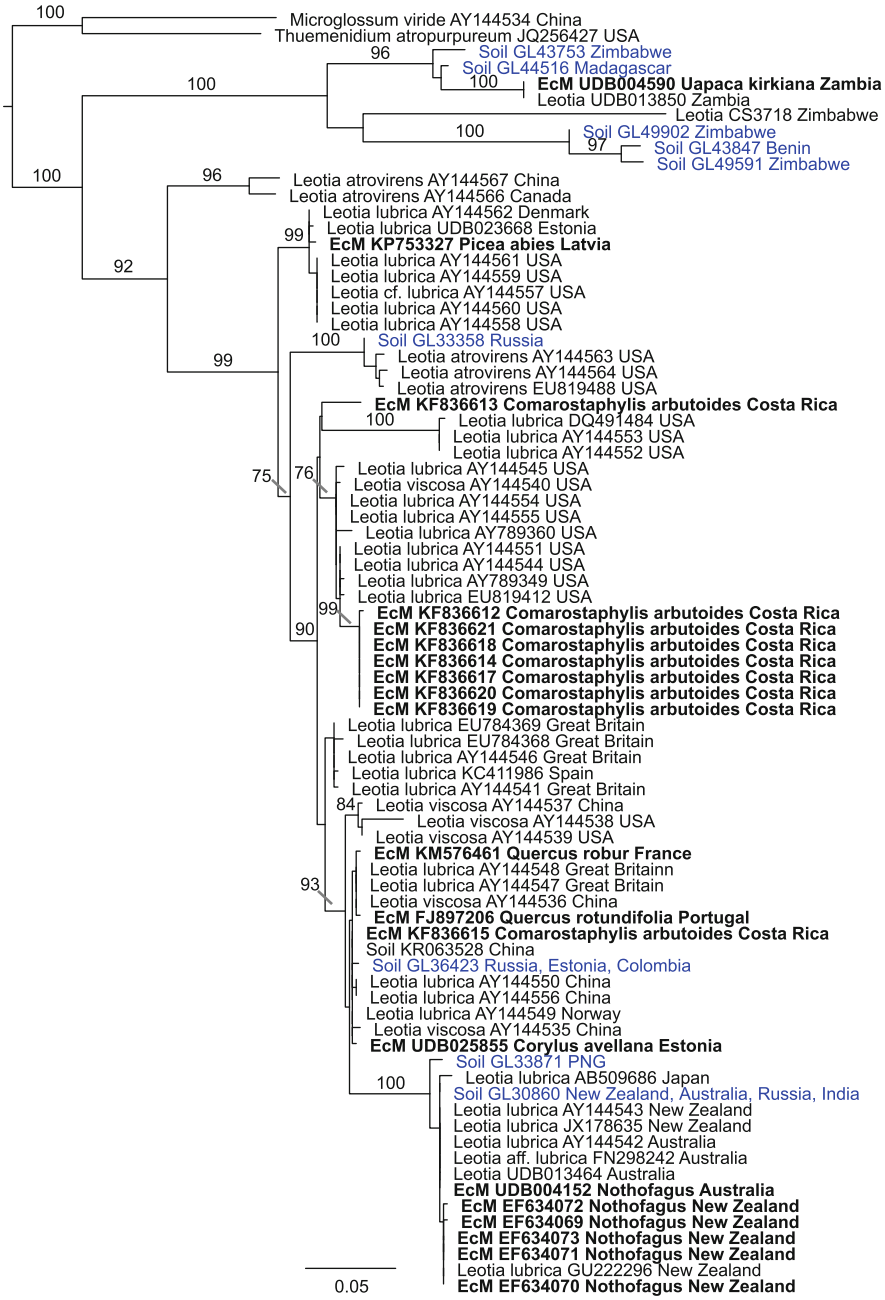

Fig. 6.1 Phylogenetic placement of sequences from ectomycorrhizal root tips (EcM; in *bold*) and soil (data from Tedersoo et al. 2014a highlighted) among *Leotia* species as based on fruit bodies. Bar, 0.05 changes per position. The ITS phylogram consists of 80 terminal taxa and 603 aligned positions, with *Thuemenidium atropurpureum* and *Microglossum viride* representing an outgroup based on closest BLASTn matches to *Leotia* spp. Note the unexpectedly high taxonomic diversity in the earliest diverging African clade

lineage is distributed in both the Northern and Southern hemispheres as well as tropical mountain regions with Fagales and Pinales and putatively with *Dicymbe* in the Guiana Shield region (M. E. Smith, personal observation).

The **/phaeocollybia lineage** is erected to accommodate species of *Phaeocollybia*. Three species of *Phaeocollybia* were reported from the roots of *Abies religiosa* in Mexico (Argüelles-Moyao et al. 2017), although the morphology of the ectomycorrhizas was not described. Many previous studies performed in the habitats of *Phaeocollybia* in North America have not detected this group on root tips. Although stable isotopes suggested the potential EcM or other biotrophic habit for *Phaeocollybia* spp., we previously considered this group non-mycorrhizal, because of long pseudorhizas being attached to long roots deep in soil (Redhead and Malloch 1985). The highest species diversity of *Phaeocollybia* (approximately 90 spp.) occurs in the temperate rainforests of western North America (Pacific Northwest), but individual endemic species are known from many regions, including Turkey, China, and northern South America (Brazil, Columbia; Coimbra et al. 2012). More work is needed to confirm that *Phaeocollybia* is a monophyletic group, to ensure that all species form EcM, and to document the morphology and exploration types of the symbiotic association.

We propose a novel **lineage /endogone3** within Mucoromycotina based on molecular identification of *Endogone* sp. (accessions LC159474-LC159479) from *Quercus* spp. root tips and anatomical descriptions of the association (Yamamoto et al. 2017). These samples comprise a novel lineage because they represent a sister group to the saprotrophic *Endogone pisiformis* (Berch and Fortin 1983a, b; Berch and Castellano 1986). They are also distantly related to the /endogone1 lineage (represented by *E. flammicorona* and *E. lactiflua*), /endogone2 (*E. aggregata*, *E. tuberculosa*, *Sclerogone eucalypti*) and /densospora (*Densospora* spp.) in a multigene phylogeny of Yamamoto et al. (2017). Unfortunately, the ITS sequences were not produced, which renders DNA barcoding-based identification of this group problematic. There are also no fruit bodies matching the sequences of these collections, and, therefore, the taxonomic identity and distribution of the /endogone3 lineage remain unknown.

6.4 New Names for Previously Known EcM Lineages

The **/guyanagarika lineage** is created here to accommodate the lineage previously referred to by Tedersoo and Smith (2013) as /agaricales1. The genus *Guyanagarika* was recently erected by Sánchez-García et al. (2016) and includes only three closely related species that all occur in the Guiana Shield region of northern South America. No sequences or sporocarps from these taxa have been collected or detected outside of this region, suggesting that this may be a narrowly endemic lineage that has evolved in the Neotropics and is restricted to endemic EcM host trees such as species of *Dicymbe* and *Pakaraimaea*. The robust multi-locus phylogenetic analysis by Sánchez-García

et al. (2016) placed this lineage within an expanded Catathelasmataceae but clearly separated from the members of the /catathelasma EcM lineage.

The /**phaeohelotium lineage** is erected to accommodate the /helotiales2 lineage that is naturally found only in the Southern Hemisphere. Dr. P. Johnston (unpubl.) first released sequences from fruit bodies of *Discinella terrestris* in New Zealand that matched closely to sequences from EcM root tips in Tasmania. The type species *D. boudieri* is only distantly related to the *D. terrestris* species complex, so *D. terrestris* was transferred to the new genus *Phaeohelotium* (Baral et al. 2013). The four described *Phaeohelotium* species are known from New Zealand and Australia and have also been documented in eucalypt plantations in Spain. Baral et al. (2013) also pointed to the observations of Warcup (1990a) that fruit bodies of *D. terrestris* sensu lato commonly co-occurred with other pyrophilic EcM and saprotrophic fungi after wildfire in Australia.

The /**tremellodendropsis lineage** is generated to accommodate the previously described /agaricomycetes1 lineage. This EcM lineage was initially erected to cover a cohesive group of Basidiomycota detected from EcM root tips especially from the Southern Hemisphere (Tedersoo and Smith 2013). A very recent fungal DNA barcoding initiative enabled to match these sequences to undescribed species of *Tremellodendropsis* from the formally monotypic order Tremellodendropsidales (Truong et al. 2017). This order forms a successive sister to Phallomycetidae, Stereopsidales, and *Clavulicium macounii* (Berbee et al. 2016). As discussed in Tedersoo and Smith (2013), not all putative species of Tremellodendropsidales are ectomycorrhizal.

6.5 Recently Revised Ectomycorrhizal Fungal Lineages

The /**cenococcum lineage** was discussed by Tedersoo et al. (2010) and Tedersoo and Smith (2013) as likely a group with only a few species and for which the sister taxon was poorly resolved. However, Spatafora et al. (2012) resolved several major lineages within /cenococcum and identified this lineage as belonging to Gloniaceae (with species of *Glonium* as the closest relatives). More recently, Obase et al. (2016) described the non-EcM *Pseudocenococcum floridanum* as a sister taxon to *Cenococcum* (see also Chap. 14).

In **Mucoromycota**, Tedersoo and Smith (2013) considered three EcM fungal lineages, viz., /endogone1, /endogone2, and /densospora. Because the two latter lineages had no fruit body sequences available, there was no information about their true taxonomic affinities. Our sequencing of Australian-type material (Tedersoo et al. 2016b) and recent phylogenetic analysis by Yamamoto et al. (2015) revealed that the EcM root tip sequences putatively assigned to the /endogone2 lineage are actually affiliated with *Densospora* in the /densospora lineage. Probably not all species of the genus *Densospora* form EcM (Warcup 1985; McGee 1996). The genus *Sphaerocreas* is also closely related to *Densospora* and affiliated EcM sequences, but its ecology is not well understood (Hirose et al. 2014). The /endogone2 lineage is comprised of the

Australian species *Endogone tuberculosa*, *E. aggregata*, and potentially *Sclerogone eucalypti* (Tedersoo and Smith 2013). Specimens of EcM species *Endogone aggregata* and *E. magnospora nom. nud.* (a putative member of this group) were recently sequenced, but these do not match closely to any sequences from EcM root tips. Specimens of *E. tuberculosa* and *S. eucalypti* have not yet been sequenced due to the age and paucity of herbarium materials. Furthermore, fruit body specimens and root tips of *Endogone* and *Densospora* are problematic to amplify and sequence because of multiple divergent ITS copies and long homopolymers (Tedersoo et al. 2016b). Endogonales resemble Glomeromycota (recently proposed as Glomeromycotina within Mucoromycota; Spatafora et al. 2016) in that they form nonseptate, multinucleate hyphae. This has been best demonstrated in pure cultures of *E. pisiformis* (Jabaji-Hare and Charest 1987). In the /endogone1 lineage, only members of the *E. flammicorona* and *E. lactiflua* species complexes (*Endogone* group B sensu Yamamoto et al. 2015) have been shown to form EcM (Warcup 1990b). Unfortunately, direct molecular evidence of EcM colonization by species in the /endogone1 and /endogone2 lineages is still lacking. The trophic status and ecophysiology of Endogonales requires urgent attention, because multiple distant clades of this group are likely recognized in the morphological species "*Glomus tenue*" *s. lat*. These have been referred to as "fine endophytes" that routinely colonize roots and form arbuscule-like structures in AM vascular plants (Orchard et al. 2017) and coils of hyphae in liverworts (Field et al. 2015).

6.6 Potential EcM Lineages that Require More Data

Several EcM lineages or putative EcM lineages still require more sampling effort to elucidate their interactions with host plants or clarify their putative trophic modes. For some of these taxa, their EcM status is suggested by the fruiting habit, associations with host plants, and/or isotopic data. However, for several groups we still lack solid data on EcM morphology and/or molecular confirmation on an EcM association.

The **/sowerbyella lineage** comprises the genus *Sowerbyella* that consists of 14 species (Yao and Spooner 2006). Members of the genus are typically found on the forest floor with EcM hosts. The rooting habit of the fruiting bodies, the fact that no member of the genus has been grown in axenic culture, and the isotopic signatures of some species (Hobbie et al. 2001) suggest that this genus may be EcM. However, there is still is no good molecular or anatomical data to show the EcM habit in this group. Hansen et al. (2013) resolved *Sowerbyella* on a branch among non-EcM relatives (e.g., *Aleuria*, *Lasiobolidium*) and suggested that *Sowerbyella* may not be EcM.

The multi-locus phylogenetic analysis of Sánchez-García et al. (2014) that separated /porpoloma from /tricholoma also proposed ***Albomagister*** as a segregate genus that is phylogenetically distinct from other Tricholomataceae. *Albomagister* was hypothesized to be EcM, because species in this genus fruit on the forest floor in association

with EcM Fagales and Pinaceae and have $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures that are similar to some other EcM fungi (e.g., members of the /catathelasma lineage) (Birkebak et al. 2013). It is possible that *Albomagister* represents another independent EcM lineage but root tips have never been sampled to test this hypothesis further.

6.7 New Additions of Genera Confirmed as Ectomycorrhizal

The genera of several EcM fungal lineages have been recently revised, resulting mostly in the splitting of large and heterogeneous genera into smaller groups. In the **/sebacina lineage**, the early diverging genus *Helvellosebacina* was separated from the rest of *Sebacina* whereas *Tremellodendron* was merged into *Sebacina* (Oberwinkler et al. 2014). The genus *Tremelloscypha* was resolved as the sister lineage to *Sebacina* and *Helvellosebacina* (but see Tedersoo et al. (2014b)), and all members of all three genera form a monophyletic group of EcM taxa. Oberwinkler et al. (2014) placed all of the non-mycorrhizal taxa in segregate genera, including *Chaetospermum*, *Craterocolla*, *Globulisebacina* (comprising *Efibulobasidium rolleyi*), and *Paulisebacina* (comprising *Sebacina allantoidea*).

The genus *Psathyloma* was provisionally included in the **/hebeloma-alnicola lineage** by Tedersoo and Smith (2013), but it was officially described only recently (Soop et al. 2016). It comprised three species, viz., *P. leucocarpum* and *P. catervatim* in New Zealand and Tasmania, and a third undescribed species from Argentina (root tip: JX316416). All known sequences and specimens of *Psathyloma* are known from Southern Hemisphere *Nothofagus* forests. In the analysis of Soop et al. (2016), the genus *Psathyloma* was resolved as the sister lineage to other taxa in the /hebeloma-alnicola lineage, suggesting the possibility of an ancient divergence between *Psathyloma* and all other genera in this group.

The **/boletus lineage** has been a subject to explosive radiation of descriptions of novel genera, several of which turn out to be non-monophyletic after addition of new taxa or information from other genes. The recent additions include *Alessioporus*, *Pulchroboletus* (Gelardi et al. 2014a), *Baorangia*, *Lanmaoa*, *Parvixerocomus*, *Rugiboletus* (Wu et al. 2015), *Binderoboletus*, *Guyanaboletus*, *Singerocomus* (Henkel et al. 2016), *Butyriboletus* (Arora and Frank 2014), *Caloboletus* (Vizzini 2014a), *Castellanea*, *Costatisporus*, *Jimtrappea* (Smith et al. 2015), *Cupreoboletus* (Gelardi et al. 2015a), *Crocinoboletus* (Zeng et al. 2014), *Cyanoboletus* (Gelardi et al. 2014b), *Exsudoporus* (Vizzini 2014b), *Imleria* (Vizzini 2014d), *Hourangia* (Zhu et al. 2015), *Neoboletus* (Vizzini 2014c), *Nigroboletus* (Gelardi et al. 2015b), *Pseudoaustroboletus* (Li et al. 2014), and *Rubroboletus* (Zhao et al. 2014).

6.8 Notes on the /Elaphomyces Lineage

The monophyly of the /elaphomyces lineage and the genus *Elaphomyces* was recently questioned by Buyck et al. (2016). Although we agree that this group warrants further taxonomic and phylogenetic research, we disagree with the suggestion that the African sequences published in Tedersoo et al. (2011b) are erroneous. We also disagree with the weak evidence that was presented for the polyphyly of this group of sequestrate hypogeous fungi. Re-evaluating the sequence data revealed that BLASTn results were meaningful only when conservative parameters (word size = 7, match score = 1, mismatch score = 3, gap opening cost = 5, gap extension cost = 2) but not MegaBLAST parameters were chosen. Buyck et al. (2016) also used only the ITS region for analysis and selected a specimen from another subclass (Chaetothyriomycetidae) as outgroup. Since the sequences of multiple clades within the /elaphomyces lineage are not alignable due to extremely high ITS sequence divergence (particularly among some undescribed tropical taxa), any phylogenetic analyses are likely to generate spurious results. We consider this study to be misleading and insufficient to suggest the polyphyly of Elaphomycetaceae or the /elaphomyces lineage. Here we do not make any changes in regard to the /elaphomyces lineage, but we do recommend caution when assigning sequences to the /elaphomyces lineage based on BLASTn searches.

6.9 Saprotrophic, Facultatively Biotrophic *Phlebopus*

In Tedersoo et al. (2010, p. 243), we discussed the mycorrhizal status of *Phlebopus* and considered this genus to be non-EcM but biotrophic. *Phlebopus* spp. readily form fruit bodies without any EcM host plants in sterile and nonsterile media and in natural conditions (Ji et al. 2011; Zhang et al. 2015; Kumla et al. 2016). In nature, *Phlebopus* spp. grow superficially and colonize the epidermal cells of AM and rarely EcM plants and associate with scale insects that form root galls in these roots (Zhang et al. 2015 and references therein). In axenic and synthesis trials in sterile and nonsterile substrate, *Phlebopus* spp. are reported to form ectomycorrhizal structures with EcM Australian *Acacia* spp. (Thoen and Ducouso 1989) and *Pinus kesiya* (Kumla et al. 2016). Although the illustrations of synthesized EcM structures are convincing in the latter study, we cannot accept *Phlebopus* as ectomycorrhizal because these associations are lacking or extremely rare in natural conditions (Zhang et al. 2015). We interpret the root-associated habit of *Phlebopus* as biotrophic but both non-mycorrhizal and non-parasitic, because the inoculated plants show no signs of decline (Kumla et al. 2016). The biotrophic associations with both roots and scale insects are likely facultative, because *Phlebopus* spp. are able to complete their life cycle saprotrophically without any of these interactions.

6.10 Recognition of EcM Fungal Lineages

Based on the criteria in Sect. 6.2, we propose specific criteria for separation of EcM fungal lineages from related non-EcM groups (Table 6.1) using the ITS2 subregion and full ITS. For ITS2 and full ITS, respectively, 45 (53%) and 60 (70%) lineages could be reliably delimited based on the BLASTn score to query sequence length (S/L) ratio alone because of a significant phylogenetic gap between EcM and closely related non-EcM groups. One quarter of lineages exhibited a small range of S/L values, where trophic assignment is unambiguous. In these cases, assignment of individual lineages should be sought for support by manual BLASTn queries and/or phylogenetic analyses for greater reliability. In general, placement tended to be relatively more ambiguous for the most diverse EcM groups such as the /russula-lactarius, /inocybe, /clavulina, and /boletus lineages but not in the /tomentella-thelephora and /cortinarius lineages. Phylogenetic analyses suggested ambiguity in cases where the non-EcM outgroup(s) was separated by a relatively short stem (e.g., /tricholoma: Sánchez-García et al. 2014), or the outgroup had a low rate of ITS evolution (e.g., /inocybe: Ryberg et al. 2010), or there in rapidly evolving clades within the EcM lineages (e.g., /clavulina: Kennedy et al. 2012; /boletus: Nuhn et al. 2013). Except for /hysterangium, /inocybe, and /clavulina, <2% of OTUs across the lineages of ambiguously delimited groups fell into the uncertain range of S/L values, indicating the overall rate for correct placement at 97–98%.

6.11 Conclusions

With the addition of the /leotia, /porpoloma, /endogone3, and /phaeocollybia lineages to information from a previous review (Tedersoo and Smith 2013), the number of EcM fungal lineages has now grown to 82–86 separate groups comprising 279–284 genera. The rate of discovery of novel EcM lineages is notably declining because the most common groups have been already described. This is due to a huge increase in the number of in situ molecular identification studies of EcM fungal communities on roots as compared to a decade ago. However, the number of profound EcM community studies tends to decline in recent years, because most laboratories have switched to HTS-based identification of EcM fungi directly from bulked root and soil samples. Since EcM fungi naturally co-occur with many other fungal and eukaryote groups, it is impossible to verify the EcM habit from these types of studies. Our overview about the parameters of semiautomatic EcM lineage recognition should enable accurate trophic assignment of 95–99% of fungal OTUs to EcM and non-EcM categories. Further developments in this field should include development and automatized application of taxon-specific sequence similarity thresholds for taxa by using expert molecular taxonomic knowledge. In the future, it will also be important to use additional, phylogenetically or functionally informative loci for HTS-based

Table 6.1 Critical values for ITS2-based separation of EcM fungal lineages from non-mycorrhizal groups

	ITS2 subregion			ITS (full length)		
	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)
/acephala macrosclerotiorum	0.30	0.46	98	nd	nd	98
/albatrellus	0.47	0.40	80	0.34	0.60	85
/aleurina	0.37	0.46	82	0.34	0.87	85
/amanita	0.39	0.40	80	0.30	0.38	77
/amphinema-tylospora	0.60	0.56	85	0.50	0.70	90
/atheliales1	0.43	0.50	80	nd	0.50	80
/atheliales2	0.50	0.65	85	nd	nd	79
/austropaxillus	0.40	0.60	90	nd	0.70	90
/boletopsis	0.55	0.55	84	nd	nd	92
/boletus	0.40	0.12	75	0.30	0.50	80
/byssocorticium	0.57	0.50	84	nd	0.70	88
/cantharellus	0.30	0.15	76	nd	0.35	75
/catathelasma	nd	nd	95	nd	nd	95
/cenococcum	0.65	0.70	95	nd	nd	95
/ceratobasidium1	0.65	0.68	90	nd	nd	90
/ceratobasidium2	0.65	0.70	90	nd	nd	90
/clavariadelphus	0.40	0.50	90	nd	nd	90
/clavulina	0.40	0.28	80	0.35	0.45	80
/coltricia	0.10	0.25	75	0.25	0.33	75
/cortinarius	0.49	0.51	85	0.55	0.60	84
/densospora	nd	nd	nd	nd	nd	80
/descolea	0.56	0.50	83	nd	nd	85
/elaphomyces	0.15	0.27	72	nd	0.50	80
/endogone1	nd	nd	nd	nd	nd	90
/endogone2	0.35	0.40	nd	0.35	0.60	85
/endogone3	nd	nd	nd	nd	nd	nd
/entoloma	0.61	0.57	86	nd	nd	88
/galactinia	0.20	0.60	84	nd	nd	85
/genea-humaria	0.30	0.25	76	0.30	0.50	80
/geopora	0.33	0.66	88	nd	0.80	90
/guyanagarica (/agaricales1)	0.31	0.41	75	0.61	0.75	85
/hebeloma-alnicola	0.56	0.52	85	0.60	0.50	86
/helotiales1	0.77	0.79	96	0.70	0.95	96
/helotiales3	nd	nd	nd	nd	nd	95
/helotiales4	nd	nd	90	nd	0.80	90

(continued)

Table 6.1 (continued)

	ITS2 subregion			ITS (full length)		
	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)
/helotiales5	nd	nd	96	nd	nd	96
/helotiales6	nd	nd	nd	nd	nd	95
/hydnellum-sarcodon	0.31	0.40	84	0.30	nd	85
/hydnotrya	nd	nd	80	nd	nd	85
/hydropus	0.75	0.85	92	nd	nd	92
/hygrophorus	0.43	0.38	80	nd	0.50	82
/hysterangium	0.20	0.09	70	nd	0.15	75
/inocybe	0.42	0.35	80	0.48	0.50	80
/laccaria	0.74	0.64	88	nd	0.85	90
/leotia	nd	0.50	85	nd	0.50	80
/leucangium	nd	nd	75	nd	nd	78
/marcelleina-peziza gerardii	0.23	0.23	65	nd	0.50	80
/meliniomyces	nd	nd	97	0.95	0.97	98
/otidea	0.34	0.38	86	0.30	0.70	88
/pachyphloeus-amylascus	0.13	0.38	75	nd	0.55	82
/paralyophyllum	0.72	0.73	90	nd	nd	93
/paxillus-gyrodon	0.40	0.60	87	nd	0.75	88
/phaeocollybia	nd	0.50	82	nd	nd	85
/phaeohelotium (/helotiales2)	nd	nd	96	nd	nd	96
/phellodon-bankera	0.30	0.56	88	nd	0.70	87
/piloderma	0.51	0.49	85	0.40	0.55	82
/pisolithus-scleroderma	0.27	0.29	79	nd	0.50	82
/porpoloma	nd	nd	92	nd	nd	89
/pseudotomentella	0.42	0.51	85	nd	0.65	86
/pulvinula	0.55	0.48	77	0.60	0.70	85
/pustularia	0.60	0.75	91	nd	0.90	93
/pyronemataceae1	0.44	0.41	78	nd	0.60	82
/pyronemataceae2	nd	nd	90	nd	nd	90
/ramaria-gautieria	0.35	0.30	70	nd	0.40	78
/rhodoscypha	0.52	0.65	88	0.30	0.70	88
/russula-lactarius	0.30	0.22	78	0.40	0.60	83
/sarcosphaera-hydnotryopsis	nd	nd	85	nd	nd	80
/sebacina	0.48	0.51	80	nd	0.60	85
/serendipita1	0.68	0.75	93	nd	nd	92

(continued)

Table 6.1 (continued)

	ITS2 subregion			ITS (full length)		
	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)	S/L highest in non-EcM fungi	S/L lowest in EcM fungi	Minimum identity (%)
/serendipita2	0.70	0.77	93	nd	nd	93
/sordariales1	0.55	0.49	85	0.45	0.60	83
/sordariales2	nd	0.70	90	nd	0.74	89
/sowerbyella	nd	nd	86	nd	nd	88
/sphaerosporella	0.49	0.56	85	nd	nd	86
/suillus-rhizopogon	0.12	0.63	82	nd	nd	80
/tarzetta	0.17	0.40	76	nd	0.70	88
/terfezia-peziza depressa	0.24	0.60	85	nd	0.60	85
/tomentella-thelephora	0.33	0.45	82	nd	0.70	88
/tomentellopsis	0.50	0.72	90	nd	0.80	90
/tremellodendropsis (agaricomycetes1)	0.65	0.63	89	nd	nd	90
/tricholoma	0.55	0.52	85	0.50	0.60	83
/tuber-helvella	0.30	0.50	80	nd	0.50	80
/tulasnella1	0.79	0.82	93	nd	nd	90
/tulasnella2	nd	nd	80	nd	nd	85
/wilcoxina	0.45	0.65	86	nd	nd	90
/xenasmatella	0.62	0.65	95	nd	nd	90

approaches beyond ITS sequencing. It should also be possible in the future to automatically assign traits and functions to the EcM fungi based on a combination of the taxonomy and what is known about reference taxa. Much has yet to be done to incorporate information about functional genes of taxa obtained from genomics studies and using probabilistic approaches rather than binary (presence/absence) functional assignments.

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Chapter 7
The Predictive Power of Ecological Niche Modeling for Global Arbuscular Mycorrhizal Fungal Biogeography

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7.1 Introduction

Arbuscular mycorrhizal (AM) fungi are globally distributed, obligate, belowground symbionts that associate with up to 80% of all plant species (Smith and Read 2008; Kivlin et al. 2011; Öpik et al. 2013; Davison et al. 2015; Soudzilovskaia et al. 2015a). Typically, AM fungi improve host plant growth by providing soil nutrients (Smith and Read 2008), water (Augé 2001), and pathogen protection (Sikes et al. 2010). In doing so, they can influence C, N, and P dynamics within ecosystems, and—given their worldwide abundance—at the global scale as well (Mohan et al. 2014; Soudzilovskaia et al. 2015b). By considering the global distribution and functions of AM fungi, we may better predict large-scale C, N, and P cycling (Brzostek et al. 2014; Treseder 2016). Additionally, because AM fungal taxa vary in their effects on plant growth and nutrient uptake (van der Heijden et al. 1998a; Maherali and Klironomos 2007; Chagnon et al. 2013; Johnson et al. 2013), it is worthwhile considering the global biogeography of individual AM taxa.

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AM fungi can be particularly sensitive to global change, because their function (Treseder 2004; Johnson et al. 2010; Kivlin et al. 2013) and community composition (Yang et al. 2013a) are affected by environmental conditions. For example, AM fungal taxa differ in their responses to climate (Kivlin et al. 2011; Davison et al. 2015), soil nutrients (Xiang et al. 2014), and plant community composition (Öpik et al. 2010). Thus, human activities that alter these conditions could, in turn, change the distribution of AM fungal taxa.

Given the importance of environmental variables in determining the distribution of AM fungal species, ecological niche models may provide robust predictions of distributions of individual AM fungal taxa. Ecological niche models, or species distribution models, use underlying variation in environmental conditions and known species occurrences to predict which unexplored areas may contain optimal habitat for a focal taxon (Phillips et al. 2006). These models are commonly used to determine potential habitat for plant and animal species (Peterson et al. 2002), define cryptic species (Raxworthy et al. 2007), predict invasion success (Peterson 2003), and model the spread of crop pests (Venette et al. 2010). However, ecological niche models do not incorporate dispersal limitation or competition, which may result in narrower realized distributions of taxa than predicted by these models (reviewed by Sinclair et al. 2010). Because there is limited evidence of short-term dispersal limitation for AM fungi (Davison et al. 2015) and competition among AM fungal taxa occurs at very small spatial scales (Maherali and Klironomos 2012), ecological niche models have the potential to accurately predict large-scale species distribution of these taxa, perhaps even better than current models for macroorganisms (Pearson and Dawson 2003). Indeed, ecological niche models have successfully modeled the niche for fungal pathogens (e.g., Baptista-Rosas et al. 2007; Reed et al. 2008); yet they have not been applied to mutualistic fungal taxa.

Once taxon-specific distributions are understood, they could then be leveraged to predict AM fungal functions across large spatial scales in cases where functions are well understood. For example, traits of AM fungi that are influential in nutrient acquisition, such as intra- and extraradical colonization rates, are phylogenetically conserved (Powell et al. 2009; Maherali and Klironomos 2012). AM fungi also exhibit generalizable and well-characterized diversity-productivity relationships (van der Heijden et al. 1998b). Thus it is relatively straightforward to link taxon distributions to well-known trait distributions for this clade. Because AM fungi are so well studied, this system provides an excellent case for linking microbial composition to ecosystem function (Treseder 2016), allowing inference of ecosystem process rates from simple community-based metrics.

7.2 Importance of Species Level Models of AM Fungal Distribution

Despite the promise that AM fungal community composition is indicative of function, we currently lack predictive models of AM fungal distribution under current or future climates. Instead, the factors affecting AM fungal composition are measured via community-wide metrics based on observational data of composition and underlying environmental conditions.

However, the community level is not the correct scale of inference to predict how AM fungi will respond to global change. Community-wide metrics, such as Bray-Curtis distance of beta-diversity among sites, are biased in their interpretation because they often favor the most abundant or widespread taxa while marginalizing the effects of rare AM fungi (Wolda 1981; Plotkin and Muller-Landau 2002). Instead, understanding the factors that affect the distribution of individual AM fungi will ultimately yield the most predictive models of AM fungal distributions, because the capability of AM fungi to disperse, adapt, or acclimate to environmental change is controlled by selection at the species level (Vellend 2010). For example, spore size varies among AM fungal taxa, which could limit the short-term dispersal ability of some large-spored species (e.g., *Gigaspora gigantea*), while other taxa (e.g., *Archaeospora schenckii*) may be less affected (Kivlin et al. 2014). In addition, local adaptation of AM fungal taxa to both soil nutrient concentrations (Johnson et al. 2010; Rúa et al. 2016) and climate (Antunes et al. 2010) suggests that AM fungal taxa may differentially respond to these drivers as well. Evidence of AM fungal acclimation is rare but can occur in response to temperature (Heinemeyer et al. 2006; Hawkes et al. 2008). Creating ecological niche models at the species level does not preclude community-level inference; once the distributions of individual fungal taxa are understood, these can be aggregated to infer potential community composition in the absence of competition at any given site (Thuiller et al. 2015). Because ecological niche models predict composition in the absence of biotic interactions, comparing models to actual communities can also help to infer the role of biotic interactions in community assembly (Wisz et al. 2013; Calabrese et al. 2014).

7.3 Testing Niche Modeling in a Common AM Fungal Taxon

Here we apply ecological niche modeling techniques to the most abundant and widespread AM fungus, *Rhizophagus irregularis* (formerly *Glomus intraradices*), to illustrate when this technique is useful for predicting where this microbial species occurs and to determine potential drawbacks of this technique. We use a presence-only modeling approach whereby environmental conditions at locations of known species occurrences are compared to environmental conditions at "background"

locations (Phillips et al. 2006). We ran three models to predict *R. irregularis* distribution: (1) a full model including all (i.e., climate and resource) variables, (2) a climate model including Bioclim variables and soil moisture, and (3) a resource model including soil resources and plant net primary productivity. We expected that *R. irregularis* distributions would be affected by both climate and soil resources given current understanding of the factors affecting AM fungi at the global scale. Despite a long tradition of determining ecological niches of plants and animals (Grinnell 1917; Elton 1927), to our knowledge, this is the first attempt to predict AM fungal niches at the global scale.

7.3.1 Species Definitions

AM fungal species in current databases (e.g., MAARJAM) are typically defined as sharing at least 97% of DNA bases in conserved 18S ribosomal subunit genes (Öpik et al. 2010). However, the most appropriate species definition of AM fungi is currently being debated (see Davison et al. 2015; Bruns and Taylor 2016; Öpik et al. 2016). Virtual taxa in the MAARJAM database may represent species complexes that more closely resemble family-level resolution in plant and animal clades (Bruns and Taylor 2016). This feature may be particularly relevant for *R. irregularis*, which is one of the most genetically diverse AM fungal morphospecies (Börstler et al. 2008). Therefore, we examined how varying the OTU definition based on sharing 95, 97, 99, or 99.5% of bases in the 18S gene affected the predicted niche of *R. irregularis*. We expected that genetic resolution could change the importance of individual drivers of *R. irregularis* distributions, but overall interpretation of the importance of climatic vs. resource drivers would not vary.

7.3.2 Spatial Resolution

Sampling effort of AM fungi to date is biased in favor of northern hemisphere locations (Kivlin et al. 2011). Consequently, global niche models may be biased to highlight only the predictive drivers of AM fungal distribution in northern latitudes. For example, because of glaciation, soil nutrients are more limiting in equatorial ecosystems (Vitousek and Howarth 1991), whereas more extreme climates are a greater constraint at temperate and boreal latitudes. These environmental drivers have been hypothesized to control the distributions of many taxa (MacArthur 1972). There is some evidence that soil resources and climate affect distribution of plant (Condit et al. 2013) and animal (Parmesan et al. 2000) species. However, a synthetic comparison of the relative importance of these drivers on species distributions at the global scale has not been conducted. Thus, we predicted that niches of *R. irregularis* in North America and Eurasia would be most affected by climate, whereas soil resources would drive niches in South America and Africa.

7.3.3 Data Acquisition

DNA sequences of the 18S gene of *R. irregularis* were collected from published studies in the GenBank database through December 17, 2015. Sequences were aligned with the MAFFT aligner (Katoh et al. 2002) using PASTA (Mirarab et al. 2015). Sequences were then separated into operational taxonomic units (OTUs) with either 95, 97, 99, or 99.5% sequence similarity using the mothur farthest neighbor algorithm in QIIME (Caporaso et al. 2010). This created two 95% OTUs, four 97% OTUs, three 99% OTUs, and one 99.5% OTU with at least ten occurrences in the dataset (Table 7.1). A representative sequence of each OTU was queried against the MAARJAM database to confirm identity to *R. irregularis* (VTX00114).

For each entry, we collected the latitude and longitude of the sample from GenBank. Locations were used to infer environmental characteristics including both climate and resource variables. Climate information was based on raster layers obtained from Bioclim (Hijmans et al. 2005), which included mean diurnal temperature range, isothermality, maximum temperature in the warmest month, minimum temperature in the coldest month, mean temperature in the wettest quarter, mean temperature in the warmest quarter, mean annual precipitation, precipitation in the wettest month, precipitation in the driest month, precipitation seasonality, precipitation of the warmest quarter, and precipitation of the coldest quarter; we further included soil moisture derived solely from climate variables (Willmott et al. 1985). Resource-related parameters were net primary productivity (NPP) (Foley et al. 1996), soil carbon (C), soil pH (IGBP-DIS), soil percent clay (Hengl et al. 2014), and soil phosphorus (P) (Yang et al. 2013b). Because Bioclim variables are highly correlated, we retained only the nonredundant variables (excluding mean annual temperature, temperature seasonality, temperature annual range, mean temperature in the driest quarter, mean temperature of the coldest quarter, precipitation of the wettest quarter, and precipitation of driest quarter) (Ricklefs and He 2016). Resolution of all raster layers was standardized to 10 arc min.

To understand the spatial variability of *R. irregularis* niches across continents, separate models were constructed on the full dataset of *R. irregularis* occurrences in Africa, Eurasia, North America, and South America, as these were the only geographic areas with over ten occurrences.

For the entire dataset and each OTU and continent, we created three main models: (1) a model that included all of the environmental (climate and soil) variables (hereafter full model), (2) a model with only nonredundant Bioclim variables (listed above; Ricklefs and He 2016) and soil moisture (hereafter climate-only model), and (3) a model with all other soil and resource variables (NPP, soil C, soil pH, percent soil clay, and soil P; hereafter resource-only model). By comparing the output of these models, we determined the relative influence of climate and resources on *R. irregularis* distributions across genetic and spatial scales.

Table 7.1 Model performance output for *R. irregularis* across genetic and spatial resolutions

	Model type	Model settings	*n*	ΔAICc	Full AUC	OR_{10}	Most influential variable
Species resolution							
VTX00114	**Full model**	**Linear/Quadratic**	**147**	**0**	**0.828**	**0.168**	**Soil moisture**
	Climate only	Linear/Quadratic	147	34.5	0.832	0.163	Soil moisture
	Resources only	Linear/Quadratic/ Hinge	147	30.1	0.782	0.181	Soil carbon
95% OTU 0	**Full model**	**Linear**	**15**	**0**	**0.961**	**0.271**	**Soil moisture**
	Climate only	Linear	15	43.2	0.984	0.625	Soil moisture
	Resources only	Linear	15	23.4	0.783	0.563	NPP
95% OTU 1	**Full model**	**Linear/Quadratic**	**95**	**0**	**0.883**	**0.155**	**Soil moisture**
	Climate only	Linear/Quadratic	95	32.0	0.866	0.139	Soil moisture
	Resources only	Linear/Quadratic	95	47.6	0.823	0.154	Soil carbon
97% OTU 2	Full model	Linear/Quadratic	36	1.0	0.931	0.233	Diurnal temp. range
	Climate only	**Linear/Quadratic**	**36**	**0**	**0.928**	**0.233**	**Soil moisture**
	Resources only	Linear/Quadratic	36	15.9	0.854	0.254	Soil carbon
97% OTU 7	Full model	Linear	41	14.4	0.902	0.170	Soil moisture
	Climate only	Linear/Quadratic	41	8.5	0.912	0.205	Soil moisture
	Resources only	**Linear/Quadratic**	**41**	**0**	**0.899**	**0.201**	**NPP**
97% OTU 9	**Full model**	**Linear**	**27**	**0**	**0.949**	**0.258**	**Isothermality**
	Climate only	Linear/Quadratic	27	0.9	0.947	0.208	Isothermality
	Resources only	Linear/Quadratic	27	2.4	0.891	0.250	NPP
97% OTU 20	Full model	Linear	27	10.3	0.863	0.183	Precip. seasonality
	Climate only	**Linear/Quadratic**	**20**	**0**	**0.913**	**0.183**	**Precip. seasonality**
	Resources only	Linear/Quadratic	20	4.6	0.736	0.483	NPP
99% OTU 2	Full model	Linear	27	10.5	0.911	0.188	Precip. seasonality
	Climate only	**Linear/Quadratic**	**27**	**0**	**0.949**	**0.182**	**Soil moisture**
	Resources only	Linear/Quadratic	27	27.9	0.824	0.223	Soil carbon
99% OTU 37	Full model	Linear/Quadratic	20	16.5	0.958	0.238	Diurnal temp. range
	Climate only	**Linear/Quadratic**	**20**	**0**	**0.963**	**0.238**	**Soil moisture**
	Resources only	Linear	20	21.0	0.809	0.113	NPP

(continued)

Table 7.1 (continued)

	Model type	Model settings	*n*	ΔAICc	Full AUC	OR_{10}	Most influential variable
99% OTU 46	Full model	Linear	10	110.0	**0.902**	0.222	Precip. seasonality
	Climate only	Linear	10	12.7	0.857	0.222	Precip. seasonality
	Resources only	**Linear**	**10**	**0**	**0.699**	**0.222**	**NPP**
99.5% OTU 2	**Full model**	**Linear/Quadratic**	**22**	**0**	**0.945**	**0.267**	**Diurnal temp. range**
	Climate only	Linear/Quadratic	22	23.7	0.936	0.267	Soil moisture
	Resources only	Linear/Quadratic	22	25.6	0.818	0.083	Soil carbon
Spatial extent							
Africa	**Full model**	**Linear**	**19**	**0**	**0.821**	**0.422**	**Mean temp. warmest quarter**
	Climate only	Linear	19	2.8	0.824	0.456	Mean temp. warmest quarter
	Resources only	Linear	19	16.3	0.670	0.067	Soil P
Eurasia	**Full model**	**Linear**	**94**	**0**	**0.854**	**0.169**	**Soil moisture**
	Climate only	Linear/Quadratic/ Hinge/Threshold/ Product	94	23.9	0.868	0.129	Precipitation seasonality
	Resources only	Linear/Quadratic/ Hinge/Threshold/ Product	94	14.3	0.833	0.137	NPP
North America	**Full model**	**Linear**	**17**	**0**	**0.740**	**0.456**	**Min. temp. coldest month**
	Climate only	Linear/Quadratic	17	5.5	0.786	0.222	Min. temp. coldest month
	Resources only	Linear/Quadratic	17	3.0	0.563	0.344	Soil P
South America	Full model	Linear	14	9.2	0.913	0.500	Mean temp. warmest quarter
	Climate only	Linear	14	2.1	0.893	0.500	Mean temp. warmest quarter
	Resources only	**Linear**	**14**	**0**	**0.818**	**0.250**	**Soil carbon**

The most influential variable affecting these distributions for each model type is presented. The best model performance parameters are bolded. The model with the lowest AICc score is bolded along with the most influential variable in that model for each genetic and spatial extent

7.3.4 Ecological Niche Model Parameters

We built ecological niche models using the MaxEnt algorithm (Phillips et al. 2006) and we used the ENMeval v 0.2.0 R package (Muscarella et al. 2014) to "tune" model parameters to balance fit and predictive ability. We used a two-stage process of model selection to first determine the optimal model complexity for each of the three main models described above and then to identify which of the three main models best described occurrence patterns for *R. irregularis*. Specifically, in the first stage, we separately evaluated a range of candidate models across a range of complexity by allowing for different possible combinations of feature classes (i.e., linear, quadratic, hinge, threshold, and product) and regularization multiplier values (Merow et al. 2013). We used *k*-fold cross validation to evaluate model performance for each combination of parameters. For this, we partitioned occurrence records and background points into testing and training bins using the "checkerboard2" method in ENMeval (using default settings for aggregation factors). We used variable importance metrics generated by MaxEnt to determine the relative explanatory power of each predictor variable in our models. Performance was assessed with AUC (Hanley and McNeil 1982), OR_{10} (Fielding and Bell 1997), and AICc (Burnham and Anderson 2004). In each case, the best fit model (full, climate-only, or resource-only) was chosen using AICc. All model runs, raster manipulations, and distribution visualizations were performed using the dismo v. 1–0.15 (Hijmans and Elith 2012) and ENMeval v. 0.2.0 (Muscarella et al. 2014) packages in R v. 3.2.4 (R Development Core Team 2009).

7.4 Model Output

At all levels of genetic resolution, both climatic and resource variables influenced the distributions of *R. irregularis* at the global scale (Table 7.1 and Fig. 7.1). However, the influence of climate was stronger in most cases. For all data points and each 95% OTU, soil moisture was the strongest predictor for *R. irregularis* occurrence, with higher probability of occurrence in wetter soils. When OTUs were delineated at 97% sequence similarity, a positive correlation with soil moisture was still the main predictive variable for one out of the four OTUs, but negative associations with precipitation seasonality and isothermality, as well as a peak at intermediate NPP, also explained some variation in occurrence of three out of the four OTUs. At 99% sequence similarity, a positive association with soil moisture and negative association with precipitation seasonality explained the variation of both two tested OTUs. The 99.5% OTU distribution was best explained by a negative correlation with diurnal temperature range.

The drivers of potential distribution of *R. irregularis* varied across continents. Potential distribution in Eurasia and North America was driven by climate—positive effects of precipitation seasonality and peaking at intermediate minimum

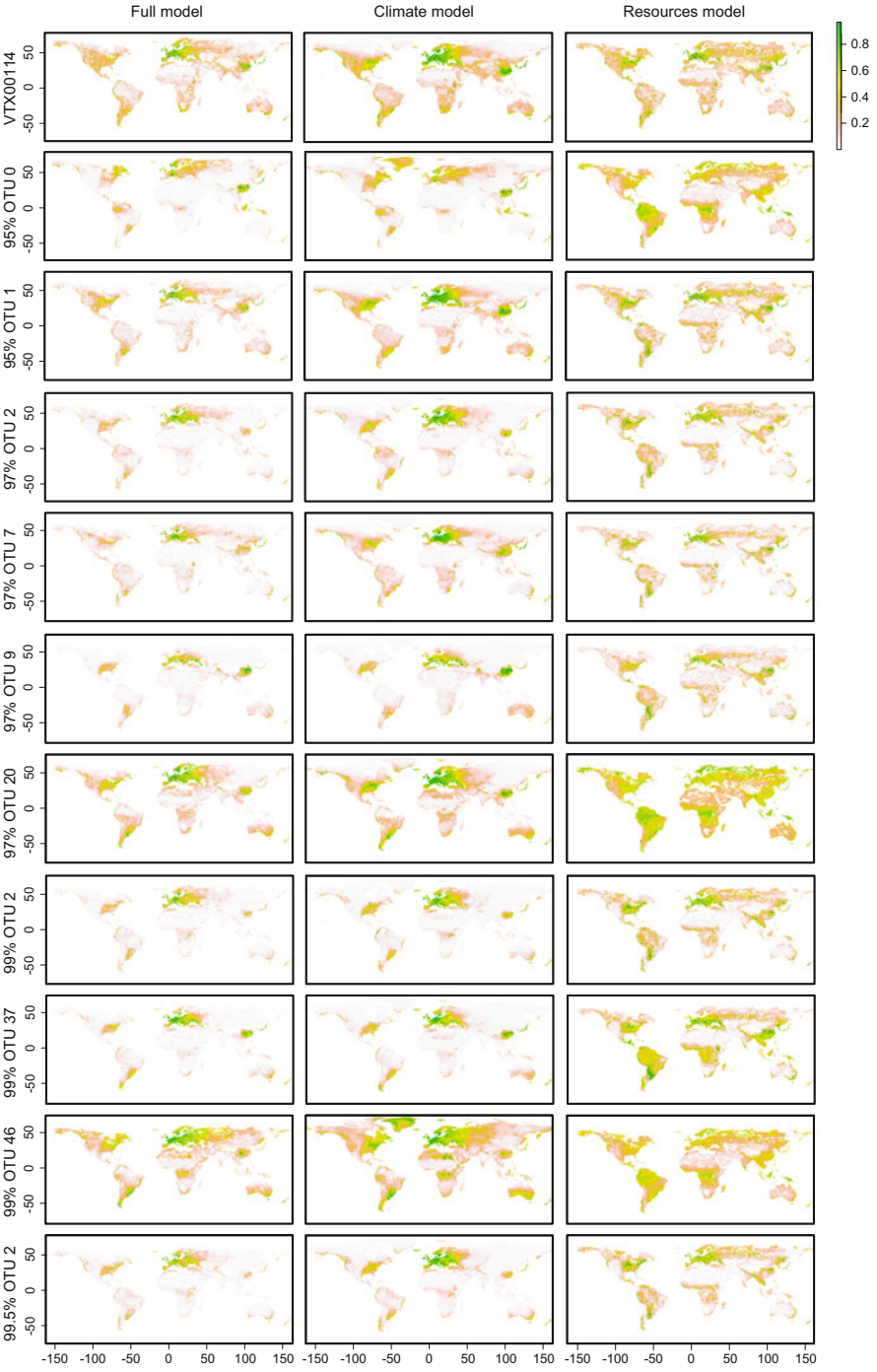

Fig. 7.1 Distribution models for *R. irregularis* at different phylogenetic resolutions for the full, climate-only, and resource-only models. *Greener* areas are more likely to contain suitable habitat for *R. irregularis*

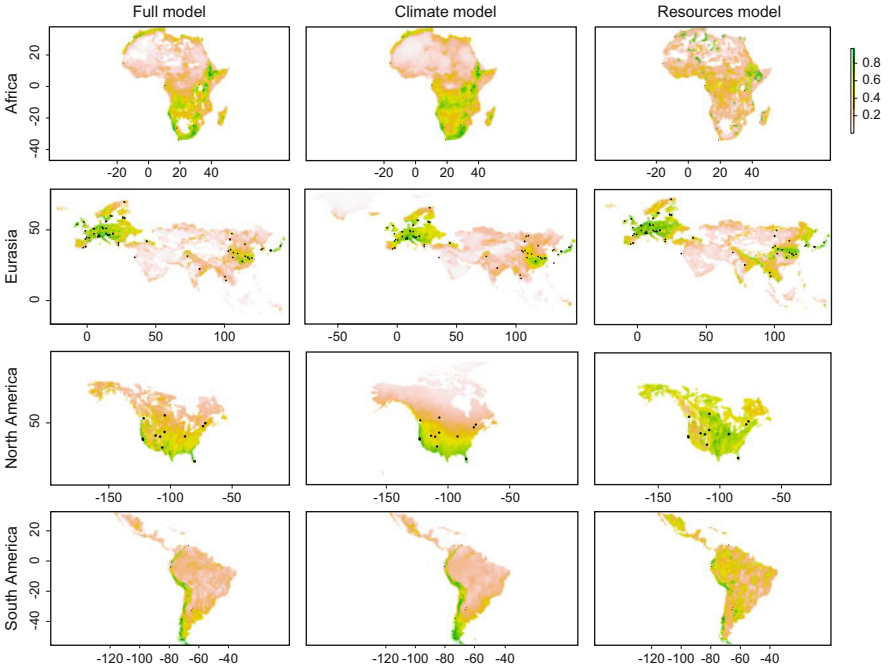

Fig. 7.2 Distribution models for all *R. irregularis* occurrences on different continents for the full, climate-only, and resource-only models. Greener areas are more likely to contain suitable habitat for *R. irregularis*. *Black points* on the map represent presences in the model

temperatures in the coldest month, respectively (Fig. 7.2). In contrast, the niche of *R. irregularis* in South America was controlled by a positive association with soil C, whereas the niche in Africa was driven by both climate (negative association with mean temperature in the warmest quarter) and resources (positive association with soil P).

Overall, based on our 15 final AICc-selected AM fungal ecological niche models, 87% had high AUC scores (i.e., AUC $>$ 0.80), indicating accurate discrimination of AM fungal presence from background points. Omission rates were also fairly low (mean OR10 $=$ 0.25), indicating that models were generally not overfit. The AICc-selected models based on different species resolution tended to have better performance than the spatial models, likely because of the higher overall sample size (e.g., species resolution models had an average AUC of 0.90 versus 0.79 for the spatial extent models). In particular, some of spatial models had high omission rates (e.g., 0.42 and 0.50 for South America and Africa, respectively), suggesting overfitting. In contrast, the average omission rate for OTU models was 0.21.

As we hypothesized, the genetic resolution of species definition for the *R. irregularis* species complex affected the relative importance of factors affecting ecological niche models. However, the most important drivers in every case were

climatic, with soil moisture dominating the distribution of 55% of OTUs. Therefore, despite the current debate about the "true" definition of AM fungal species, current databases of virtual taxa still provide relevant information about the importance of climatic versus resource-related drivers of AM fungal distributions.

The spatial scale of the ecological niche models affected AM fungal distribution much more than genetic resolution. As expected, ecological niche models constructed in mostly temperate and boreal latitudes reflected the influence of climate on AM fungal distribution, whereas those from mostly tropical regions highlighted the influence of soil resources. The congruence of these models with previous modeling attempts for plants and animals suggests that tropical nutrient limitation and temperate climatic variability may also affect mycorrhizal life forms. This is also consistent with community-level mycorrhizal fungal patterns (e.g., Tedersoo et al. 2014). However, we have only examined a single complex AM fungal taxon; additional work will be needed to generalize these patterns.

7.5 Limitations of Ecological Niche Models

Despite the promise of ecological niche models to infer the factors affecting microbial distribution, they do not capture several dynamic aspects that may influence microbial biogeography. For example, dispersal is not explicitly represented in ecological niche models (Soberón 2007). If Glomeromycota dispersal indeed is not limiting (Davison et al. 2015), this constraint may not be meaningful. However dispersal of AM fungi remains poorly understood. In addition, for obligate plant symbionts, such as the AM fungi modeled here, host distribution and association preference are not taken into account. While AM fungi are mostly host species generalists (Öpik et al. 2013), variation in function among AM fungal hosts (Rúa et al. 2016) may affect both fungal and host fitness, with implications for AM fungal niches. These models also assume that species are at equilibrium in the environment (Yackulic et al. 2015), which may not be true since suitable habitat space fluctuates regularly for reasons as varied as seasonality, disturbance, plant succession, and global change. There was also a substantial sampling bias of both AM fungal composition and underlying environmental layers toward northern hemisphere locations that may skew the interpretation of our models. For example, only 33 of the 147 occurrences of *R. irregularis* in the current dataset were in South America or Africa. The models based on these records suffered from overfitting, and further work will be required to generate robust estimates of species ecological niches, particularly in these areas. As appreciation of this sampling bias is realized, more geographically explicit sampling schemes can only improve the resolution of global ecological niche models for microorganisms. Finally, by their nature, MaxEnt models only model occurrence records and do not take into account true absences. It is currently difficult to assess true absences of microbial species due to low sequencing effort and primer bias, but as sequencing methodology and depth improve, future distribution modeling may benefit from presence and absence data.

7.6 The Future of Ecological Niche Models of AM Fungi

Ultimately, AM fungal ecological niche models should be combined with similar models of their plant hosts. If both AM fungi and their hosts are affected by climate, and dispersal limitation does not limit migration, we can project future ranges based on our current understanding of climate change projections (with caveats as mentioned above). Attempts to predict biogeographical ranges are common for plants at large scales (Bakkenes et al. 2002), but only two localized studies (Pellissier et al. 2013; Bueno de Mesquita et al. 2015) have incorporated fungal symbionts that may hinder or ameliorate plant environmental stress tolerance and only under current environmental conditions. In addition, understanding not only the distribution but also the demographic rates of symbiotic fungi across environmental gradients will aid in determining the future distributions of these species (Merow et al. 2014). For example, if current ecological niche models indicate that soil moisture is the most influential variable for current AM fungal distribution, but temperature is more influential on AM fungal spore production and fitness (Schenck and Smith 1982; Zhang et al. 2016), then future AM fungal populations may not track current drivers of biogeography. Integrating performance-based metrics of microbial population dynamics into spatially explicit ecological niche models will be necessary to capture these processes.

Nevertheless, current datasets across broad spatial scales and taxonomic levels are ushering in a new age of microbial biogeography. By comparing distribution patterns of individual AM fungal taxa, we can predict simple macroecological patterns for these, for example, range size. Furthermore, because computationally stacking distribution patterns of individual AM fungal taxa can predict their diversity and community composition, these models can also be used to elucidate community-level patterns, such as latitudinal gradients in diversity or species turnover across environmental gradients. The macroecological hypotheses generated from ecological niche modeling techniques can then be tested with molecular surveys, allowing for a predictive microbial biogeography framework.

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Chapter 8
Biogeography of Orchid Mycorrhizas

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8.1 Introduction

The mycorrhizal interaction between plants and fungi is probably one of the most important symbiotic associations of terrestrial ecosystems (van der Heijden et al. 2015) and is one that has the longest evolutionary history for terrestrial plants (Selosse et al. 2015). In this mutualism, the soil fungus contributes mineral nutrition and water to the plant that, in turn, contributes photosynthetically fixed carbon back to the fungus, by way of a dual organ made of roots colonized by fungal hyphae, the mycorrhiza (Smith and Read 2008). While many studies have shown that plant species are mycorrhizal generalists, in that they can interact with many taxonomically disparate mycorrhizal taxa, there are also cases of plants that are mycorrhizal specialists (van der Heijden et al. 2015). Hence, it is widely assumed that coevolutionary patterns between plants and fungi are weak or nonexisting.

Some plant groups have reversed the mycorrhizal nutrient exchange and obtain carbon from their fungal partner for at least a portion of their life cycle, a nutritional strategy called "mycoheterotrophy." Orchids are all mycoheterotrophic on germination. Their minute seeds are devoid of nutritional resources (endosperm), and the undifferentiated embryo relies on a fungus for its nutrition, including water, mineral salts, and carbon supply (Rasmussen 1995; Merckx 2013). During further

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development, seedlings often become autotrophic and subsequently revert to usual mycorrhizal functioning (Cameron et al. 2008). Yet, some species from forest environments remain mycoheterotrophic at adulthood. Some orchids develop partial photosynthetic capacity but still rely 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 their fungus for nutrition. This nutritional mode, which has evolved >30 times independently in orchids (Merckx 2013), is termed "obligate mycoheterotrophy."

Given the reliance of orchids on OMF, it is increasingly important to understand how OMF affect orchid distribution to accurately predict whether orchids can colonize new habitats or become threatened in others under conditions of rapid environmental change. While it has traditionally been assumed that broad-scale geographical ranges of taxa are determined mainly by abiotic variables (e.g., annual rainfall, mean spring temperature; Grinnell 1917), it is becoming clear that interactions between organisms (e.g., competition for nutritional resources or symbiont availability) also play a role in shaping the distribution of taxa. Yet, because biotic interactions are often only measured on local (population) scales, they are often seen as not playing as an important role in determining the larger-scale geographical distributions of taxa. Indeed, the realized niche of a particular species is often referred to in the context of habitat suitability, determined by resources available and the environmental conditions it can tolerate (Soberón 2007). It is therefore important to recognize the current extent of orchids' geographical distributions to understand whether mutualists, such as OMF, can affect their distributions and whether this will be affected by environmental change. Fortunately, there has been a recent rapid increase in the availability of biodiversity databases, niche modeling software, and geographical information system (GIS) techniques, and it is becoming increasingly accessible to understand the determinants of the ranges of taxa under various environmental scenarios (e.g., Lurgi et al. 2015).

Despite this, basic observational data about the distribution of mutualists are needed and informative experimental manipulations in field settings to test the reliance of organisms on mutualists are still required. Afkhami et al. (2014) demonstrated the importance of soil mutualists in determining species distribution. They showed that fungal endophytes ameliorated drought stress and broadened the range of their host grass *Bromus laevipes* by thousands of kilometers. This highlights that the current observed distribution of a species can be affected by many factors. Further work is needed to test whether biotic interactions, such as mutualists, in tandem with abiotic factors, are important in determining range limits of species. This is particularly important in a plant group such as the Orchidaceae as they not only rely on pollinators to set seed but also on mycorrhizal fungi to germinate and establish seedlings (Smith and Read 2008; Rasmussen and Rasmussen 2009).

With an estimated 27,000 species (Dressler 2005), the Orchidaceae represents one of the most species-rich plant families. Orchids occur across the entire globe, except Antarctica, and occupy a wide range of habitats including tropical and temperate rainforest, dry tropical forest, savannas, temperate forest, temperate grasslands, Mediterranean shrubland, and even arctic tundra (Givnish et al. 2016).

In the Southern Hemisphere, the most southern populations occur on the subantarctic Macquarie Island (Brown et al. 1978; Clements and Jones 2007). The widespread occurrence of orchids across the globe suggests that the OMF that are necessary for orchid germination and establishment are widespread and not especially limited by biogeographical regions. However, we know little of the biogeography of OMF. Without such information, it is difficult to assess how the distributions of both OMF and orchids will respond to a rapidly changing environment.

In this chapter, we investigate biogeographic patterns in orchid mycorrhizal fungi from the global scale to the population level. We first give an overview of the most important mycorrhizal fungi associating with orchids, and then investigate whether biogeographic patterns in the distribution of OMF exist. Finally, we discuss the population-scale interactions that can determine orchid co-occurrence.

8.2 Main Orchid Mycorrhizal Fungal Symbionts

8.2.1 Rhizoctonias

Stemming from the pioneering work of Noël Bernard (see Selosse et al. 2011), OMF were traditionally classified as "rhizoctonias." However, rhizoctonias belong to three distinct basidiomycete families, namely, Tulasnellaceae and Ceratobasidiaceae (both from the order Cantharellales) and Serendipitaceae (Sebacinales). Although they have a reputation of being saprotrophic fungi (Rasmussen 1995; Smith and Read 2008), they may also be endophytic in non-orchid roots (e.g., Selosse and Martos 2014), meaning that they grow diffusely within living plant tissues, without apparent infection symptoms or forming symbiotic organs termed mycorrhizas. Members of Tulasnellaceae are enigmatic basidiomycetes (Cruz et al. 2016; Chap. 12), whose genome displays wide saprotrophic enzymatic abilities (Kohler et al. 2015), but some also have endophytic abilities (Girlanda et al. 2011). Members of Ceratobasidiaceae encompass endophytic, plant-parasitic, and free-living (perhaps saprotrophic) species. Indeed, phylogenetic analysis of Ceratobasidiaceae has shown that species that are OMF tend to be closely related (Veldre et al. 2013). Serendipitaceae (formerly called Sebacinales clade B) tend to have saprotrophic capacities (Kohler et al. 2015) but are well known as endophytes of non-orchid plants (Weiss et al. 2016). The well-studied root endophyte model *Serendipita* (= *Piriformospora*) *indica* is indeed orchid mycorrhizal (Oliveira et al. 2014). Members of this group are often described as "*Sebacina*" spp. incl. "*Sebacina vermifera*," but recently the species complex has been transferred into the genus *Serendipita* (Weiss et al. 2016). Despite the recent increase in our knowledge of the taxonomy of these mycorrhizas, the exact distribution of rhizoctonias as saprotrophs or endophytes, beyond

their association with the relatively few investigated orchid taxa, remains to be determined.

8.2.2 *Other Mycorrhizal Fungi*

While orchids have a long, likely plesiomorphic evolutionary history of association with rhizoctonias (Yukawa et al. 2009; Dearnaley et al. 2013), many more mycorrhizal fungi have been found recently, each associated with a limited number of orchids. The study of OMF in obligate mycoheterotrophic and partially mycoheterotrophic orchid species has revealed a large diversity of ectomycorrhizal fungi (Merckx 2013), including some ascomycete taxa (Selosse et al. 2004) and saprotrophic fungi from the Psathyrellaceae and Mycenaceae (Selosse et al. 2010; Dearnaley et al. 2013). This suggests that the same fungi could form both ectomycorrhizal and orchid mycorrhizal associations depending on their host. Autotrophic orchids may associate with a variety of different taxa, including Atractiellomycetes (Kottke et al. 2010) and Pezizomycetes such as *Tricharina* and *Peziza* (Waterman et al. 2011). Moreover, some photosynthetic orchids associate with saprotrophic fungal species from the Mycenaceae (Zhang et al. 2012) or the Psathyrellaceae (Yagame et al. 2013) as well.

8.3 Biogeographic Distribution of Orchid Mycorrhizal Fungi

Based on taxonomic similarities, the Russian botanist Armen Takhtajan (1986) recognized six floral kingdoms, which he further subdivided in 12 subkingdoms and 37 floral regions. These kingdoms essentially depict large-scale regions on earth that have distinct floras. This subdivision went back to earlier attempts to identify biogeographical regions that differed in their endemic plants (de Candolle 1820; Engler 1879; Good 1974). In 2001, Cox revised Takhtajan's subdivision and proposed some major modifications. As a result, five major biogeographic regions of floral kingdoms have been retained and have been referred to as the Australian, African, Indo-Pacific, South American, and Holarctic Kingdom. It is tempting to assume that the major divisions in floral kingdoms are also reflected in fungal communities, since the major processes shaping variation in floras may be similar to the processes shaping variation in fungal communities.

At present, no formal studies have compared OMF diversity and community composition between biogeographic regions. Here we present an overview of some major studies that have investigated variation in orchid mycorrhizal communities between species growing in particular biogeographic regions. The major fungal families that associate with orchids (Tulasnellaceae, Ceratobasidiaceae, and

Serendipitaceae) are ubiquitous across the entire globe (Fig. 8.1). Members of Tulasnellaceae have been found associated with orchids in every biogeographic region and sometimes tend to dominate the OMF communities (e.g., Suárez et al. 2006; Waterman et al. 2011; Yuan et al. 2010; Martos et al. 2012; Chap. 12; Fig. 8.2). Similarly, members of Ceratobasidiaceae occur in every biogeographic region (Veldre et al. 2013). While some Australian orchids associate with members of Ceratobasidiaceae (e.g., Warcup 1981; Bougoure et al. 2005, 2009; Irwin et al. 2007; Graham and Dearnaley 2012) or Tulasnellaceae (Roche et al. 2010; Phillips et al. 2011), a large number of species predominantly associate with closely related representatives of Serendipitaceae (Swarts et al. 2010; Davis et al. 2015; Phillips et al. 2016). Members of Serendipitaceae appear to be major symbionts in the

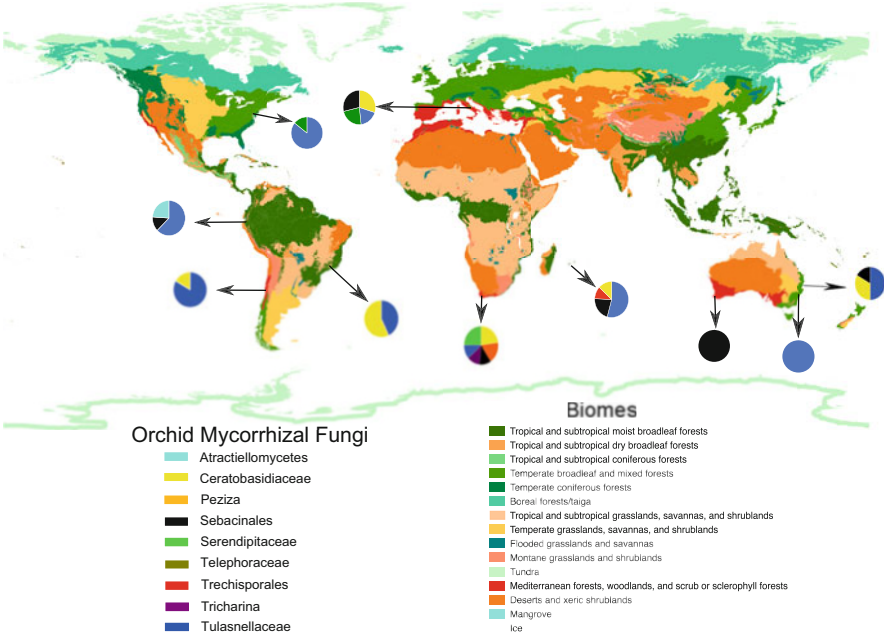

Fig. 8.1 Biogeography and diversity of orchid mycorrhizal fungi (OMF) in the major biomes of the world. Pie charts represent the relative proportion of OMF taxa of the major mycorrhizal families. Depicted on the map is a selection of studies that provided a broad representation of OMF for each biome and that sampled more than one orchid species. Not depicted on the map, for clarity, are the cosmopolitan distributions of Tulasnellaceae and *Ceratobasidium* that have been found at various locations in either China, Europe, Japan, Malaysia, Puerto Rico, Russia, Taiwan, and the United States (e.g., Otero et al. 2002; Shan et al. 2002; Ma et al. 2003; Shefferson et al. 2007, 2010) and Russulaceae that are associated predominately with non-photosynthetic orchids (e.g., Dearnaley 2006; Girlanda et al. 2006). Ecuador = Kottke et al. (2010); Chile = Herrera et al. (2016); Brazil = Pereira et al. (2005); the United States = McCormick et al. (2004); Italy = Jacquemyn et al. (2014); South Africa = Waterman et al. (2011); La Réunion = Martos et al. (2012); Perth = Swarts et al. (2010); Sydney = Roche et al. (2010), south Queensland = Bougoure et al. (2005)

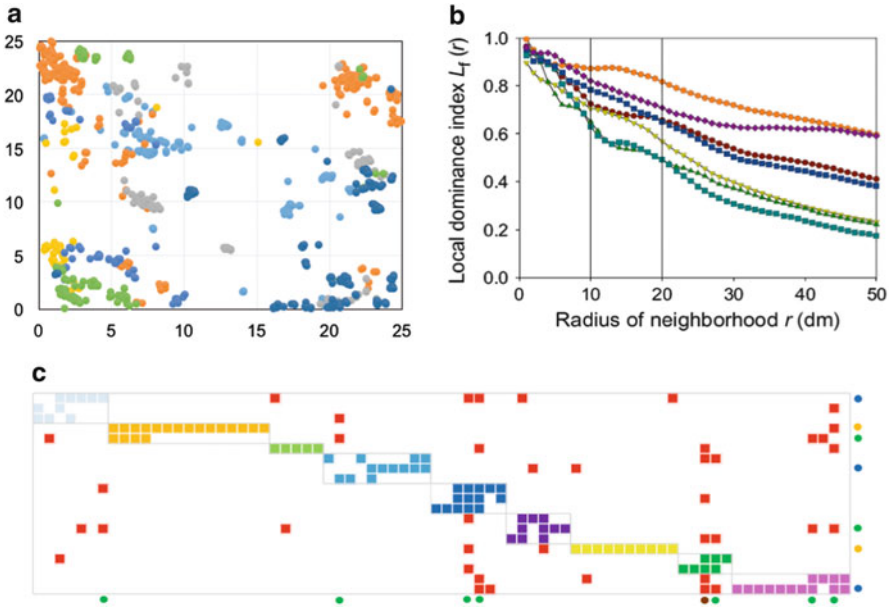

Fig. 8.2 Spatial distribution and community structure of orchid mycorrhizal fungi (OMF) in co-occurring orchid species in Mediterranean shrubland. (**a**) Spatial distribution of seven orchid species in a 25 × 25 m plot in southern Italy. *Colors* indicate different species. (**b**) Indices of local dominance estimating the extent of spatial clustering of the same set of orchid species. Local dominance of orchid species *f*, $L_f(r)$, was estimated as the mean proportion of conspecific neighbors within neighborhood of radius *r* (dm) of the individuals of the focal orchid species. Local dominance of all investigated orchid species was high, ranging from 0.63 to 0.87 at 1 m neighborhood and from 0.49 to 0.81 at 2 m neighborhood. This was caused by the strong clustering of individual orchid species and little overlap among orchid species. (**c**) Matrix representation of an orchid mycorrhizal network encompassing 20 orchid species co-occurring in Mediterranean shrubland (rows) and 96 fungal operational taxonomic units (OTUs) (columns). Different colors represent different modules. *Red* cells represent species links between an orchid and a fungal OTU that join the nine modules together into a coherent network, and *non-red* cells represent links within modules. Species acting as module hubs (*yellow*, provincial hubs; *blue*, connector hubs) and connectors (*green*, non-hub connectors; *brown*, non-hub kinless nodes) are shown outside the matrix border with *small dots*. Peripheral nodes are not shown. Data from Jacquemyn et al. (2014, 2015a, b)

Australian orchid flora, but in the other floral realms, they have been found less frequently, and the reason for their success in Australia remains unexplained so far.

Although the major fungal groups occur in multiple continents, the level of specialization differs largely between biogeographic regions, especially among the non-rhizoctonia partners. A review of the level of OMF specialization exhibited by orchids in Australia (Ramsay et al. 1986, 1987; Perkins and McGee 1995; Irwin et al. 2007; Bougoure et al. 2009; Huynh et al. 2009; Roche et al. 2010; Wright et al. 2010; Phillips et al. 2011, 2016; Davis et al. 2015) suggests that the orchid flora in this region has a relatively high incidence of mycorrhizal specialization compared with the terrestrial orchid flora of South Africa (that, together with the rest of

Africa, remains poorly studied), Eurasia, and North America. For example, Davis et al. (2015) recently investigated the continent-wide distribution of mycorrhizal fungi associating with the terrestrial orchid *Pheladenia deformis*. Their results showed that with the exception of one isolate, all fungi used by *P. deformis* belonged to a single OTU from the Serendipitaceae. Similarly, Roche et al. (2010) showed that six *Chiloglottis* species associated with a narrow clade of *Tulasnella* fungi. In contrast, orchids on the European continent seem to associate with a much wider range of mycorrhizal fungi from different families. For example, a low specificity occurs in *Epipactis* roots, associated with various ectomycorrhizal fungi (Selosse et al. 2004; Jacquemyn et al. 2016a), whereas species of the terrestrial orchid genus *Orchis* are associated with at least ten different mycorrhizal strains that encompassed four different fungal families (Jacquemyn et al. 2011). Similarly, orchids of the European genus *Dactylorhiza* associate with a large number of fungal OTUs, mainly from the Tulasnellaceae (Jacquemyn et al. 2016b).

Although the exact reasons for the high levels of specialization on the Australian continent remain unclear, Phillips et al. (2011) suggested that the prevalence of relatively old, stable landscapes in Australia affords the opportunity for specialization on a single or few orchid mycorrhizal fungi that are best adapted to the edaphic conditions. Waterman et al. (2011) found similar results in a range of oil-secreting orchid species of the subtribe Coryciinae in the Cape fynbos region. The investigated orchid species encompassed five different genera (*Disperis*, *Pterygodium*, *Corycium*, *Ceratandra*, and *Evotella*). Although the studied orchids associated with a large number of fungal OTUs from different families, including Tulasnellaceae, Ceratobasidiaceae, and Serendipitaceae, associations appeared to be strongly phylogenetically conserved, also suggesting a high level of specialization. For example, all species from the genus *Disperis* exclusively associated with members of the Ceratobasidiaceae, whereas species of the genus *Corycium* almost exclusively associated with members of the ascomycetous genus *Peziza*.

8.4 Orchid Mycorrhizal Fungi and Islands

Islands represent interesting study systems to investigate the ways in which spatial isolation, island area, and time since colonization result in adaptation between orchids and fungal communities. Because of their minute seeds and presumed high dispersal capacity, orchids are often disproportionally well represented on islands compared with other plant taxa. For example, orchids comprise about 25% of the endemic flora of La Réunion (Jacquemyn et al. 2005a). Similarly, the native flora of New Zealand contains a relatively high proportion of orchids (McGlone et al. 2001). However, surprisingly few studies are available that have studied mycorrhizal associations in orchids on islands. Probably the most complete study to date investigating mycorrhizal associations on islands was on Réunion Island (Martos et al. 2012), a volcanic island that formed about 3 million years ago in the Indian Ocean. Analyzing a total of 77 different orchid species, Martos et al. (2012)

identified 95 rhizoctonia OTUs, of which 58 belonged to the Tulasnellaceae, 23 to the Serendipitaceae, and 14 to the Ceratobasidiaceae. The mycorrhizal fungi were not randomly distributed across orchids but rather showed a modular structure, which strongly coincided with the growth habit of the orchids. Terrestrial orchids associated with different mycorrhizal OTUs than epiphytic orchids, although the three main rhizoctonia taxa were present in both terrestrial and epiphytic environments. Mycorrhizal specialization was low and most species associated with several OTUs. It may be that mycorrhizal specialization is rare on young islands, due to migration of nonspecialist orchid clades from Africa and subsequent selection against specialization due to resource (mycorrhizal) limitations post-colonization. Yet, as this study was from only one island in the subtropics, we do not know what the general effects of islands are on mycorrhizal communities and diversity, and this speculation deserves further study in other islands across the globe. For instance, it remains to be tested whether there are differences in mycorrhizal community composition and diversity between continental and oceanic islands, between young and old islands, or with varying distances from the nearest mainland. In this context, identifying the mycorrhizal fungi associating with orchids in very remote islands (e.g., Macquarie Island) can reveal interesting information about the distribution of OMF across large spatial scales.

8.5 Distribution of Orchid Mycorrhizal Fungi Across Biomes

We used the 14 major biomes of the world, based on 867 ecoregions, as the biogeographic framework for this review. The classification of biomes is based on biogeographic distributions for major plant and animal groups and hence should be representative for orchids and fungi (Olson et al. 2001). Since most of the research on OMF is based on the analysis of a single or few orchid species (with few exceptions; Kottke et al. 2008; Martos et al. 2012; Jacquemyn et al. 2014, 2015a, b) and since some parts of the world are under-sampled and no information is available for some biomes (e.g., savanna), comparisons between biomes are difficult to make. Despite this, some broad trends emerge from our comparative analysis of OMF across the major biomes of the world.

8.5.1 Mediterranean Shrublands

The Mediterranean shrublands of Europe, the Cape region of South Africa (the fynbos), Australia, South America, and the United States are among the most species-rich vegetation on Earth, containing about 25% of all plant species but occupying only 5% of the total land surface. Orchids are an important part of the

flora of this vegetation type (Rossini and Quitadamo 2003; Liltved and Johnson 2012), and several orchid genera (e.g., *Disa*, *Neotinea*, *Ophrys*, *Orchis*, *Pterygodium*, *Disperis, Caladenia*) almost exclusively occur in this habitat, although there may be some exceptions. For example, some species of *Caladenia* or *Orchis* can also occur in woodlands, whereas members of *Disa* can also be found in montane grasslands. Most species are tuberous orchid species that emerge in late winter, flower in early spring, and disappear again before the summer heat. Natural densities of orchids can be high and species from different genera often coexist (Waterman et al. 2011; Jacquemyn et al. 2014, 2015a; Fig. 8.2). Most species from Mediterranean shrublands associate with a large number of mycorrhizal partners, the most prominent fungal families being Tulasnellaceae and Ceratobasidiaceae (Phillips et al. 2011; Girlanda et al. 2011; Waterman et al. 2011; Jacquemyn et al. 2014, 2015a). Members of other fungal families are normally ectomycorrhizal on trees, including Cortinariaceae, Sebacinaceae, Russulaceae, and Thelephoraceae. Pezizaceae and other ascomycetes have been observed as well, either sporadically in autotrophic orchids (Swarts et al. 2010; Waterman et al. 2011; Girlanda et al. 2011; Jacquemyn et al. 2015a) or as main associates in obligate mycoheterotrophic and mixotrophic species (Girlanda et al. 2006).

Investigation of the interaction network between orchids and mycorrhizal fungi in 20 coexisting orchid species indicated that the network was significantly modular (Jacquemyn et al. 2015b; Fig. 8.2), suggesting that different orchid species associate with different subsets of mycorrhizal fungi. Similar results have been reported for coexisting orchids in the fynbos region of South Africa (Waterman et al. 2011). This modular network structure may contribute to niche partitioning and coexistence of orchids (Waterman et al. 2011; Jacquemyn et al. 2014; Waud et al. 2016a).

8.5.2 Temperate Deciduous Forests

Temperate deciduous forests occur in three disjunct areas of the Northern Hemisphere: Europe, Eastern North America, and Eastern Asia. These forests usually grow on rather young soils formed since the most recent glaciation. The forest structure includes several layers that allow little light penetration to soil. Temperate deciduous forests contain a wide range of orchid species that have adapted to the low light levels, often evolving mixotrophy or obligate mycoheterotrophy. Species of *Epipactis*, *Cephalanthera*, and *Neottia* are predominant in these forests in Europe (Delforge 2006), whereas species of *Goodyera*, *Platanthera*, *Liparis*, and *Tipularia* can be regularly encountered in temperate deciduous forests of Eastern North America (McCormick et al. 2004; Diez 2007). Unlike species from more open habitats, species that grow in the understory of temperate forests often form mycorrhizal associations with obligate ectomycorrhizal basidiomycetes, including *Cortinarius*, *Hymenogaster*, *Inocybe*, *Tomentella*, and *Thelephora* (Bidartondo et al. 2004; Selosse et al. 2004; McCormick et al. 2004; Julou et al. 2005). In addition, associations with a range of ectomycorrhizal ascomycetes (e.g., *Tuber*,

Wilcoxina) may occur (Bidartondo et al. 2004; Selosse et al. 2004; Bidartondo and Read 2008; Těšitelová et al. 2012), with rhizoctonias sometimes present. The shift from an autotrophic to mixotrophic or obligate mycoheterotrophic lifestyle is related to a shift from rhizoctonias to ectomycorrhizal partners (Selosse and Roy 2009; Motomura et al. 2010; Kagame et al. 2016). For example, the mycoheterotrophic *Neottia nidus-avis* associates primarily with ectomycorrhizal fungi of the Sebacinaceae (Selosse et al. 2002; McKendrick et al. 2002), whereas its photosynthetic relatives mainly associate with rhizoctonia fungi belonging to Serendipitaceae (that are a sister clade to Sebacinaceae in the Sebacinales), although occasionally ectomycorrhizal fungi are present in the roots as well (Těšitelová et al. 2015; Jacquemyn et al. 2015b; Kagame et al. 2016).

Detailed investigations of mycorrhizal communities in both root and soil samples across nine populations of *N. ovata* have further shown that orchid mycorrhizal communities can vary substantially across populations. Similarity in mycorrhizal communities was higher in the roots than in the soil, suggesting that the orchid tends to associate with a subset of the available potentially mycorrhizal fungi (Jacquemyn et al. 2015b). Nonetheless, the overall similarity index was low, although adjacent populations within the same forest complex tended to have higher similarities in mycorrhizal fungal communities. Mantel tests further showed that there was no significant relationship between pairwise similarity in mycorrhizal communities and geographic distance. These results are similar to those of Pandey et al. (2013), who also found large variation in mycorrhizal communities between populations in the terrestrial orchid *Piperia yadonii*. However, differences in OMF communities were to some extent related to soil characteristics, most importantly soil moisture content and pH (Jacquemyn et al. 2015b), suggesting that local environmental conditions may affect OMF community composition.

8.5.3 Boreal Forests

Boreal forests cover an enormous area of the circumpolar subarctic, encompassing approximately 12 million km^2. Boreal forests are generally dominated by only a few species of coniferous trees from the genera *Larix*, *Picea*, *Abies*, or *Pinus*. Orchid diversity is lower compared with other biomes. Typical examples are *Arethusa bulbosa*, *Calypso bulbosa*, *Corallorhiza maculata*, and *C. trifida* and several species from the genus *Cypripedium* (e.g., *Cypripedium pubescens*, *C. passerinum*), *Malaxis* (e.g., *Malaxis monophyllos*, *M. paludosa*), *Neottia* (e.g., *N. cordata*, *N. borealis*, and *N. camtschatea*), and *Platanthera* (e.g., *Platanthera dilatata*, *P. hyperborea*, *P. obtusata*, and *P. orbiculata*). Recent investigations in *Neottia* have shown that species from boreal zones frequently associate with representatives of ectomycorrhizal Sebacinaceae (Oja et al. 2015; Těšitelová et al. 2015). McKendrick et al. (2000) showed that *Corallorhiza trifida* mainly associated with ectomycorrhizal Thelephoraceae. In both studies, members of the Tulasnellaceae and Ceratobasidaceae were only sporadically observed or absent.

On the other hand, species of *Cypripedium* appeared to associate with a limited number of closely related *Tulasnella* spp. and perhaps sporadically with ectomycorrhizal fungi (Shefferson et al. 2007). Thus, boreal forests follow the trend found in temperate forests, i.e., a gradient ranging from autotrophic, rhizoctonia-associated orchids to mixotrophic orchids associated with ectomycorrhizal fungi.

8.5.4 Tropical Forests

Tropical rainforests are among the most diverse and productive ecosystems on the planet, where the bulk of orchid diversity is found, including many epiphytes. Although a lower percentage of tropical orchid species have been investigated compared to other regions, two trends are emerging. First, rhizoctonias are the dominant orchid mycorrhizal taxa in tropical forests on all continents, often with *Tulasnella* as the most frequent taxon (e.g., Suárez et al. 2006; Kottke et al. 2008; Yuan et al. 2010; Martos et al. 2012). This applies to both terrestrial and epiphytic orchids (Martos et al. 2012), despite the fact that their ecology strongly differs. Tropical montane forests harbor more epiphytes and reveal similar dominance of rhizoctonias (Kottke et al. 2008, 2010). Second, the taxonomic diversity of OMF associated with photosynthetic orchids appears to be higher and some unexpected taxa have been observed along with rhizoctonias—Atractiellomycetes in Andean montane forest (Kottke et al. 2010) and Mycenaceae in Asia (Zhang et al. 2012). Mycoheterotrophic orchids have been found to associate with ectomycorrhizal fungi as in temperate regions (Roy et al. 2009) or with saprotrophic fungi from the Mycenaceae, Psathyrellaceae, or other basidiomycete families (Martos et al. 2009; Selosse et al. 2010; Lee et al. 2015). Since non-rhizoctonia saprotrophics have been occasionally found in some mycoheterotrophic orchids from moist subtropical forests (Ogura-Tsujita et al. 2009), it may be that high humidity or high temperatures are required to allow saprotrophic fungi to be sufficiently active and gain enough carbon to support obligate mycoheterotrophic orchids (Martos et al. 2009).

8.6 Symbiont-Driven and Propagule-Driven Dispersal Limitation

The broad-scale biogeographical distribution of orchid mycorrhizal fungi (Fig. 8.1) suggests that representatives of the major clades of OMF are ubiquitous and may explain why orchids occur in most regions of the world. Therefore, it is reasonable to assume that the distribution of OMF taxa per se is not a limiting factor for the distribution of orchids. Nonetheless, orchids are declining worldwide (Jacquemyn

et al. 2005b; Kull and Hutchings 2006; Swarts and Dixon 2009) and a better understanding of what drives orchid distribution is urgently needed. The most direct means of testing the relative importance of mycorrhizal fungi in affecting the distribution of orchid species is to conduct seed germination experiments (Turnbull et al. 2000). Ideally, seeds are added to sites where the species occurs and to sites where the species does not occur, and the numbers of protocorms or seedlings that emerge are compared between occupied and unoccupied sites (McCormick and Jacquemyn 2014). Because of the minute size of orchid seeds, most seed introduction experiments are conducted using seed packets in nylon mesh bags (Rasmussen and Whigham 1993; Brundrett et al. 2003). In multiple studies seed germination did not differ significantly between sites where the orchid occurred and sites where it did not occur (reviewed in McCormick and Jacquemyn 2014). One reason for this may be that the seeds of many orchids are indiscriminate toward the fungi that initiate germination. Bidartondo and Read (2008) showed that OMF observed in germinating seeds constituted a much broader range than in seedlings. Similarly, Waud et al. (2017) showed that the OMF communities associating with *Liparis loeselii* varied among sites and life cycle stages, but did not affect seed germination. This occurred regardless of *L. loeselii* presence and was rather affected by soil moisture content. These results indicate that germinating seeds may associate with a broad range of mycorrhizal fungi and illustrate the opportunistic association of some orchids on their OMF.

Molecular identification provides an alternative way of testing how OMF affect the distribution of orchids. Voyron et al. (2017) recently showed that OMF are unevenly distributed within orchid populations. Some OMF taxa that associate with orchids were undetected in soil. Similarly, Waud et al. (2016a) investigated spatial variation in the community of OMF within the roots of three co-occurring orchid species and the surrounding soil in an orchid-rich calcareous grassland in Southern Belgium. They showed that OMF were broadly distributed in the soil, although variation in community composition was strongly related to the proximal host plant. The diversity and frequency of sequences corresponding to OMF in the soil also declined with increasing distance from orchid plants. More detailed analyses using quantitative PCR (qPCR) further showed that fungal abundance declined rapidly with distance from adult host plants (Waud et al. 2016b). This raises the possibility that OMF have limited dispersal in soil and that orchid roots may maintain OMF in particular habitats.

Recent work by Nurfadilah et al. (2013) and Fochi et al. (2017) has further shown that *Tulasnella* fungi cannot take up soil nitrate. This is expected to have direct consequences to the occurrence of orchids that depend on *Tulasnella* spp., particularly in human-modified habitats that experience severe eutrophication. In addition, Těšitelová et al. (2012) investigated seed germination in four *Epipactis* species both at sites that were occupied by adult plants and sites that were not. They found that germination was not limited by fungal availability, even at sites that were deemed unsuitable. The authors speculated that the transition from mycoheterotrophic, subterranean germination to an epigeous autotrophic life is a drastic transition, which may be fatal to many orchid individuals. Therefore, more

work is required to tease apart the effects of habitat quality, nutrient resource, and OMF availability in the colonization of new habitat.

8.7 Future Perspectives

In this chapter, we have shown that the mycorrhizal fungal families associating with orchids occur in a wide variety of habitats and that some species have a very wide distribution. This suggests that orchid distribution is generally unlimited by fungal availability. Our review also suggests that the diversity of some OMF taxa is higher in the tropics both in terms of species and functional guild richness, although it remains unclear how this affects orchid diversity and abundance. A major caveat in our current understanding of the biogeographical distribution of OMF is that most of the available data are very fragmentary. Often a few populations within a restricted area are sampled, making it impossible to draw any general conclusions about the distribution of fungi that can facultatively associate with orchids across larger scales. To our knowledge, only a few studies (Taylor et al. 2004; Irwin et al. 2007; Otero et al. 2007; Davis et al. 2015) have attempted to sample the continent-wide distribution of mycorrhizal fungi associating with a particular orchid species. This "Wallacean shortfall," i.e., the lack of knowledge of the geographical distributions of OMF, makes it nearly impossible to make any firm predictions about the potential distribution ranges of OMF across large geographic areas or to predict changes in distribution under conditions of environmental change. Moreover, we do not know whether the OMF encountered at one part of the range of an orchid are able to sustain orchids at another part of their range. It is reasonable to assume, based on often preferential or specific orchid associations, that OMF communities show spatial turnover, resulting in adaptation of orchid populations to local fungal communities and the formation of ecotypes. However, this information is crucial if we want to make accurate predictions about the distribution of a particular orchid species in changing environments or to propose management interventions aiming at restoring populations of threatened orchid species (Reiter et al. 2016). This is further complicated by the fact that we currently lack an acceptable phylogenetic framework that allows adequate assessment of the phylogenetic relationships between fungal strains, leading to a proliferation of fungal species (OTUs) within a certain genus or family (especially for Tulasnellaceae). Indeed, in order to further understand diversity within OMF clades, we need to develop a robust species concept for OMF (Linde et al. 2014). Given that the majority of recent studies use molecular tools to identify and delineate taxa, the phylogenetic species concept (e.g., Faith 1992) is probably the most appropriate. The use of different gene markers in combination with recent improvements in sequencing techniques and the wide variation in orchid mycorrhizal strains (especially large variations in ITS regions; Linde et al. 2014) makes it rather challenging to set up a general phylogenetic framework for assessing fungal distribution ranges. Nonetheless, better taxonomic delimitation of OMF lineages,

combined with better distribution data of OMF, is needed if we want to predict the impact of changing environments on the distribution of orchid species.

It also needs to be stressed that most of our knowledge of OMF associations comes from adult plants. OMF taxa that associate with adult plants are not necessarily also the fungi that stimulate initial germination or seedling growth. Bidartondo and Read (2008) showed that fungi supporting protocorms represented a subset of the taxa supporting seeds and adult plants. Germination experiments under lab conditions have shown that certain strains may be favorable to promote growth, whereas others sustain subsequent growth (Rasmussen 1995). As long as in situ data are lacking, we still do not know whether these "additional" fungi initiate further growth and development in juvenile and adult orchids: if they did, it would imply that our current understanding of the role of OMF diversity on the distribution of orchid species is limited. Presumably, this will further complicate attempts to accurately predict the distribution of orchids based on location records and climatic variables only, as the interplay between mycorrhizas and orchids may be more complex in the life cycle of the orchid, depending on the species. Considering the effects of different mutualists (i.e., pollinators and OMF; Selosse 2014) on orchid distribution will considerably increase the ability of niche models to predict how orchids will respond to changing environments in the future.

Based on our findings, we first recommend that future research should focus on the systematics of OMF and develop a robust species concept for rhizoctonias. Second, broad-scale surveys are needed to assess whether there is turnover of OMF families or OTUs or both across geographical ranges of orchid species and investigate whether certain abiotic variables (e.g., rainfall, geological substrate) govern the distribution of OMF communities over geographical ranges. Combined with experimental assessments of the effect OMF diversity has on individual orchids in natural communities, this will not only lead to a better understanding of the geographical distribution of OMF but also to a better understanding of how mycorrhizas can either limit or broaden the range of orchids. Third, detailed investigations about the environmental threats to OMF are needed to test the hypothesis that nutrient eutrophication destroys OMF populations with subsequent extirpation of orchid populations. Such research would go a long way to help us understand the relative importance of mycorrhizas in determining the distribution of orchids and allow us to improve orchid conservation and restoration projects in the future.

In summary, OMF are an important biotic determinant of the life cycle of orchids with many of the major clades found in every geographic region in the world. Yet we know little of OMF species identity and function in natural habitats, especially in the tropics. In the face of ongoing habitat loss and environmental change, such information is crucial to make accurate predictions of orchid distribution in the future.

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Chapter 9
Biogeography of Ericoid Mycorrhiza

Petr Kohout

9.1 Ericoid Mycorrhizal Symbiosis

Mycorrhizal symbiosis is a mutualistic partnership between plants and fungi that represents one of the oldest and the most widespread symbioses on earth (Redecker et al. 2000). It has been estimated that approximately 80% of vascular plant species form symbiosis with mycorrhizal fungi (Brundrett 2009). Mycorrhizal fungi play a crucial role in water and nutrient uptake to the host plant. They also enhance host plant defense mechanisms against pathogens and facilitate their growth in environments with high levels of heavy metals. In return, mycorrhizal plants provide carbohydrates, such as glucose and sucrose, to their symbiotic partners (Smith and Read 2008).

Several mycorrhizal types exist that have evolved independently multiple times for the last 400 million years. This chapter will focus on the youngest type, viz., ericoid mycorrhizal (ErM) symbiosis. Ericoid mycorrhiza is a mutualistic relationship between several lineages of the Ericaceae family and diverse group of soil fungi. The first appearance of Ericaceae-like plants dates back to 90–75 Ma. (Nixon and Crepet 1993; Carpenter et al. 2015). It has been hypothesized that ErM symbiosis may have evolved in the same time frame (Cairney 2000). Ericoid mycorrhiza (one of the so-called endomycorrhizal types) is characterized by the intensive fungal colonization of the outermost root cell layer. Mycorrhizal fungi form a coiled intracellular hyphal complex. The fungal hyphae within the plant cell

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are usually hyaline with a thin cell wall. The plant plasma membrane of the root cells invaginates to envelope the fungal structures, but it is separated from the fungal cell by an interfacial matrix. This represents the interface between the two symbionts, where nutrients exchange takes place (Smith and Read 2008).

Ericoid mycorrhizal plants often occur on extremely poor soils, where most of the nutrients are locked up in complex forms of soil organic matter, with restricted biological availability. The ErM symbiosis represents a key evolutionary adaptation of ErM plants to mobilize the nutrients from such recalcitrant substrates (Kerley and Read 1998). However, ericoid mycorrhiza remains largely overlooked compared to the more common mycorrhizal types, such as arbuscular mycorrhiza (AM) and ectomycorrhizal (EcM), and a broader general understanding of the ErM symbiosis is lacking. This chapter aims to summarize current knowledge of global distribution and biogeography of ericoid mycorrhizal plants and their mycorrhizal fungi.

9.2 Ericoid Mycorrhizal Plants

9.2.1 Ericoid Mycorrhizal Plant Diversity

The Ericaceae family comprises 9 subfamilies, 124 genera, and approximately 4250 species (Kron et al. 2002; Christenhusz and Byng 2016). In this review, I follow the revised phylogenetic classification of Ericaceae established by Kron et al. (2002) and modified by Freudenstein et al. (2016). According to these studies, the Ericaceae are divided into nine subfamilies. Only the basal evolutionary lineages of the Ericaceae, namely, Enkianthoideae, Arbutoideae, Pyroloideae, and Monotropoideae, lack the capability to form ErM. Instead, species of the Monotropoideae subfamily form the so-called monotropoid mycorrhizal symbiosis (characterized by ectendomycorrhizal anatomical structures) with specific groups of EcM fungi from the Basidiomycota phylum (Hynson and Bruns 2009), while members of the Arbutoideae and Pyroloideae subfamilies host a wide spectrum of EcM mycobionts in their roots (Krpata et al. 2007; Chap. 19). The earliest diverging lineage Enkianthoideae, represented by the sole genus *Enkianthus*, forms arbuscular mycorrhizal symbiosis (Gorman and Starrett 2003; Abe 2005). Recently, Obase and Matsuda (2014) documented the presence of well-known ErM fungal (ErMF) symbionts in roots of *Enkianthus campanulatus*, which might evoke potential capabilities of some Enkianthoideae species to form ErM. Resynthesis experiments involving the mycobionts and host plants *E. campanulatus* are essential to determine the character of both formerly described interactions. So far, the only confirmed ErM plant species belong to the Cassiopoideae, Ericoideae, Harrimanelloideae, Styphelioideae (formally known as Epacridaceae), and Vaccinioideae subfamilies. Okuda et al. (2011) described a symbiosis resembling ErM in *Schizocodon soldanelloides* (Diapensiaceae) roots, but this requires independent confirmation.

The Harrimanelloideae is the smallest ErM-forming clade of the Ericaceae family. This subfamily is represented by a single genus and two species, with distribution restricted to arctic and boreal regions of North America and arctic region of Eurasia. The second smallest Ericaceae clade is the Cassiopoideae subfamily, represented by a single genus *Cassiope* with 12 species. The distribution range of *Cassiope* spp. is, similar to *Harrimanella* spp., restricted to high latitudes of the Northern Hemisphere, but it also covers the Himalayas. The remaining three Ericaceae subfamilies are considerably larger in terms of taxonomic richness. The Styphelioideae, represented by 35 genera with approx. 545 species, is the only Ericaceae subfamily with distribution concentrated mostly in the Southern Hemisphere. Its disjunct distribution around the southern Pacific Rim (namely, Australia, New Zealand, Papua New Guinea, New Caledonia, Chile) is rather a result of recent dispersal than common origin in the Gondwana supercontinent, as revealed by phylogenetics. Molecular dating methods suggest that some of the New Caledonian and New Zealand Styphelioideae taxa (e.g., *Dracophyllum*) dispersed from Australia in recent times. The ancestors of *Dracophyllum* most likely arrived by long-distance dispersal long after these lands had separated from Gondwana (Wagstaff et al. 2010). Considering the number of described genera, the Vaccinioideae is the richest of all Ericaceae subfamilies, and its members can be found on all continents except Antarctica. *Vaccinium* and *Gaultheria* have very broad distribution spanning over several continents. On the contrary, the species-rich genus *Agapetes* (with around 180 species) has a relatively restricted distribution with a range spanning the Himalayas to northern Australia. Many species of the Vaccinioideae subfamily have high economic as well as ecological importance. The most well-known Ericaceae subfamily with known ErM plants is that of the Ericoideae. Species from the Ericoideae subfamily are distributed throughout the world except Antarctica and Australia. It is the most species-rich Ericaceae subfamily, with more than 1700 species. It comprises many broadly distributed genera, such as *Empetrum* or *Rhododendron*, as well as the most species-rich genus *Erica*.

9.2.2 Ericoid Mycorrhizal Plant Biogeography

Although the ericoid mycorrhiza represents ecologically important symbiotic partnership, its global distribution has never been assessed. To visualize the putative distribution of ErM plants on a global scale, I have compiled information about the global distribution pattern of each ErM-forming Ericaceae subfamily from Kron and Luteyn (2005) to a single figure (Fig. 9.1). It is clear that, although the distribution of the ErM plants is very broad (all continents except Antarctica), there are also many areas where ErM plants are missing, such as large parts of South America, SW Asia, and much of Africa and Australia. Considering the ecosystem types, ErM plants are largely absent from Neotropical and African lowland rainforests. The same habitat hosts only a few ErM plant species in New Guinea.

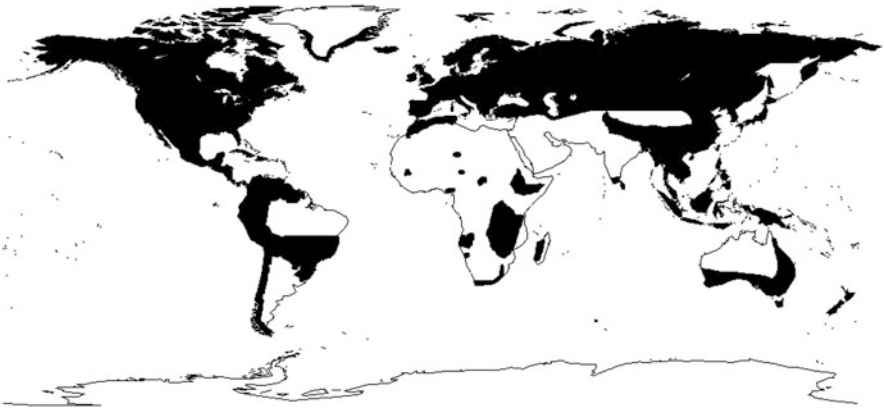

Fig. 9.1 Global distribution of putatively ericoid mycorrhizal plants, based on the distribution of Ericaceae subfamilies (Cassiopoideae, Ericoideae, Harrimanelloideae, Styphelioideae, and Vaccinioideae) with ericoid mycorrhizal lifestyle. Compiled following Kron and Luteyn (2005)

This strongly contrasts with adjacent New Guinean Highlands, where ErM plants reach one of the highest levels of diversity on earth (Stevens 1982). While only plants adapted to low light can grow in forest floor in lowland tropical forests, ecosystems in high mountains may harbor species-rich understory vegetation, because of favorable light conditions. Besides the light conditions, lowland and highland tropical sites also differ in their soil conditions. While lowland tropical soils suffer by significant leaching and therefore they are very poor in nutrients and soil organic matter, soils in high altitudes (e.g., New Guinean Highlands or Andes) are more nutrient-rich and characterized by accumulation of soil organic matter binding most of the nutrients (Townsend et al. 1995). Soil conditions of high tropical mountains are therefore probably more suitable for ErM plants, because many of their symbiotic, ericoid mycorrhizal fungi possess the ability to gain nutrients from hardly decomposable components of the soil organic matter. These nutrients can be subsequently transported to their host plants (Kerley and Read 1998). Altogether, specific environmental conditions of high mountains (e.g., the Andes and Himalayas as well as African and New Guinea Highlands) support higher species diversity of ErM plants than can be found in lowland sites in tropical regions.

Tropical high mountains also harbor most of the Ericaceae biodiversity hot spots. The species diversity hot spot of Vaccinioideae lies in the Neotropical region, with the highest species number concentrated around the equator between 1000 and 3000 m elevation in the Andes. About 800 Ericaceae species with very high endemicity are divided into 46 genera in this region (Luteyn 2002). Ericoideae has its diversity hot spot in the Cape Floristic Region (CFR), the smallest but also one of the most floristically diverse provinces on Earth (Goldblatt and Manning 2002). Although the genus *Erica* is the only representative of the Ericoideae subfamily in CFR, it diversified into an astonishing number of species, with more than 800 species

(of which 690 are endemic) recorded in this region, representing one of the epitome of local plant biodiversity (Pirie and Oliver 2011; Pirie et al. 2016). Such extremely high plant biodiversity has been ascribed to favorable climatic conditions and topographical stability in Cape flora throughout the Pleistocene and Holocene, leading to high diversification and low extinction rates (Cowling et al. 2015). The third hot spot of the Ericaceae biodiversity occurs in mountains of New Guinea. In contrast to the previous two regions, New Guinean highlands host all three Ericaceae subfamilies that naturally occur in the Southern Hemisphere. The high phylogenetic diversity in this area can be explained by historical biogeography of the Ericaceae family. While species of the Styphelioideae subfamily have their ancestors in Australia, members of the other two subfamilies Vaccinioideae and Ericoideae are closely related to South Asian Ericaceae. Members of all these three groups migrated by long-distance dispersal to New Guinea where they currently co-occur (Stevens 1982). The highest diversity of ErM plants is observed at an altitudinal range of 1000–3750 m, but especially in the upper mountain and subalpine vegetation on New Guinean highlands that harbor >400 species in total. Mountains of New Guinea support especially high species diversity of *Rhododendron*, with approx. 200 described species (Heads 2003).

Although the ErM plant species diversity is rapidly decreasing toward poles, ErM plant relative abundance increases. ErM plants represent an important component of vegetation in boreal forest and tundra biomes. ErM plants even dominate in many habitats of these regions, for example, as an understory in coniferous forests or the main component of peat bog vegetation (Read 1991). Interestingly, even though most of the ErM Ericaceae subfamilies are globally distributed, clear latitudinal trends in ErM plant species diversity are apparent.

9.3 Ericoid Mycorrhizal Fungi

9.3.1 Ericoid Mycorrhizal Fungal Diversity

Compared to more common mycorrhiza types, such as AM and EcM, our knowledge about the diversity of ErM fungi is very superficial. While AM fungi have a monophyletic origin, the ability to form ErM as well as EcM evolved independently multiple times in several fungal lineages (Smith and Read 2008; Chap. 6). Earlier attempts to determine fungal diversity were based on direct observations of macroscopic (fungal fruit bodies) as well as microscopic (e.g., spores) structures. These methods allowed researchers to classify AM fungi to morphospecies based on their chlamydospore anatomy. Similarly, EcM fungi were classified based on the morphology and anatomy of the EcM colonization structure formed by each individual unique plant-fungal species combination (Agerer 1987–2006). Although these early methods suffered from many drawbacks, their implementation enabled us to classify uncultured fungal species, which would have been completely overlooked and

uncommunicated otherwise. Subsequent implementation of molecular methods for fungal species determination boosted up our knowledge of AM as well as EcM fungal diversity (Öpik et al. 2014; Tedersoo et al. 2010; Tedersoo et al. 2014). On the other hand, research focused on the ErM fungal diversity suffered from much more serious drawbacks. Determination of the ErM fungal lifestyle can neither be based on the phylogenetic affinity to any known lineage as it is the case of AM fungi and EcM fungi to some extent (Chap. 19) nor can ErM lifestyle be defined based on the occurrence of fungal species in Ericaceae root segments, because Ericaceae roots can also harbor non-mycorrhizal fungi (Bougoure and Cairney 2005a). Therefore, mycorrhizal resynthesis experiments are needed to describe the character of the association between the host plant and mycobiont and to sufficiently prove the ericoid mycorrhizal lifestyle of Ericaceae-associated mycobionts (Leake and Read 1991). Anatomical features of ErM symbiosis were described above (Sect. 9.1) as well as more specifically in Smith and Read (2008). Alternatively, methods applying transmission electron microscopy associated with molecular methods of fungal detection can be used in specific cases (Selosse et al. 2007).

To date, there are only a few fungal taxa for which the ErM habit has been indisputably confirmed (Tedersoo et al. 2011). *Rhizoscyphus ericae* (formerly known as first *Pezizella ericae*, subsequently *Hymenoscyphus ericae*) was the first described ErM fungal species (Pearson and Read 1973). Several fungal species with high phylogenetic affinity to the *R. ericae* (Helotiales) were later described and placed within the *R. ericae* aggregate (REA; Vrålstad et al. 2000; Hambleton and Sigler 2005). This group comprises mostly plant root-associated fungal taxa. The potential ErM lifestyle of these fungi were repeatedly examined and discussed because of their close phylogenetic affinity to *R. ericae*. However, only *M. variabilis* has been consistently detected in Ericaceae roots in nature (e.g., Bougoure et al. 2007; Grelet et al. 2010; Ishida and Nordin 2010, Lukešová et al. 2015) and shown to form typical ErM fungal structures in host plant roots in resynthesis experiments (Grelet et al. 2009; Vohník et al. 2013). Grelet et al. (2009) further documented bidirectional nutrient and carbon flows between *M. variabilis* and its *Vaccinium* host. Evidence for the ErM lifestyle is much more unclear in case of other REA species such as *M. vraolstadiae*, *M. bicolor*, and *Cadophora finlandica*. Although their ability to form typical ericoid mycorrhizal structures with ericaceous roots has been shown in resynthesis experiments (Villarreal-Ruiz et al. 2004; Grelet et al. 2009; Vohník et al. 2013), these fungal species have been only rarely, if ever, detected in Ericaceae roots under natural conditions.

Oidiodendron maius and *Cairneyella variabilis* are two other well-known ErM fungal taxa (Couture et al. 1983; Dalpe 1986; Midgley et al. 2016). Both species have shown the ability to form ericoid mycorrhizal structures in resynthesis experiments. Similar to *R. ericae*, *O. maius* and *C. variabilis* have been also repeatedly detected in Ericaceae roots in natural environments (e.g., McLean et al. 1998; Bougoure and Cairney 2005b; Chambers et al. 2008). Ericaceae roots may harbor many other fungal taxa with the ability to form structures resembling ErM colonization in the resynthesis experiment, for example, *Capronia* sp. (Allen et al. 2003), *Cryptosporiopsis* sp., and *Lachnum* sp. (Walker et al. 2011). However, these

associations might represent only opportunistic colonization by non-mycorrhizal fungi (Leopold 2016).

Besides Ascomycota, several ErM fungal lineages are also known from the phylum Basidiomycota. Decades ago, Seviour et al. (1973) suggested the ErM lifestyle for *Clavaria* sp. However, the provided evidence of bidirectional nutrient flow between *Rhododendron* sp. host plant and *Clavaria* sp. was inconclusive (Englander and Hull 1980; Mueller et al. 1986). Subsequently, Berch et al. (2002) and Allen et al. (2003) detected fungi with an affinity to Sebacinales in roots of *Gaultheria shallon*. Based on the combination of ultrastructural features of Ericaceae root-associated mycobionts and molecular techniques, Selosse et al. (2007) discovered that the interaction between Sebacinales and Ericaceae roots can be considered as mycorrhizal. These findings were also proven in the resynthesis experiment (Vohník et al. 2016). Recently, the ErM lifestyle was discovered in another group of unidentified basidiomycete lineage with an affinity to Trechisporales (Vohník et al. 2012). Although the ErM lifestyle of *Clavaria* remains questionable, it has been sufficiently proven for other basidiomycetous lineages.

9.3.2 Ericoid Mycorrhizal Fungal Biogeography

Compared to ErM plants, much less is known about the distribution and global biogeography of their root-associated mycorrhizal symbionts. Because of the lack of host plant-mycobiont specificity in ericoid mycorrhizal symbiosis (e.g., Kjøller et al. 2010; Walker et al. 2011), the distribution of the Ericaceae species or lineages can hardly be used to estimate the ErM fungal biogeography. Traditionally, most of the studies focused on the diversity and community ecology of ErM fungi were performed on the Northern Hemisphere, particularly in Europe and North America. The lack of data about ErM fungal diversity from diversity hot spots of the Ericaceae (listed above) is especially problematic. Therefore, our knowledge about the global diversity and distribution or ErM fungi is very superficial. Fortunately, ErM fungi may act as common root endophytes of non-Ericaceae plants (Curlevski et al. 2009; Tedersoo et al. 2009). Studies focused on the diversity of root-associated fungi, and surveys of soil fungal diversity can therefore serve as substantial source of information about the distribution of ErM fungi worldwide. Importantly, most studies focused on the diversity of Dikarya (where all known ErM fungal taxa belong) employed the molecular methods of fungal taxa detection, based on the sequencing of ITS region of rDNA (Schoch et al. 2012). Therefore, fungal ITS databases, such as UNITE (Abarenkov et al. 2010), represent an important source of information about the occurrence and distribution of ErM fungi.

The most well-known ErM fungus, *R. ericae*, was originally described from the United Kingdom (Pearson and Read 1973). Since then, *R. ericae* was detected multiple times in Ericaceae roots in Canada (Gorzelak et al. 2012), Sweden

(Ishida and Nordin 2010), Norway (Vrålstad et al. 2002), Germany (Horn et al. 2013), Ireland (Hazard et al. 2014), Portugal (Turnau et al. 2007), and Japan (Usuki et al. 2003). Besides Ericaceae, *R. ericae* is also known as a root endophyte in non-Ericaceae species as well as a symbiont of lower plants, such as liverworts. *R. ericae* has been detected as an endophyte in non-ErM host plants in the United States (Deslippe et al. 2011), Lithuania (Menkis et al. 2004), Norway (Vrålstad et al. 2000), Chile (Pressel et al. 2008), as well as Antarctica (Upson et al. 2007). Besides the largely undersampled regions in Africa, *R. ericae* is so far missing only in Australia. Therefore, *R. ericae* represents the most widespread fungus with ErM lifestyle.

Compared to *R. ericae*, *M. variabilis*, another member of the REA with ErM lifestyle, has more restricted distribution. The vast majority of *M. variabilis* records originate from the Northern Hemisphere. This group of species has been repeatedly detected in Ericaceae roots in Sweden (Kjøller et al. 2010), Canada (Gorzelak et al. 2012), the United Kingdom (Grelet et al. 2010), Czechia (Vohník et al. 2013), and Norway (Vrålstad et al. 2000). Besides its association with Ericaceae roots, *M. variabilis* often interacts with non-Ericaceae plants as an endophytic symbiont. Although the ability to colonize non-Ericaceae host plants is a common feature of ErM fungi, *M. variabilis* seems to be particularly frequently associated as an endophyte with woody plant species. For example, *M. variabilis* has been found in Pinaceae roots in Germany (Ducic et al. 2009), Czechia (Vohník et al. 2013), Canada (Jones et al. 2012), the United States (Deslippe et al. 2011), the United Kingdom (Grelet et al. 2010), Sweden (Marupakula et al. 2016), and Japan (Yoshida et al. 2014). Compared to the well-documented occurrence of *M. variabilis* in the Northern Hemisphere, evidence about its distribution to the south of the equator is questionable. Hawley et al. (2008) provided the only report of *M. variabilis* in the Southern Hemisphere, where this fungus was detected in roots of introduced *Pinus patula* in a plantation in South Africa. Considering the fact that *M. variabilis* has never been detected in roots of native plants in the Southern Hemisphere, it is highly probable that its presence in *P. patula* roots represents an example of plant-associated fungal co-introduction. It remains questionable, if the putative non-native *M. variabilis* would be able to switch from the introduced host plant to the local Ericaceae species. South Africa hosts one of the Ericaceae diversity hot spots, so the potential changes in the local ErM fungal species pool may have serious consequences on local diversity.

Besides the members of the REA, *Oidiodendron maius* is considered to be a putative cosmopolitan species (Leopold 2016 and references therein). However, the phylogenetic placement of sequences with an affinity to *O. maius* is questionable (Hambleton et al. 1998). To disentangle the phylogenetic relationships among the sequences related to *O. maius*, I downloaded all available ITS sequences with similarity >97% to *O. maius* from the UNITE database (accessed 25 July 2016), aligned them and performed a neighbor joining analysis (Fig. 9.2a). The phylogram reveals two well-supported clades within the *O. maius* complex. Clade A is comprised of 137 sequences, and it contains sequences obtained from morphologically determined *O. maius* cultures. This clade is identical with SH216987.07FU,

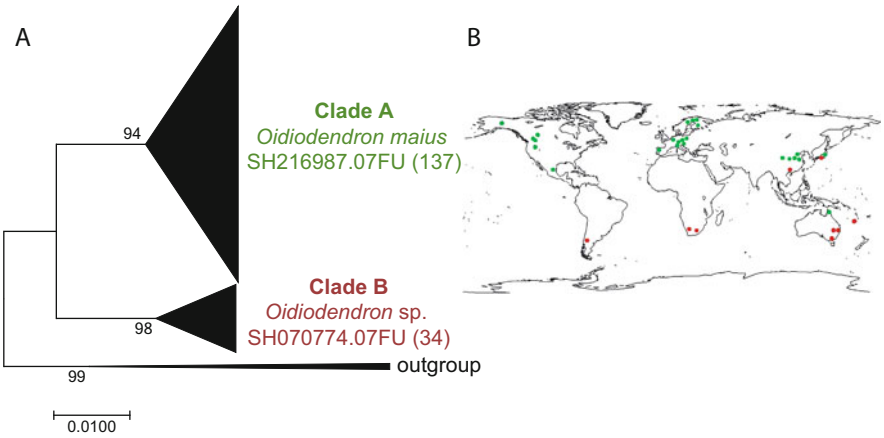

Fig. 9.2 (**a**) Phylogenetic tree of ITS1, 5.8S, and ITS2 rDNA sequences (483 characters) with >97% similarity to *Oidiodendron maius*. The numbers next to branches denote neighboring-joining bootstrap values from 1000 replications. The tree was rooted by sequences of *O. griseum* (AF062793), *O. pilicola* (AF062787), and *O. echinulatum* (AF062791). Numbers in brackets indicate sequence numbers in each clade. According to the naming system for fungal sequences (Kõljalg et al. 2013), Clade A is identical with SH216987.07FU and Clade B with SH070774.07FU. (**b**) Map indicating geographic distribution of sequences from the two clades

according to the Species Hypothesis molecular classification proposed by Kõljalg et al. (2013, 2016). Clade A is almost exclusively distributed in the Northern Hemisphere (136 out of 137 sequences). The only exception is that of a single sequence from *Rhododendron lochiae* in Australia (Bougoure and Cairney 2005a). Clade B (SH070774.07FU) is comprised of 34 sequences. None of these have been detected in North America, Europe, or Western and Central Asia. Clade B is mostly distributed on the Southern Hemisphere (30 out of 34 sequences). The only exception is represented by two records from Japan and China (Obase and Matsuda 2014). Clade B is comprised of sequences associated with ErM hosts from many continents (Africa, Asia, Australia, and South America). Interestingly, there are several sequences obtained from non-ErMF host plants in the Clade B. To conclude, it is likely that the currently recognized *O. maius* comprises two geographically segregated species (Fig. 9.2b) rather than a single cosmopolitan species.

Cairneyella variabilis has so far the most restricted distribution among all known ErM fungi. All records of this species are derived from the Ericaceae roots (McLean et al. 1999; Williams et al. 2004; Bougoure and Cairney 2005a, b; Midgley et al. 2016) as well as roots of other woody plant species (Chambers et al. 2008) in Australia. It seems that ErM fungal communities in Australia differ from those in other continents. However, much more data, especially from South America, Africa, and Asia, are needed to draw more general conclusions.

So far, the only basidiomycetous fungi with undisputable ErM habit belong to an unidentified basidiomycete lineage within *Agaricomycetes* (Vohník et al. 2012)

and to a lineage within the serendipitoid (Serendipitaceae; formerly Sebacinales Group B) group (Vohník et al. 2016). Members of the former lineage were so far detected only on *Vaccinium* spp. roots in Norway. Such a limited distribution range might rather be caused by template mismatches (three nucleotides mismatch) with the most commonly used fungal universal primer ITS1F (Vohník et al. 2012) than rarity. The serendipitoid ErM fungal lineage has been so far described from Norway (Vohník et al. 2012), Sweden (Clemmensen et al. 2013), Germany (Garnica et al. 2013), the United Kingdom (Bougoure et al. 2007), China (Selosse et al. 2007), Panama (S. Setaro, unpublished), Ecuador, and the United States (Setaro and Kron 2011). Almost all of the sequences (28 out of 29 sequences) with high similarity (>97%) to the proven ErM fungus were described from Ericaceae roots. Therefore the ability to interact with non-ErM plants remains questionable in this ErM fungal taxon.

9.4 Conclusions

Ericoid mycorrhizal symbiosis occurs on all continents, except Antarctica. Although ErM plants species richness is the highest in tropical and subtropical regions, they also represent an important vegetation component in temperate and arctic regions. Compared to well-known biogeography of ErM plants, we have very limited knowledge about the diversity and distribution of ErM fungi. Preliminary insights indicate that some ErM fungal species (*Rhizoscyphus ericae*) have a very broad distribution range. On the contrary, some species have much narrower distribution range restricted to a single hemisphere (*Meliniomyces variabilis*) or continent (*Cairneyella variabilis*).

To elucidate the global distribution and biogeography of ErM fungi, more data should be first obtained from regions with high Ericaceae diversity such as the Cape region in South Africa, New Guinean Highlands, and Neotropical Andes. Further implementation of Species Hypothesis concept as proposed by Kõljalg et al. (2013, 2016) might help to link the previously gained information about the ErMF distribution from Sanger sequencing-based studies with raising number of next-generation sequencing-based studies. Although the novel molecular approaches of fungal detection boosted up our understanding of fungal community ecology, studies providing the evidence of the ecological lifestyle of the Ericaceae-associated fungal isolates are still highly valuable.

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Chapter 10
Biogeography of Root-Associated Fungal Endophytes

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10.1 Introduction

Fungal endosymbionts colonize living roots of all plants and across all surveyed terrestrial ecosystems. Generally considered benign intracellular inhabitants of plant roots, these hidden players inside plants may control plant productivity and community assembly, and thus ultimately the function of ecosystems (Bever et al. 2010, 2012). In addition to the better-known and more extensively studied mycorrhizal symbionts, a diverse group of non-mycorrhizal, nonpathogenic, endophytic fungi also occupies root tissues (Mandyam and Jumpponen 2005; Rodriguez et al. 2009; Porras-Alfaro and Bayman 2011). However, presence of a fungus in the root system does not make it an endophyte (Jumpponen et al. 2011): some superficial inhabitants may be casual colonizers from the soil environment, whereas others are adapted to the root environment—colonizing roots persistently and maintaining some metabolic or molecular interaction with the plant host (Hardoim et al. 2008). As a result, healthy plant roots often host complex and heterogeneous fungal communities (Vandenkoornhuyse et al. 2002; Glynou et al. 2016; Porras-Alfaro et al. 2008)

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that seem abundant in all plants across terrestrial ecosystems (Mandyam and Jumpponen 2005; Sieber and Grünig 2013).

Although the presence of endophytes is widely acknowledged for a range of habitats and hosts (e.g., Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005; Kageyama et al. 2008), the characterization of the root-associated endophyte functions in symbiosis, particularly in natural environments, remains poorly resolved (Mandyam and Jumpponen 2005; Newsham 2011; Mayerhofer et al. 2013). As a result, our understanding of endophyte habitat requirements and their distribution, ecology, diversity, and contribution to plant community feedbacks is currently superficial at best (Mandyam and Jumpponen 2005; Mandyam and Jumpponen 2015). Similarly to the mycorrhizal fungi (see Wilson and Hartnett 1998; Hartnett and Wilson 1999; van der Heijden 2002), inter- and intraspecific variability in host responses may, in part, structure the plant communities (Mandyam et al. 2012), although only sparse empirical evidence exists for such community modulation by the root-associated endophytes (but see Reininger et al. 2012; Aguilar-Trigueros and Rillig 2016). In addition, root-associated endophytes may alter biogeochemical processes, including the breakdown of organic forms of nitrogen (Upson et al. 2009; Mahmoud and Narisawa 2013; Yang et al. 2015). Further, a recent meta-analysis also highlighted their roles in protecting plants against drought and climate warming (Kivlin et al. 2013).

Root endophyte communities include diverse fungi that represent a range of taxa and ecological roles (Mandyam and Jumpponen 2005; Mayerhofer et al. 2013). Some clearly benefit host plants, whereas others may compromise plant performance (Saikkonen et al. 1998; Mandyam and Jumpponen 2015; Bonfim et al. 2016). While some interpret the parenchymatous net and/or the labyrinthine tissue that helotialean endophytes possess when colonizing woody plants (see Jumpponen and Trappe 1998, Lukesová et al. 2015) as a potential site for nutrient exchange, such structures are far from universal and may not form with nonwoody hosts (see Yu et al. 2001). In addition to the common absence of such well-defined, physiological interface that would provide a distinct site for nutrient exchange (Yu et al. 2001), the reported necrotic cytoplasm and cell death evidenced in detailed microscopic investigations of intracellular colonization (Deshmukh et al. 2006; Peterson et al. 2008) further challenge deciphering the host–endophyte interaction. This contrasts with mycorrhizal fungi whose definitions strongly rely on morphological and structural attributes of the fungus–host dual organ (Bonfante 1984, 2001; Smith and Read 2008) and—particularly for the arbuscular mycorrhizal fungi—includes the development of a defined and distinct interface for resource exchange (Bonfante 2001; Genre et al. 2008; Smith and Read 2008).

Distinguishing and identifying the host–fungus interfaces is not a simple task and is additionally complicated by the many organisms that simultaneously inhabit the root tissues. For example, Vági et al. (2014) visualized simultaneous colonization of root tissues and cells by arbuscular mycorrhizal fungi and fungal root endophytes suggesting that endophyte colonization does not necessarily lead to cell death (compare Deshmukh et al. 2006; Peterson et al. 2008). Further, the distinction of plant–fungus interaction may not clearly fall into a single facet of

known ecologies and may be inconsistent even within a strain: Lukesová et al. (2015) observed a helotialean endophyte to form microsclerotia typical to endophytes as well as coils resembling those of ericoid mycorrhizae when colonizing a *Vaccinium* species that is more commonly known to form ericoid mycorrhizae with its fungal partners. It is the combination of these complexities and inconsistencies that crumble the foundations of making simple generalizations about endophytes and their interactions with the host.

Similarly to the complexity of explicitly defining the host–endophyte interface, the functional attributes of these interactions have eluded simple and general functional categorization. Several underlying mechanisms have been proposed to explain host responses to the ubiquitous endophytes (see Mandyam and Jumpponen 2005; Rodriguez et al. 2008; Newsham 2011), and empirical evidence is starting to accumulate for, e.g., endophyte regulation of nutrient uptake, phytopathogen suppression, and control of environmental tolerances. Recently, Yang et al. (2014) observed that endophytic *Phomopsis liquidambari* upregulates genes related to nitrogen uptake and metabolism. These regulatory responses coincided with greater biomass accumulation and nitrogen content in inoculated plants compared to non-colonized controls. Such findings are particularly interesting because of the diverse enzymatic capacities of root endophytic taxa and strains (Caldwell et al. 2000; Mandyam et al. 2010; Knapp and Kovács 2016), which may be crucial for the maintenance of diverse ecosystem functions. Similarly, even though precise mechanisms still often remain unclear (but see review by Hamilton et al. 2012), fungal endophytes present some promising candidates for biocontrol and either antagonize or suppress phytopathogens (see, e.g., Harman et al. 2004; Chen et al. 2016; Terhonen et al. 2014a, b). Finally, endophytes can alter plant ecophysiological performance and thus also the environmental tolerances of their hosts (Kivlin et al. 2013). Recent studies suggest that endophyte inoculation can increase net photosynthesis and water use efficiency, improving drought tolerance (Molina-Montenegro et al. 2016). These findings from independent empirical studies support earlier speculation that endophytic fungi may produce phytohormones or secondary metabolites that promote host performance (Mandyam and Jumpponen 2005), defend against antagonists (e.g., Braun et al. 2003; Hamilton et al. 2012), alter host stress responses, or control host metabolism—particularly carbon and nitrogen metabolism—leading to changes in biomass allocation and/or improved performance (Mandyam and Jumpponen 2014).

As diverse as the root-associated endophyte communities can be phylogenetically and functionally, they appear adapted to the root environment: the endophyte communities are distinct from those that inhabit the adjacent soil and other plant organs (Herrera et al. 2010; Porras-Alfaro et al. 2011, Maciá-Vicente et al. 2012). Yet, a large proportion of the endophyte communities remains poorly known (Mandyam and Jumpponen 2005; Coleman-Derr et al. 2016). These root-associated communities—or at least their studied components—have been proposed to improve plant fitness (Mandyam and Jumpponen 2005; Newsham 2011), albeit the experimental evidence for the mechanisms is rather sparse or inconclusive (but see Aguilar-Trigueros and Rillig 2016) highlighting the potential environmental or

biotic context dependency of host responses (Kivlin et al. 2013; Mandyam and Jumpponen 2015).

Here, we draw from the available data on distributions of root-associated endophyte communities and explore questions examining the primary determinants of those communities. As a result of our current research focus on endophytes and rhizobiomes of grasses and the wide diversity of fungi that have been described as root-associated endophytes, we primarily focus on fungi associated with grasses. We fully acknowledge the findings of recent studies that suggest that endophyte colonization is controlled by biotic (host), edaphic, climatic, or spatial (location) factors (Zubek et al. 2009; Ranelli et al. 2015; Bokati et al. 2016), but propose that different endophyte groups are under different controls or selection pressures (Ruotsalainen et al. 2004; Ranelli et al. 2015). While some effort exists to map and better understand the biogeography of the better understood mycorrhizal endosymbionts—even on global scales (e.g., Öpik et al. 2010; Põlme et al. 2013; Davison et al. 2015)—very little is known about the controls of the distribution of the diverse fungal endophytes that seem universally present in most plant roots (Queloz et al. 2011).

We ask questions about whether or not the efforts to seek universal drivers for the endophyte community assembly are likely to prove productive. We approach these issues from two distinct perspectives:

First (from the whole community perspective): is there evidence for distinct communities across broad geographical scales?

Second: is there any evidence that the most well-studied endophyte taxa (i.e., the helotialean endophytes that commonly colonize the roots of woody plants in temperate and boreal forests and the pleosporalean endophytes that are emerging as the common grass associates in the temperate grassland systems) carry any biogeographic signal?

We acknowledge that the data to evaluate such questions are sparse. Thus, by definition, our discussion is largely speculative. We posit, similarly to Glynou et al. (2016), that the organismal functions should be tightly linked to their habitat and thus the ecological roles can be derived from the location of the focal organisms. If endophyte occurrence is correlated with abiotic (environmental conditions such as precipitation) or biotic (host phenotypes or phylogeny) drivers (see Maciá-Vicente et al. 2008, 2012; Glynou et al. 2016), that may facilitate efforts to elucidate endophyte functional roles.

10.2 Biogeographic Signal in Endophyte Communities

In general terms, geography, dispersal, environment, and organismal interactions determine the current and observable biogeographies (Prosser et al. 2007). However, the Baas-Becking hypothesis (Baas-Becking 1934) posits that microorganisms—including the fungal endophytes—are globally cosmopolitan and have high diversity locally but only limited beta-diversity. This is a result of their great

dispersal potential and large population sizes (Fitter 2005), leading to the environmental selection that the Baas-Becking hypothesis suggests. Clearly, a large body of current evidence challenges the "everything is everywhere, but, the environment selects" hypothesis (Baas-Becking 1934) and implies that, in addition to the environmental drivers, dispersal limitations also control the assembly of root-associated fungal communities (e.g., Peay et al. 2012; Peay and Bruns 2014). The relative importance of the environmental drivers and dispersal limitations may be context dependent and differ among fungal guilds. For example, root endophytes may possess distinct biogeographies (Glynou et al. 2016), whereas aboveground (foliar), and other root-associated (mycorrhizae) plant symbionts may not (Tedersoo et al. 2012; U'Ren et al. 2012). Either this indicates that different drivers control the assembly of the different fungal communities (Tedersoo et al. 2012), that some fungal groups have received more research attention than others, or that our understanding of the process of fungal community assembly is far from complete. To exemplify the last point, we highlight the contrast between the results of Queloz et al. (2011) versus Glynou et al. (2016). Whereas the former—focusing on the *Phialocephala fortinii* sensu lato *Acephala applanata* species complex (*Phialocephala–Acephala* complex, hereafter PAC) characterized by cryptic species—explained that stochastic effects are primarily responsible for PAC community composition, the latter highlighted the strong influence of the local environment in determining root endophyte community composition. Clearly, the jury is out on the importance of environmental or habitat filtering of root endophyte communities.

Detecting a biogeographic signal in the heterogeneous root-associated symbiont communities is a challenging undertaking. Efforts to elucidate the drivers that result in the observed organismal distribution pose an even greater challenge. Glynou et al. (2016) suggested that climatic drivers may be more important than dispersal limitation or soil variables in influencing the assembly of a root-colonizing fungal endophyte community. Additional variables that may include a set of other environmental, historical, or biotic variables were also considered influential under a combined "spatial effect" variable in that research effort. Interestingly, Glynou et al. (2016) observed no evidence for strict distance-decay effect (see Green et al. 2004; Peay et al. 2007) suggesting that it is not the geographic distance—and therefore not dispersal limitation—but instead the site-relevant environmental attributes, and thus the endophyte and host plant environmental tolerances, that are the primary filters that control the endophyte community assembly. These findings are congruent with Kivlin et al. (2014), who similarly concluded that fungal communities in soil and those collected from air currents had no compositional shifts over distance, but rather seemed structured by environmental filtering. Because these authors observed community commonalities among sites that were very distant from each other, it seems that the soil-inhabiting and endophyte communities may distribute propagules abundantly and over great distances. However, contrastingly, Glynou et al. (2016) observed that sites separated by greater distances tended to be more similar than those adjacent to each other, suggesting that some environments may strongly inhibit the establishment of some propagules.

These latter conclusions are similar to the Baas-Becking hypothesis and to empirical results from studies of shoot-colonizing endophytic fungi (U'Ren et al. 2012).

Jumpponen and Egerton-Warburton (2005) attempted to summarize components that define community assembly by liberally adopting Diamond's environmental filtering model (Diamond 1975) for mycorrhizal communities. A similar approach can be used for root-associated endophytes. In this model, local and regional propagule pools represent a transient community, from which persistent community members are selected, possibly based on abiotic filtering (see Kivlin et al. 2014). Only those members from the available pool that can establish under the prevailing environmental conditions may become members of the endophyte community, given that they locate compatible hosts to colonize. Among those that establish, biotic interactions (competition and facilitation) select individuals and species that remain and persist in the community. These persistent community constituents then enrich the local propagule pools with abundant short distance dispersal that can be initiated from the relatively few propagules that had dispersed over larger distances. This model would lead to a core community enriched with locally adapted taxa along with numerous transient components that persist in the system for only limited periods of time under the current prevailing environmental conditions. Although such filter models may overly simplify community assembly and dynamics, they provide a starting point for dissecting processes that lead to biogeographic signals in endophyte communities.

What then constitutes the local or regional propagule pools that permit the long-range dispersal of root-associated endophytic fungi and upon which the environmental selection may act? Many root endophytes rarely sporulate and thus lack the abundant dispersal propagules (Jumpponen and Trappe 1998; Addy et al. 2005) that would best explain the absence of distance-decay effects described in Glynou et al. (2016) and Kivlin et al. (2014). It is possible that the endophytes, or some constituents of the root endophyte community, would share dispersal strategies similar to those of vertically transmitted foliar (clavicipitalean) grass endophytes that colonize the seed and thus the emerging plant at the time of germination (Clay and Schardl 2002; Saikkonen et al. 2004). However, to our knowledge, there is no strong evidence supporting such seed-borne vertical transmission, although some endophytes can be isolated from both above- and belowground tissues, including the seed coat (Redman et al. 2002). In fact, the endophyte communities seem quite distinct between the above- and belowground plant compartments, albeit both may be recruited from the same soil inoculum pool (see Bodenhausen et al. 2013). But, there are some possible exceptions, which show commonalities in composition between roots and shoots (Rodriguez et al. 2009; Herrera et al. 2010; Porras-Alfaro et al. 2014b). Other possible dispersal mechanisms may include vector-mediated propagule transport and deposit. Two lines of evidence support this possible dispersal mechanism. First, some endophytes commonly develop structures that are resistant to the environment, as exemplified by the common microsclerotia of the so-called dark septate endophytes (Jumpponen and Trappe 1998; Currah et al. 1993; Kageyama et al. 2008; Porras-Alfaro et al. 2008). Second, some studies have reported that fungal communities present in the herbivore dung include a

considerable proportion that overlap with root-associated fungal communities (see Hawkins 1996, 1999; Porras-Alfaro et al. 2008; Herrera et al. 2011a and references therein). It is not only the mammalian herbivores that may carry inoculum. Bultman and Leuchtmann (2008) summarized data from clavicipitalean fungi and concluded that insects are likely dispersers of propagules for foliar endophytes. Taken together, herbivore-mediated dispersal combined with the persistent propagules that resist environmental decay may to a degree explain the lack of dispersal limitations. Finally, dispersal mechanisms common in soil-inhabiting fungi (see Kivlin et al. 2014), e.g., wind dispersal combined with adhesion to soil particles, may also underlay the observed broad distribution and effective dispersal of the root endophytes.

10.3 Biogeographic Signal in the Commonly Observed Endophyte Taxa

One challenge in identifying a biogeographic signal in populations of root-associated endophytes is the difficulty of strict and explicit taxon delineations. Currently, the efforts to identify endophyte community constituents are hindered by the lack of a consistent taxonomic and phylogenetic framework. In other words, many of the constituent taxa may still remain undescribed and new to science. Fortunately, recent morphological and molecular systematic work has begun to elucidate these issues for some pleosporalean taxa (Knapp et al. 2015). These studies circumscribed three novel genera that are related to other common endophytes in grassland biomes (Mandyam et al. 2010, Porras-Alfaro et al. 2008) and clearly highlight the lack of understanding of the endophyte taxon distribution even at the coarsest spatial levels. Advances have also been achieved for the helotialean endophyte taxa. For example, the development and use of restriction fragment length polymorphism (RFLP) probes, inter-simple sequence repeat (ISSR) (Grünig et al. 2001), and microsatellite markers (Queloz et al. 2010) have assisted in taxon assignments and spatial and/or temporal dynamics of the cryptic PAC taxa. Combined, these efforts have elucidated spatial dynamics of genotypes over extended periods of time in forest tree plantations (Stroheker et al. 2016) indicating that—once established—endophyte communities shift over space and time and that few genotypes maintain persistent colonization. These studies on defined spatial scales highlight the dynamic nature of endophyte communities and populations and contrast with those that highlight a lack of biogeographic signal in endophyte communities on larger spatial scales (Queloz et al. 2011).

Although the PAC fungi have been successfully assigned to a number of molecularly distinct, but morphologically indistinguishable and thus cryptic species, this is not the case for all root-inhabiting endophytes that still lack tools permitting reliable taxon assignments. The lack of a morphological taxonomic framework, unreliable production of taxonomically indicative morphological

structures (Jumpponen and Trappe 1998; Addy et al. 2005; Sieber and Grünig 2006), and existence of many closely related cryptic taxa that possess some degree of host preference (Grünig et al. 2004, 2008a, b; Queloz et al. 2008, 2010) all complicate taxon identification. Combined, these challenges severely hinder a better understanding of the biogeography of endophytes and their communities.

A further challenge in seeking broad biogeographic patterns of plant-associated organisms is that host plants are not globally distributed. As a result, separating host-mediated effects from environmental and dispersal effects becomes increasingly challenging as the spatial scale increases. Detecting geographic range limits of host-specific fungi would require co-modeling the distribution of the host plants in order to separate dispersal limitation from limitations due to host plant availability. Consequently, many of the existing studies that aim to tackle these challenges focus on different host species that are not present in all sampled locations (e.g., Maciá-Vicente et al. 2008). Those few studies that have succeeded in meeting the challenges presented by distribution of the hosts provide evidence that root-associated endophyte communities colonizing conspecific hosts across larger geographic ranges do indeed shift across large spatial scales and carry a biogeographic signal (Herrera et al. 2010; Glynou et al. 2016).

In those studies, Herrera et al. (2010) compared cultured, root-associated fungal communities of blue grama grass (*Bouteloua gracilis*) along a transect from Mexico to Canada. Although the communities differed in the less common members, many taxonomically related groups commonly occurred at all sites (including fungi in the Pleosporales, Agaricales, and Hypocreales). Because shared members of the dominant groups resulted in communities that were more similar among adjacent sites, the community and geographic distances were negatively correlated—consistent with the distance-decay models (see Green et al. 2004; Peay et al. 2007). Similarly, Glynou et al. (2016) analyzed cultured root endophytes of non-mycorrhizal plants in the genus *Microthlaspi* across 52 plant populations in Europe. These studies revealed that climate—along with geographic controls—best explained endophyte community composition. Corroborating the findings of Herrera et al. (2010), Glynou et al. (2016) also observed a few common taxa in the orders Pleosporales and Hypocreales, and also Helotiales, that altogether represented approximately half of the collected isolates. Taken together, these studies suggest that while common endophytes may occur ubiquitously across large geographic ranges, the communities as a whole can be strongly influenced by environmental drivers. However, additional research efforts are necessary to expand the geographic reaches of studies, even if they must rely on naturalized, non-native taxa such as *Arabidopsis* (e.g., Lundberg et al. 2012; Bodenhausen et al. 2013).

As a comprehensive treatise of the distribution of root endophytes would be an exhausting exercise in futility, we broadly target the commonly observed helotialean PAC endophytes in temperate and boreal forested ecosystems (Grünig et al. 2008b; Queloz et al. 2011) and the pleosporalean endophytes that appear common in grassland ecosystems in both North America (Porras-Alfaro et al. 2008; Mandyam et al. 2010) and Europe (Knapp et al. 2012, 2015). We fully acknowledge that we are likely to combine several biological species into super-taxa. At the same

time, our speculation and broad conclusions are not sensitive to cryptic species or inaccurate taxon delineations. Despite our very broad grouping of taxa commonly observed as endophytes, some patterns still emerge. In a nutshell, while our data use coarse categories for focal taxa, it appears that the members of PAC are commonly and abundantly present in temperate (Ahlich and Sieber 1996; Queloz et al. 2005) and boreal coniferous forested ecosystems (Summerbell 2005; Kernaghan and Patriquin 2011; Vohnik et al. 2013; Terhonen et al. 2014a) as well as in arctic tundra ecosystems (Björbäkmo et al. 2010; Walker et al. 2011; Dean et al. 2013). This pattern is in stark contrast with the near absence of these common fungi in temperate grassland ecosystems in North America (Porras-Alfaro et al. 2008; Mandyam et al. 2010) and Europe (Knapp et al. 2012). Instead of the common helotialean components, these grassland ecosystems host a large pleosporalean component. Interestingly, these pleosporalean isolates, when inoculated on either native or model hosts, produced morphological structures quite similar to those reported for the helotialean fungi from forested systems (Mandyam et al. 2010, 2012; Knapp et al. 2012). Taken together, these observations seem to suggest that there is some level of biome specificity in the constituent taxa that colonize hosts in distinct grassland and forested biomes. Although our discussion here is quite speculative, we propose that the hypothesis of biome specificity serves as a starting point for more detailed studies, including perhaps common garden or reciprocal transfer experiments that would permit better and more rigorous testing.

Our speculation utilized studies that relied exclusively on efforts that isolated fungi into pure culture. We considered this important as only few community-level studies fulfill Koch's postulates, which we consider mandatory to confirm whether any isolate forms endophyte symbiosis (see Jumpponen et al. 2011). However, these pure culture studies are also burdened by a potential shortcoming. There is a question whether the pure culture data are a result of culturing bias (see, e.g., Walker et al. 2011). However, our choices for targeted systems are a result of existing available information, and our cursory synthesis leads to conclusions that while some biogeographic signals may distinguish the forested and grassland systems, we may still be far from being able to argue for biome-specific, root-associated endophyte guilds.

10.4 Drivers of the Root-Associated Endophyte Communities

The few studies that have focused on identifying the drivers that structure root-colonizing fungal communities rather consistently imply some degree of importance for edaphic or climatic conditions, or both (e.g., Bokati et al. 2016; Glynou et al. 2016), in addition to some control by the host taxon (e.g., Bokati et al. 2016). Naturally, edaphic and climatic drivers are not geographically randomly distributed but often strongly correlate with each other leading to complex problems in

selecting the best explanatory models and variables. It is also unlikely that a single strong driver governs the fungal community dynamics. Rather, the community dynamics are near certainly under control of multiple, interacting variables that affect which species successfully colonize host roots. To exemplify, Bokati et al. (2016) recently concluded that soils play a primary role in structuring the root-associated fungal communities in maize, wheat, and their progenitors. Despite the soil's primary role, there were some similarities in the communities that were best explained by host species identity (Bokati et al. 2016). These results are congruent with others that conclude that soil microbiomes are originators of root microbiomes (e.g., Edwards et al. 2015; Zarraonaindia et al. 2015) and biotic and/or abiotic filtering likely takes place during the community assembly of host-associated microbiomes. This soil-driven community assembly would produce root communities that are effectively subsets of soil and rhizosphere communities. Further, although aerial dispersal likely dominates, as perhaps implied from the Baas-Becking hypothesis, other factors, such as vector-mediated dispersal, may also control how endophyte communities assemble. Long-range transmission by insects (as is the case for clavicipitaceous endophytes; Bultman and Leuchtmann 2008) or herbivores (see discussion in Porras-Alfaro et al. (2008); Herrera et al. 2011a) provides evidence that some of the transmission—and thus also assembly—may be vector-driven and not exclusively airborne. However, these alternate dispersal hypotheses are challenging to test given that roots likely filter endophyte communities from the more diverse microbial community in the surrounding soils.

Some data describing fungal communities colonizing Poaceae suggest that many of the grassland species harbor a suite of cosmopolitan root-associated taxa (Herrera et al. 2010; Knapp et al. 2012, 2015). In some cases, some of these taxonomic clades vary over geographic space or environmental conditions (Herrera et al. 2010, 2011b). Recent and unpublished data examining the root communities in five different grass species over geographic distances suggested that there are some modest distinctions among sites (see case study below), but none among hosts. Similarly, provisional microscopy assessment also indicated that the fungal endophytes colonize different grass species at about the same rate, although some of the grasses responded to water amendments by, for example, quickly increasing the proportion of some clades (Herrera et al. 2011b). This evidence suggests that the root-associated communities are not stable in time or across environmental stressors, but may—indeed—rapidly respond to changes in environmental conditions and shift dramatically over very short periods of time. Although speculative, these data suggest that additional research is needed to ascertain the effects of localized edaphic and environmental conditions on root-associated fungal communities, in addition to identifying drivers on broader geographical scales. Similarly, while the colonization frequency by different endophytes may differ among host species (Tejesvi et al. 2013), there appears to be no strict host specificity wherein some endophytes would prove incapable of colonizing a host species or even a guild of hosts in either natural or manipulative experimental settings (Mandyam et al. 2012). Collectively, there is some support for conclusions that the root-associated fungal communities are not specifically bound to any one host but rather are generalists, as suggested in previous synthetic efforts (Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005), and that these

communities may be transient and/or respond to environmental drivers either over spatial or temporal scales.

10.5 Case Study

As a part of an ongoing investigation of root-associated mycobiomes (fungal rhizobiomes) of common graminoids in central and south central United States, we evaluated the use of ITS2 barcodes to identify unknown cultured fungal isolates. Our approach parallels that described in Shokralla et al. (2015), wherein the authors MiSeq-analyzed PCR-amplified cytochrome c oxidase DNA barcodes that spanned 658 bp from 1010 specimens representing eleven orders of arthropods. That approach proved successful, and the authors argued that the use of next-generation sequencing of taxon barcodes permitted superior data generation at reduced cost compared to the more conventionally used Sanger sequencing.

In the course of our ongoing rhizobiome research, we isolated fungi into pure culture from a total of 23 sites located in Colorado, Kansas, Nebraska, New Mexico, Oklahoma, Texas, and Wyoming (Fig. 10.1). Our goal was to establish replicated latitudinal gradients to enable robust generalizations that capture natural and multifactor climatic contexts. We aimed to address three specific hypotheses on root endophyte communities: (1) root endophyte communities are distinct among the host species, i.e., shaped by the host identity (Hartmann et al. 2009; Prescott and Grayston 2013); (2) root endophyte communities decrease in similarity with increasing geographic distance; and (3) root endophyte communities correlate with environmental gradients, i.e., are structured by environmental drivers and thus likely driven by the environmental tolerances of the constituent species (see Jumpponen and Egerton-Warburton 2005). We take this opportunity to evaluate the first two hypotheses with early emerging data from this project to better refine these hypotheses and to provide a basis for future discussion on potential drivers. Although we list three hypotheses here, the sparse data matrices resulting from the initial ITS2 barcode evaluation provide inadequate data to compare models with a great variety of environmental drivers that may be correlated with each other. As a result, we address only the first two hypotheses here.

For this rhizobiome research project, within each of the selected sites, we targeted grasses that are dominant, widely used in restoration, and span the major tribes of Poaceae in Central US grasslands: *Andropogon gerardii* (big bluestem); *Bouteloua gracilis* (blue grama), *B. eriopoda* (black grama), *Buchloe dactyloides* (buffalograss), and *Schizachyrium scoparium* (little bluestem). Many of these grasses also host a variety of root endophytic fungi as indicated by earlier data (Barrow 2003; Porras-Alfaro et al. 2008; Herrera et al. 2010; Jumpponen et al. 2011; Mandyam et al. 2012), making them prime targets for rhizobiome surveys. A total of twelve whole plants were excavated with a transplanting shovel as described in Mandyam et al. (2012), and root systems were sampled for culturing at Western Illinois University (WIU) by Porras-Alfaro's group.

Fig. 10.1 Map of sites included in the current field survey of rhizobiomes in the common and dominant grasses. *Black dots* with site identifiers are those included in the case study here; *gray dots* identify additional samples not included in the preliminary barcode trial analyses. Sites are CAD, Caddo and Lyndon B. Johnson National Grassland, Texas; DMT, Davis Mountains State Park, Texas; GMT, Guadalupe Mountains National Park, Oklaholma; KNZ, Konza Prairie Biological Station, Kansas; LBJ, Ladybird Johnson Wildflower Center, Texas; SCP, Spring Creek Prairie Aubudon Center, Nebraska; UHC, University of Houston Coastal Center, Texas. Additional information on the sites is available in Table 10.1

To culture fungi from excised root tissues, surface-sterilized roots of the replicate individuals were plated on malt extract agar (MEA) on 48-well plates. A subset of the surface-sterilized roots was pressed against the media to confirm the effectiveness of the surface sterilization—these press controls remained largely free of any fungal colonies, indicating successful surface sterilization. Fungi emerging from roots were aseptically transferred to MEA, and representatives of the cultures are currently maintained at the WIU Fungarium and at UNM in cryovials with sterile water.

From >2000 pure cultures generated thus far, we selected 768 early emerging isolates to preliminarily evaluate the utility of barcode analyses. In this experiment, our primary goal was to test the utility and expedience of the barcode identification for a large number of cultures and to assign them to provisional OTUs for more detailed screening. These analyses and conclusions will be further confirmed with Sanger sequence data once the culturing efforts are completed. From the selected pure cultures, DNA was extracted using a Wizard genomic DNA purification kit (Promega, Madison, Wisconsin) and adjusted to 2 ng μl^{-1} concentration. Similarly to the approach described by Shokralla et al. (2015), we chose a barcode of life locus that has been proposed for fungi (Schoch et al. 2012) and the PCR-amplified internal transcribed spacer 2 (ITS2) locus using primers that flank the target region

(Ihrmark et al. 2012). A total of 20 ng of each template DNA was PCR-amplified in 50 μl reactions using fITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1990) primers, 192 of each carried 12 bp DNA-tags that differed in a minimum of two nucleotides. The PCR conditions and protocols were identical to those described earlier (Oliver et al. 2015a, b), and except that for expedience, we omitted the primary PCR without the DNA-tagged primers. This approach included 192 pure cultures in each of four MiSeq Libraries, to each of which Illumina TruSeq adapters were ligated using a GeneRead DNA Library I Core Kit (Qiagen, Hilden, Germany; catalog #180432) at the Integrated Genomics Facility at Kansas State University. The four libraries were paired-end sequenced using a MiSeq Reagent Kit v3 (Illumina, San Diego, California) with 2 × 300 cycles in a combined run, from which ~10% of the anticipated total yield—or roughly two million raw reads—were expected for each of the four libraries.

Our barcode libraries yielded a total of 6,495,500 raw sequences across the four libraries (or ~1.6M reads per library); 758 of the 768 isolates (98.7%) yielded some sequence data. The paired raw sequences were contiged and quality screened as described previously (Oliver et al. 2015b) using mothur software (v. 1.33.1; Schloss et al. 2009): sequences with no exact match to primers or DNA-tags, with long homopolymers (>8), or with ambiguous bases were omitted. The sequences from the four MiSeq libraries were then merged to expedite downstream analyses and truncated to 236 bp—a length equal to the shortest resultant read—to facilitate pre-clustering of near identical (99.2% similarity) sequences and reduce potential sequencing bias (Huse et al. 2008). These data were screened for chimeras (uchime; Edgar et al. 2011), and 1,254 putative chimeras were omitted. A total of 4,588,780 reads passed quality screening and included a total of 15,188 nonidentical sequences, suggesting considerable heterogeneity in the dataset characterizing the collection of isolates. These data were used to estimate a pairwise distance matrix (conservative nearest neighbor clustering), based on which the sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity. The OTUs were assigned to putative taxon identities using the Naïve Bayesian classifier (Wang et al. 2007) with UNITE taxonomy reference (http://unite.ut.ee/repository.php). To improve data integrity, rare OTUs (OTUs with sequence count $\leq$ 10) were omitted from each DNA-tag-identified sample (Brown et al. 2015; Oliver et al. 2015a). This resulted in a total of 740 isolates with sequence data.

Of the 740 isolates that yielded sequence data passing our quality control, a total of 417 (56.4%) resulted in unambiguous single OTUs and thus potential barcode identification. The remaining 43.6% of the isolates resulted in more than one OTU, compromising thus the unambiguous identification. Reasons for the multiple OTUs resulting from presumably monospecific isolates are unclear but may include mixed cultures; multiple divergent ITS copies within an isolate (Thiery et al. 2012; Zhao et al. 2015); PCR-induced mutations (Qiu et al. 2001); stochastic generation of chimeric sequences (Fonseca et al. 2012; Shin et al. 2014) that remained undetected in our screening; cross contamination during DNA extraction, plate manipulation, PCR, or subsequent cleanup steps; polymerase errors (Eckert and Kunkel 1991; Oliver et al. 2015a); DNA-tag switching (Carlsen et al. 2012); and/or sequencing

artifacts (Medinger et al. 2010; Brown et al. 2015). To make use of these data as well, we assumed that the most abundant read for each isolate was the most likely representative of the template DNA of the intended isolate. Our ongoing research efforts include Sanger sequencing to evaluate the value and reliability of the barcoding approach. Regardless of the low percentage of isolates that yielded only one OTU for an isolate, we learned two important lessons from this exercise. First, generating data in a laboratory with an easy and inexpensive access to sequencing is very fast—generating these four libraries ready for data generation required less than 1 week of work in the laboratory. We conclude that this NGS approach serves as an expedient tool for preliminary screening of large isolate collections. Based on these efforts, the most interesting—or most common—isolates can be selected for more detailed analyses.

The 740 isolates retained in our analyses were assigned to a total of 132 OTUs that we presume to represent species level resolution at 97% sequence similarity. These OTUs represented 120 putative species, 71 genera, 48 families, and 26 orders. The close similarity of OTU and putative species numbers suggests that the OTUs likely approximate species level. However, ITS2 barcode-inferred OTUs may fail to resolve some closely related species, such as those exemplified by OTUs assigned to genus *Fusarium* (e.g., *F. oxysporum* and *F. redolens* in our dataset) or its sexual states (genus *Gibberella*) (Geiser et al. 2004). A large majority of isolates belonged to phylum Ascomycota (91.4%) followed by a small proportion of Basidiomycota (6.6%) and a few unclassified OTUs (~2%). On the order level, Hypocreales (36.9%) and Pleosporales (29.5%) dominated, although a few members of Agaricales (5.5%), Eurotiales (4.1%), Sordariales (3.2%), Xylariales (3.1%), and Helotiales (2.8%) were also present. Although the taxon rankings may differ, these order level data corroborate those of others. Glynou et al. (2016) observed that Pleosporales and Hypocreales (also Helotiales) represented a large proportion of isolates acquired from *Microthlaspi*, and Herrera et al. (2010) observed that Pleosporales, Agaricales, and Hypocreales were dominant orders in their isolates from *Bouteloua*. Notably, our data also included some Helotiales, but they were a rather minor component (~ 3% of the isolates). These helotialean isolates represented a few different putative species: uncultured *Lachnum* (14 isolates), *Acephala* (3 isolates), *Chalara* (3 isolates), and *Cryptosporiopsis ericae* (1 isolate). The small proportion of the helotialean taxa that have been confirmed endophytes strongly indicates that helotialean taxa are indeed rare in these grassland systems, supporting our earlier speculation on biome-defined endophyte guilds. Based on the congruence with the observations in Glynou et al. (2016) and Herrera et al. (2010), we conclude that barcode identification has the potential to serve as an expedient method for assigning large numbers of specimens into clusters approximating conspecific groups. However, this approach may suffer from issues emerging from operator errors and limited resolution in available databases.

Overall, in these barcode identified data, consistently with other published studies (e.g., Maciá-Vicente et al. 2008), the most common genera included *Fusarium* (20.0%) and its sexual teleomorph state *Gibberella* (3.1%) for a total of 23.1%

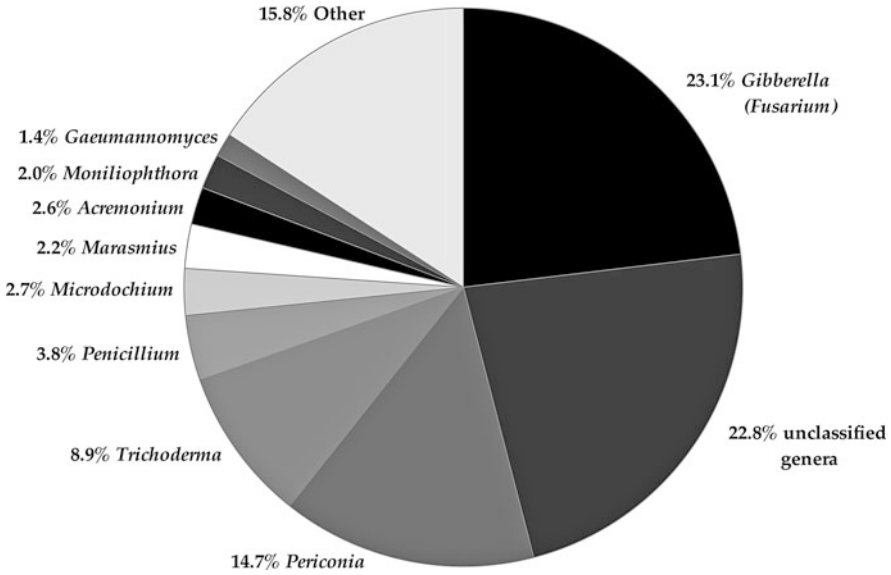

Fig. 10.2 Genera isolated in the current field survey of rhizobiomes (Fig. 10.1) in the common and dominant grasses. The *pie chart* includes ten most common genera—the remaining 61 genera are combined under "other"

of all isolates (Fig. 10.2). The second largest group was a mixture of isolates that lacked generic affinities (unclassified genera; 22.8% of all isolates). These were isolates placed into higher taxonomic levels including Pleosporales (10.1%) and Sordariales (2.0%) plus others that constituted <1% of all isolates. Interestingly, consistent with other studies from grassland ecosystems based on pure culture analyses (see Mandyam et al. 2010; Knapp et al. 2012), OTUs assigned to genus *Periconia* (Pleosporales) were also common and represented the second largest group of isolates with generic affinities in our analyses (14.7%). Other common taxa included some typically common soil and rhizosphere fungi (Fig. 10.2).

From these data, we selected those that permitted inferences on the phylogeography of the rhizobiome. Because the selection of isolates for our barcode trials was not specifically stratified to address these questions, we set some a priori thresholds for sample selection. We omitted all sites that did not yield a minimum of five isolates for a host sampled at that site. This resulted in a data matrix with seven sites and two to four hosts present per site, for a total of 19 observations (Table 10.1). As a result of this selection of samples, the number of included OTUs was reduced to 84.

We converted the sequence counts to presence/absence data and estimated the Bray–Curtis pairwise distances for use in nonmetric multidimensional scaling (NMS) in PC-ORD (v. 6.19; McCune and Mefford 2011). A two-dimensional solution ($k = 2$; Fig. 10.3A) resulted in stress (0.18) significantly lower for each axis than that derived from randomized data ($P < 0.05$). The two axes represented

Table 10.1 Site descriptions, site names, locations, elevations and host species sampled for the analyses of cultured fungal communities in the ITS2 barcode trials

Site	Name	Latitude	Longitude	Elev (m)	Grassland type	Hosts
UHC, TX	University of Houston Coastal Center, Texas	29.40N	95.05W	6	Coastal tallgrass	*Andropogon gerardii* (1), *Schizachyrium scoparium* (1)
LBJ, TX	Ladybird Johnson Wildflower Center, Texas	30.18N	97.87W	2554	Mixed grass	*Andropogon gerardii* (1), *Buchloe dactyloides* (1), *Schizachyrium scoparium* (1)
DMT, TX	Davis Mtns/ Mimms Ranch, Texas	30.63N	104.17W	2660	Desert, shortgrass	*Bouteloua eriopoda* (1), *Schizachyrium scoparium* (1)
CAD, TX	Caddo–LBJ National Grassland, Texas	33.42N	97.63W	272	Mixed grass	*Andropogon gerardii* (2), *Schizachyrium scoparium* (1)
KNZ, KS	Konza Prairie Biological Station, Kansas	39.10N	96.56W	415	Tallgrass	*Andropogon gerardii* (2), *Bouteloua gracilis* (1), *Buchloe dactyloides* (1), *Schizachyrium scoparium* (2)
SCP, NE	Spring Creek Prairie Audubon Center, Nebraska	40.69N	96.85W	406	Tallgrass	*Andropogon gerardii* (1), *Bouteloua gracilis* (1)

The short, *three letter codes* refer to the site names in Fig. 10.1. *Numbers in parentheses* following the host taxon binomials indicate the number of host individuals remaining in the analyses after applying the a priori thresholds for experimental unit retention in the case study. Note that these preliminary analyses did not permit inclusion of nested or interactive terms as a result of low number of included experimental units

10.4% and 53.6% of the variation (Fig. 10.3A). To test for differences among the host species and sites, we used multi-response permutation procedure (MRPP) suitable for unbalanced experimental designs like ours. The MRPP analyses indicated that while the hosts did not differ in the rhizobiomes detected in our isolation effort ($T = –0.923$; $P = 0.1757$), the rhizobiomes at the seven sites did ($T = –2.65$; $P = 0.0066$). These results are consistent with others (Bokati et al. 2016), who concluded that hosts were of lesser importance than the site/soil properties in root-associated fungal endophyte communities. Our subsequent pairwise comparisons indicated that Ladybird Johnson Wildflower Center site in Southern Texas was distinct from other sites ($P \leq 0.0318$) and that Guadalupe Mountains National Park in Texas was distinct from Konza Prairie Biological Station in Kansas ($P = 0.0197$). It is of note that the low number of replicates in these analyses did

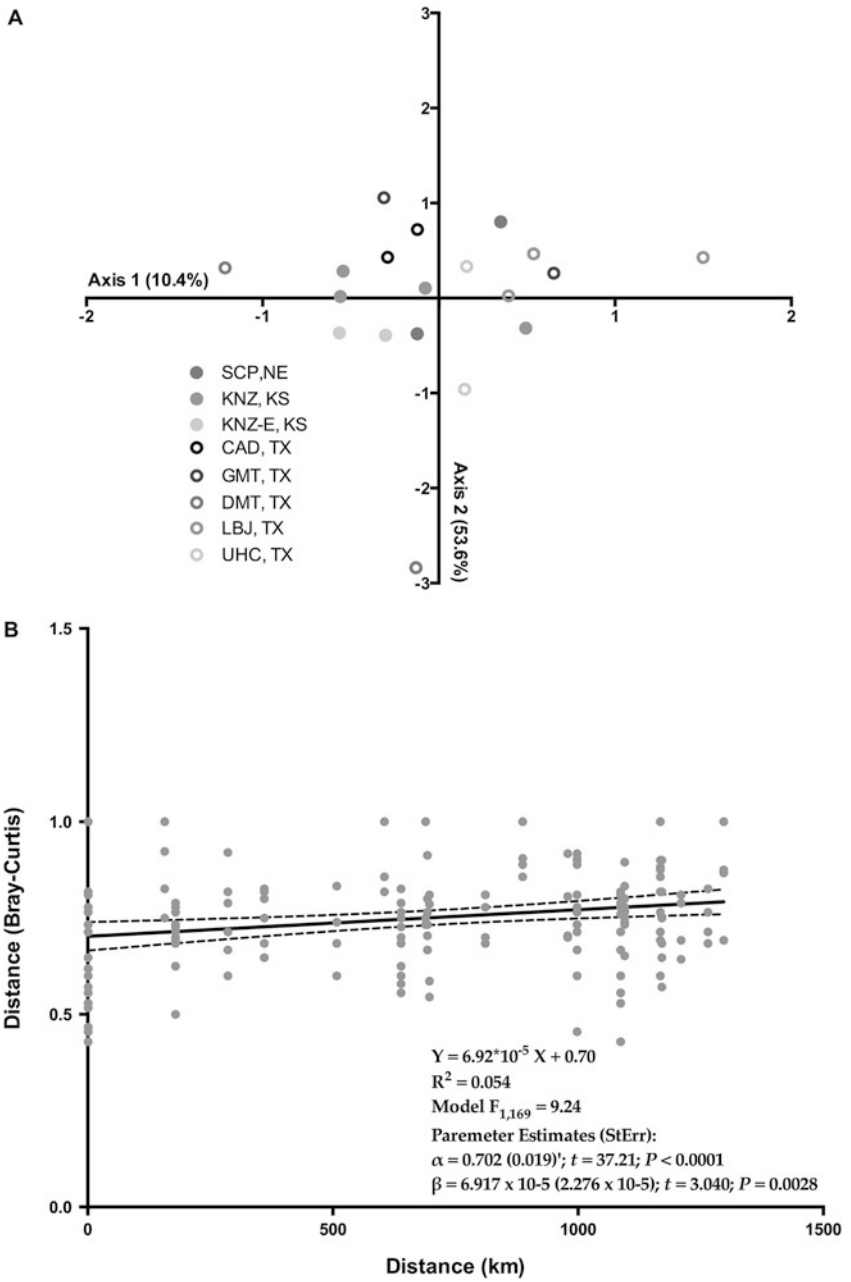

Fig. 10.3 A: Nonmetric multidimensional scaling (NMS) ordination of the 19 observations of cultured communities included in the ITS2 barcode trials. Multiple response permutation procedure (MRPP) indicated that while the host plants do not differ in their isolated fungal communities ($T = -0.923$; $P = 0.1757$), the sampled sites do ($T = -2.65$; $P = 0.0066$). B: The pairwise community distances (Bray–Curtis dissimilarity) plotted against geographic distance between the sites. Regression analyses indicate small (slope $= 6.92 \times 10^{-5}$) but significant ($P = 0.0028$) increase in the community distance with increasing geographic distance

not permit all possible pairwise comparisons. Yet, we argue that our data clearly suggest a geographic distinction among the analyzed rhizobiomes, whereas the host species were of lesser importance.

To further evaluate this geographic distinction, we analyzed the distance-decay effect (Green et al. 2004; Peay et al. 2007) using linear regression analysis of the Bray–Curtis distances and the geographic distances among the included sites (Fig. 10.3B). Interestingly, this analysis indicated a slight but positive correlation (slope $= 6.9 \times 10^{-5} \pm 2.3 \times 10^{-5}$) between the pairwise geographic distances and the pairwise community distances. The effect was rather weak, as indicated by the small slope ($-6.9 \times 10^{-5} \pm 2.3 \times 10^{-5}$) and low R^2 (=0.05) but highly significant ($T = 3.040$; $P = 0.0001$). However, one should bear in mind that our data matrix is preliminary and rather sparse. Thus, additional data are necessary to refine our observations and to shed further light into the dispersal limitations in these communities.

10.6 Challenges of Biogeographical Studies of Root Endophytes

We briefly touched on the challenges of broadscale studies on the root-associated endophytes resulting from poorly defined taxonomic frameworks and potential challenges of locating conspecific hosts over large geographic ranges. Here, we return to some additional challenges that stem from our lack of understanding of the ecology of root-associated fungi and the coinciding poor annotation of references in available databases.

Rhizosphere environments harbor a diversity of fungi with a range of potential interactions with their hosts (Vandenkoornhuyse et al. 2002; Glynou et al. 2016). However, presence of a fungus in the root system does not make it an endophyte. Although there are means to fulfill Koch's postulates to confirm endophytes isolated from roots (Jumpponen et al. 2011), they are rarely employed because the manipulation of symbiotic systems is challenging and tedious. Studies that inoculate acquired fungal isolates back to native hosts (Walker et al. 2011; Mandyam et al. 2012, Lukesová et al. 2015), non-model (Mugerwa et al. 2013; Knapp et al. 2012), or model plants (Mandyam and Jumpponen 2015) have observed indicative fungal morphologies within the roots and permitted simultaneous confirmation of endophytic colonization as well as evaluation of host growth responses to inoculation. These studies establish a model for the effort required for confirmation of endophytic association. Such experiments are particularly demanding with native plants, whose germination rates can be dismal and growth rates painfully slow. Fortunately, recent syntheses that summarize data and conclusions from model and native plant experiments strongly suggest that the model plant systems that are more simple to execute can serve as reasonable proxies to infer

colonization and host responses in native plant systems (Mandyam and Jumpponen 2015).

Both pure culture and direct environmental sequencing studies rely heavily on available reference databases such as UNITE (http://unite.ut.ee/; Tedersoo et al. 2011) or RDP (https://rdp.cme.msu.edu; Cole et al. 2014) for assigning isolates or phylotypes to taxa. However, although these databases and the automated classifiers (e.g., Naïve Bayesian classifier; Wang et al. 2007) make taxon annotations expedient and objective, additional assignments to ecological roles are lagging far behind. Further, transfer of information on the confirmed endophyte taxa to the existing databases usually works with a lag and requires some substantial community involvement. Such efforts are already underway for some groups of fungi (Nilsson et al. 2014) and lay a foundation for database annotations of additional functional roles. Alternatively, independent tools for ecological annotation, exemplified by FUNGuild (Nguyen et al. 2016), are likely to simplify sharing the emerging ecological information, but do similarly rely extensively on third party annotation of the database entries. Concerted community efforts to update and maintain these databases would likely expedite improved use of and greater insight into the data on endophyte communities and their phylogeographies. Some efforts to initiate curated databases for root endophytic fungi are underway (Gábor M. Kovács and Dániel G. Knapp, personal communication). The plans include crosslinking these databases with other fungal and sequence databases with oversight by advisory boards drawn from the community of endophyte researchers.

Another issue is data compatibility. Although ITS regions have been proposed as the primary barcode for fungi (Schoch et al. 2012), some studies have chosen the use of large subunit (LSU) as a target (e.g., Amend et al. 2010; Rigdon et al. 2013) in environmental analyses of fungal communities, whereas others choose alternative markers because of inadequate resolution within their target groups (e.g., Maciá-Vicente et al. 2008). Although many of the examined markers—such as the LSU and ITS regions—yield comparable data (Brown et al. 2014; Porras-Alfaro et al. 2014a), use of the different targets makes direct comparisons across datasets impossible. Similarly, the use of different subregions of the proposed universal fungal barcode—the ITS (Schoch et al. 2012)—compromises such direct comparisons. However, the choice of a marker is not straightforward: some have concluded that the ITS2 region is superior in high-throughput applications (Tedersoo et al. 2015), whereas others have claimed that ITS1 provides a superior resolution for Eukarya (Wang et al. 2015). Additionally, while ITS is commonly used, it is unreliable for distinguishing *Fusarium* species (Geiser et al. 2004), whose identification relies on other loci (e.g., translation elongation factor 1α—TEF-1α) that are also common in phylogenetic analysis within this group (Seifert 2009). As a result, each research group makes decisions on selecting the primary target region, resulting in datasets for which comparisons are possible only based on annotated sequence data at generic levels, at best. We cannot provide a recommendation for the target locus selection here, but wish to draw attention to the problem posed by heterogeneity in accumulated data. A potential solution is the use of multiple marker genes, as is common in phylogenetic studies (e.g., James et al. 2006).

However, single copy genes are difficult to deploy in direct environmental sequencing approaches, and additional genes linearly increase sequencing costs if used to identify collections of pure isolates.

In addition to the problems resulting from marker selection, data generation, and sparse information on the ecology of the fungi that reside within the rhizobiomes, there are gaps in our understanding of the distribution of endophyte taxa. Above, we highlighted two endophyte guilds—grassland endophyte communities that appear to host a large Pleosporales component and the distinct forested ecosystem communities that host a large Helotiales component. This is agreeably quite a coarse resolution to infer either distributions or commonalities within the endophyte communities. Yet, these coarse distinctions at least serve as a starting point for developing more defined hypotheses on the distribution of endophyte communities and their constituent taxa and eventually also the primary drivers that define those communities, be it host species, dispersal limitations, or edaphic and climatic environmental controls. What becomes apparent, however, is the urgent need to execute large-scale field studies to broaden the range of parameters that can be used to select the most likely controls for the assembly of endophyte communities. Although our cursory case study focusing on a subset of pure cultures isolated from grasses from widely distributed field sites strongly suggest distinctions among the sites, it falls short of identifying possible environmental drivers. Our goal—upon completion of the culture-based and culture-independent analyses—is to provide further insight into the primary drivers of the root endophyte communities.

10.7 Conclusions

We summarized some of the data available on the distributions of some of the better-known groups of root-associated fungal endophytes. Without aiming to be comprehensive in our treatise, we arrived at a conclusion—at the coarsest level of resolution—that at least two distinctly distributed guilds of root-associated endophytes seem to exist. One consisting mainly of pleosporalean culturable taxa appears common and perhaps dominant in the grassland ecosystems and another consisting of helotialean culturable taxa seems similarly common in forested and other northern ecosystems. Agreeably, such biome-level coarse syntheses leave many unanswered questions. However, we sincerely hope that the hypotheses that we pose spark greater interest in resolving questions about the distribution of fungal taxa that establish endophyte symbioses with their hosts.

We presented preliminary data that we generated in a trial of high-throughput sequencing of ITS2 barcodes to identify fungi in pure culture libraries. While these trials were only a partial success, they did nonetheless provide a limited dataset that permitted us to identify a number of common root-associated fungi from grassland ecosystems. These data suggested that, while the hosts were seemingly similar in their culturable rhizobiome communities, the sites were distinct. These results beg the obvious questions on the drivers of such distinctions. Our data provided some

support for distance decay, with greater dissimilarities among communities that were more distantly located. However, some of these sites were also located >1100 km apart, and several edaphic and climatic conditions differ among them. Because of the limited data matrices thus far, we did not explore environmental drivers—such as gradients in precipitation or annual mean and maximum temperatures. We hope these data provoke interest in studies that broadly address the composition of endophyte communities and rhizobiomes over large geographical scales. Clearly, there is evidence for geographic distinctions, but the underlying reasons remain open questions.

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Chapter 11
Global Patterns of Mycorrhizal Distribution and Their Environmental Drivers

Nadejda A. Soudzilovskaia, Stijn Vaessen, Maarten van't Zelfde, and Niels Raes

11.1 Introduction

Mycorrhizal symbiosis is an important driver of carbon (Averill et al. 2014; Godbold et al. 2006; Read et al. 2004; Read and Perez-Moreno 2003) and nutrient (Phillips et al. 2013; Read and Perez-Moreno 2003; Terrer et al. 2016) cycling. Mycorrhizal fungi enhance plant nutrient acquisition by creating large mycelial networks that can both access mobile and immobile forms of soil nutrients (Smith and Read 2008; Smith and Smith 2011). Furthermore, fungi themselves constitute an important carbon pool and control biogeochemical soil transformation processes (Leake et al. 2004; Soudzilovskaia et al. 2015b). However, the mechanisms and magnitude of involvement of mycorrhizal symbiosis in soil carbon and nutrient transformation processes differ among distinct types of mycorrhiza. Currently, six main types of mycorrhizal symbiosis are recognized: ectomycorrhiza (EcM), arbuscular mycorrhiza (AM), ericoid mycorrhiza (ErM), arbutoid, monotropoid and orchid mycorrhiza (Smith and Read 2008). Each of the mycorrhizal types feature distinct fungal and plant species involved in symbiosis, with seldom exceptions of dual colonization, i.e. when the same plant individual is colonized by two types of fungi (Chilvers et al. 1987; McGuire et al. 2008; Wang and Qiu 2006). Among these mycorrhizal

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types, AM, EcM and ErM are the most geographically widespread, featured by ca 85% of the Earth's plant species (Brundrett 2009). They are predominant across the majority of the terrestrial vegetated areas (Brundrett 2009; Read et al. 2004; Smith and Read 2008). Read (1991) proposed to roughly divide the Earth into biomes dominated by AM plants (e.g. temperate grasslands, savannahs), EcM plants (e.g. boreal taiga, temperate forest) and ErM plants (e.g. tundra) and the zones possessing vegetation of mixed mycorrhizal type (e.g. Mediterranean vegetation, tropical forests). Since then, not much progress has been achieved in our understating of global distribution of mycorrhizas.

Recently, a number of studies have demonstrated that distinct mycorrhizal types are associated with fundamentally different patterns of nutrient and carbon cycling (Brzostek et al. 2014; Godbold et al. 2006; Read et al. 2004; Read and Perez-Moreno 2003; Shi et al. 2015; Terrer et al. 2016), which results in more intensive carbon accumulation in EcM-dominated vegetation stands compared to AM-dominated ones (Averill et al. 2014; McGuire and Treseder 2010; Phillips et al. 2013; Soudzilovskaia et al. 2015b). Moreover, the susceptibility of the soil carbon stock to future environmental changes, such as induced by increased temperatures and drought, might be mediated by the predominant type of mycorrhiza (Creamer et al. 2015; Mohan et al. 2014; Peltoniemi et al. 2015).

Our progress in understanding global patterns of mycorrhizal impacts on biogeochemical processes, however, is seriously hindered by the lack of accurate data on geographical distribution patterns of the different mycorrhizal types. So far, the map of Read (1991) remains our best estimation of the distribution of plants with distinct types of mycorrhizal infections at the global scale. The map has a relatively low geographical accuracy as a result of expert estimations of plant distribution ranges rather than actual collection records of species occurrence and abundance. Neither does the map account for human-driven land use change, such as forest logging, urbanization and agricultural practices, the latter, for instance, having nearly entirely replaced mostly ectomycorrhizal temperate forests by arbuscular mycorrhizal crops across North America (Swaty et al. 2016) and Europe. Thus, in order to better understand the impacts of mycorrhizal fungi on terrestrial biogeochemical processes and ecology of terrestrial ecosystems, we urgently need accurate data on current distribution of mycorrhizal types.

Mycorrhizal research has a tendency to examine impacts of distinct mycorrhizal types on ecosystem functioning without specifying relative abundance of a given mycorrhizal type in an ecosystem examined. Likewise, the available data on mycorrhizal distributions describes distribution patterns of dominant plants possessing distinct mycorrhizal types. However, such division of vegetation into mycorrhizal types is potentially misleading, because the majority of the vegetation type host plants of several mycorrhizal types in different proportions (Akhmetzhanova et al. 2012; McGuire et al. 2008; Soudzilovskaia et al. 2015b), though some types are dominating (i.e. the above-mentioned boreal forests are dominated by ectomycorrhizal trees but typically have ericoid vegetation in the understory). Thus, all mycorrhizal types simultaneously affect biogeochemical cycling in such ecosystems, and their cumulative impacts differ depending on their relative abundances. Recently, Soudzilovskaia et al. (2015b) suggested to follow Grime's biomass-ratio theory (Grime 1998) in order to

obtain a comprehensive understanding of the effects of distinct mycorrhizal types on soil carbon and nutrient cycling. They suggest to take actual abundances of mycorrhizas in ecosystems into account and to develop methods that estimate the relationships between abundances and mycorrhizal impacts on ecosystem functioning and biogeochemical cycling. However, being a mutualistic relationship, mycorrhiza poses a challenge for quantification establishing patterns of geographical distribution. The abundance of a given mycorrhizal type in the soil is ultimately characterized by three parameters: (1) abundance of mycorrhizal fungi in soil (extraradical mycorrhizal fungal mycelium); (2) abundance of mycorrhizal fungi in plant roots, i.e. mycorrhizal colonization level of plant roots; and (3) root abundance of mycorrhizal host plants. For the latter two parameters, ecological science has accumulated reasonable data on global distribution patterns and knowledge on mechanisms controlling these patterns (Iversen et al. 2017; Soudzilovskaia et al. 2015b). Given that the relations between these three parameters are virtually unknown, the data for each of the parameters is incomplete, and thus global patterns of abundance of mycorrhizal fungal extraradical mycelia in the soil are poorly understood. Therefore the entire picture of global patterns of mycorrhizal distributions remains unclear. Here we review the current knowledge with respect to parameters that determine global mycorrhizal distribution patterns and provide recommendations to improve our understanding of mycorrhizal biogeography and its environmental drivers.

11.2 Distribution Patterns of Extraradical Mycorrhizal Fungal Abundance

Global patterns of fungal richness, including that of mycorrhizal fungi, are relatively well understood (Davison et al. 2015; Öpik et al. 2013; Tedersoo et al. 2014). This understanding is based on global datasets (such as Öpik et al. 2006; Tedersoo et al. 2012), which hold information on the genetic diversity of mycorrhizal fungi. In contrast, our knowledge about the actual biomass of extraradical mycorrhizal fungal mycelium in soils of distinct biomes is limited, and our knowledge on factors that control it is premature. Probably this is related to the fact that examination of abundance of fungal mycelium in soil is labour- and time-consuming, especially so for ectomycorrhizal fungi [see Leake et al. (2004) for method review]. In view of the absence of such data at global and regional scale, and following the widely accepted biomass-ratio concept (Grime 1998), current investigations typically presume that biomass of mycorrhizal mycelium of a given mycorrhizal type in the soil is roughly proportional to biomass of host plants. Recent works of Finer et al. (2011a) and Mariotte (2014), however, have shown that plant species' aboveground biomass at stand level is not a good predictor for species' fine root biomass, where mycorrhizal colonization mostly takes place. Finer et al. (2011a) have shown that at a level of an individual tree species, fine root biomass correlates with a tree basal area. This discrepancy is probably due to the significant amount of fine roots formed by understory vegetation. Also, fungal species richness patterns do not always follow

patterns of plant species richness, especially so for ectomycorrhizal plants and fungi (Tedersoo et al. 2014). How these discrepancies are reflected in the relationship between aboveground biomass of mycorrhizal plants and biomass of extraradical mycorrhizal fungal mycelium is yet unclear.

Finally, individual species of mycorrhizal fungi of a given type differ in morphology and physiology, with subsequent effects on plant nutrition and ecosystem functioning processes. These interspecific differences are especially known to be strong among EM fungi (Bodeker et al. 2014; Clemmensen et al. 2015; Falconer et al. 2007; Hobbie and Agerer 2010; Hobbie et al. 2013; Koide et al. 2014; Koide and Malcolm 2009; Martin et al. 2008; Rineau et al. 2012), although AM fungi have been shown to have different traits as well (Hart and Reader 2002; Sikes et al. 2010; Veresoglou and Rillig 2012). This suggests that for certain types of assessments on the role of mycorrhizas in ecosystem functioning, data on the distribution of particular mycorrhizal fungi might be of greater importance than that of the distribution of the entire mycorrhizal type.

First attempts to examine AM fungal species distribution and their environmental drivers have been done recently (Chap. 7). Bouffaud et al. (2016) showed that soil properties (textural characteristics, pH, organic C, total N, Mg and Na content), land use intensity and seasonal variability explain ca 25% of variation in AM fungal distribution. At the same time, these authors point out that stochastic events such as population dynamics can be a stronger driver of AM fungal community composition than environmental factors (Bouffaud et al. 2016). Analyses of fungal distribution in extreme conditions of polar areas suggest, however, that in harsh environments, soil and climate conditions might be stronger drivers of fungal distribution than dispersal and population dynamics (Cox et al. 2016). Using high-throughput sequencing, Cox et al. (2016) showed that a large number of fungal species found in Antarctica and Arctic have a bipolar distribution, indicating a prevailing role of environmental filtering in assemblages of fungal community in polar environments. Assembly of databases containing information on the geographical distribution of biomass of extraradical mycorrhizal fungal mycelium of each mycorrhizal type, as well as biomass of individual mycorrhizal fungal species, linking species to traits, and analyses of environmental factors that underpin these distribution patterns, is an important topic for future research.

11.3 Distribution Patterns of Plant Root Colonization Intensity by Mycorrhizal Fungi

Intensity of root colonization by mycorrhizal fungi is among the best available quantitative measures of the intimate relationship between plants and fungi and therefore serves as proxy of the fungal role into the above-belowground processes (Treseder 2013). The extent to which roots of particular plant species are colonized by mycorrhizal fungi depends on species identity as well as on environmental conditions, i.e. for a given plant species, the extent of its root colonization under natural conditions

constitutes a variable plant trait (Soudzilovskaia et al. 2015b). Global patterns of plant root colonization intensity by arbuscular mycorrhizal fungi are presented in the work of Treseder and Cross (2006). These authors found that the mean percentage of plant root length colonized by mycorrhizal fungi ranged from 22.6% of the total plant root length in temperate forests to 66.3% in savannas. For EcM and ErM, such analyses have not been conducted yet. Notably, Treseder and Cross (2006) were the first to simultaneously analyse root colonization intensity and fine root length, providing the first estimates of actual biomass of AM fungi in roots for the major Earth biomes.

The role of environmental drivers (soil and climate) as factors underpinning global patterns of AM and EcM fungal root colonization has been recently assessed by Soudzilovskaia et al. (2015a). The environmental conditions explained ca. 50% of variation in plant root colonization by mycorrhizal fungi, with temperature, seasonality and soil fertility (C/N ratio) being the main drivers of arbuscular fungal colonization intensity and pH and soil C/N controlling EcM colonization intensity. These relations, however, were non-linear (Fig. 11.1) and suggest an optimum response of the environmental control over intensity of mycorrhizal fungal colonization.

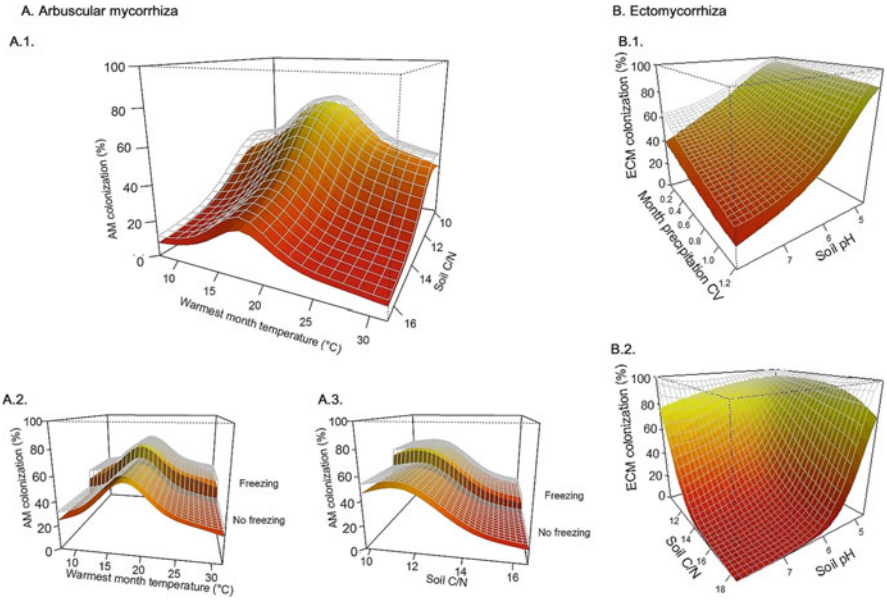

Fig. 11.1 Relationships between environmental predictors and site-averaged plant root colonization intensity by (**A**) arbuscular mycorrhizal fungi (AM, $R^2_{adj} = 0.49$, $n = 233$ sites) and (**B**) ectomycorrhizal fungi (EM, $R^2_{adj} = 0.51$, $n = 92$ sites). (A.1.) Relationship between root colonization by AM fungi, mean temperature of warmest month and soil carbon-to-nitrogen ratio (soil C/N). (A.2.) Relationship between root colonization by AM fungi, mean temperature of warmest month and the presence of freezing period. (A.3.) Relationship between root colonization by AM fungi, soil carbon-to-nitrogen ratio (soil C/N) and the presence of freezing period. (B.1.) Relationships between root colonization intensity by EM fungi, seasonality in precipitation expressed as coefficient of variation (CV) of monthly precipitation and soil pH. (B.2.) Relationships between root colonization intensity by EM fungi, soil C/N and soil pH. Coloured surface represents predicted relationship; *grey mesh* indicates standard error of prediction. Modified from Soudzilovskaia et al. (2015a)

11.4 Distribution Patterns of Mycorrhizal Plant Roots

In order to assess geographical distribution patterns of mycorrhizal types in an ecosystem, we need data on distribution patterns of plants that host a given type of mycorrhiza, as well as on root traits of these plants (i.e. biomass and length of fine roots, where mycorrhizal colonization primarily takes place; Guo et al. 2008). Information on species-specific standing fine root biomass and length has been recently assembled into large databases of root traits FRED (Iversen et al. 2017) and more general plant traits TRY (Kattge et al. 2011). Besides, the data on per plant species fine root biomass are available through individual publications (Beyer et al. 2013; Birouste et al. 2012; Comas and Eissenstat 2004, 2009; Gu et al. 2014; McCormack et al. 2012; Pregitzer et al. 2002; Wang et al. 2006; Yuan and Chen 2010). Furthermore, solid data on fine root biomass exists at the biome level (Finer et al. 2011a, b; Jackson et al. 1997). Relationships between root biomass or length and their plastic responses to nutrient concentrations in the soil have been established (Chapman et al. 2012; Chen et al. 2013; Valverde-Barrantes et al. 2013), which may be used to refine database-derived estimates.

Obtaining solid data on global distribution of plants featuring distinct mycorrhizal types remains a challenge. Estimations of global geographic patterns of mycorrhizal plant distribution with particular mycorrhizal types and their ecological drivers have been published in the preceding decades (Read 1991; Read and Perez-Moreno 2003). These analyses established that ectomycorrhizal and ericoid mycorrhizal plants dominate vegetation stands on nitrogen-poor, acidic soils at harsh, often cold and wet (EcM plants), climates, while AM plants are typically associated with nutrient-rich soils and warmer climates. Although these analyses qualitatively draw a generalized figure of mycorrhizal distributions and their drivers, they do not provide quantitative data on relationships between mycorrhizal types and climatic conditions and soil nutrient availability.

Quantitative data on abundance of plants featuring distinct mycorrhizal types is lacking at a global scale, although first attempts to get better data on distribution of mycorrhizal plants are forthcoming. For example, Swaty et al. (2016) and Fisher et al. (2016) have mapped vegetation of mycorrhizal types in the USA. Although these studies did not include ecological drivers, this study is the first attempt to describe mycorrhizal distribution patterns at continental scale. A quantitative approach with plant mycorrhizal types has been used by Menzel et al. (2016), who have targeted obligatory AM, facultative AM and non-mycorrhizal plants and their drivers at the regional scale (Germany).

Through decades of botanical and ecological surveys, plant species-per-plot data is available for a number of sites. Publicly available resources therefore include the Global Index of Vegetation-Plot Databases (GIVD, http://www.givd.info, Fig. 11.2) and, particularly for the Americas, the Botanical Information and Ecology Network (BIEN, http://bien.nceas.ucsb.edu/bien/about/). However, these data are limited to particular regions making extrapolations to a global scale at best questionable, if not impossible. The only currently available global source of data on plant occurrence is

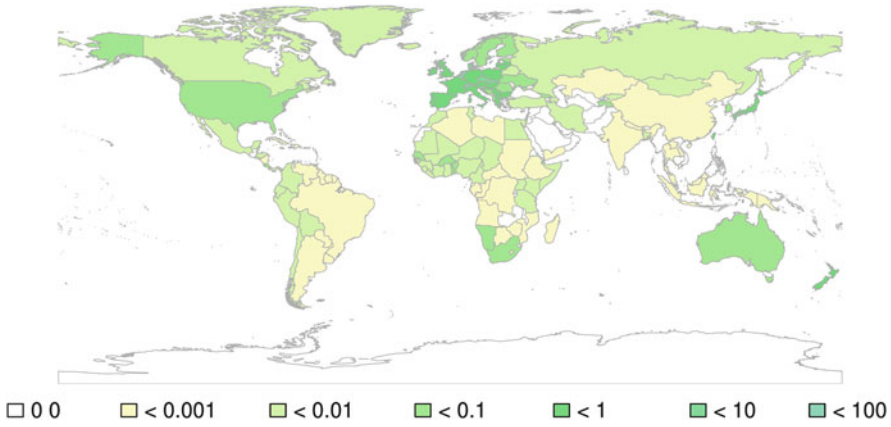

Fig. 11.2 Density of vegetation plots registered in GIVD database across the globe, measured as number of individual vegetation plots divided by country area in (km^2). Modified from Global Index of Vegetation-Plot Databases (http://www.givd.info/givd/faces/index.xhtml)

the Global Biodiversity Information Facility (GBIF, http://www.gbif.org), which provides loads of data for plant species occurrence across the globe. These data can be used to estimate the probability of occurrence for species per site using species distribution modelling techniques. The idea behind these techniques is that by knowing the environmental conditions at sites where a given species is known to occur, one can predict the probabilities of species occurrence in other sites across gradients of environmental conditions in the region of interest (Beaumont et al. 2005; Guisan and Zimmermann 2000).

Using GBIF data and an exhaustive literature search, we conducted an analysis aimed to estimate availability of data on global distribution of plants featuring arbuscular, ecto- and ericoid mycorrhizas. We combined the recently published lists of plant mycorrhizal associations (Akhmetzhanova et al. 2012; Harley and Harley 1987; Hempel et al. 2013; Soudzilovskaia et al. 2015a; Wang and Qiu 2006), removed duplications and performed an exhaustive search through the Web of Science and Google Scholar search engines for missing papers that contain data on plant associations with arbuscular, ecto- and ericoid mycorrhizas. Our dataset contains 9700 individual plant species assigned as AM, EcM, ErM or NM (non-mycorrhizal). We performed a taxonomical and synonymy check of the species names using The Plant List taxonomic engine (http://www.theplantlist.org/) and extracted from GBIF the data on geographical occurrences of species with accepted taxonomic names. We mapped the resulting dataset (AM records, 46.3 million observations, 6337 species; EcM records, 3.3 million observations, 631 species; ErM records, 800,000 observations, 83 species; Fig. 11.3). A handful of plant species featuring mixed colonization by fungi of several mycorrhizal types were excluded from this analysis.

The maps (Fig. 11.3) show a strong sampling bias towards West Europe, the Americas and Australia and indicate large areas that are in need of assessments of

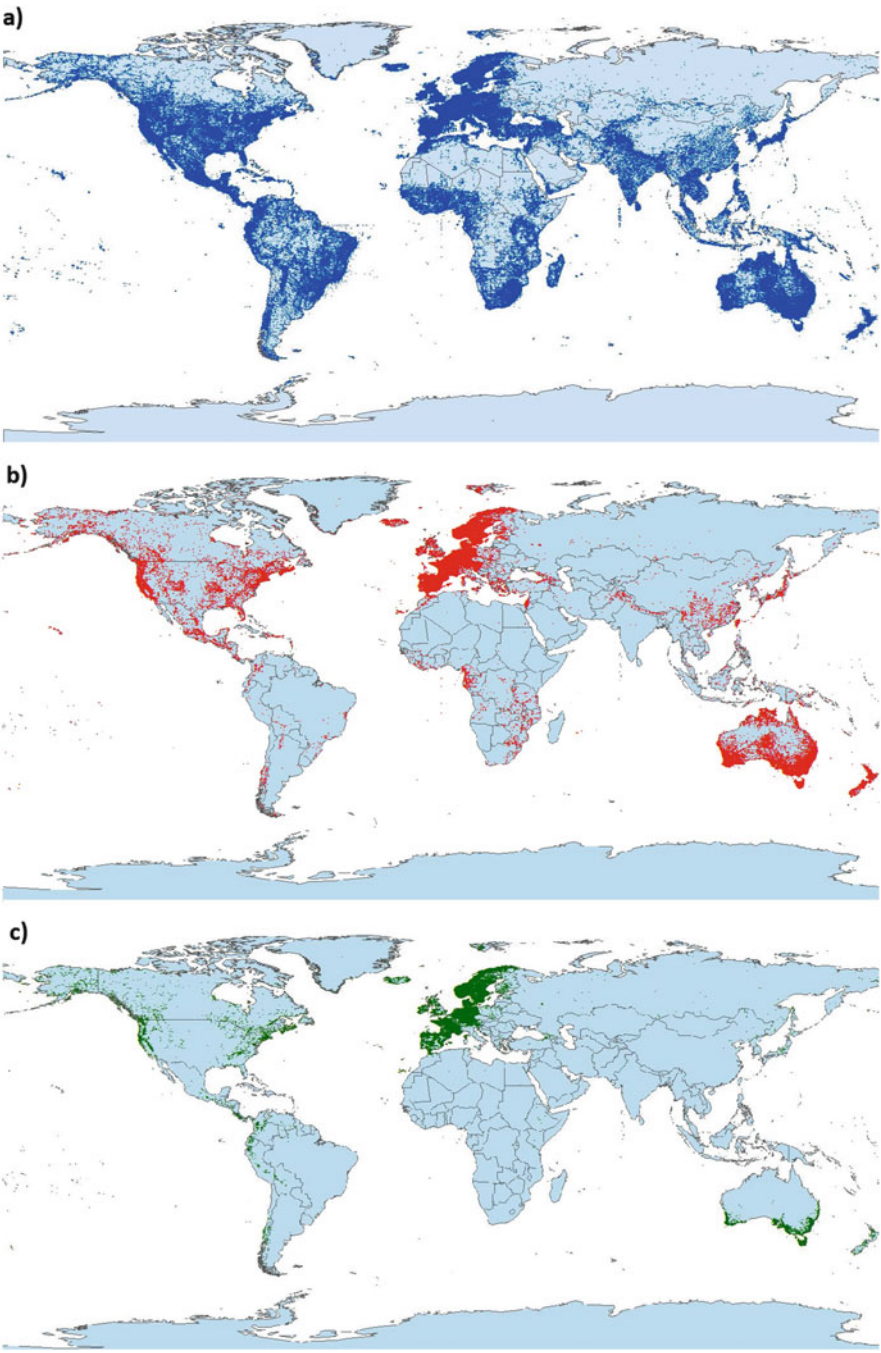

Fig. 11.3 Records of (**a**) AM, (**b**) EcM and (**c**) ErM plant species in GBIF (coloured dots)

plant distribution with different mycorrhizal associations (Russia, East Asian countries, Africa). Some countries in these areas have a rich botanical collection history (Russia, China, http://www.cvh.ac.cn/), as well as vegetation and forestry mapping. Those, rather than GBIF records, should probably constitute a basis for our further assessments of mycorrhizal distributions in the regions. Within the well sampled areas, our maps (Fig. 11.3) generally confirm the paradigm of Read (1991) that temperate and boreal forests are dominated by ectomycorrhizal plant species, tundra and alpine zones by ericoid and some ectomycorrhizal plants and grasslands being dominated by arbuscular mycorrhizal plants. Asian and African tropical zones show mixtures of AM and EM plant species, while the South American tropics have only a few records of EcM plants. Given the wide taxonomic range of AM plant species—85% of all plant species are AM (Brundrett 2009)—those species occur across all terrestrial biomes of the Earth (Fig. 11.3a). It is important to realize, however, that our data do not show abundance (i.e. biomass) of plants, but only occurrences of plant species, and it suffers from limitations known for GBIF data (i.e. the data of plant occurrence is not exhaustive, but reflects sampling efforts of collectors; data of common plants might be underrepresented, as data collectors often look for rare species; easy-to-access areas are sampled better than the ones difficult to access). Taken together, this is the first attempt to assemble available data on mycorrhizal distribution, which opens further possibilities for detailed analyses on ecological drivers of distributions of AM, EcM and ErM species. Further research should aim to establish an explicit connection between plant-specific trait data on roots and on filling gaps in the global distribution of plant species and their abundance.

11.5 Synthesis

While qualitative and some quantitative data on the possible (i.e. given climate and environment) geographical distributions of mycorrhizal types are available, better understanding of mycorrhizal involvement in ecosystem functioning at regional and global scale requires further improvement of our quantitative data on mycorrhizal distribution. The distribution maps should account for human land use, especially the conversion from forest to agricultural use and urbanization. Decomposition of mycorrhizal abundance measure into three individual components, (1) extraradical fungal abundance, (2) intensity of plant root colonization by mycorrhizal fungi and (3) abundance of host plant fine roots, may constitute a useful basis to analyse, in which environmental drivers determine each of these components individually and thereby enhance our understanding of the ecology of mycorrhizas and their role in ecosystems. The nature of the relationships between these components of mycorrhizal abundance, however, has not yet been investigated, hindering our understanding of the patterns of mycorrhizal distributions across the globe and the environmental drivers that determine their

distributions. Currently available GBIF collections combined with global soil and climate data may provide a basis for analyses of distribution of mycorrhizal plant species and environmental drivers of those distribution patterns. However, these records should be used with a caution, because they represent species presence data but not species abundance and the records show sampling bias towards West Europe, the Americas and Australia. Future research should serve as basis to understand the role of mycorrhizas in ecosystem functioning and biogeochemical cycling at regional and global scale.

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Chapter 12
Biogeography and Ecology of Tulasnellaceae

Franz Oberwinkler, Darío Cruz, and Juan Pablo Suárez

12.1 Introduction

Schröter (1888) introduced the name *Tulasnella* in honour of the French physicians, botanists and mycologists Charles and Louis René Tulasne for heterobasidiomycetous fungi with unique meiosporangial morphology. The placement in the Heterobasidiomycetes was accepted by Rogers (1933), and later also by Donk (1972). In Talbot's conspectus of basidiomycetes genera (Talbot 1973), the genus represented an order, the Tulasnellales, in the Holobasidiomycetidae, a view not accepted by Bandoni and Oberwinkler (1982). In molecular phylogenetic studies, Tulasnellaceae were included in Cantharellales (Hibbett and Thorn 2001), a position that was confirmed by following studies, e.g. Hibbett et al. (2007, 2014).

12.2 Systematics and Taxonomy

Most tulasnelloid fungi produce basidiomata on wood, predominantly on the underside of fallen logs and twigs. Reports on these collections are mostly published in local floras, mycofloristic listings, or partial monographic treatments.

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Unfortunately, the ecological relevance of *Tulasnella* fruiting on variously decayed wood or on bark of trees is not understood. It would appear plausible to assume that *Tulasnella* species are involved in wood decay, and that they may function in anamorphic stages as mycobionts in close by habitats. Therefore it seemed imperative to include in this overview of tulasnelloid mycobionts also reports on basidiomata.

Though some well developed *Tulasnella* species can be recognized in the field by the experienced mycologist with some certainty, correct identification of the genus was only possible microscopically in pre-molecular times. Most tulasnelloid fungi were sampled by collectors interested in corticiaceous fungi, Reports on these collections are mostly published in local floras, mycofloristic listings, or partial monographic treatments. Some of these publications are used to document biogeographical patterns on continental scales (Table 12.1). Because of considerable taxonomic difficulties and inaccuracies in traditional microscopic identification of *Tulasnella* morphospecies, they cannot be used for an attempt to disentangle their distribution areas. However, molecular data may help to overcome this bottleneck.

In several *Tulasnella* species the hymenial surface has a rosy to faintly violaceous tint (Fig. 12.1). Basidiomata consist of a few basal hyphae with or without clamps. Normally a simple but rarely considerably thickened hymenium is developed. Subhymenial structures may be lacking, and consequently single generative hyphae produce meiosporangia. Such growth forms or developmental stages cannot be detected in the field. These are only detected microscopically by chance, growing on the surface of other fungi, especially their hymenia. The growth can be intrahymenial, e.g. in *T. inclusa* (*Gloeotulasnella i.*, Christiansen 1959), or, rather exotically, parasitising on amoebae (*T. zooctonica*, Drechsler 1969).

The anamorphic stage of *Tulasnella* has been named *Epulorhiza* (Moore 1987), and it has been often used in mycorrhiza studies. Since the concept "One fungus = one name" was implemented at the International Botanical Congress XVIII, Melbourne, July 2011 (McNeill and Turland 2011; McNeil et al. 2012), the name *Epulorhiza* became synonymous. Nevertheless, articles dealing with *Epulorhiza* are included in our review, even when it appears uncertain in several cases, whether or not *Tulasnella* is involved. For the reason of taxonomic clarity in the following text, a short comment on the *Ceratobasidium-Rhizoctonia* complex is included here. In various treatments, the formal taxonomy of the so-called "form genus *Rhizoctonia*" has been dealt with (e.g. González Garcia et al. 2006; Yang and Li 2012). As pointed out by Oberwinkler et al. (2013), the name *Ceratobasidium* can only be applied for *Ceratobasidium calosporum* and the genera *Koleroga*, *Oncobasidium*, *Uthatobasidium*, and *Ypsilonidium* have to be put under synonymy of *Rhizoctonia*. The latter one has priority over *Thanatephorus*. Unfortunately, these taxonomic re-arrangements were widely ignored in a recent paper by Gónzalez et al. (2016).

Micromorphological characteristics of *Tulasnella* species include unique basidia with strongly swollen sterigmata (Fig. 12.1), also called epibasidia, which is a misleading term. After meiosis in the basidium, haploid nuclei and the basidial cytoplasm migrate through the sterigmata into the terminally developing basidiospores. In the basal position, the sterigmata become secondarily septate. Apically

Table 12.1 Compilation of perfect stages of Tulasnellaceae species, arranged according to Fig. 12.2

Regions		Europe					Asia		Af	America			Pac	Aus
Subdivisions		N	W	C	E	S	te	tr		N	C	S		
Species	**Spores**													
T. eichleriana	Globose–elliptical	●	●	●	●	●	●				●	●		●
T. violea		●	●	●			●					●		
T. zooctonia										●	●			
T. cystidiophora		●	●	●										
T. pacifica													●	
T. bourdotii			●	●										
T. subglobispora		●		●										
T. hyalina			●	●										
Pseudotulasnella guatemalensis											●			
T. guttulata														
T. traumatica			●							●				
T. conidiata			●							●				
T. valentini	Oblong–elliptical			●										
Stilbotulasnella conidiophora													●	
T. albida		●	●	●	●	●								
T. pinicola			●	●						●				
T. thelephorea		●	●	●	●	●								
T. asymmetrica														●
T. pruinosa		●	●	●		●								
T. dissitispora	Phaseoli-form-subcylin-drical		●											
T. tomaculum		●	●	●	●	●					●	●		
T. andina												●		
T. irregularis														●
T. fuscoviolacea				●	●	●								
T. rubropallens			●	●		●							●	
T. griseorubella		●		●										
T. bifrons			●							●				
T. robusta												●		
T. cruciata				●										●
T. kirschneri							●							
T. pallidocremea		●												
T. balearica	Sigmoid					●								
T. deliquescens		●		●										
T. quasiflorens		●												
T. curvispora	Allantoid			●										
T. permacra			●											
T. allantospora		●	●	●						●		●		●
T. danica			●	●						●				
T. saveloides		●	●		●									
T. aggregata										●				
T. anguifera	Spiral		●											
T. interrogans			●	●										
T. falcifera			●											
T. helicospora			●	●								●		
T. calospora	Fusiform-subfusi-form		●	●	●	●			●	●		●		●
T. eremophila									●					
T. kongoensis									●					
T. brinkmannii				●										
T. pallida		●	●	●	●	●								
T. echinospora		●	●				●							

● records arranged geographically. *C* central, *E* east, *N* north, *S* south, *te* temperate, *tr* tropical, *W* west. Literature: **Europe:** Bresadola (1903), Bourdot and Galzin (1927), Pearson (1928), Strid (1975), Torkelsen (1977), Hjortstam (1978), Wojewoda (1978, 1983, 1986), Hauerslev (1989), Roberts (1992, 1993a, b, 1994a, b, 1996, 1999, 2003), Dueñas (1996, 2001, 2005), Van de Put and Antonissen (1996), Roberts and Piątek (2004), Ordynets (2012), Kunttu et al. (2015), Polemis et al. (2016). **Asia:** Doğan and Kurt (2016). **Africa:** Crous et al. (2015). **North America:** Rogers (1933), Olive (1946). **Central America:** Roberts (2006). **South America:** Martin (1939), Lopez (1987), Greslebin and Rajchenberg (2001), Cruz et al. (2011, 2014, 2016), Nouhra et al. (2013). **Pacific area:** Olive (1957), Bandoni and Oberwinkler (1982). **Australia:** Warcup and Talbot (1967, 1971, 1980). Orig

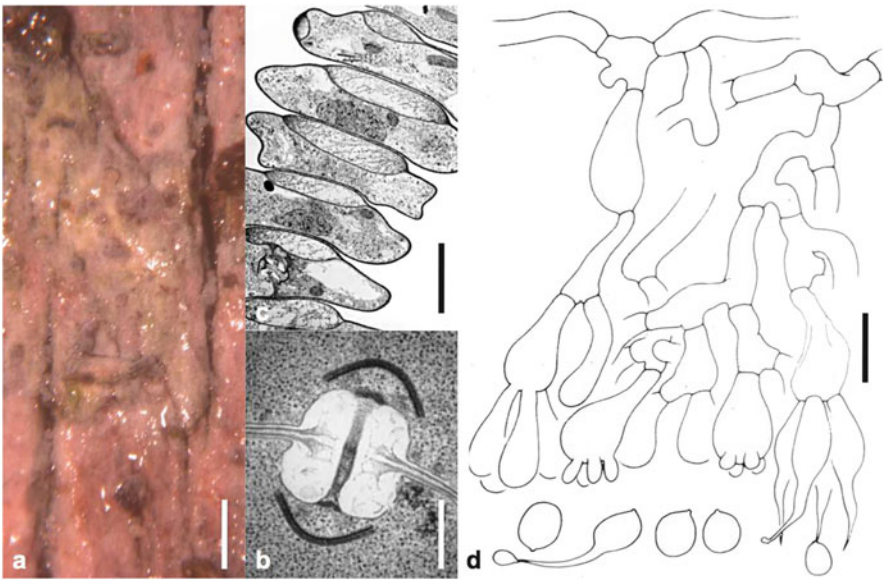

Fig. 12.1 *Tulasnella violea* (**a**, **d**) and *Tulasnella* spp. (**b**, **c**): (**a**) hymenial surface, bar 5 mm; (**b**) dolipore with continuous parenthesomes, bar 0.1 μm; (**c**) spirally growing hypha with cell wall extensions (*arrows*), bar 2 μm; (**d**) section through basidiome with basidia and basidiospores, one forming a secondary spore, bar 5 μm. From Oberwinkler (2012)

partly septate basidia have been reported for *Pseudotulasnella guatemalensis* (Lowy 1964). Basidiospores germinate by hyphae or secondary ballistospores. Dolipores with continuous parenthesomes are a constant ultrastructural feature in *Tulasnella* (Fig. 12.1). However, parenthesomes could not be found in dolipores of *Stilbotulasnella conidiophora* (Bandoni and Oberwinkler 1982). Other apparently unique ultrastructural features include cell wall expansions filled with amorphous matrix (Fig. 12.1). It is unknown whether this character is representative in all or most of *Tulasnella* species. Morphological and ultrastructural characters were indicative of a separate systematic position in former heterobasidiomycetous fungi, but precise phylogenetic position of *Tulasnella* within Basidiomycota remained unsettled.

There is a set of micromorphological characters in *Tulasnella* species, which appear to be applicable for circumscribing taxa. However, even in the case of very accurate microscopic work, there remains much uncertainty about the variability of structural features. This explains at least partly why reliable species identification is difficult and quite often questionable. This situation became strikingly evident, when molecular analyses showed that morphospecies were often not verifiable or included cryptic taxa (Taylor and McCormick 2008; Cruz et al. 2014). Whether the finding of Linde et al. (2013) in Australian orchid mycorrhizae, that an eight-locus analysis is broadly congruent with the solely ITS based result, can be generalized, remains questionable. For taxonomic details and nomenclature of *Tulasnella*

species we refer to Cruz et al. (2014, 2016). Table 12.1 provides an overview about the basic morphological features and distribution of Tulasnellaceae morphospecies.

12.3 Phylogenetic Position of *Tulasnella*

A sequence database for the identification of ectomycorrhizal basidiomycetes included also *Tulasnella* (Bruns et al. 1998). Tulasnelloid orchid associates clustered with good support within the cantharelloid clade. In an attempt to identify single pelotons of *Dactylorhiza majalis* using single-strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences, Kristiansen et al. (2001) found two taxa, *Tulasnella*, and a second one, distantly related to *Laccaria*. As sister of the *Tulasnella* cluster, *Sebacina* sp. was found, and both together appeared in a neighbour position to cantharelloid fungi. An expanded taxon set of basidiomycetes was used by Bidartondo et al. (2003) to resolve the phylogenetic placement of *Aneura* (*Cryptothallus*) associated fungi (see Sect. 12.5.1). They were phylogenetically well supported with *T. asymmetrica* as a sister taxon and *T. obscura* and *T. calospora* in the same clade. Similar results were obtained by Kottke et al. (2003), focusing on the mycobiont of *Aneura pinguis*, and Weiß et al. (2004) in an approach covering most of heterobasidiomycetous genera sequenced at that time. Resupinate homobasidiomycetes were analyzed molecularly by Binder et al. (2005), again fitting *Tulasnella* species to the cantharelloid clade but without substantial support. The results of Moncalvo et al. (2006) in analyzing the cantharelloid clade were also ambiguous concerning *Tulasnella* in nuc-rDNA and RPB2 together with mtSSU genes. Shimura et al. (2009) sequenced the Japanese *Cypripedium macranthos* mycobiont and found a weakly supported sister relationship to *Cantharellus* spp. and related taxa, including *Sistotrema* sp., in a very limited sampling. In a comprehensive analysis of publicly available sequences of Ceratobasidiaceae s.l. and related taxa, Veldre et al. (2013) included also some anamorphic tulasnelloid strains and *T. cystidiophora*. Both groups clustered in a sister relationship and were positioned in the Cantharellales. Also in the review on Agaricomycetes of Hibbett et al. (2014), the Tulasnellaceae are included in the Cantharellales.

12.4 The Presumable Age of *Tulasnella* and Evolution of Plant Associations

Taylor and Berbee (2006) dated Basidiomycota between 1489 and 452 Mya, the huge timespan resulting from the uncertainty in determining the age of the ascomycetous fossil *Paleopyrenomycites*. A maximum age of the evolutionary root in Marchantiophyta is calculated for 450 Mya by Clarke et al. (2011), 520–470 Mya

by Cooper et al. (2012), and 475 Mya by Sun et al. (2014). In a detailed time scale, Cooper et al. (2012) mark a divergence time of 100–50 Mya for *Aneura pinguis* and *A. mirabilis*. It may be concluded that *Tulasnella* mycobionts share the same age of their liverwort photobionts. The second calibration approach of Taylor and Berbee (2006) was used by Garnica et al. (2016) to determine divergence times in Sebacinales and other taxa of Basidiomycota. For Cantharellales they found 317–128 Mya with an average of 203 Mya. With some caution, a similar age interval may be adopted for Tulasnellaceae. Orchids originated approximately 100–80 Mya before present (Givnish et al. 2015), thus indicating a similar age of their mycobionts, including *Tulasnella*.

Yukawa et al. (2009) summarized the occurrence of ORM mycobionts in major clades of the Orchidaceae. Tulasnellaceae were reported from Apostasioideae, Vanillinae, Cypripedioideae, Disinae, Orchidinae, Goodyerinae, Prasophyllinae, Diuridinae, Caladeniinae, Neottieae, Dendrobiinae, Malaxideae, Calypsoeae, Pleurothallidinae, and Cymbidiinae.

12.5 Biotrophic Associations of *Tulasnella*

12.5.1 Tulasnella *Associated with Liverworts*

Liverwort mycobionts were examined in the course of an extensive study of biodiversity in a tropical cloud forest in South Ecuador (Kottke et al. 2003). *Aneura pinguis* was associated with *Tulasnella* species related to *T. asymmetrica* (Fig. 12.2), while Jungermanniales (*Lophozia* spp. and *Calypogeia muelleriana*) involved sebacinoid mycobionts. The same sequence group of *T. asymmetrica* (AY152406) was recovered in a study on the enigmatic hepatic *Aneura mirabilis* (as *Cryptothallus mirabilis*, Wickett and Goffinet 2008) mycobionts in Europe by Bidartondo et al. (2003). *Aneura mirabilis* is a mycoheterotrophic liverwort and specialized as an epiparasite on *Tulasnella* species that form ectomycorrhizae with surrounding trees like *Alnus glutinosa*, *Betula pubescens*, *Pinus pinaster*, *P. muricata* or *Salix aurita* and *S. cinerea* (Bidartondo et al. 2003). In a geographically strongly expanded study on liverwort-fungal symbioses, Bidartondo and Duckett (2010) reported Aneuraceae-associated *Tulasnella* from Europe, North and South America, East Asia and New Zealand.

Thallose European and Andean species of Aneuraceae (Metzgeriales) host *Tulasnella* mycobionts of high diversity especially in the European samples (Nebel et al. 2004; Pressel et al. 2010; Preußing et al. 2010). These interactions were considered by Krause et al. (2011) as a model of early evolved symbiotic associations. It is most likely that specific *Tulasnella* species occur together with the hosts throughout their distribution range.

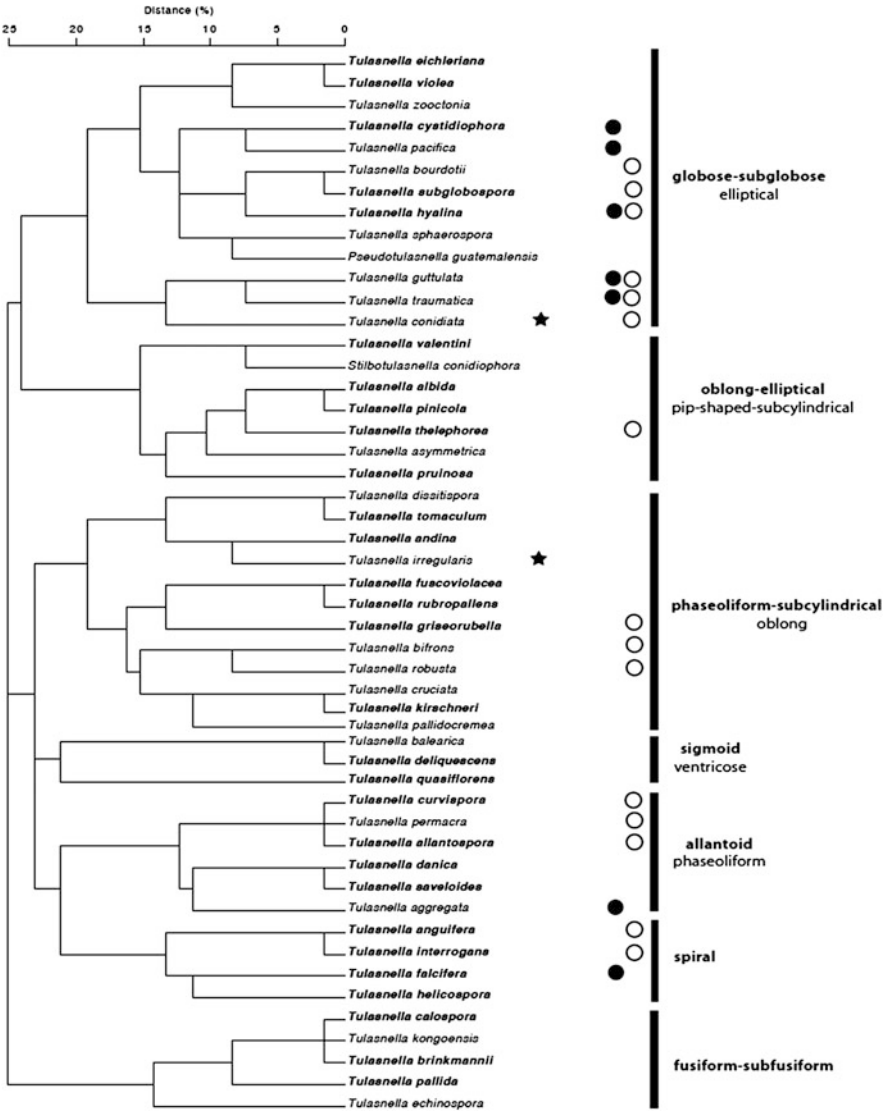

Fig. 12.2 Dendrogram of Tulasnellaceae species inferred by Jaccard analysis of all available structures from 48 taxa, including the new species *Tulasnella andina* and *T. kirschneri*. Names of species presented in detail by Cruz et al. (2016) are written in *bold*. Seven groups are defined, based on basidiospore morphology. Other characters are indicated by symbols: clamp connections (*unfilled circles*), cystidia (*filled circles*), chlamydospores (*filled stars*). From Cruz et al. (2016)

12.5.2 *Ectomycorrhiza (EcM)*

The ectomycorrhizal lifestyle in fungi, including *Tulasnella*, and dealing with diversity, distribution and evolution, was reviewed by Tedersoo et al. (2010). In a study on ectomycorrhizal liaisons between forest orchids and trees in the Bavarian northern Frankenalb, Bidartondo et al. (2004) mention *Tulasnella* and tulasnelloid fungi as "lineages that contain some ectomycorrhizal strains", however, without further explanation.

In a wet Tasmanian sclerophyll forest, Tedersoo et al. (2008a) report several unidentified *Tulansella* species associated with *Eucalyptus regnans* (Myrtaceae), *Nothofagus cunninghamii* (Nothofagaceae), and *Pomaderris apetala* (Rhamnaceae). The authors mention that *Tulasnella* is commonly observed in Tasmania but seldom recorded in the Northern Hemisphere as EcM mycobionts. This comment appears hardly probable for the real ECM occurrence of *Tulasnella*, but matches literature information at present. Nevertheless, when studying the community composition of *Picea abies* and *Betula pendula* seedlings in three Estonian old-growth forests, Tedersoo et al. (2008b) found that "ordination analyses suggested that decay type determined the composition of EcM fungal community in dead wood". In fact, in this study, *Tulasnella* EcMs were verified for the first time in the Northern Hemisphere besides the experimental synthesis study of Bidartondo et al. (2003).

12.5.3 Tulasnella *Orchid Mycorrhiza (OM)*

In seed germination experiments of orchids, Bernard (1899, 1909) and Burgeff (1909, 1932, 1936) detected the importance of fungal mycobionts during the early developmental stages. At that time, identification of the mycobionts was impossible. In addition, Burgeff (1932) treated the biology of symbiosis in tropical orchids extensively. After a review of OMs by Rasmussen (2002), Dearnaley (2007) updated new publications in this field. The trophic relationships in orchid mycorrhizae, including Tulasnellaceae, and their implications for conservation were summarized by Rasmussen and Rasmussen (2007). In a review on mutualistic, root-inhabiting fungi of orchids, Kottke and Suárez (2009) compiled also reports of tulasnelloid mycobionts, some of them associated with epiphytic tropical orchids. The complex of requirements of germination and seedling establishment in orchids, including tulasnelloid mycobionts, were comprehensively treated by Rasmussen et al. (2015). Suárez and Kottke (2016) summarized their overview on ORMs in tropical mountain forests in Ecuador that main fungal partners, including *Tulasnella*, correspond to findings in other biomes. Partial genome sequences of two *Tulasnella* mycobionts, originating from Australian *Chiloglottis* and *Drakaea* orchid species, may allow to obtain insight in evolutionary trends of tulasnelloid OM (Ruibal et al. 2013).

12.6 Biogeography of *Tulasnella*

12.6.1 Europe

Europe has the most abundant records of *Tulasnella* as fruit-bodies and in molecular identification events from plant roots (Fig. 12.3). Hadley (1970) reported no specificity of *Tulasnella calospora* in symbioses tests with European orchids, *Coeloglossum viride*, *Dactylorhiza purpurella*, *Goodyera repens* and the tropical *Cymbidium canaliculatum*, *Epidendrum radicans*, *Laeliocattleya* cv., *Spathoglottis plicata*, and considered it as a potential universal orchid symbiont. Dijk et al. (1997) stated that "*Epulorhiza repens* has been isolated from a vast amount of terrestrial orchids, and is considered a ubiquitous orchid endophyte". *Tulasnella* was the predominant mycobiont in 59 root samples of seven European and North American ***Cypripedium*** species (Shefferson et al. 2005). In addition, mycorrhizal specificity of 90 populations of 15 *Cypripedium* taxa across Europe, Asia, and North America was quantified by Shefferson et al. (2007). The orchids were associated almost exclusively with Tulasnellaceae mycobionts.

The mycobiont septal structure of native terrestrial French ***Dactylorhiza*** *majalis* (Strullu and Gourret 1974) and Italian *D. maculata*, *D. sambucina*, and *Platanthera bifolia* (Filipello Marchisio et al. 1985) was studied with the transmission electron microscope. They authors found dolipores with continuous parenthesomes, suggesting *Sebacina* and/or *Tulasnella* mycobionts, which were finally identified by Andersen (1990) as *T. deliquescens* and *T. calospora*, respectively. A remarkable experimental approach was carried out by Smreciu and Currah (1989), who studied symbiotic and asymbiotic germination of seeds of north temperate terrestrial orchids in Europe and

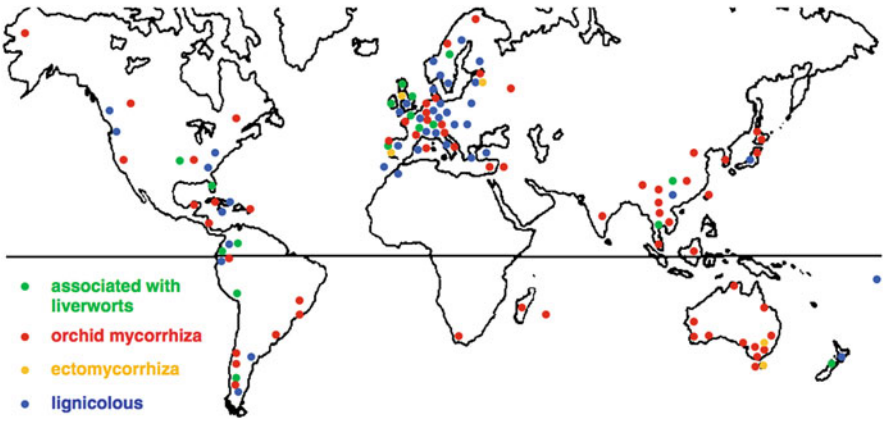

Fig. 12.3 Sampling localities for *Tulasnella* spp., extracted from literature. Tulasnelloid associates with liverworts are marked with *green dots*. Orchid mycorrhizae (*red dots*) summarize isolates of *Tulasnella* from orchid roots and molecularly identified samples. Tulasnelloid ectomycorrhizae are marked with *yellow dots*. Lignicolous (*blue dots*) means that basidiomata were collected on wood

North America. The European species included *Dactylorhiza maculata*, *D. sambucina*, *Epipactis palustris*, *E. purpurata*, *Gymnadenia conopsea*, *G. odoratissima*, *Neottia nidus-avis*, *Nigritella nigra*, and *Orchis morio*. It appears that mycobionts of these mostly widespread orchids were predominantly tulasnelloid fungi, except in *N. nidus-avis* and *E. purpurata*. Rasmussen and Rasmussen (1991) tried to identify experimentally the environmental conditions for germination and seedling development in *D. majalis* together with *T. calospora*. A stimulating effect of *Tulasnella* (*Epulorhiza repens*) and *Rhizoctonia* (*Ceratorhiza* sp.) on the growth of Dutch *Dactylorhiza* spp. and *Orchis morio* was reported by Dijk and Eck (1995). Single-strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences were used by Kristiansen et al. (2001) to identify *T. deliquescens* and *Laccaria* sp. as *D. majalis* mycobionts from single pelotons. Various fungal strains, isolated from non orchid sources were used to test symbiotic germination of British *D. fuchsii* (Salman et al. 2001). Besides *Ceratobasidium cornigerum*, also *T. helicospora* stimulated germination of the orchid seeds and promoted seedling growth. From a wetland of Bavaria, Bidartondo et al. (2004) reported *Tulasnella* as a mycobiont of *D. majalis*. Unidentified *Tulasnella* OM symbionts were found in *D. baltica*, *E. atrorubens*, and *O. militaris* in Estonian mine tailing hills and pristine sites (Shefferson et al. 2008). Most likely the seed germination experiments of the boreal-alpine *D. lapponica*, collected from the Solendet Nature Reserve in Central Norway, were enhanced by tulasnelloid mycobionts (Øien et al. 2008). In analyzing the mycobionts of five *Dactylorhiza* species in Belgium, Jacquemyn et al. (2012) concluded that orchid rarity is related to mycorrhizal specificity and fungal distribution. In an extensive study of 114 sampled individuals from one to three populations of 14 species of *Dactyorhiza* in Belgium, France, Italy, Portugal, Sweden and the United Kingdom, Jacquemyn et al. (2016b) suggested that habitat-driven variation occurs in mycorrhizal communities in which *Tulasnella* plays an essential role.

Tulasnelloid mycobionts of ***Epipactis*** *palustris* were reported from Northeast Bavarian wetlands (Bidartondo et al. 2004). Multiple independent colonization events of former lignite mining areas in Eastern Germany by *E. palustris* were documented by Esfeld et al. (2008) and observed in different rockgarden areas of Tuebingen Botanical Garden by the first author between 1975 and 1995 (unpubl). In a comparative study of *E. helleborine*, *E. neerlandica*, and *E. palustris* in Belgium, *Tulasnella* was only retrieved from the latter photobiont (Jacquemyn et al. 2016a). In ten North American and European ***Goodyera*** species, *Tulasnella* was only found in *G. pubescens* and *G. repens* in the USA (McCormick et al. 2004; Shefferson et al. 2010). In their study on carbon and nitrogen exchange in *Goodyera repens*, Liebel et al. (2015) found *Tulasnella* and *Ceratobasidium* as the most frequent mycobionts of the orchid species.

Fungi from the roots of the common terrestrial orchid ***Gymnadenia*** *conopsea* included typical ORMs of the Tulasnellaceae and Ceratobasidiaceae as well as several ectomycorrhizal taxa of the Pezizales (Stark et al. 2009). In this orchid, Těšitelová et al. (2013) found evidence that polyploidization can be associated with a shift in their tulasnelloid mycorrhizal symbionts. Among a variety of ascomycetous and

basidiomycetous associates of ***Himantoglossum*** *adriaticum*, Tulasnellaceae were identified in two protected areas of Central Italy (Pecoraro et al. 2013).

Liparis *loeselii* and ***Hammarbya*** *paludosa* are wetland specialists associated with tulasnelloid mycobionts in Hungary (Illyés 2011). In situ and in vitro germination of *L. loeselii* were studied by Illyés et al. (2005). They found *Tulasnella* (*Epulorhiza*) and *Ceratobasidium* (*Rhizoctonia*) as mycorrhizal partners. Broader samplings with *Dactylorhiza incarnata*, *Epipactis palustris*, *Gymnadenia conopsea*, *Ophrys oestrifera*, *Op. sphegodes*, and *Orchis militaris*, *Or. palustris*, and *Or. purpurea* indicated *Tulasnella* associations to prefer wetter habitats (Illyés et al. 2009), or to tolerate a wide spectrum of water availability (Illyés et al. 2010). Here, the question arises, what constrains the distribution of orchid populations (McCormick and Jacquemyn 2014), a question that should better be modified into what constrains the distribution of orchid-mycobiont associations. Recently Jacquemyn et al. (2015b) reported Tulasnellaceae in the roots and the soil of the green ***Neottia*** *ovata* (*Listera ovata*) in eastern Belgium. It is noteworthy to mention that tulasnelloid mycobionts have not been found in the achlorophyllous *N. nidus-avis* (e.g. Selosse et al. 2002).

The mycorrhizal fungal diversity of ***Orchis*** *militaris*, including tulasnelloid associates, detected in some Hungarian habitats, is considered to be essential for the wide ecological range of the orchid species (Ouanphanivanh et al. 2007). In a multidisciplinary approach of the simultaneously investigated mediterranean *Orchis simia*, *O. anthropophora*, and their hybrid *O.* × *bergonii*, Schatz et al. (2010) compared leaf growth, seed viability, emitted scent, and mycorrhizal species and their rate of infection. The mycobionts were unidentified *Tulasnella* species. Five *Orchis* species, *O. anthropophora*, *O. mascula*, *O. militaris*, *O. purpurea*, and *O. simia*, sampled from the Netherlands to Italy by Jacquemyn et al. (2010), contained a majority of *Tulasnella* mycobionts. In three closely related and hybridizing species, *O. anthropophora*, *O. militaris*, and *O. purpurea*, the influence of mycorrhizal associations on reproductive isolation of the orchids appeared to be of minor importance (Jacquemyn et al. (2011a). Girlanda et al. (2011) reported *Tulasnella calospora* mycobionts in the mediterranean meadow orchids ***Ophrys*** *fuciflora*, *Anacamptis laxiflora*, *O. purpurea*, and *Serapias vomeracea*. In a comprehensive survey of 16 European and Mediterranean *Orchis* species, Jacquemyn et al. (2011b) found dominating *Tulasnella* OMs from the Netherlands, Belgium, France, Portugal, Italy, Cyprus, and Israel. For the persistence and rarity of *A. morio* and *Dactylorhiza fuchsii* in Belgian habitats, Bailarote et al. (2012) suggested that fungal diversity with dominating *Tulasnella* are not necessarily related. Studies conducted in the Gargano National Park in southern Italy by Jacquemyn et al. (2014, 2015a) comprised ***Anacamptis*** *pyramidalis*, *A.* (*Orchis*) *morio*, *A. papilionacea*, *Neotinea maculata*, *N. ustulata*, *Orchis anthropophora*, *O. italica*, *O. pauciflora*, *O. provincialis*, *O. quadripunctata*, *Ophrys apulica*, *Op. biscutella*, *Op. bombyliflora*, *Op. sphegodes*, *Op. sicula*, *Op. tenthredinifera*, *Serapias bergonii*, *S. cordigera*, *S. lingua*, and *S. vomeracea*. The mycobionts of coexisting orchid species had distinct mycorrhizal communities and were predominantly recruited by *Tulasnella* and *Rhizoctonia* ("Ceratobasidiaceae"). A broad

spectrum of mycobionts, including *Tulasnella*, were found to be associated with *O. tridentata* in Central Italy by Pecoraro et al. (2012). The temporal variation in mycorrhizal diversity of *A. morio* from North Italian meadows was analysed by Ercole et al. (2014). The fungi, manually isolated from pelotons, were common *Tulasnella* in autumn and winter, the pezizacean clade very frequent in spring, and *Ceratobasidium* more frequent in summer. In 16 Mediterranean orchid species of the genera *Anacamptis*, *Ophrys*, *Orchis*, and *Serapias*, Pellegrino et al. (2014) found 18 operational taxonomic units (OTUs) of *Tulasnella* and "Ceratobasidiaceae". Mycobiont analyses of the mediterranean *Op. bertolonii* revealed *Tulasnella* as the dominant fungal partner (Pecoraro et al. 2015). The fine-scale spatial distribution of OM fungi, including *Tulasnella*, in soils of host-rich mediterranean grasslands of northern Italy was screened by Voyron et al. (2016) and found to be extremely sporadic. The spatially tight dependency of tulasnelloid associates of orchids was clearly documented in populations of *A. morio*, *Gymnadenia conopsea*, and *O. mascula* in Southern Belgium (Waud et al. 2016a). Also in Belgium, the majority of mycobionts of *O. mascula* and *O. purpurea* appeared to be *Tulasnella* (Waud et al. 2016b).

Bidartondo et al. (2004) reported *Tulasnella* as mycobiont of ***Platanthera*** *chlorantha* from the Bavarian Frankenalb. In a study on the evolution of endemic Azorean orchids, also ORMs were analyzed, and *T. calospora* and *Tulasnella* spp. were found in *Platanthera* species (Bateman et al. 2014). Kohout et al. (2013) studied the fungal communities associated with ***Pseudorchis*** *albida* in the Šumava National Park, Czech Republic. The mycobionts of the orchid were four unnamed *Tulasnella* strains. In protocorms of *P. albida*, also from this country, and in ***Serapias*** *parviflora* from Sardinia, *Tulasnella* spp. were detected by Stöckel et al. (2014). Protocorms of the mediterranean orchid *Serapias vomeracea* were colonized by *Tulasnella calospora* in an experimental study of Balestrini et al. (2014).

12.6.2 Temperate Asia

Whole rDNA analyses of roots and leaves of ***Bletilla*** *ochracea* from a mountain near Guiyang in Guizhou Province, China, provided a high number of fungal OTUs, dominated by ascomycetes (Tao et al. 2008). In addition, also *Epulorhiza* sp. could be identified. Eom (2012) isolated *T. calospora*, *T. irregularis*, and *Tulasnella* sp. from terrestrial Korean *Bletilla striata*, *Calanthe discolor*, *Cymbidium goeringii*, and *Pogonia minor*. Eom (2015) identified *T. calospora* and *Tulasnella* sp. in ***Cephalanthera*** *falcata*, *C. longibracteata*, ***Platanthera*** *chlorantha*, and *P. mandarinorum* in Korea. Jiang et al. (2011) isolated *Tulasnella* spp. from ***Changnienia*** *amoena*, an orchid distributed in various provinces of Central China.

Lee and You (2000) identified *Tulasnella repens* in the native Korean ***Cymbidium*** *goeringii*. Korean species of *Cymbidium* were successfully inoculated with *Tulasnella repens* by Lee et al. (2001). In a comparative study, Ogura-Tsujita et al. (2012) tried to find a correlation in mycobiont's association in *Cymbidium* during

the evolution of autotrophy to mycoheterotrophy. *Tulasnella* dominated in the autotrophic *C. dayanum*, were less frequent in mixotrophic *C. goeringii* and *C. lancifolium* and absent in mycoheterotrophic *C. macrorhizon* and *C. aberrans*. In five Korean terrestrial orchids, *C. goeringii*, *Spiranthes sinensis*, *Calanthe discolor*, *Bletilla striata*, and *Pogonia minor*, Youm et al. (2012) identified *Tulasnella calospora*, *T. irregularis*, *T. sp.*, and *Sebacina vermifera*.

The mycobiont of the threatened orchid ***Cypripedium*** *macranthos* var. *rebunense*, from Rebun Island northwest of Hokkaido was identified as *Tulasnella* (Shimura et al. 2009). Mycobionts of six endangered slipper orchid species from Southwestern China, ***Paphiopedilum*** *micranthum*, *P. armeniacum*, *P. dianthum*, *Cypripedium flavum*, *C. guttatum*, and *C. tibeticum*, were identified as *Tulasnella* spp. by Yuan et al. (2010). Hayakawa et al. (1999) isolated *Tulasnella deliquescens* from naturally occurring protocorms, seedlings, and adult Japanese *Dactylorhiza aristata*. Most of the OM fungi in ***Dendrobium*** *fimbriatum* and *D. officinale* from Guangxi were identified as members of the Tulasnellaceae by Xing et al. (2013). Tan et al. (2014) used their *Tulasnella* isolates of *D. officinale* from Yunnan to carry out seed germination experiments. They found different interactive capacities in two fungal strains.

As mycobionts of ***Epipactis*** *thunbergii*, Eom and Kim (2013) identified i. a. *T. calospora* and *Tulasnella* sp. *E. thunbergii* and *Habenaria radiata* were colonized by the ecologically adapted, associated with various mycobionts in manmade wetlands in the Hiroshima Prefecture, Japan (Cowden and Shefferson 2013). While a diverse suite of fungal symbionts was found in *H. radiata*, *E. palustris* was nearly exclusively inhabited by *Tulasnella* spp. Based on the morphology and cultures of isolates with anastomoses, Uetaka et al. (1999) identified *Epulorhiza repens* in the Japanese terrestrial orchids ***Gymnadenia*** *camtschatica*, ***Platanthera*** *tipuloides* and ***Pogonia*** *japonica*. In nine species of the genus ***Holcoglossum*** from Yunnan and Guangxi, *T. calospora* and the anamorphic tulasnelloid *Epulorhiza* were found (Tan et al. 2012). From different populations of ***Liparis*** *japonica* in Northeast China, Ding et al. (2014) identified fungi of the *T. calospora* species group. In situ and in vitro specificity between mycobionts and ***Spiranthes*** *sinensis* var. *amoena* was analyzed by Masuhara and Katsuya (1994). The germination was mainly induced by *Tulasnella* (as *Rhizoctonia repens*).

12.6.3 Subtropical and Tropical Asia

Apostasioideae are considered the basal group of the Orchidaceae (Chase et al. 2003). Five studied ***Apostasia*** species had *Botryobasidium* and *Ceratobasidium* mycobionts, and the related *Neuwiedia veratrifolia* was associated with *Ceratobasidium* and *Tulasnella* (Yukawa et al. 2009). Most of the mycobiont isolates of ***Neuwiedia*** *veratrifolia*, collected in Borneo, could be assigned to *Tulasnella* by Kristiansen et al. (2004).

The mycobiont of the "Chinese King Medicine Orchid", ***Anoectochilus*** *roxburghii*, was identified as *Epulorhiza* sp. and was successfully used in co-culture experiments to improve the growth of the host plant (Li et al. 2012). Dan et al. (2012) found that eight of 42 OM fungal strains tested including three *Epulorhiza* spp. enhanced the growth of the host plantlets. The endophyte promoting the growth and contents of kinsenosides and flavonoids of *A. formosanus* was identified as *Epulorhiza* sp. by Zhang et al. (2013). Likewise, in seven localities of Taiwan, Jiang et al. (2015) isolated mycobionts of this medicinally used orchid. No increase in orchid seed germination was found when *Tulasnella* strains were applied that clustered in clade III of their study. Mycobionts of the Chinese medicinal orchid ***Dendrobium*** *officinale* were identified as *Epulorhiza* sp. and inoculation of the fungus resulted in promoted seedling growth (Jin et al. 2009). For symbiotic seed germination of *D. draconis* and ***Grammatophyllum*** *speciosum*, native orchids of Thailand, the anamorph of *Tulasnella calospora* proved to be most effective to stimulate protocorm development (Nontachaiyapoom et al. 2011). In contrast, Salifah et al. (2011) found that seed germination rates in this orchid were best when co-cultured with *Fusarium* sp. Five *Tulasnella* isolates of four *Dendrobium* species from Chiang Rai Province of Thailand showed different promoting effects on seed germination (Swangmaneecharern et al. 2012). The in situ seed baiting of the epiphytic *D. aphyllum* from the Xishuangbanna tropical Botanical Garden in South Yunnan, studied by Zi et al. (2014), revealed *Tulasnella* spp. as mycobionts. In contrast, Agustini et al. (2016) isolated *Rhizoctonia*-like fungi from *D. lancifolium* var. *papuanum* and *Calanthe triplicata* from Papua, which was considered of "*Ceratobasidium*" relationship. Khamchatra et al. (2016a) isolated *T. violea* and *Epulorhiza repens* from the Thai epiphytic *D. friedricksianum*. Under in vitro culture conditions, Wang et al. (2016) found promoted *D. catenatum* seedling growth from Hainan with dual inoculation of *Epulorhiza* and *Enterobacter* or *Herbaspirillum* bacteria.

Commercially grown Thai species and hybrids of ***Cymbidium***, *Dendrobium*, and *Paphiopedilum* were used by Nontachaiyapoom et al. (2010) for isolation of mycobionts. They identified *Tulasnella* anamorphs. *Tulasnella* spp., isolated from wild and horticulturally grown *Cymbidium* spp. in SW-China, were used to test growth differences in co-cultures with *C. hybridum*, an important pot ornamental orchid (Zhao et al. 2014a). In addition, deep sequencing-based comparative transcriptional profiles of these photo- and mycobionts were carried out (Zhao et al. 2014b). The positive experiments were indicative for application in *Cymbidium*'s commercial cultivation. Mycobionts of *C. faberi*, *C. goeringii*, and *C. goeringii* var. *longibracteatum*, also from SW-China, included *Tulasnella* spp. (Huang and Zhang 2015). Yu et al. (2015) isolated and identified endophytes, and *Tulasnella* ORMs from roots of *C. goeringii* and *C. faberi*.

The germination and development of the terrestrial *Arundina chinensis*, ***Spathoglottis*** *pubescens*, and *Spiranthes hongkongensis* from various locations of Hong Kong were found to be strongly stimulated by *Epulorhiza* isolates (Shan et al. 2002). Isolated *E. repens* from the Thai terrestrial *S. plicata* enhanced seed germination in vitro considerably (Athipunyakom et al. 2004a). From this orchid species

of Papua, Sufaati et al. (2012) reported *Tulasnella* mycobionts. In a study on mycorrhizal associations and root morphology of 31 terrestrial and epiphytic orchids species of the Western Ghats, southern India, also *S. spicata* was included (Sathiyadash et al. 2012). Regarding the mycobionts, there is only the single remark that the orchids "had moniliform structures resembling those of *Tulasnella calospora* (*Epulorhiza repens*) in the cortical and root hair cells".

In the endangered epiphytic Thai slipper orchid ***Paphiopedilum*** *villosum*, *Tulasnella* sp. could be identified as mycobiont (Khamchatra et al. 2016b). A highly compatible *Epulorhiza* strain was used to demonstrate promotion of seed germination and protocrom development in ***Papilionanthe*** *teres* from Xishuangbanna, South China (Zhou and Gao 2016). In seed germination and seedling development of the Thai terrestrial orchid ***Pecteilis*** *susannae*, the incubation of *Tulasnella* enhanced growth considerably (Chutima et al. 2011). Isolates from the tropical orchids ***Arachnis*** sp., *Arundina graminifolia*, *Dendrobium crumenatum*, *Diplocaulobium enosmum*, *Oncidium* hybr., *Vanda* hybr., and *Spathoglottis plicata* in Singapore comprised both *Sebacina* and *Tulasnella* mycobionts (Ma et al. 2003). Mycobionts isolated from pelotons of ***Calanthe*** *rubens*, Ca. *rosea*, *Cymbidium sinense*, *Cy. tracyanum*, *Goodyera procera*, *Ludisia discolor*, *Paphiopedilum concolor*, *P. exul*, *P. godefroyae*, *P. niveum* and *P. villosum* were identified as *Epulorhiza calendulina*, *E. repens*, *and Tulasnella* sp. among multiple mycobionts (Athipunyakom et al. 2004b). Suryantini et al. (2015) reported on *Epulorhiza* and *Tulasnella* spp. associated with epiphytic Ca. *vestita* and *Bulbophyllum beccarii* from West Kalimantan. Seed germination of the epiphytic, therapeutically valuable orchid ***Coelogyne*** *nervosa*, endemic to south India, was higher when inoculated with *Epulorhiza* sp. (Sathiyadash et al. 2014).

12.6.4 North America

Rhizoctonia anaticula was described by Currah (in Currah et al. 1987), based on five isolates of native Alberta orchids, and later transferred into the tulasnelloid anamorphic genus *Epulorhiza* (Currah et al. 1990). The same mycobiont was also isolated from ***Calypso*** *bulbosa* and *Platanthera obtusata* sampled in various locations of Alberta (Currah and Sherburne 1992; Currah et al. 1988). The TEM micrographs indicate tulasnelloid fungi (Currah and Sherburne 1992). Smreciu and Currah (1989) recovered potentially high percentage of tulasnelloid mycobionts in symbiotic and asymbiotic germination of seeds of north temperate terrestrial orchids *Amerorchis rotundifolia*, Ca. *bulbosa*, *Coeloglossum viride*, *Corallorhiza maculata*, *Co. trifida*, *Cypripedium calceolus*, *Goodyera repens*, *Platanthera hyperborea*, *P. obtusata*, and *P. orbiculata*, four of them also occurring in Europe. So far, it remains unsettled what *Ceratobasidium cereale*, a mycobiont of *G. repens*, is (Peterson and Currah 1990). In germination experiments of *P. hyperborea* seeds, mycobionts of uncertain taxonomic position, like *Rhizoctonia cerealis* or *Ceratorhiza goodyerae-repentis*, were used (Richardson et al. 1992).

The orchid–mycobiont association was studied in detail in ***Goodyera*** *repens*, a terrestrial orchid of the eastern United States (McCormick et al. 2006). It was found that protocorms and adult orchids were able to switch with closely related *Tulasnella* fungi. In germination tests of seeds of *Goodyera discolor*, *Liparis liliifolia* and *Tipularia discolor*, McCormick et al. (2012) used fungal strains isolated from adult orchids and found that *Tulasnella* was involved in all cases.

Shefferson et al. (2005) detected *Tulasnella* spp. in root samples of ***Cypripedium*** *californicum*, *C. fasciculatum* and *C. montanum* in California; *C. candidum* and *C. parviflorum* in Illinois and Kentucky, *C. guttatum* in Alaska. Whitridge and Southworth (2005) reported Tulasnellaceae associated with *Cypripedium fasciculatum*, and with *Piperia* sp. One of the rarest North American terrestrial orchids, ***Piperia*** *yadonii*, showed non-specific ORMs, including Tulasnellaceae (Pandey et al. 2013). In ***Encyclia*** *tampensis* of South Florida, Zettler et al. (2013), reported *T. irregularis* as mycobiont and essential fungal partner during seed germination. The symbiotic germination of ***Spiranthes*** *lacera*, with a naturally occurring endophyte, *Ceratorhiza* cf. *goodyerae-repentis*, and with *Epulorhiza repens* was tested by Zelmer and Currah (1997). The orchid occurs in the eastern, northern and central parts of North America. The symbiotic germination of *S. brevilabris* showed *Epulorhiza* mycobionts, and the reintroduction of the endangered orchid, native to Florida, was discussed by Stewart et al. (2003).

In an integrated approach to *Rhizoctonia* taxonomy, Mordue et al. (1989) succeeded in taxonomically separating orchid isolates, i.e. tulasnelloid mycobionts from other *Rhizoctonia*-like fungi. A key and notes for the genera of fungi, mycorrhizal with orchids, and a new species in the genus *Epulorhiza*, was provided by Currah and Zelmer (1992). *Ceratorhiza pernacatena* and *Epulorhiza calendulina* were described as mycorrhizal fungi of terrestrial orchids in the Canadian prairies by Zelmer and Currah (1995), tulasnelloid mycobionts at least in one case. *Epulorhiza inquilina* was proposed for the mycobiont of the mature orchids ***Platanthera*** *clavellata*, *P. cristata* and *P. integrilabia* in Canada (Currah et al. 1997). For the propagation of the auto-pollinated terrestrial *P. clavellata* in the southern Appalachians, *Epulorhiza* spp. strains were applied in vitro by Zettler and Hofer (1998). In *P. praeclara* of midwestern prairies, *Epulorhiza* and *Ceratorhiza* were found and used in symbiotic seed germination and coinoculations by Sharma et al. (2003a, b). Also in the endangered Hawaiian endemic *Platanthera leucophaea*, *Epulorhiza* was found as mycobiont (Zettler et al. 2005).

Seeds of the endangered epiphytic orchid ***Epidendrum*** *nocturnum* from Florida were germinated in vitro with *Epulorhiza repens* (Massey and Zettler 2007; Zettler et al. 2007). Mycorrhized seedlings could successfully be reintroduced in the Florida Panther National Wildlife Refuge. Symbiotic seed germinations of three semi-aquatic orchids, ***Habenaria*** *macroceratitis*, *H. quniqueseta*, and *H. repens* from Florida had *Epulorhiza* mycobionts (Stewart and Zettler 2002). Later, in *H. macroceratitis*, Stewart and Kane (2006) isolated six *Epulorhiza* strains. *Epulorhiza* sp. was present in seed germination of *H. repens* in situ beyond its range in southern North America (Keel et al. 2011).

12.6.5 Central and South America

Unfortunately, in their study on basidiomycetous endophytes from the roots of epiphytic orchids in La Selva, Costa Rica, Richardson et al. (1993) use the generic names *Moniliopsis* and *Ceratorhiza* for the isolates. Though it is most likely that *Tulasnella* is included in these fungi, verification is impossible. Otero et al. (2002) isolated *Rhizoctonia*-like fungi inclusive of *Tulasnella* from orchids in Puerto Rico. They included the epiphytic species *Campylocentrum fasciola*, *C. filiforme*, *Ionopsis satyrioides*, *I. utricularioides*, *Psychilis monensis*, *Tolumnia variegata*, and the terrestial *Erythrodes plantaginea*, *Oeceoclades maculata*, and *Oncidium altissimum*. In Brazil, *Epulorhiza epiphytica* was isolated from mycorrhizal roots of epiphytic orchids and described as a new tulasnelloid anamorph by Pereira et al. (2003), and additional ORMs from neotropical orchids were characterized morphologically and molecularly by Pereira et al. (2005b), and for Laeliinae by Almeida et al. (2007).

Kottke et al. (2008) used sequence data of *Tulasnella* and other mycobionts to interprete fungal networks between diverse photobionts, including epiphytic orchids and Aneuraceae. Mosquera-Espinosa et al. (2010) studied 12 fungal isolates of eight Colombian orchids and reported *Ceratobasidium* spp. as mycobionts. However, a proper taxonomic identification was not achieved. Mycorrhizal networks with prominent *Tulasnella* OM mycobionts were considered to promote and stabilize the neotropical mountain rain forest (Kottke et al. 2013). Cruz et al. (2014) analyzed the variability of micromorphological features of basidiomata and the genomic polymorphism of *Tulasnella* ORMs in South Ecuadorian orchid species of the genera *Elleanthus*, *Maxillaria*, *Pleurothallis*, *Prostechea*, and *Stelis*. From five terrestrial orchids of Córdoba, Argentina, *Aa achalensis*, *Cyclopogon elatus*, *Habenaria hexaptera*, *Pelexia bonariensis*, and *Sacoila australis*, Fernández Di Pardo et al. (2015) isolated various mycobionts, including *Epulorhiza*. Suárez and Kottke (2016) summarized main mycobionts, including *Tulasnella*, and their specificities in neotropical orchids of South Ecuadorian rain forests. In an Andean cloud forest of South Ecuador, Suárez et al. (2006) found that diverse tulasnelloid fungi form mycorrhizae with epiphytic ***Pleurothallis*** *lilijae*, ***Stelis*** *concinna*, *S. hallii*, and *S. superbiens*. A study of Suárez et al. (2016) in Ecuador revealed that ***Teagueia*** spp. were associated with members of Tulasnellaceae, corresponding to four OTUs. All detected mycobionts had a wide geographical distribution.

Experiments for a symbiotic propagation to reintroduce endangered Mexican terrestrial ***Bletia*** *urbana*, *B. campanulata*, and *Dichromanthus aurantiacus* were carried out by Ortega-Larrocea and Rangel-Villafranco (2007), applying anamorphic *Tulasnella* strains. Ovando et al. (2005) isolated and screened endophytic fungi from the roots of the epiphytic orchids ***Brassavola*** *nodosa*, ***Cattleya*** *skinneri*, and *C. aurantiaca* from Tuzantán, South Mexico. The isolated strains were assigned to 11 fungal genera. Eight strains, used for germination experiments, did not show any promoting effects. However, three strains, including *Epulorhiza*, provided mycorrhizal characteristics in *C. aurantiaca*. A new tulasnelloid anamorph, *Epulorhiza*

amonilioides, lacking monilioid hyphae in pure culture, was isolated from *Brassavola* and ***Encyclia*** species and described by Almeida et al. (2014) from Bahia, Brazil. When analyzing three sympatric epiphytic Cymbidieae, ***Cyrtochilum*** *flexuosum*, *C. myanthum*, and *Maxillaria calantha* from two sites of South Ecuadorian mountain rain forests, Cevallos et al. (2016) concluded that these orchids have site-adjusted OM communities with keystone mycobionts, including *Tulasnella*. In testing seed germination and protocorm development of ***Cyrtopodium*** *glutiniferum* from Brazil, Pereira et al. (2015) found promotion by mycorrhizal fungi of the tulasnelloid anamorphs *Epulorhiza* spp. In roots of four *Vanilla* species from Puerto Rico, Costa Rica and Cuba, Porras-Alfaro and Bayman (2007) found mycobionts of *Ceratobasidium*, *Thanatephorus* and *Tulasnella*.

Epulorhiza spp. was isolated from various Brazilian ***Epidendrum*** species (Pereira 2009, Pereira et al. 2009, 2011a, b, 2014a). From the epiphytic *E. stamfordianum*, *Erycina crista-galli*, and *Stelis quadrifida* from Southeast Chiapas, Mexico, *Ceratorhiza* and *Epulorhiza* mycobionts were reported by Cruz Blasí (2007). Two different *Tulasnella* species were found to be associated with South Ecuadorian *E. rhopalostele*, an orchid preferably growing on dead trees (Riofrío et al. 2013). Populations of *E. firmum* in Costa Rica had highly diverse and spatially heterogeneous mycobionts, including six *Tulasnella* strains (Kartzinel et al. 2013). The mycobionts of *E. secundum*, a widespread Brazilian orchid, were identified as *Tulasnella* spp. by Pereira et al. (2014a) and as *T. calospora* by Nogueira et al. (2014). In vitro seed germination and protocorm development of Brazilian *Oncidium flexuosum* was studied with mycobionts of *Epulorhiza* and *Ceratorhiza*, earlier isolated from this orchid (Pereira et al. 2005a, c), and Da Silva Coelho et al. (2010) reported regeneration and production of the fungal protoplasts.

Epulorhiza epiphytica was isolated from ***Polystachya*** *concreta* and the African ***Oeceoclades*** *maculata*, naturalized in the Neotropics, by Pereira et al. (2005b). Nine unnamed morphotypes of fungi, associated with *O. maculata*, were isolated from the understory of Avocado in Brazil by Teixeira et al. (2015).

In the mycorrhizal association of the terrestrial Chilean orchid ***Bipinnula*** *fimbriata* also tulasnelloid ORMs were present (Steinfort et al. 2010). Mujica et al. (2016) found that mycorrhizal diversity, including *Tulasnella*, decreased in habitats of *B. fimbriata* and *B. plumosa* with higher N, but increased with P availability in *B. fimbriata*. Morphological and molecular characterization confirmed that Chilean ***Chloraea*** *collicensis* and *C. gavilu* mycorrhizal partners belong to *Tulasnella* (Pereira et al. 2014b). In contrast, Atala et al. (2015) reported mycobionts with possible *Thanatephorus* teleomorphs from the critically endangered Chilean *C. cuneata*. However, the data presented cannot exclude tulasnelloid associates. In a study by Herrera et al. (2016), in six *Chloraea* species and *Bipinnula fimbriata* from Chilean Coastal Range and Andes. *Tulasnella* spp. were found as dominating mycobionts. Fracchia et al. (2014) found promoted see germination through tulasnelloid and *Ceratobasidium*-like fungi in ***Gavilea*** *australis*, an endangered terrestrial orchid from south Patagonia.

12.6.6 Africa

Martos et al. (2012) identified a bipartite network including 73 orchid species and 95 taxonomic units of mycorrhizal fungi across the natural habitats of Reunion Island. 58 tulasnellaceous OTUs were found in 73 orchid species, thus representing the most frequent OM mycobionts. In their study on the evolution of endemic Azorean orchids, Bateman et al. (2014) reported also the mycorrhizal association of *Tulasnella* aff. *Calospora* with ***Platanthera*** *algeriensis* in Morocco. Most of the OM fungi of the Itremo region in the Central Highlands of Madagascar were identified as *Tulasnella* (Yokoya et al. 2015). The symbiotic seedling development of the terrestrial ***Cynorkis*** *purpurea*, also from the Itremo area, has been tested experimentally by Rafter et al. (2016). Though epiphyte-derived *Sebacina* cultures had the strongest influence, also *Tulasnella* appeared as an advantageous mycobiont. *Disa bracteata* of South Africa was associated with *Tulasnella* spp. in West and South Australia as in its country of origin (Bonnardeaux et al. 2007). In an attempt to elucidate the impact of above- and belowground mutalisms in South African orchid diversification, an irregular pattern of fungal associates, including 35, unspecified *Tulasnella* individuals, were detected (Waterman et al. 2011). The authors concluded that "shifts in fungal partner are important for coexistence but not for speciation" of the host plants.

12.6.7 Australia

When Warcup and Talbot (1967) succeeded to isolate and cultivate OM fungi from terrestrial Australian orchids, and finally obtained perfect states of Rhizoctonias, a new era of experimental mycology and especially of studies in symbiotic systems began. *Tulasnella calospora* was found to be the perfect state of three cultures considered to be *Rhizoctonia repens*. Isolates were obtained from South Australia (*Acianthus exsertus*, *Caladenia reticulata*, *Cymbidium canaliculatum*, *Dendrobium* sp., *Diuris longifolia*, *D. maculata*, and *Thelymitra antennifera*). *Tulasnella asymmetrica* was described as a new species and as mycobiont of *Thelymitra luteocilium* from the Australian Mt. Lofty Range. In a second contribution of the authors (Warcup and Talbot 1971), the description of *Tulasnella asymmetrica* was emended and further orchid hosts were reported from the Mt. Lofty Range: ***Thelymitra*** *aristata* (also Cape Jervis), *T. grandiflora*, and *T. pauciflora*. Additional hosts were *Th. epipactoides* (Eyre Peninsula), and *Dendrobium tetragonum* from North Queensland. The basidial stage of the morphotype of *T. allantospora* with clamps was obtained from Mt. Lofty isolates of *Corybas dilatatus*, and basidiocarp samples without clamps were collected on fallen *Eucalyptus* wood in the same locality. The perfect stage of *T. violea* developed from an isolate obtained from *Th. aristata*, collected in Uley, Eyre Peninsula. *Tulasnella cruciata* was introduced as new to science, isolated from the Mt. Lofty Range orchids *Acianthus caudatus* and *Th. pauciflora*, while the strain of *Th. fusco-lutea* originated

from Pomonal, Victoria. In the third joint effort of Warcup and Talbot (1980) to obtain perfect states of OM mycobionts they succeeded with *T. irregularis* sp. nov., isolated from ***Dendrobium*** *dicuphum*, sampled near Darwin, Northern Territory. In studying the specificity of ORMs in Australian terrestrial orchids, Warcup (1971) reported that *Th. aristata* is at least associated with three species of *Tulasnella*. In the "Orchids of South Australia" (Bates and Weber 1990), *T. calospora* is listed as mycobiont in orchid species of the genera *Acianthus*, *Diuris*, *Orthoceras*, and *Thelmytra*. For the latter one and *Acianthus*, also *T. cruciata* is mentioned. The symbiotic germination of some Australian terrestrial orchids was analyzed by Warcup (1973) who reported that various isolates of *T. calospora* differed markedly in the efficiency with which they stimulated germination of the ***Diuris*** and *Thelymitra* photobionts. A close association of this mycobiont with *Diuris* and *Orthoceras* orchids was confirmed by Warcup (1981). The mycorrhizal specificity of *D. fragrantissima* with *Tulasnella* spp. and persistence in a reintroduced population west of Melbourne was studied by Smith et al. (2007, 2010). In *D. magnifica* and *Prasophyllum giganteum*, *T. calospora* was found, and in *Pyrorchis nigricans* isolates *T. danica* were identified (Bonnardeaux et al. 2007).

A narrow group of monophyletic *Tulasnella* symbiont lineages is associated with multiple species of ***Chiloglottis*** in New South Wales and the Australian Capital Territory (Roche et al. 2010). For *Tulasnella* OM species delimitation in the Australian orchid genera *Chiloglottis*, *Drakaea*, *Paracaleana* and *Arthrochilus*, Linde et al. (2013) used six nuclear loci, two mitochondrial loci, the photo- and mycobiont association and sampling locations in an integrated approach. They found that the *Chiloglottis* isolates belong to one species, and those from *Drakaea* and *Paracaleana* to a sister taxon, a result in accordance with previous ITS analyses. Boddington and Dearnaley (2009) reported a putative mycorrhizal *Tulasnella*-like fungus in the tropical epiphytic *Dendrobium speciosum* of Queensland. In studies of ***Drakaea*** species in Southwest Australia, Phillips et al. (2011, 2014) found no evidence that *Tulasnella* specificity contributed to the rarity of the orchids.

According to Brundrett (2007), most West Australian orchids studied have highly specific mycorrhizal associations with fungi in the *Rhizoctonia* alliance, most likely including *Tulasnella* spp. The nutrient-acquisition patterns of ORMs, inclusive of *Tulasnella*, appear to explain the diversification in terrestrial orchids in this biodiversity hotspot (Nurfadilah et al. 2013).

Milligan and Williams (1988) obtained 27 tentatively identified *Tulasnella calospora* isolates from ***Microtis*** spp. at seven sites in the Sydney region. The specificity of associations between *M. parviflora* and *Epulorhiza* spp. was studied by Perkins et al. (1995). The compatibility webs of brief encounters, lasting relationships and alien invasions of West Australian terrestrial orchids were studied by Bonnardeaux et al. (2007), documenting that *M. media*, together with the invasive *Disa bracteata*, had the most ORMs. Mycorrhizal preference apparently promotes habitat invasion of *M. media* in Western Australia (De Long et al. 2013). When studying the effects of endophytic fungi on New Zealand terrestrial *M. unifolia*, *Spiranthes novae-zelandiae*, and *Thelymitra longifolia*, Frericks

(2014) obtained *Tulasnella calospora* isolations and compared them with strains of various geographical origins.

The rare subterranean, achlorophyllous orchid ***Rhizanthella*** *gardneri* from western Australia lives in a more than triple association with autotrophic and heterotrophic partners in which, apparently, two *Tulasnella* species are involved (Warcup 1985). In a taxonomic study and an experimental approach to grow *Rhizanthella gardneri* together with *Melaleuca scalena* (Myrtaceae), Bougoure et al. (2009a, b) used as mycobiont an unidentified, so-called "*Ceratobasidium*" with the positive result that 5% of carbon fed to *Melaleuca* as $^{13}CO_2$ was transferred to *R. gardneri*. Further studies are needed to clarify the taxonomy and whether diverse mycobionts are involved in this association.

12.7 Conclusions

Our literature search for *Tulasnella* on a global scale confirmed that distribution patterns are biased by sampling. Nevertheless, there is unequivocal documentation that *Tulasnella* as a group and certain morphological species have global distribution. Furthermore, it appears obvious that the world-wide distribution of orchids may reflect a similar occurrence of their mycobionts, for which *Tulasnella* species play a crucial role. The same may be true for *Tulasnella* associates of certain liverworts. In addition, lignicolous basidiomata of *Tulasnella* are reported from collecting areas of mycologists, interested in corticioid fungi. Apart from these restrictions, a more adequate interpretation of *Tulasnella*'s biogeography is the distribution pattern of suited habitats which appear to occur in a nearly world-wide range.

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Chapter 13
Biogeography of the Ectomycorrhizal Mushroom Genus *Laccaria*

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13.1 Introduction

The patterns and processes that describe the biogeography of ectomycorrhizal (EcM) fungi are as complex as they are diverse. From the delimitation of species, through identification of genetic barriers that circumscribe population boundaries and define distributions, to the ecological factors that shape these boundaries, mycologists have to accumulate evidence from a variety of sources in order to describe the biogeographic and evolutionary history of EcM fungi. The EcM genus *Laccaria* has been a model for understanding EcM fungal ecology. As a result, it is one group where the accumulated evidence can be used to provide insights into its complicated biogeographic history (Fig. 13.1).

Even though *Laccaria* has long been a model for understanding the biology of ectomycorrhizal symbiosis, it is significant that the forces responsible for intercontinental distributions shaping *Laccaria's* diversity have not been clearly identified. In many ways, the accumulated data on *Laccaria* indicates how far understanding of EcM ecology has come, but also how much more there is to learn.

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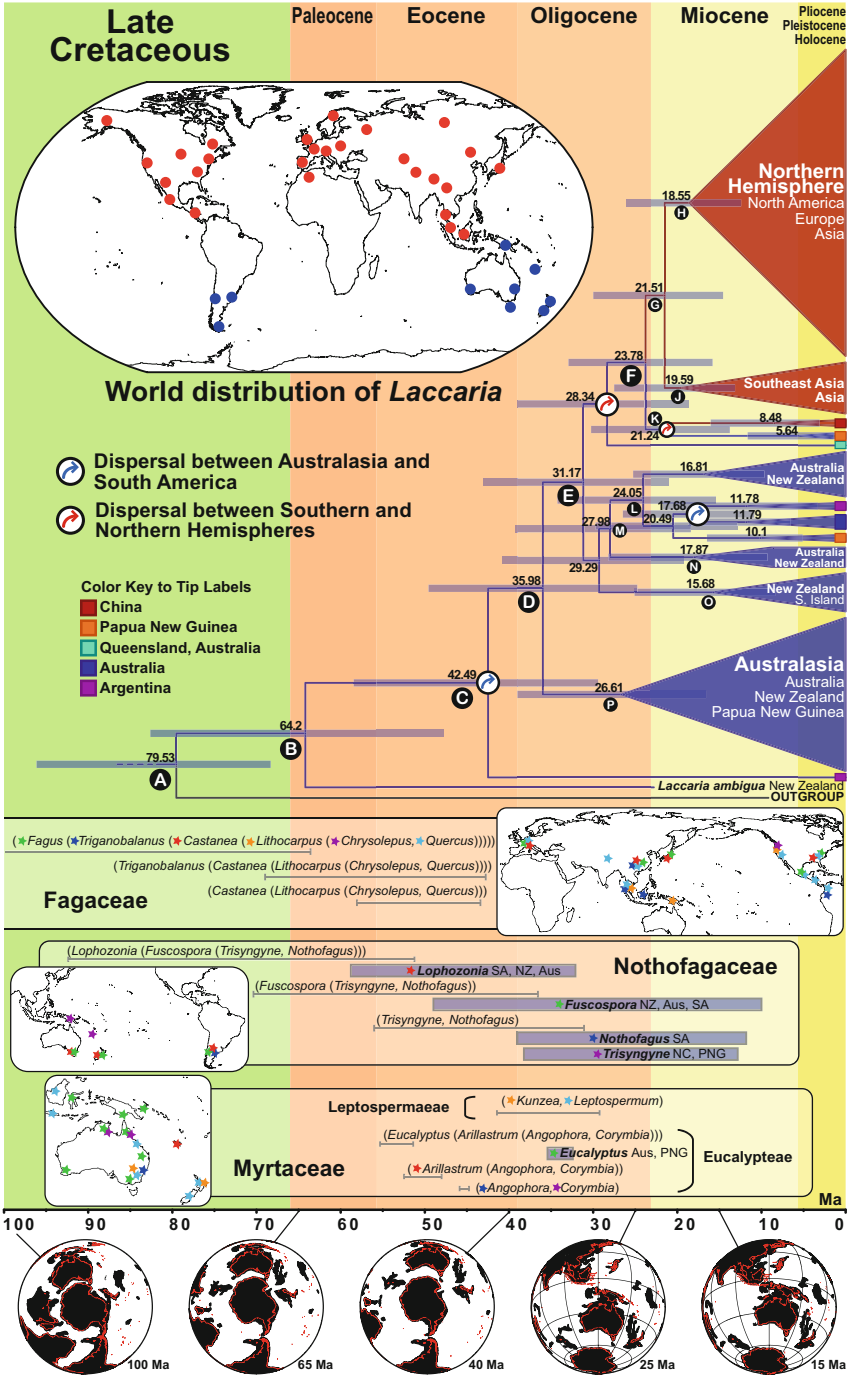

Fig. 13.1 Global *Laccaria* biogeography. World map presents the locations where *Laccaria* has been collected. Collapsed phylogeny is modified from Wilson et al. (2016), and describes the

In this chapter *Laccaria* is treated as inclusive of the sequestrate (truffle-like) genera *Hydnangium* and *Podohydnangium*. These genera nest well within the clade comprising the agaricoid members of the genus in phylogenetic studies, with multiple origins of the sequestrate form (Sheedy et al. 2013, 2015; Wilson et al. 2017). However, formal transfers have not been made pending conservation of the later name *Laccaria* over the earlier name *Hydnangium*.

The goal of this chapter is to review the science relevant to understanding the patterns and processes shaping the known distribution of *Laccaria*. In doing so, this chapter will present the current ecological and evolutionary evidence relevant to the biogeography of *Laccaria*. It will also suggest the direction that research must continue in order to complete this evolutionary picture. The next section of this chapter reviews studies that have helped describe *Laccaria* diversity and highlights best practices for delimitation of *Laccaria* species.

In the third section, *Laccaria* biogeography is explored from the micro-evolutionary perspective in relation to population genetics and life history. Several of the most important population genetic studies of Agaricomycetes have been performed on species of *Laccaria*. These studies not only help us understand the extent and direction of gene flow in mushroom-forming fungi, but also present a starting point from which we can begin to explore the ecological factors that divide EcM populations and promote speciation.

The fourth section is a discussion and review of how species of *Laccaria* engage in EcM associations. Like all agaricomycete EcM lineages, *Laccaria* evolved the capacity to associate with plant hosts from its non-EcM ancestors. As a result it has developed its own particular form of EcM symbiosis that has been characterized in numerous ecological studies. These behaviors are reviewed with an interest in understanding their potential role in the distribution of *Laccaria*.

The fifth section reviews the biogeographic studies of known *Laccaria* EcM host families. Understanding the history of EcM plant hosts is critical to understanding the evolutionary history of an obligate EcM fungal symbiont.

The last three sections deal with the phylogenetic diversity of *Laccaria* in a global context. This expands upon Wilson et al. (2017), which describes *Laccaria's* Southern Hemisphere origins (Australasia and temperate South America), and attempts to contextualize this distribution with regard to its known EcM hosts.

Fig. 13.1 (continued) systematic distributions of *Laccaria* taxa along with the ages of nodes. For both map and clades, *red* identifies Northern Hemisphere, and *blue* identifies Southern Hemisphere *Laccaria*. Arrows at nodes signify transitions between continental landmasses (*blue* = South America and Australasia) or between hemispheres (*red* = between Southern Hemisphere and Northern Hemisphere). Gray bars in EcM host families represent the most recent common ancestor for multiple genera, while *blue* rectangles are the most recent common ancestor of individual genera. Divergence times were taken from Sauquet et al. (2012) (Nothofagaceae and Fagaceae), and Thornhill et al. (2015) (Myrtaceae). Maps along the bottom assess potential dispersal routes of *Laccaria* taxa and their hosts by estimating the proximity of post-breakup Gondwanan landmasses and proximity of Southeast Asian and Australasian landmasses at specific ages

This is followed by a hypothesis that explains the dispersal of *Laccaria* from the Southern to the Northern Hemisphere. Lastly, the Northern Hemisphere is discussed with regard to its diversity and distribution of *Laccaria* taxa. The chapter concludes with a discussion on how future studies can provide further understanding of the processes that drive the diversification, dispersal, and distribution of *Laccaria*.

13.2 *Laccaria* Diversity and Species Delimitation

Delimiting *Laccaria's* species is a key step to understanding its biogeographic history. While certain *Laccaria* species are easily differentiated from others, there are many species that overlap in their morphological characters. May (1991) delimited eight Australian species based on macro- and micromorphological characteristics, but certain characters had enough overlap between collections that multivariate analysis was needed to distinguish the species. Multivariate analysis of morphological characters was also utilized by Mueller (1991) to assist in separation of species in the *Laccaria laccata* complex.

Since then, molecular systematics has enabled mycologists to effectively recognize fungal species from a phylogenetic standpoint (Chap. 1). Genealogical concordance phylogenetic species recognition (GCPSR), initially elaborated for fungi by Taylor et al. (2000), is an essential tool for species delimitation. Application of GCPSR to *Laccaria* has revealed cryptic phylogenetic species that are morphologically indistinguishable. While some of the morphological species of May (1991) were confirmed as phylogenetic species by Sheedy et al. (2013), others were found to contain up to four distinct phylogenetic species (albeit with some represented by single collections).

Once species are delimited across multiple markers, it may be possible to identify an effective barcode region, where intra-specific (i.e., within species) variation under a designated threshold is used as an indicator of conspecificity. The nuclear ribosomal internal transcribed spacer region (ITS) is currently the most efficient barcode across fungi, and is the "official" fungal barcode (Schoch et al. 2012). However, Sheedy et al. (2013) found that certain phylogenetic markers showed higher discrimination ability than others when identifying *Laccaria* molecular species from Australasia. The authors evaluated the ability of three molecular markers to serve as a barcode by comparing the distribution of intra- and inter-specific uncorrected pairwise distances for species identified under GCPSR. Using the ITS region, they did not find a clear gap in the distribution between intra-specific and inter-specific pairwise distances (Fig. 13.2). However a clear gap in pairwise distances was present in both *RPB2* and *TEF1α* sequences.

The ITS region has been useful for identifying new species of *Laccaria* using phylogenetic methods despite harboring low inter-species variation (Osmundson et al. 2005; Wilson et al. 2013; Popa et al. 2014, 2016; Montoya et al. 2015). The wide application of ITS in fungi, the availability of fungal specific ITS primers, and the relative ease in which the region is amplified using PCR, make

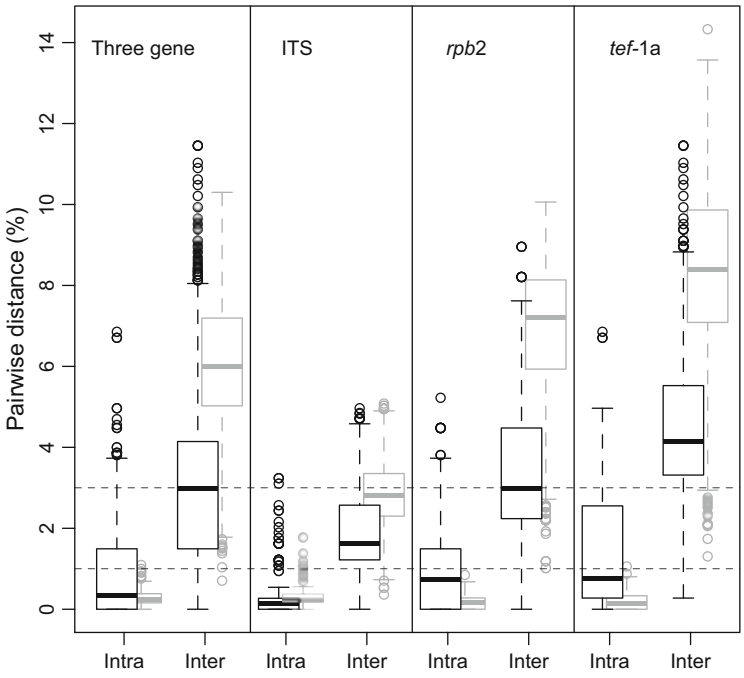

Fig. 13.2 A comparison of uncorrected pairwise distances between Northern Hemisphere and Southern Hemisphere *Laccaria* across three genes. *Black boxplots* in the foreground represent Northern Hemisphere *Laccaria*. *Gray boxplots* represent Southern Hemisphere *Laccaria* data from Sheedy et al. (2013)

it useful for initial screening of fungal diversity (Schoch et al. 2012). But the identification of phylogenetic species boundaries within *Laccaria*, using a barcoding approach (phenetic vs. phylogenetic), requires further study using additional markers and specimens to replicate and establish confidence in the identification of new species.

Wilson et al. (2017) used the GCPSC method across four markers to describe global *Laccaria* biodiversity. This study identified at least 116 molecular species of *Laccaria* which is a more than 50% increase in known *Laccaria* diversity (Kirk et al. 2008). This dramatic increase in species does not include several recently described species or species that are awaiting documentation from under-sampled parts of the world. The major clades for *Laccaria* and their global geographic distributions are presented in Fig. 13.1.

One of the observations in this study was that while the multi-gene phylogeny was able to effectively delimit Southern Hemisphere species of *Laccaria*, delimitation of *Laccaria* species in the Northern Hemisphere was not as easy. This is likely due to the difference in the age of the Northern Hemisphere's most recent common ancestor (MRCA) relative to the Southern Hemisphere's. Hypothetically, the younger MRCA in the Northern Hemisphere provides less time for molecular

variation to accumulate between the markers used for species delimitation. To evaluate this hypothesis the gene regions ITS, *RPB2* and *TEF1α* of Northern Hemisphere *Laccaria* species were evaluated in the same manner as Sheedy et al. (2013). In their study, inter and intra-species gap in pairwise distances between Southern Hemisphere *Laccaria* sequences was approximately 2.0% for ITS, 7.0% for *RPB2*, and 8.0% for *TEF1α*, with a 5.5% gap using a mix between all three genes. To perform a similar analysis on Northern Hemisphere *Laccaria*, a total of 310 sequences (ITS dataset = 128 sequences, *RPB2* = 125, *TEF1α* = 65) were obtained from 132 specimens. These specimens represent a total of 41 species: 39 species are represented in the ITS dataset, 39 in *RPB2*, and 33 in *TEF1α*. The results show that gaps between intra- and inter-species distances in Northern Hemisphere *Laccaria* turned out to be narrower (approximately 1.5%, 2.3%, 3.3%, and 2.5% respectively; Fig. 13.2) than those from the Southern Hemisphere.

As a caveat, the above exercise simply demonstrates the differences in inter- vs. intra-species variation between Northern and Southern Hemisphere *Laccaria* based on sequences of common phylogenetic markers in Agaricomycetes. Pairwise distances are not phylogenetic distances. While the ITS region has been useful for the initial detection of new *Laccaria* species, further work is needed to identify the most effective set of regions for carrying out GCPSR and for revealing patterns of diversity, as well as identification of appropriate single-gene barcode regions. In the meantime, it is advised that researchers draw their conclusions from the corroboration of multiple phylogenetic markers in regards to *Laccaria* phylogenetic species recognition.

13.3 *Laccaria* Population Genetics and Life History

Knowledge of the patterning of infra-specific genetic variation across space can illuminate processes of interest for biogeographic studies (Chap. 2). Infra-specific genetic variation in fungi has been studied using markers such as microsatellites ('population genetics') and also tree-based approaches, such as coalescence analysis ('phylogeography'). The population genetics of ectomycorrhizal fungi was reviewed by Douhan et al. (2011) who tabulated published and unpublished (see Vincenot et al. 2012) studies on various species of *Laccaria*. Subsequent research on *Laccaria* population genetics includes investigations of above and below ground population structure in *L. amethystina* in Europe (Hortal et al. 2012), genet dynamics of two Japanese species (Wadud et al. 2014), and population structure of an Australian species (Sheedy et al. 2015). These three studies used microsatellite markers, with Hortal et al. (2012) also analyzing variation in the rDNA intragenic space. Wilson et al. (2015) explored an alternative approach to generating markers, using restriction-site associated DNA (RAD) sequencing to detect and compare single nucleotide polymorphisms (SNPs) among *L. bicolor* specimens from North America and Europe.

Studies of European *L. amethystina s. lat.* and *L. laccata s. lat.* showed that individuals formed small genets (on the basis of haplotypes, up to 2 m, rarely larger, and often smaller), with high temporal turnover. Although there may be some spatial autocorrelation at small scales, there is low genetic differentiation at large scales, up to several thousand kilometers (once putative cryptic species are discounted—see caveat below). In contrast, the Southern Hemisphere population genetic study of *Laccaria*, focused on the phylogenetic species *Laccaria* sp. 'A', found significant genetic structure across a range of 700 km. There was genetic differentiation between populations in Tasmania and mainland Australia, which were separated by the Bass Strait; but also geographically isolated populations in southern Australia separated by around 150 km due to non-continuous distribution of the host. In this study, *Laccaria* sp. 'A' and its host, *Nothofagus cunninghamii*, were observed to be more or less co-extensive over their entire ranges. Interestingly, the pattern of genetic variation in the fungus was fairly similar to that of the host, indicating that both responded similarly to historical events such as Pleistocene glaciations (Sheedy et al. 2015). A 6 m diameter 'fairy ring' of *Laccaria* sp. 'A' sporcarps around a *Nothofagus* tree exhibited the same haplotype, which suggests that genets can be large and long-lived (Sheedy et al. 2013).

Life history characteristics (i.e., size, growth rate and age of individual genets, rate of establishment and death of genets, and spore dispersal dynamics) are crucial features relevant for biogeography, but are often unknown in fungal species. Generation time is an overlooked factor that has potential impacts on diversification, because shorter generation times mean more frequent sexual recombination and opportunities for adaptation and speciation. For *Laccaria*, differences in genet size indicate potential differences in generation time. Presumably, if many genets are long-lived, there is a lower rate of new genets being established and hence, longer mean generation times. The rate of establishment of new genets is related to the availability of suitable colonization sites. For example, south temperate *Nothofagus* rainforests appear to be more stable than adjacent sclerophyll vegetation dominated by *Eucalyptus*. These latter communities are frequently disturbed by wildfires, which are soon followed by large flushes of *Laccaria* sporocarps. A comparison of the genetic structure between species that experience different disturbance regimes would be instructive.

Dispersal is strongly inferred in disparate lineages of fungi from current distributions in combination with dated phylogenies (Lumbsch et al. 2008; May 2017). In the Southern Hemisphere, fungal lineages are often too young for their distribution to be explained by vicariance at the continental scale. Better understanding of spore movement within species could illuminate the role of dispersal in biogeographic patterns. A conundrum for most Basidiomycota, including EcM fungal species is that outcrossing requires two monokaryons for successful creation of a competent dikaryon. However, while *Laccaria* has a tetrapolar mating system, there are some interesting variations in reproductive cycles. Two-spored species are common, some of which have been shown to produce dikaryotic spores with compatible nuclei (Tommerup et al. 1991; Mueller et al. 1993). If dispersal is a rare event, genetic bottlenecks would ensue. A comparative study of genetic

variation across closely related species, combined with the identification of putative historic dispersal events, is necessary to detect the signature of genetic bottlenecks.

Given that morphologically cryptic species of *Laccaria* are known to be common, the caveat of rigorous species delimitation is emphasized. Otherwise, the observed genetic variation may be due to the mixing of phylogenetic species rather than true infra-specific variation. Sheedy et al. (2015) carried out an initial multigene concordance study to confirm that the subject of their population study was a single phylogenetic species. Phylogenetic analysis has revealed that several model species (actually 'species complexes') of *Laccaria* are comprised of more than one phylogenetic species, e.g., *Laccaria bicolor* (Wilson et al. 2015, 2017) and *L. amethystina* (Vincenot et al. 2012). For the latter species complex, large genetic distances between European and Asian populations, associated with separate clades across ITS and nuclear markers, certainly suggest multiple phylogenetic species. For the French populations of *L. amethystina* analyzed by Roy et al. (2008), there was a focus on establishing conspecificity, using gene flow rather than genealogical concordance. For Polish populations of *L. amethystina*, Hortal et al. (2012) found two genetically distinct but co-occurring 'genetic clusters', with a low proportion of admixed individuals. They suggested that these clusters 'may correspond to two distinct ecotypes', but they also introduced the possibility of sympatric speciation.

There is much scope for comparative study of infra-specific genetic variation across species of *Laccaria* with different reproductive strategies, host relationships, disturbance tolerances and geographic patterns and ranges. Increased knowledge of life history characteristics is required to accurately interpret population genetic and evolutionary spatial patterns and to determine how these relate to biogeographic processes. Future studies will benefit from a foundation of multigene concordance and coalescent analyses, in order to rigorously delimit species, and also to detect incipient speciation.

13.4 Ectomycorrhizal Associations in *Laccaria*

To adequately study the biogeographic story of *Laccaria* it is necessary to identify the host associations that are formed by these species. However, identification of plant hosts in EcM relationships is challenging on a number of levels. Traditionally, the hosts attributed to fungal EcM species are the co-occurring dominant over-story tree species, but this data is speculative and subjective, whereas certainty of the association is needed to infer evolutionary relationships (Wilson et al. 2012b). There are a number of ways in which researchers can establish associations between host and fungi. Culture studies in vitro can establish potential host association between fungi and a chosen host within a controlled greenhouse setting. While in vitro experiments demonstrate what kinds of EcM associations are possible, they do not identify naturally occurring EcM associations. Identification of the fungus and host involved in EcM associations in vivo (in nature) requires direct sampling and identification of EcM root tips. Some studies have endeavored to actually trace

the mycelium from the basidiomes to the root on which they are growing (Watling et al. 1995). Another method used by Agerer in Colour Atlas of Ectomycorrhizae (Agerer 1987–1996) attempted to document morphological species of fungi from EcM root tips. However neither of these methods are considered practical because: (1) tracing mycelium from fruit bodies to the roots of their host is extremely taxing and requires further tracing of the roots to identify the host tree/shrub; and (2) since determination of morphological species from basidiomes is difficult enough (see Sect. 13.2 on species delimitation), accomplishing species level identification using relatively character-poor EcM roots is not considered feasible. Currently, identification of hosts in vivo is best accomplished through molecular analysis of EcM root tips. In this process, DNA sequence data from the fungal symbiont and host can be acquired from EcM root tips using fungal and plant specific primers. Resources for these are provided in Chap. 1.

Species of *Laccaria* can be either EcM generalists (associated with many hosts) or EcM specialists (species only associated with a single or limited taxonomic group of hosts). On the generalized end of the spectrum, Molina et al. (1992) used an in vitro method with *Laccaria amethystina*, *L. bicolor*, *L. laccata*, *L. montana*, and *L. proxima* and found them to have broad host ranges. The problem is that the *Laccaria* taxa used in this and similar studies were identified morphologically and several of these names have now been shown to represent species complexes. As a result, *s. lat.* interpretation of these species is the most prudent. However, while there is room to question the specific identity of these *Laccaria*, it is important to note that these studies demonstrate the ability of *Laccaria* species (as represented by the cultures used for inoculation) to associate with multiple hosts in greenhouse experiments.

Molecular population genetic studies of *L. amethystina s. lat.* describe the species as a generalist, growing in forests containing a wide variety of hosts in Europe (Roy et al. 2008; Vincenot et al. 2012) and in Japan (Vincenot et al. 2012). In contrast, the distribution of *Laccaria* sp. 'A' populations is restricted to *Nothofagus cunninghamii* forests in the Australian territories of Tasmania and Victoria (Sheedy et al. 2015). For species such as *Laccaria* sp. 'A', the limits of their ability to associate with other EcM hosts have yet to be fully explored.

The problem with the specialist vs. generalist dichotomy is that it ignores scale and the possibility that specialization can be measured in degrees. By definition, EcM fungi are "specialized" to grow on an EcM host, but for a particular species of EcM fungus specialization may be on a single host species, while another species might be specialized to a host genus, or perhaps even to a host family. Other species might be true generalists and able to associate across orders of plant hosts (e.g. associate with both *Quercus* and *Pinus* hosts). As a result, the degree to which *Laccaria*, either from the Southern or Northern Hemispheres, engage in generalized or specialized associations needs to be thoroughly examined for most species. Knowing the potential host associations that individual *Laccaria* taxa can form would help researchers better understand their fundamental niche and put into context the ecological forces that shape their realized niche.

The historical relationships between *Laccaria* species and their hosts require further study, but to do this requires adequate identification of the species involved in the EcM association. Most reported *Laccaria* associations are based on observation of the EcM hosts dominating the canopy, or in vitro synthesis of EcM associations, which as stated earlier are not ideal when trying to address actual relationships in nature. Future research in *Laccaria* biogeography should attempt to use molecular methods to identify fungus/plant relationships. A pilot study is currently underway to determine the efficacy of sampling *Laccaria* EcM roots from soil cores collected directly beneath *Laccaria* sporocarps. Preliminary results successfully recovered EcM root tips from seven out of nine *Laccaria ochropurpurea* soil cores sampled over five different plots in the same forest. To effectively document fungal-host associations, this should be performed multiple times per species from different habitats. For *Laccaria* this will help determine the degree to which a *Laccaria* species functions as a "specialist" or "generalist", and help fill the gaps in understanding the evolution of EcM associations in the genus.

13.5 Known *Laccaria* Hosts and Their Biogeography

The exploration of *Laccaria* biogeography would advance with continued study of how species distributions correlate with the biogeographic history of their hosts. The current literature on the biogeography of several important *Laccaria* EcM host families reveals histories that can be used to construct a biogeographic theory of *Laccaria's* evolution.

The Nothofagaceae are an important EcM host for *Laccaria* in the Southern Hemisphere (McNabb 1972; McKenzie et al. 2000; Sheedy et al. 2015; Wilson et al. 2017). The family has long been a model for understanding the biogeography of disjunctively distributed Southern Hemisphere flora. Early study of Southern Hemisphere biogeography assumed that floral and faunal distributions were largely the result of vicariance due to the breakup of Gondwana (Raven and Axelrod 1972). Later phylogeographic studies of Southern Hemisphere fauna and flora (including *Nothofagus s. lat.*) began to explore the validity of vicariance as the mechanism shaping species distributions. Earlier studies would reconstruct the biogeographic history of the group using vicariance to describe distributions of Nothofagaceae (Manos 1997; Sanmartín and Ronquist 2004). As molecular data and computational capacity increased, so did the ability to assess molecular divergence times in comparison with the fossil and geological record. As a result of the age and fossil record for the Nothofagaceae, the current distribution of Nothofagaceae genera cannot be described without long-distance dispersal (Knapp et al. 2005; Sauquet et al. 2012).

Recent taxonomic study has divided *Nothofagus s. lat.* into four genera, two of which are distributed within continuous biogeographic regions while the other two contain taxa found in disjunct regions of the Southern Hemisphere (Heenan and Smissen 2013). The genus *Nothofagus s. str.* consists of five species that are

currently limited to southern South America. In contrast, the genus *Trisyngyne* has 25 species distributed across West Papua and Australasian regions of Papua New Guinea and New Caledonia. The seven species that comprise the genus *Lophozonia* are split between two biogeographic regions: one representing South America, and the other representing Australia and New Zealand. The genus *Fuscospora* has a similar distribution, but with only one species (*F. alessandri*) from South America and two in Australia. The remaining seven *Fuscospora* species are found in New Zealand. Some of these patterns of Nothofagaceae biogeography are also seen in the distributions of *Laccaria*. The molecular divergence times and distributions for these four genera are presented in Fig. 13.1 to compare with those of major *Laccaria* lineages.

The Myrtaceae genera *Eucalyptus*, *Angophora*, *Baeckea*, *Kunzea*, *Leptospermum*, and *Melaleuca* are known to form EcM relationships (Wang and Qiu 2006). *Laccaria* species such as *L. laccata* sensu *lato* and *Hydnangium carneum* form EcM with several species of *Eucalyptus* in synthesis studies (Malajczuk et al. 1982). *Laccaria* are also known to associate with *Kunzea* and *Leptospermum* (McNabb 1972; McKenzie et al. 2006). The degree to which *Laccaria* taxa can form EcM relationships with Myrtaceae other than *Eucalyptus*, *Kunzea* and *Leptospermum* remains to be fully explored.

The Myrtoideae group—which includes *Eucalyptus*, *Kunzea* and *Leptospermum*—was split from the African distributed Psiloxyloideae group in the first half of the Late Cretaceous (~85 Ma [73–93.2 Ma]) (Thornhill et al. 2015). The genera *Eucalyptus*, *Angophora*, and *Corymbia*—also known as the 'eucalypts'—form the core group of the Eucalypteae along with *Arillastrum*, *Stockwellia*, and *Eucalyptopsis* (Thornhill et al. 2015). The 'eucalypts' are widespread across Australasia and have the largest species diversity among the Myrtaceae genera (Ladiges et al. 2003) (Fig. 13.1). Both *Eucalyptus* (>600 species) and *Corymbia* (>100 species) are widespread across Australia. *Angophora* has the fewest number of species (13), and its distribution is limited to Eastern Australia. The EcM status of *Corymbia* is unknown, but as part of the 'eucalypts', its relationship with *Eucalyptus* and *Angophora* makes EcM associations likely. Species of *Laccaria* are typically reported within *Eucalyptus* groves but identification of the host species is notoriously difficult using field identification. The genus *Arillastrum* is represented by the single known species *A. gummiferum*, which is endemic to New Caledonia (Ladiges et al. 2003). The ability of *Laccaria* to associate with *Arillastrum* is unclear, but considering that *Laccaria* species have been collected in New Caledonia associated with *Trisygyne* (Nothofagaceae; K. Hosaka, personal communication), and their ability to form EcM with *Eucalyptus*, the potential for *Laccaria* taxa to form EcM relationships with *Arillastrum* is worth investigating.

The oldest Australian 'eucalypts' fossil is at least 48 million years old (Greenwood 1991). Thornhill et al. (2015) used extant pollen morphology and the oldest fossil pollen to calibrate a Bayesian estimation of divergence times in the Myrtaceae. Within the Eucalypteae, the split of *Eucalyptus* from the clade containing *Arillastrum*, *Angophora* and *Corymbia* occurred at the beginning of the Eocene epoch (Fig. 13.1).

The widespread and diverse nature of the 'eucalypts' creates a challenge in identifying patterns of EcM relationships between them and *Laccaria*. A study by González-Orozco et al. (2014) focused on 798 'eucalypt' species occurring in phytogeographical regions that were shaped largely by climate. The study was able to identify three centers of species richness and 14 regions of endemism for Australian and Malaysian 'eucalypts'. Understanding *Laccaria* species richness and patterns of distribution both inside and outside of these regions could provide a sense of the importance of 'eucalypt' hosts to *Laccaria* diversity. Given the diversity of *Laccaria* in Australia, accurate species identification will be crucial for establishing relationships between *Laccaria* and their eucalypt hosts.

Australian *Laccaria* have been detected via molecular analysis on the roots of the genus *Pomaderris* in the Rhamnaceae (Tedersoo et al. 2008). However, *Laccaria's* relationship with this genus is unclear as *Pomaderris* co-occurs with eucalypts and *Nothofagus*, complicating determination of host associations. *Casuarina* and *Allocasuarina* in the Casuarinaceae are further potential EcM hosts for *Laccaria* in Australasia as demonstrated through in vitro synthesis by Theodorou and Reddell (1991).

Ectomycorrhizal members of the Fabaceae are represented in Australasia through the Mimosoideae (*Acacia*) and Papilionoideae (*Mirbelia*, *Oxylobium*, *Gastrolobium*, and *Jacksonia*) (Smith et al. 2011). *Acacia* and *Pultenaea* (Papilionoideae) are known to benefit from mycorrhizal associations in inoculation experiments (Warcup 1980). Most Australian Fabaceae are small to medium sized shrubs, or small trees in *Eucalyptus* or *Nothofagus* forest understory. However, these leguminous plants are not often recorded in the vegetation details on fungi specimen labels. *Laccaria* certainly occurs quite frequently in Australian forests that also contain some of these Fabaceae, but assessment of connections with *Laccaria* will require isolation of DNA from roots.

Another important tropical EcM group is the Dipterocarpaceae. This family has EcM members in paleotropical Asia (*Shorea*, *Hopea*, *Dipterocarpus*, *Vateria*, *Vateriopsis*, and *Vatica*), Africa (*Monotes* and *Marquesia*) and in neo-tropical South America (*Pseudomonotes*). *Laccaria* has been documented on paleo-tropical dipterocarps (Phosri et al. 2012), but not on neotropical ones. This pattern potentially stems from the biogeographic history of *Laccaria* in each of these tropical regions. The longer presence of *Laccaria* in the paleotropics than in the neotropics may have allowed more time for host switching to the Dipterocarpaceae to take place in this part of the world.

There are several Northern Hemisphere plant families that are hosts to *Laccaria*. These include the Fagaceae, Betulaceae, Salicaceae, and Pinaceae, each of which have been documented as important EcM hosts (Wang and Qiu 2006), and some have well documented biogeographic histories.

The Fagaceae has a number of important genera that form EcM associations. These include *Castanea*, *Castanopsis*, *Fagus*, *Lithocarpus*, and *Quercus* (Wang and Qiu 2006). Of these *Quercus* (~600 species), *Lithocarpus* (~340 species, including *Notholithocarpus*), and *Castanopsis* (~130 species) have the greatest species diversity. These genera have distributions in Asia (Fig. 13.1). *Quercus* and

Notholithocarpus extend into North and Central America (via Beringia), and *Quercus* extends west from Asia into Europe and parts of North Africa (Manos and Stanford 2001). The pattern of distribution of *Castanea* and *Fagus* is similar to that of *Quercus*, but these groups consist of only 12 and 10 species respectively. *Castanopsis* and *Lithocarpus* have centers of diversity in Asia, while the center of diversity for *Quercus* is in North America. Other genera can be found outside of Asia (*Chrysolepis* and *Trigonobalanus*), but their relative diversity is small (2 and 3 species respectively). As a result, it is not surprising that Manos and Stanford (2001) concluded that the ancestral range for the Fagaceae was centered in Asia. This conclusion makes the Fagaceae a critical component in a theory that explains *Laccaria's* dispersal from Australasia to the Northern Hemisphere, which will be discussed later in this chapter.

Laccaria species are known associates of Salicaceae genera *Salix* and *Populus*. The genus *Salix* consists of nearly 400 species and is distributed among all continents except Australasia and Antarctica. However, like the Fagaceae, the diversity of *Salix* subgenera is concentrated in Asia with *Salix s. lat.* extending into the Indonesian archipelago (Wu et al. 2015). Using a molecular phylogeny based on plastid data, Wu et al. (2015) estimated the split of *Salix* from *Populus* at approximately 48 Ma (Fig. 13.1). The genus initially diversified in the middle Eocene, around 44 Ma, with continued diversification increasing through the latter half of the Tertiary (35–3 Ma). The genus *Populus* has only 25–35 known species. There are several studies of *Populus* that focus on the distributions and population genetics of individual species, but apparently none that addresses broader questions such as the age or ancestral range for the genus.

In the Betulaceae *Laccaria* are found in forests containing *Betula*, *Carpinus*, and *Corylus* (Roy et al. 2008; Vincenot et al. 2012). *Betula* has up to 60 known species, while *Carpinus* has 40, and *Corylus* has 18. These genera are largely restricted to the Northern Hemisphere, each with numerous taxa in Asia. *Carpinus* and *Corylus* each only have a few species represented in Europe and North America, while *Betula* is more widespread with up to 15 species occurring in North America. The genus *Alnus* is another important EcM genus with around 35 known species and a wide distribution across the Northern Hemisphere, which also extends down into Central America and south along the Andes to the southern end of Peru and northern tip of Chile. Species of *Alnus* are interesting due to their capacity to harbor nitrogen-fixing bacteria on their roots. European *L. purpureobadia* is noted by Kibby (2010) as growing with *Salix* and *Betula* but also 'commonly' under *Alnus*. In addition, Bogar and Kennedy (2013) recovered *L. laccata* sensu *lato* on *Betula* and *Alnus* root tips. However, *Laccaria* is an uncommon occurrence in *Alnus* EcM communities (Tedersoo et al. 2009; Põlme et al. 2013; Kennedy et al. 2015). Additionally, in vitro synthesis studies formed incomplete mycorrhizas between *L. laccata* and several *Alnus* species (Molina 1981).

It is notable that species of *Laccaria* have never been reported in association with lowland Neotropical or tropical African EcM hosts, e.g., Caesalpinioideae, *Coccoloba* (Polygonaceae), Nyctaginaceae, and *Pakaraimaea* (Dipterocarpaceae) (Tedersoo et al. 2010b, 2011; Smith et al. 2013; Bâ et al. 2014). This lack of host

association partially explains why the genus has not been reported from these regions.

The capacity for host switching in *Laccaria* is not yet well understood. While necessary to explain *Laccaria's* current distribution, there are clearly limits to host switching that prevent the genus from becoming truly cosmopolitan. Regardless, introductions of *Laccaria* EcM hosts may facilitate host switching of exotic EcM species to native hosts. Australian *Laccaria fraterna* has been introduced to the Iberian peninsula through the transplanting of *Eucalyptus* (Díez 2005) while North American *L. bicolor* has been observed on the continent as well (Dickie et al. 2016). Monitoring whether exotic *Laccaria* species engage in EcM associations with native hosts after a recent introduction would provide a better understanding of *Laccaria's* ability to disperse through host switching.

Ultimately it is important to understand the limitations under which host associations influence EcM biogeography. While *Laccaria* are clear EcM partners with *Eucalyptus*, *Laccaria* do not occur wherever there is *Eucalyptus* within Australasia. Members of the Fagaceae (*Quercus*, *Lithocarpus*, *Castanopsis*) are important EcM hosts but the ranges of each associated *Laccaria* species that associate with the Fagaceae is discrete and limited to a subset of hosts. This relates to the classic understanding of ecological niche space where the host distributions represent the fundamental niche of an EcM fungal species, but the presence of factors, abiotic or biotic, shape its realized niche. An understanding of what shapes the distribution and diversity of EcM fungal species and populations.

13.6 Early Evolution in the Southern Hemisphere

The global study by Wilson et al. (2017) presents the systematic evolution of *Laccaria* based on a multi-gene phylogenetic dataset of over 230 specimens. The study's 'Global *Laccaria*' dataset represents 116 species. A streamlined version of this phylogeny with molecular age estimates for important nodes is given in Fig. 13.1 along with contrasting divergence ages for important EcM hosts and maps with theorized dispersal routes for *Laccaria* lineages. The phylogeny demonstrates the origin of *Laccaria* in the Southern Hemisphere. All Northern Hemisphere *Laccaria* are derived from a single lineage identified by the most recent common ancestor at node F (Fig. 13.1). Dispersal to the Northern Hemisphere is believed to have occurred from Australasia, through Southeast Asia, and into China before spreading to other parts of the Northern Hemisphere. The remaining sections of this chapter review the evidence for the biogeographic origins of *Laccaria* (this section), discuss how dispersal to the Northern Hemisphere might have occurred (Sect. 13.7), and give an overview of the current understanding of Northern Hemisphere *Laccaria* biogeography as well as the challenges and opportunities that lie ahead for future research on this group (Sect. 13.8).

The global *Laccaria* phylogeny (Wilson et al. 2017) has overstory host association data for >80% of the specimens in the dataset. While this provides a good overview of host associations across the phylogeny, it does not have the depth of detail that is needed to truly deconstruct the *Laccaria* host associations that drive distribution patterns. However, overstory observations can potentially survive scrutiny as long as observed host associations are consistently repeated. Across the Southern Hemisphere, where *Laccaria* occurs, either Nothofagaceae or Myrtaceae EcM hosts are always observed (with the exception of *L. ambigua*).

The early evolutionary history of *Laccaria* is fascinating, because the original diversification occurred between the Late Cretaceous and early Paleogene (47–82 Ma) (Fig. 13.1). This most recent common ancestor to all *Laccaria* is identified as node B, and diversified into two lineages, one representing >99% of EcM *Laccaria*, and the other represented by a single species of *Laccaria* collected from New Zealand. This second species is named *Laccaria ambigua*, due to a distinct ^{13}C and ^{15}N profile that is unique among *Laccaria* species (Wilson et al. 2017). The unique physiology and unknown ecology of *L. ambigua* is derived from the most recent common ancestor to all *Laccaria*, while displaying the morphological hallmarks of the genus including stature and echinulate spore ornamentation. While the most recent common ancestor to all *Laccaria* at node B (Fig. 13.1) bifurcates to *L. ambigua* in New Zealand, the other lineage leads to the next most recent common ancestor for all remaining *Laccaria* (node C). The ancestor at node C bifurcates to a lineage containing all other Australasian *Laccaria* (node D), while the other leads to *L. galerinoides*, which is endemic to temperate South American. This phylogenetic grade that alternates between Australasia, South America, and back to Australasia, makes predicting ancestral range for the ancestor to all *Laccaria* somewhat difficult. It is more challenging to address how the genus became distributed between the distant and unconnected landmasses of Australasia and South America.

The results suggest that *Laccaria* dispersed between Australasia and South America between 29 and 82 Ma (the error range for *Laccaria* ancestors at nodes B and C in Fig. 13.1). If these dates are accurate, then some dispersal over water would have had to occur as no continuous connection between Australia, New Zealand, Antarctica, and South America existed more recently than 40 Ma (Kroenke 1996). However, the same problem exists for the distribution of the Nothofagaceae, which is not known to have long-distance dispersal capabilities, yet molecular dating analysis demonstrates that this group must have dispersed at a time when there was no intact land connection between Australasia and South America (Knapp et al. 2005). The degree to which this EcM association helped facilitate the distribution of *Laccaria* is difficult to assess. However, Nothofagaceae are host to other fungal species with similar distributions, e.g., the pathogenic fungal genus *Cyttaria* (Peterson et al. 2010). Other Agaricomycetidea genera share this Southern Hemisphere distribution. The genera *Descolea* and *Austropaxillus* are distributed in both Australasia and South America (Tedersoo et al. 2010a). For *Austropaxillus* this distribution likely developed after the breakup of Australasia, Antarctica, and South America from each other during the

Oligocene to early Miocene (Skrede et al. 2011). This suggests that fleshy basidiomycetes other than *Laccaria* have undergone long-distance dispersal to achieve distributions between Australasia and South America. In addition, the biotrophic association of *Cyttaria* and *Austropaxillus* with the Nothofagaceae demonstrates the significance of these plants to Southern Hemisphere fungal distributions.

Of the approximately 50 species that represent Southern Hemisphere *Laccaria* in the Global *Laccaria* phylogeny of Wilson et al. (2017), only three are from South America (Fig. 13.1). They are commonly collected from the Nothofagaceae forests of southwestern South America in both Chile and Argentina. In the phylogeny, temperate South American *Laccaria* is polyphyletic. *Laccaria galerinoides* is resolved near the base of the tree, while the other two South American *Laccaria* species are derived from the middle of the Southern Hemisphere grade (see the blue arrows at the nodes in Fig. 13.1). Additional unidentified *Laccaria* species are likely to occur in the region. Mueller (1992) placed a number of *L. laccata* and *L. tetraspora* varieties described from temperate South America by Singer into synonymy based on the broad morphological species concept then being used. Other than these temperate South American species, the remaining *Laccaria* taxa within clade L are all found in Australasia. This suggests that a more recent instance of long-distance dispersal occurred between 11 and 26.5 Ma (Fig. 13.1). This took place around the late Oligocene to middle Miocene (around 15–30 Ma) when the Southern Hemisphere had experienced significant climate shifts that resulted in warmer temperatures, smaller ice sheets, and high sea levels fueled by elevated CO_2 in the first half of the Miocene epoch (Foster et al. 2012). The second half of the Miocene (5.3–15 Ma) experienced cooling temperatures that accompanied the expansion of ice sheets in the Antarctic (Lewis et al. 2008; LaRiviere et al. 2012). Considering that fossil evidence shows that the Nothofagaceae were in Antarctica as early as the Late Cretaceous (66–100 Ma) (Hill 1991; HaoMin and ZheKun 2007), and that the Antarctic tundra existed up until the middle Miocene (13–14 Ma) (Lewis et al. 2008), the optimal time for the dispersal of *Laccaria* between Australasia and South America, using Antarctica EcM hosts as a "stepping stone," would have been the late Oligocene to early Miocene (around 15–30 Ma).

Detailed descriptions of species-level host associations in South American *Laccaria* taxa are limited. Three of four Nothofagaceae genera (*Lophozonia*, *Fuscospora*, and *Nothofagus*) are known to occur in South America. The most recently diverging lineage, *Nothofagus* split from *Trisingyne* (AKA subgenus *Brassospora*) by at least 31 Ma (Sauquet et al. 2012). How *Laccaria* may have followed this route is not clear, but this puzzle could potentially be pieced together if specific Nothofagaceae hosts were identified. If different South American *Laccaria* species associate with different Nothofagaceae genera, then it would be possible to correlate species-host relationships with molecular dating of respective host lineages and potentially explain how *Laccaria* dispersed between Australasia and South America. In essence, the current hypothesis using shallow seas around Antarctica as a "stepping stone" to explain the disjunct distribution of Nothofagaceae (Hill 1996; Knapp et al. 2005) may be a viable explanation for the

dispersal of *Laccaria* between Australasia and South America as well. Further study of Southern Hemisphere associations between *Laccaria* and their specific Nothofagaceae hosts may help provide evidence for this or alternative biogeographic hypotheses.

Fossils of *Eucalyptus* have been documented from South America (Hermsen et al. 2012; Gandolfo et al. 2011). This provides potential for *Eucalyptus* hosts to act as dispersal vectors for *Laccaria* to the continent as soon as the early Eocene, which is the same time frame as the earliest Australian 'eucalypts' which are around 48 Ma in age (Greenwood 1991). In any case where *Laccaria* were to have traveled to South America on *Eucalyptus* hosts, further host switches to South American Nothofagaceae genera are required prior to the extinction of *Eucalyptus* on the continent.

The remaining Southern Hemisphere *Laccaria* are dispersed throughout Australasia. *Laccaria* species display a high level of endemism, with 53 of 57 species being reported from only one of four major Australasian regions: Australia, New Zealand, Papua New Guinea, and New Caledonia (Wilson et al. 2017). Within the Southern Hemisphere grade are several Australasian lineages and species with diverse geographic distributions and host associations. One of the most notable is the clade identified as node O (Fig. 13.1). This clade represents up to seven *Laccaria* species all associated with Nothofagaceae native to New Zealand's South Island. While other New Zealand *Laccaria* species occur with Nothofagaceae within the Southern Hemisphere grade, this clade is particularly interesting given the unusually high diversity recovered from such a small geographic area. For this reason it is worth exploring the ecological phenomenon that may explain this diversity.

As mentioned in the section on species delimitation one of the major challenges to understanding Australasian *Laccaria* biogeography is the abundance of cryptic diversity that remains to be described. Since the study by Sheedy et al. (2013), continued use of GSPCR to study the diversity of Australasian *Laccaria* has expanded the current number of phylogenetic species from this part of the world to >70, much of which is based upon singletons. This research is part of a web-based *Laccaria* biodiversity project (www.laccaria.org), which demonstrates the extent of Australasian *Laccaria* diversity using molecular sequence data.

The origins of *Laccaria* and its EcM ecology in the Southern Hemisphere evolved at a time and place that produced the genus we see today. From a biogeographic standpoint this includes its conspicuous absence from regions like sub-Saharan Africa and neotropical South America. Geologically speaking, the genus did not evolve until long after the African continent broke off from Gondwana (~120 Ma), after which the proximity between the continent and Australasia made long-distance dispersal improbable. The current observation is that *Laccaria* species do not extend into sub-Saharan Africa, nor into neotropical regions of South America other than those inhabited by *Quercus*. As mentioned previously, species of *Laccaria* have not been found in association with the typical EcM hosts of these areas, e.g., caesalpinioid legumes, neotropical dipterocarps, *Coccoloba* (Polygonaceae), and members of the Nyctaginaceae. This is in contrast

to other EcM fungal genera—such as *Cantharellus* and *Craterellus* (Wilson et al. 2012a; Henkel et al. 2014), *Amanita* (Sánchez-Ramírez et al. 2015), Inocybaceae (Matheny et al. 2009), and *Russula* (Looney et al. 2015)—that have various distributions and associations with hosts from sub-Saharan Africa and tropical South America.

13.7 Dispersal of *Laccaria* to the Northern Hemisphere

In the global *Laccaria* phylogeny (Wilson et al. 2017), node F represents the most recent common ancestor to all Northern Hemisphere *Laccaria*. What was the likely ancestral range for this species, and what can that tell us about how *Laccaria* got to the Northern Hemisphere? Several pieces of evidence are presented here that support a hypothesis describing the dispersal of *Laccaria* from the Southern hemisphere through Southeast Asia into the temperate Northern Hemisphere. This route is complex due to the unlikely possibility that Australasia and Asian landmasses were connected when this dispersal took place during the Oligocene and early Miocene.

In the Global *Laccaria* phylogeny, the sister to the ancestor at node F is a single taxon from the state of Queensland, Australia, which is directly south of Papua New Guinea (Fig. 13.1). The most recent common ancestor at node F diversifies into two groups, one leading to node G and the other to node K. Node K is a relatively small group containing taxa from Papua New Guinea and China. Node G is the ancestor to >95% of the Northern Hemisphere *Laccaria* species in the global *Laccaria* data set. Node J is derived directly from node G and it represents the ancestor to all Southeast Asian *Laccaria* taxa. The pattern that early diverging lineages, associated with node F, represented by *Laccaria* species collected from Southeast Asia and adjacent regions (Queensland, Australia, and Papua New Guinea to the south, and China to the north) is consistent with the theory of *Laccaria's* dispersal through Southeast Asia. But this may be coincidental unless the plausibility of this dispersal route can be established through other evidence.

Two of *Laccaria's* most important Australasian EcM hosts, the Myrtaceae and Nothofagaceae, have distributions into Papua New Guinea that could have facilitated the transition to Southeast Asia and points north from Australasia. In the middle to late Miocene (14–23 Ma) when global climate experienced a cooling and ice sheets began to grow in the Antarctic (Lewis et al. 2008; LaRiviere et al. 2012), there is evidence that this cooling resulted in the lowering of sea levels. This created a potential for land bridges to form between Australia, Papua New Guinea and the Sunda plate—a plate that encompasses several Southeast Asian landmasses including most of Indonesia and the Philippines, along with Malaysia, Thailand, Laos, and Vietnam (Hall 2001).

When evaluating the fossil record of "primitive" Angiosperms in Asia and Australasia, Morley (2001) describes the Nothofagaceae first occurring north of 5° south latitude during the middle of the Miocene (5.3–23 Ma). In addition, Morley

shows that potential hosts in the Fagaceae (*Quercus*, *Castanopsis* and *Lithocarpus*) were also present in the same equatorial tropical forests during this time. Papua New Guinea is an important crossroads for Southern and Northern Hemisphere EcM hosts, as noted by Horak (1983) in his description of the co-occurrence of southern Fagaceae (e.g. Nothofagaceae) and northern Fagaceae (*Castanopsis* etc.) in that country. Extant *Laccaria* species in Papua New Guinea are collected from *Trysingne* dominated forests as well as forests containing *Castanopsis* and *Lithocarpus*. One potential outcome of the dispersal of *Laccaria* with the Nothofagaceae (and/or Myrtaceae) was the co-occurrence of EcM hosts in the Fagaceae that facilitated a host switch and the ability of *Laccaria* to disperse into forests north of the equator.

Another potential scenario that should be considered is the dispersal of *Laccaria* together with Myrtaceae into Southeast Asia. Extant distributions of *Eucalyptus* extend to Sulawesi (Ladiges et al. 2003), providing opportunities for Australasian *Laccaria* to disperse farther north before engaging in host switching. Association with both Fagaceae and Myrtaceae in Southeast Asia is observed in EcM *Calostoma* (Boletales). Ectomycorrhizal root tips colonized by *C. sarasinii* have been recovered from a *Lithocarpus* or *Castanopsis* (Fagaceae) host in peninsular Malaysia, as well as *C. retisporum*-colonized roots belonging to a Myrtaceae host identified as either *Tristaniopsis*, *Eugenia*, or *Myrtus* in Malaysian Borneo (Wilson et al. 2012b). Such evidence suggests that it may have been possible for *Laccaria* to make a host switch from the Myrtaceae to the Fagaceae due to the proximity of these hosts within Southeast Asian forests. Given that the Fagaceae has its origins in forests of Southeast Asia (Manos and Stanford 2001), a switch to these hosts would give *Laccaria* an opportunity to disperse into the temperate Northern Hemisphere and its mesophytic forests during the Miocene.

The dispersal of fungi from their Southern Hemisphere origins is not unprecedented. Bonito et al. (2013) studied the biogeographic history of the Tuberaceae and demonstrated that they shared *Laccaria's* Southern Hemisphere distribution between Australasia and South America. There is similar evidence that dispersal of the Tuberaceae likely occurred through Asia before further radiation in the Northern Hemisphere. The genus *Descolea* is native to Australasia but it also extends into Southeast Asia. Systematic analysis of the relationship between Australasian and Southeast Asian *Descolea* species is needed to better understand this biogeographic pattern.

13.8 Northern Hemisphere *Laccaria* Diversity and Biogeography

When compared to the Southern Hemisphere, the Northern Hemisphere provides more area and habitats for EcM fungi, with an abundance of opportunities for diversification. In the global *Laccaria* phylogeny, the clade identified by node G

consists solely of temperate Northern Hemisphere *Laccaria* (Fig. 13.1). *Laccaria* spp. are widely dispersed throughout the Northern Hemisphere, but the group's lack of morphological variability, relative youth, and wide dispersal makes it challenging to evaluate biogeographic patterns.

The low level of morphological variation within groups of Northern Hemisphere *Laccaria* (e.g., *L. bicolor s. lat*, *L. ohiensis*, and *L. laccata s. lat.*) has limited the discovery of new species using the morphological species concept. As a result, the application of molecular methods has become necessary for the discovery and description of cryptic Northern Hemisphere *Laccaria* species from the Eastern Himalayas (Wilson et al. 2013) to southern China (Popa et al. 2014), and in Central America from Mexico (Montoya et al. 2015) to Panama (Popa et al. 2016). Despite the low levels of inter-specific sequence variation described earlier in the chapter, molecular methods should continue to be the preferred practice in describing Northern Hemisphere *Laccaria* species, but with greater emphasis on the genealogical concordance phylogenetic species recognition technique.

Some *Laccaria* species in the Northern Hemisphere may be classified as endemics. A group of Southeast Asian and Asian *Laccaria* form a distinct clade identified by node J (Fig. 13.1). Based on sampling and the resulting phylogenetic analysis, none of these taxa are expected to be encountered elsewhere. Because this group represents *Laccaria* that remained during the dispersal of the genus from the Southern Hemisphere, an exploration into the identities of their EcM hosts may yield clues that would test the previously described Southeast Asian dispersal hypothesis.

In looking at scales of endemism within Northern Hemisphere *Laccaria*, a clade identified by node H contains a number of species that may be categorized as endemics. A species that is endemic to particular habitats is *L. trullisata*, which is restricted to sand dunes of the Eastern and Midwestern United States. In contrast, *L. ochropurpurea* may be categorized as a regional endemic as this species occurs in mixed woodlands from the Midwest to the Northeastern United States. Another potential regional endemic in Asia is *L. himalayensis*, which is described from, and named after, the Himalayan alpine region. However, too few specimens have been studied to adequately establish the limits of this species' range, which can also be said for many other Northern Hemisphere *Laccaria* species.

13.9 Concluding Remarks on the Future of *Laccaria* Biogeographic Study

Laccaria is an excellent genus for further exploration of EcM fungal biogeography. The genus is large, but not so diverse as to prevent the sampling of most of its diversity. Its absence in tropical South America and sub-Saharan Africa greatly simplifies rigorous geographic sampling. An extensive base of morphological and molecular species delimitation has been established. In addition, the contrast

between Southern and Northern Hemisphere species creates a rich template from which to explore evolutionary questions regarding diversification and distribution in EcM fungi. Future research should continue to identify and define species using the GCPSR approach and settle upon an appropriate barcode region in which to screen the species identity of collections and further knowledge of their distributions. Lastly, linking species and their distributions to specific hosts will provide a framework to help mycologists and other evolutionary biologists understand how *Laccaria* species distributions are shaped by their EcM ecology.

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Chapter 14
Progress and Challenges in Understanding the Biology, Diversity, and Biogeography of *Cenococcum geophilum*

Keisuke Obase, Greg W. Douhan, Yosuke Matsuda, and Matthew E. Smith

14.1 Introduction

Cenococcum geophilum Fr. (Fries 1825) (syn. *C. graniforme* Ferd. and Wing.; Ferdinandsen and Winge 1925) was described as an anamorphic, melanized fungus characterized by the production of jet-black, hard, spherical sclerotia in forest soils (Massicotte et al. 1992). Since Linhell (1942) found that *C. geophilum* forms ectomycorrhizal associations with woody plants, the combination of the sclerotia, the ectomycorrhizal nutritional mode, and the distinct morphology of the ectomycorrhizas (Agerer and Gronbach 1988; Ingleby et al. 1990; Agerer and Rambold 2004–2016) have been accepted as crucial characters to identify *C. geophilum* (Fig. 14.1).

C. geophilum was one of the first ectomycorrhizal fungi to be studied in great detail. In a seminal work, Trappe (1964) showed that *C. geophilum* has an extremely wide host range and forms ectomycorrhizas with gymnosperms (such as species of Pinaceae) and angiosperms (such as species of Fagaceae, Betulaceae,

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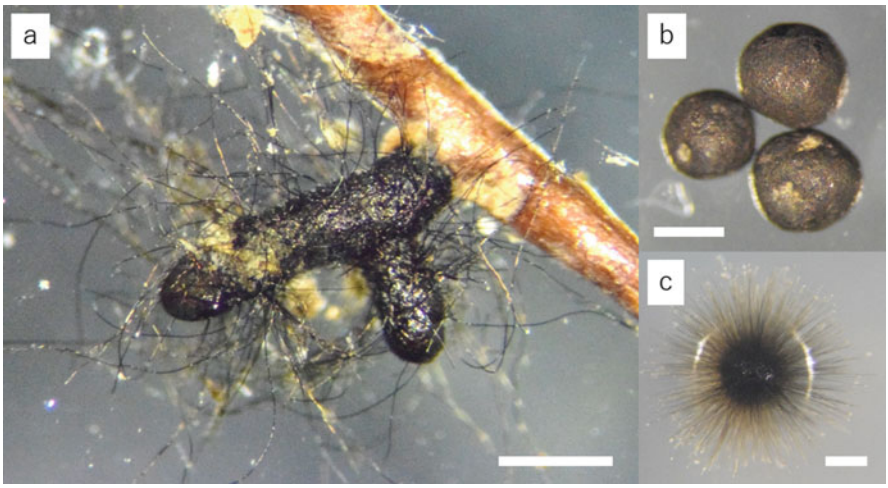

Fig. 14.1 *Cenococcum geophilum*. (**a**) An ectomycorrhizal root of *Betula ermanii* that has been colonized by *C. geophilum*. (**b**) Fresh sclerotia of *C. geophilum* after they were extracted from forest soils. (**c**) Hyphae extending from a *C. geophilum* sclerotium as it begins to grow in axenic culture on an agar plate. Bars = 0.5 mm

and Salicaceae). *C. geophilum* is also widely distributed in boreal, temperate, and subtropical regions where compatible host plants grow and is also often a major component of ectomycorrhizal fungal communities (Trappe 1962, 1964; Dahlberg et al. 1997; LoBuglio 1999; Horton and Bruns 1998). It is no exaggeration to say that *C. geophilum* is ubiquitous on roots of ectomycorrhizal woody plants in most natural and second-growth forests, although it appears that *C. geophilum* may be absent or rare on ectomycorrhizal trees in some tropical biomes (Tedersoo et al. 2010; Smith et al. 2011).

At the global scale, *C. geophilum* is considered one of the most ubiquitous ectomycorrhizal fungi in forest soils and on woody plant roots. Despite the fact that *C. geophilum* is common across many habitats on multiple continents, no sexual or asexual spores have ever been convincingly recorded for this fungus and we do not actually know how the fungus spreads in nature. Fernández-Toirán and Águeda (2007) recorded a cleistothecium that they considered to be a fruitbody of *C. geophilum*. However, the identity of this cleistothecium was not confirmed based on direct physical connection between mycorrhizas and fruitbody or using molecular tools, and therefore remains open to interpretation and doubt. The available evidence suggests that *C. geophilum* disperses clonally via sclerotia and hyphal growth between root tips and should therefore be limited to short-distance dispersal. Although the short-distance exploration type of mycorrhizas (Agerer 2001), limited hyphal growth, and sclerotia formation in *C. geophilum* suggest the likelihood of short-distance dispersal, these morphological observations contrast with the fact that *C. geophilum* is abundant and ubiquitous in many forests. These contradictory observations suggest that population biology studies using

molecular tools are needed to elucidate the biology of *C. geophilum*, explain how it is dispersed, and determine if it undergoes sexual recombination.

The inability to mate strains of *C. geophilum* in the lab has previously hampered accurate identification of the fungus and limited our understanding of genetic variation and genetic spatial patterns in this fungus. However, starting in the 1990s advances in molecular techniques clarified the phylogeny, ecology, and systematics of *C. geophilum*. Phylogenetic studies have recently shown that *C. geophilum* is a member of the Gloniaceae (Dothideomycetes, Ascomycota; Spatafora et al. 2012). Molecular approaches can also convincingly identify samples of *C. geophilum* (Matsuda et al. 2015) to genotype level and this approach will be critical to understand the dispersal mode of this fungus as well as elucidating the population structure at various spatial scales from soil cores to regions to continents (e.g., Wu et al. 2005; Douhan et al. 2007a; Matsuda et al. 2015). Evidence from population studies and from phylogenetic analyses all suggested that there is some cryptic recombination process that occurs in *C. geophilum* (LoBuglio and Taylor 2002; Douhan et al. 2007b; Bourne et al. 2014; Matsuda et al. 2015). Moreover, a recent study found a sex-related gene (MAT1-1-1) and genes encoding pheromone response proteins that are involved in the formation of fruiting bodies in the genome of a *C. geophilum* isolate (Peter et al. 2016). This suggests that *C. geophilum* likely is able to form fruiting bodies and reproduce sexually.

Simultaneously, however, molecular data have clarified several critical issues for understanding the spatial genetic distribution and population biology of *C. geophilum*. First, *C. geophilum* is monophyletic but either an extremely heterogeneous species or (more likely) a species complex (LoBuglio et al. 1991). A series of studies indicated high local and global genetic diversity within *C. geophilum* and the presence of several cryptic lineages that are likely distinct species (Douhan and Rizzo 2005; Douhan et al. 2007a; Matsuda et al. 2015; Obase et al. 2016a). Because all of these lineages look essentially identical in the morphology of their sclerotia, root tips, and axenic cultures, there is an accidental risk of including phylogenetically distant isolates in population genetic analyses. As pointed out by Douhan and Rizzo (2005), there have also been cases where melanized, sclerotia-forming fungi from outside of the monophyletic *C. geophilum* lineage have accidentally been included in population studies and may have generated spurious results. Another factor to consider is that genetic diversity is occasionally high even within individual soil cores due to co-occurrence of distinct lineages at a small spatial scale. The co-occurrence of multiple distantly related lineages within individual soil cores means that the results of population genetic and phylogenetic diversity studies will be directly related to the amount of sampling effort that is expended. An additional but related issue is that the *C. geophilum* genotype pools detected from ectomycorrhizas may be systematically different from the pool of isolates obtained from sclerotia (Obase et al., unpublished). In addition to those issues, there are relatively few studies that have examined the diversity of *C. geophilum* outside of the USA, Europe, and Japan so our global view of this group of fungi is still limited to certain regions. The populations and lineages of *C. geophilum* in Africa, South America, Australasia, and most of central Asia remain almost completely unknown.

In this chapter we revisit the host range and global distribution of *C. geophilum*, which has not been compiled in a review since the overview provided by Trappe (1964). We will also discuss the challenges for understanding the biogeography of *C. geophilum* in light of the high number of cryptic species, the co-existence of multiple lineages at small spatial scales, and the unknown aspects of the lifecycle of *C. geophilum*. We discuss the implications of the most recent in-depth studies that revealed spatial genetic structure of one lineage of *C. geophilum* at larger geographical scales in Japanese pine forests. Finally, we discuss future research directions that will be needed to understand the spatial genetic structure of a common but enigmatic ectomycorrhizal fungus, *C. geophilum*.

14.2 Host Range and Distribution

The global host range and distribution of *C. geophilum* was summarized by Trappe (1964) but a large number of mycorrhizal studies and an excellent review (LoBuglio 1999) have been published since that landmark paper. As a host for *C. geophilum*, Trappe (1964) listed 129 species/variations/hybrids in Pinaceae (*Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, *Tsuga*), Betulaceae (*Alnus*, *Betula*, *Carpinus*, *Corylus*), Ericaceae (*Arctostaphylos*), Fagaceae (*Castanea*, *Castanopsis*, *Fagus*, *Lithocarpus*, *Nothofagus*, *Quercus*), Myrtaceae (*Eucalyptus*), Rosaceae (*Cercocarpus*, *Chamaebatia*, *Rosa*, *Sorbus*), Salicaceae (*Populus*, *Salix*) and Tiliaceae (*Tilia*). In addition to these original reports, *C. geophilum* has been reported on a large number of temperate or arctic-alpine woody host genera: *Adenostoma*; Allen et al. (1999), *Arbutus*; Molina and Trappe (1982), *Cassiope*; Väre et al. (1997), *Cathaya*; Vaario et al. (2006), *Cedrus*; Bakshi et al. (1968), *Chosenia*; Hashimoto and Higuchi (2003), *Dryas*; Haselwandter and Read (1980), *Ostryopsis*; Bai et al. (2003), and *Photinia*; Grand (1971). *C. geophilum* has also been reported from several genera of tropical and subtropical woody hosts in Dipterocarpaceae [*Dipterocarpus*; Phosri et al. (2012), *Dryobalanops*, *Hopea*, *Parashorea*; Brearley et al. (2003), *Shorea*; Brearley et al. (2007), *Tristaniopsis*; Alexander and Högberg (1986)] as well as in *Coccoloba uvifera* (Séne et al. 2015). In addition to the genera of trees and shrubs mentioned above, *C. geophilum* has also been shown to form ectomycorrhizas with herbaceous species in Cistaceae (*Helianthemum*, *Hudsonia*, *Lechia*) (Malloch and Thorn 1985; Dickie et al. 2004), Cyperaceae (*Kobresia*) and Polygonaceae (*Polygonum*) (Massicotte et al. 1998; Mühlmann et al. 2008) as well as with mycoheterotrophic plants in the genera *Hemitomes*, *Pleuricospora*, and *Pterospora* (Castellano and Trappe 1985).

As we can deduce from the exceedingly wide host range, *C. geophilum* is widely distributed in boreal, temperate, and subtropical regions (Trappe 1962, 1964; Dahlberg et al. 1997; LoBuglio 1999; Horton and Bruns 1998). Most ectomycorrhizal studies in tropical regions have reported that *C. geophilum* is absent or infrequent in ectomycorrhizal fungal communities (Diédhiou et al. 2010; Tedersoo et al. 2010; Smith et al. 2011; Corrales et al. 2016). However, several recent studies have documented *C. geophilum* from tropical biomes, suggesting that novel and

undiscovered host associations are likely to be found from locations where the ectomycorrhizal fungal communities were not sufficiently surveyed (Morris et al. 2008; Phosri et al. 2012; Dokmai et al. 2015; Séne et al. 2015).

C. geophilum has been found in contrasting climatic regions, including arctic (Fujiyoshi et al. 2011) and subarctic (Hrynkiewicz et al. 2009) to tropical (Phosri et al. 2012) and subtropical regions (Trappe 1962; Obase et al. 2016a). *C. geophilum* is also found across wide elevational gradients from coastal forests near sea level (Matsuda et al. 2009a, b; Obase et al. 2009, 2011; Séne et al. 2015) to alpine habitats at or near treeline (Hasselquist et al. 2005). *C. geophilum* is often dominant in ectomycorrhizal fungal communities that are exposed to high drought stress, including pine forests on sand dunes (Matsuda et al. 2009a, b; Obase et al. 2009, 2011) and seasonally dry woodlands and savannahs (Smith et al. 2007). Also, *C. geophilum* is found in serpentine soils that are known to have phytotoxic levels of Mg and/or Ni (Panaccione et al. 2001; Moser et al. 2009). Factors involving the wide ecological niche have not been fully investigated but one possibility is that the high levels of melanin in fungal cell walls may contribute to tolerance of environmental stress such as drought (Fernandez and Koide 2013) and toxicity from heavy metals. This result has been previously shown in melanized pathogenic fungi (Gómez and Nosanchuk 2003). Recently, a genomic study of *C. geophilum* uncovered patterns that may partly explain the wide ecological niche of the fungus. *C. geophilum* has a larger genome (ca. 178 Mb) compared with other Dothideomycetes owing to the high content of transposable elements (Peter et al. 2016). Transposable elements are correlated with the plasticity and adaptability of fungi to their environment (e.g., Casacuberta and González 2013). Last, sclerotia of *C. geophilum* are excellent resting structures that may remain active for several years (Trappe 1962; Miller et al. 1994). These structures act as a spore bank and readily colonize host plant roots in response to disturbance like other disturbance-adapted ectomycorrhizal fungi such as *Rhizopogon* spp. Indeed, *C. geophilum* sclerotia are the most resistant structures of ectomycorrhizal fungi and they can survive long-lasting drought treatments and readily survive soil heating of 45–60 °C (Izzo et al. 2006; Glassman et al. 2015; Miyamoto and Nara 2016).

14.3 Phylogenetic Diversity in the *C. geophilum* Species Complex

The phylogenetic position and the closest relatives of *C. geophilum* have been unknown until recently, because no sexual and asexual spores were recorded. Based on similar morphological characteristics of sclerotia, anatomical features in hyphae and the ability to form ectomycorrhizas with woody plants, *C. geophilum* was historically hypothesized to be an anamorphic stage of *Elaphomyces* (Eurotiales, Ascomycota) (Ferdinandsen and Winge 1925; Trappe 1971). Co-occurrence of *C. geophilum* and *Elaphomyces* spp. in several forests also supported this idea.

However, LoBuglio et al. tested the hypothesis using rDNA hybridization (LoBuglio et al. 1991) and phylogenetic analysis of the 18S rDNA (LoBuglio et al. 1996). They found that *C. geophilum* is genetically distinct from *Elaphomyces* spp. and is not a close relative. More recently, Spatafora et al. (2012) analyzed the phylogenetic relationships of *C. geophilum* with other members of Dothideomycetes (Ascomycota) based on five loci. They found that *C. geophilum* is closely related to the genus *Glonium* and is an isolated ectomycorrhizal lineage not closely related to any other known mycorrhizal fungi. The ecology of *Glonium* is not well understood, but species in this genus are likely non-mycorrhizal saprobes that inhabit soil or decaying wood (Kantvilas and Coppins 1997). Interestingly, species of *Glonium* form darkly pigmented, carbonaceous ascomata (modified hysterothecia—Boehm et al. 2009). It is not known if *Glonium* species form sclerotia in soils. A BLAST search based on the ITS sequence of *Glonium stellatum* deposited in MycoCosm (Grigoriev et al. 2014; http://jgi.doe.gov/fungi) revealed that similar sequences have been detected from ericaceous plant roots and suggest that some members of the genus may be able to colonize roots. Obase et al. (2016a) recently discovered a fungus that was isolated from surface-sterilized *Cenococcum*-like sclerotia from soil but this fungus was not resolved within the *C. geophilum* lineage. This species was described as *Pseudocenococcum floridanum* K. Obase, G.W. Douhan, Y. Matsuda and M.E. Smith and is genetically closer to *C. geophilum* than to species of *Glonium*. *P. floridanum* is morphologically similar to *C. geophilum* but grows faster in culture and did not form ectomycorrhizas with pine and oak seedlings. The fungus is likely a saprobe and the closest known relative of *C. geophilum*. The discovery of these close relatives of *C. geophilum* (*Glonium* and *Pseudocenococcum*) suggests that the ancestor of *C. geophilum* was morphologically similar to *P. floridanum* and *C. geophilum,* grew in forest soil and formed sclerotia but was probably not ectomycorrhizal.

Due to the lack of closely related ectomycorrhizal fungi and the distinct morphological characteristics of ectomycorrhizas, *C. geophilum* is regarded as a unique ectomycorrhizal fungus that can be identified reliably to the 'species' level based solely on morphological characteristics of ectomycorrhizal roots. However, previous studies have often found diverse cultural and physiological characteristics among *C. geophilum* isolates (LoBuglio 1999), indicating genetic diversity and/or the presence of cryptic species in *C. geophilum*. LoBuglio et al. (1991) were the first to document high genetic variation among *C. geophilum* isolates from geographically divergent locations in the USA and Europe. This was the first evidence to indicate that *C. geophilum* was either an extremely heterogeneous species or a species complex. Even though the ITS region is rather conserved within *C. geophilum* (Shinohara et al. 1999), high genetic diversity was nonetheless detected in a series of studies using a variety of molecular biology methods that sampled at various spatial scales from forest stands to regions to continents (Panaccione et al. 2001; Portugal et al. 2001; Jany et al. 2002; Douhan and Rizzo 2005; Wu et al. 2005; Chen et al. 2007; Gonçalves et al. 2007; Bahram et al. 2011; Spatafora et al. 2012; Matsuda et al. 2015; Obase et al. 2016a). For example, Douhan and Rizzo (2005) found three phylogenetically distinct lineages within

C. geophilum populations from one oak stand in California based on glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the ITS region, the mitochondrial SSU (mit SSU), and an intron in the 18S rDNA. Douhan et al. (2007b) further examined genetic variation using the GAPDH gene sequence by adding isolates of *C. geophilum* from Europe but found that phylogeographic inference was obscured and that the backbone nodes of this larger phylogeny had poor bootstrap support. Interestingly, however, isolates from different continental origins were often intermingled in the phylogenetic tree. Obase et al. (2016a) revisited the phylogenetic diversity of *C. geophilum* at an intercontinental scale by using new data from Florida (USA) with existing data from Douhan et al. (2007b) and Japan (Matsuda et al. 2015) based on two loci (ITS and GAPDH). The combination of the two loci resolved six well-supported lineages and some of them included isolates from different geographical regions, as shown in Douhan et al. (2007b). Re-analysis of the phylogeny of a smaller subset of isolates with more genes (ITS, GAPDH, SSU, LSU, TEF, RPB1, and RPB2) confirmed the uniqueness of the six cryptic lineages but also resolved some higher-level relationships among them (e.g. clades 1, 2 and 4 are clustered together with a 87% bootstrap support—Fig. 14.2).

Although the ITS region is not the ideal locus for delineating lineages within *C. geophilum* sensu lato (Obase et al. 2016a), this DNA region can nonetheless be used to identify additional phylogenetic diversity within *C. geophilum* (Bahram et al. 2011) by using the massive sequence data that are deposited in the UNITE database (Kõljalg et al. 2013). Three-hundred-forty-four ITS sequences from putative *C. geophilum* were available by searching with the query "*Cenococcum*" in the UNITE database (https://unite.ut.ee/, accessed October 2016). These sequences originate from various geographic regions, including North and South America, Europe and Asia. They can be divided into 12 groups based on 97% sequence similarity cutoff. Most of the ITS sequences were unified into one putatively monophyletic group that includes sequences from different geographical regions ($n = 318$). However, a few sequences from North America (Canada and USA), Asian countries (China, Thailand, Pakistan) and Sweden were resolved into distinct groups. In addition, several unique *C. geophilum* ITS groups that are delineated by 97% sequence similarity were detected in forests across various geographical regions (e.g., Ge et al. 2012; Huang et al. 2014). Although it is possible that these distinct groups of ITS sequences could be chimeras generated during PCR or artefacts of low read quality, cloning, or sequencing, it is also possible that these could be unique, undescribed species that are more distantly related to *C. geophilum* sensu lato. We expect that extensive sampling of ectomycorrhizal roots and sclerotia from different geographical regions and with phylogenetically unique host plants is likely to yield a large number of unique lineages of *C. geophilum* that match these unique ITS sequences, much like we found in our intensive studies in Florida, USA (Obase et al. 2016a).

All available evidence suggests that *C. geophilum* is a species complex. Therefore, it is extremely important not to include phylogenetically-distinct lineages of *C. geophilum* together in analyses of spatial genetic structure and population biology. When unrelated *C. geophilum* lineages are inadvertently mixed together for these types

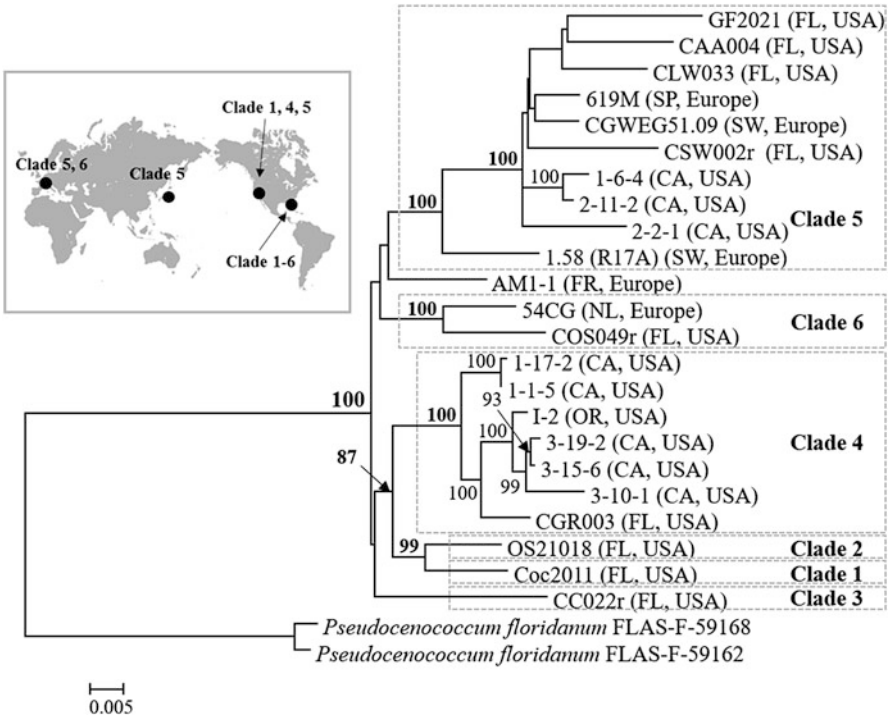

Fig. 14.2 The optimum phylogenetic tree of *C. geophilum* based on maximum likelihood analysis of seven concatenated loci (ITS, SSU, LSU, TEF, RPB1, RPB2 and GAPDH). The placement of each lineage is highlighted and clades 1–6 are named according to Obase et al. (2016a). Inset in the *upper left corner* shows the known global distribution of each lineage based on multi-gene data (although vast areas of the globe have not been sampled). Isolates of *P. floridanum* are included as outgroups. CA: California, USA, FL: Florida, USA, FR; France, NL: Netherland, OR: Oregon, USA, SP: Spain, SW: Switzerland

of analyses it is inevitable that we will infer erroneous spatial genetic patterns. Multilocus phylogenetic analysis, even based on only two loci (Obase et al. 2016a), is an important option for determining the phylogenetic affinities of *C. geophilum* samples and then selecting adequate data for the analysis of spatial genetic structure. The procedure is also useful for excluding unrelated dematiaceous fungi that can be accidentally obtained from sclerotia of *C. geophilum* as contaminants (Douhan et al. 2007a; Obase et al. 2016b) or non-ectomycorrhizal fungi that are related to *C. geophilum,* such as *P. floridanum* (Obase et al. 2016a). For example, Jany et al. (2002) documented high genotypic diversity of *C. geophilum* at the scale of individual soil cores (10 × 10 × 10 cm) and a pattern of isolation by distance in five beech forests in northeastern France across approximately 250 km, using PCR/RFLP of the ITS region and sequence characterized amplified region (SCAR1) with the *Hinf*I endonuclease. However, a subsequent study found that some samples in the Jany et al. (2002) study were likely not *C. geophilum* based on LSU-rDNA or GAPDH gene sequences

and that their sampling probably included isolates from multiple lineages of *C. geophilum* (Douhan et al. 2007b). This makes interpretations regarding population genetic issues difficult.

14.4 High Genetic Diversity at Small Spatial Scales

We have a quite limited view of the genetic diversity of ectomycorrhizal fungi at a fine spatial scale (Douhan et al. 2011; Chap. 2). Many fruiting ectomycorrhizal fungi are known to form genets with extending hyphae from several centimeters to meters in soils (Douhan et al. 2011). One genotype often dominates within the soil samples collected from the central area of the genet (e.g., *Tricholoma matsutake*; Lian et al. 2006; *Tuber melanosporum*; Murat et al. 2013). In the case of *C. geophilum*, high genetic diversity has often been detected at the centimeter scale. For example, Douhan and Rizzo (2005) found sclerotia formed by three distinct lineages of *C. geophilum* from a single one-liter soil sample in oak forests in California, USA. Matsuda et al. (2015) studied several widely spaced coastal pine forests in Japan and found that one multi-locus genotype was present on *Pinus thunbergii* ectomycorrhizal roots in most soil samples (3 cm in diameter and 30 cm in depth). One-third of the samples, however, contained several different genotypes. Obase et al. (2016a) classified isolates of *C. geophilum* obtained from mixed pine-oak forests in Florida and Georgia (USA) into genotypes based on GAPDH sequences. They found that 75% of soil samples ($7 \times 7 \times 10$ cm) contained more than one genotype. Half of the samples included 2–3 genotypes but in the remaining 25% of the soil cores up to nine different genotypes were found to co-exist in these small samples.

Mechanisms that are involved in structuring such high genetic diversity at a fine spatial scale remained unclear. However, our recent research has explored one possible factor. Several previous studies that have focused on genetic diversity of *C. geophilum* have used either sclerotia (Portugal et al. 2001; Douhan and Rizzo 2005; Gonçalves et al. 2007) or ectomycorrhizal roots (Panaccione et al. 2001; Matsuda et al. 2015) for molecular analyses. Although either approach is valid as a way of detecting diversity within *C. geophilum*, it is possible that unique pools of genotypes are present in sclerotia versus ectomycorrhizal roots due to different turnover rates. Sclerotia are excellent resting structures that can remain viable in soil for a long period of time (several years; Trappe 1962), while ectomycorrhizal roots likely remain active for much less time (Fernandez et al. 2013). Obase et al. (unpublished) compared genotypic diversity in *C. geophilum* isolates from sclerotia and from ectomycorrhizas that were collected in the same $7 \times 7 \times 10$ cm soil samples (see Obase et al. 2016a for sampling details). They found that many genotypes were unique to sclerotia or ectomycorrhizas and >50% of genotypes were unique to only one of the sources in most samples. Rarefaction analysis indicates that genotypic diversity was significantly higher in sclerotia than in ectomycorrhizas. This finding suggests that the pool of genotypes that are actively

growing on ectomycorrhizal roots are a more limited subset of the local genotypic diversity than the genotypes found as sclerotia. Furthermore, this suggests that different life forms (e.g. sclerotia versus ectomycorrhizas) play different roles in structuring the high genetic diversity of *C. geophilum*. The results also indicate that (1) sampling both sclerotia and ectomycorrhizas is optimal to maximize the detection of genetic diversity in *C. geophilum* at a fine spatial scale and that (2) intensive sampling effort is probably required in many habitats to adequately assess the genetic diversity of *C. geophilum* due to the complexity at a fine spatial scale (Obase et al. 2016a).

14.5 Patterns in *C. geophilum* at Larger Geographic Scales

Many ectomycorrhizal fungi produce sporocarps and disperse large numbers of spores via wind or mammalian mycophagy to colonize new habitats and increase genetic diversity at the landscape scale. Both spore dispersal and vegetative hyphal growth play important roles for structuring the spatial genetic structure of ectomycorrhizal fungi (e.g., Douhan et al. 2011). In contrast to other ectomycorrhizal fungi, *C. geophilum* has been considered a putatively asexual fungus, and no spores have ever been convincingly discovered. It has been suggested that the vegetative sclerotia and extending hyphae of *C. geophilum* are the only means of dispersal for this fungus. In theory, these dispersal mechanisms should be less efficient than the large numbers of microscopic spores that are produced by most fungi and distributed by wind, water, or animals (e.g., Maser and Maser 1987). Because there are no other known anamorphic ectomycorrhizal fungi for which spatial genetic structures have been studied, it is difficult to predict the spatial genetic structure that should be hypothesized for *C. geophilum*. However, if sclerotia and hyphal growth are truly the only dispersal mechanisms for *C. geophilum* then we might expect highly localized populations with evidence of limited gene flow.

However, many recent studies found evidence of cryptic recombination in organisms that were previously considered to be asexual (Kück and Pöggeler 2009). For example, the human-pathogenic fungus *Aspergillus fumigatus* was considered to be asexual but were later shown to undergo cryptic recombination (O'Gorman et al. 2009). Several authors have recently suggested that *C. geophilum* may also undergo cryptic recombination (Douhan et al. 2007a; Bourne et al. 2014; Matsuda et al. 2015; Peter et al. 2016). If present, cryptic recombination would certainly alter the spatial genetic structure in *C. geophilum* populations (as compared to a completely asexual lifecycle based only on clonal propagation).

Only a handful of studies have conducted detailed population-level research in *C. geophilum*. Wu et al. (2005) investigated the genetic structure of four *C. geophilum* populations in *Salix reinii* patches across several kilometers in an early successional volcanic desert on Mount Fuji in Japan using five microsatellite markers. They found that genotypic assemblages of *C. geophilum* were spatially

heterogeneous. Two of the populations harbored many common genotypes whereas the remaining populations did not share any overlapping genotypes. Wu et al. (2005) inferred that frequent avalanches may transfer sclerotia of *C. geophilum* with scoria to lower positions on the slope and therefore contribute to the shared genotypes between some patches. They also found that two geographically close populations of *C. geophilum* did not harbor common genotypes and they suggested that a small valley between the populations could act as a barrier for gene flow. Next, they suggested that recombination in the population was absent or rare due to the fact that there were no 'intermediate' genotypes between the two distinct groups of genotypes in each population. These results indicate that spatial distance and other physical barriers may contribute to spatial genetic structuring of *C. geophilum* at the spatial scale of kilometers. One problem with the Wu et al. (2005) study is that they did not account for the fact that *C. geophilum* is a species complex and it is therefore likely that they sampled a mixed population that included several cryptic species. Accordingly, it is difficult to fully interpret the results of this study and to determine whether the results might change if the cryptic phylogenetic species of *C. geophilum* had been recognized.

Matsuda et al. (2015) studied the spatial population structure of *C. geophilum* but they accounted for cryptic species by selecting one dominant lineage using GAPDH barcoding followed by phylogenetic analysis. They found significant genetic variation and no significant spatial autocorrelation within each stand of *P. thunbergii* coastal forests (1–5 ha). In most cases, although identical genotypes were not detected from adjacent soil samples within each stand, they were infrequently detected from samples that were 10–50 m apart (and in some cases even >100 m apart), indicating that genet size may be small or genets may be spatially fragmented. It is possible that the high genetic diversity of *C. geophilum* is maintained by cryptic recombination processes at the landscape scale. Indeed, linkage disequilibrium tests favored recombination as a more likely explanation for the genetic variation rather than clonal reproduction. Next, genetic distance among the populations was weak but significantly correlated with geographic distance (17–1364 km), suggesting a pattern of isolation by distance (Fig. 14.3). The result indicates that unknown migration events might influence spatial distribution and genetic structure of *C. geophilum* in coastal pine forests at the regional scale. The study by Matsuda et al. (2015) therefore suggests that the spatial genetic structure of *C. geophilum* is actually somewhat similar to the genetic structure of other ectomycorrhizal fungi that disperse predominantly via spores and less via mycelial growth.

Even though *C. geophilum* is found in forests on many continents, no population genetic studies have been conducted at the global scale. However, Obase et al. (2016a) inferred some broader patterns in the genetic diversity of *C. geophilum* at the continental scale. The combination of ITS and GAPDH resolved several well-supported phylogenetic clades which included isolates from different geographical regions in North America and several European countries. This suggests that some *C. geophilum* lineages have dispersed widely within and between continents or that cryptic long-distance dispersal is ongoing via some unknown method. On the other

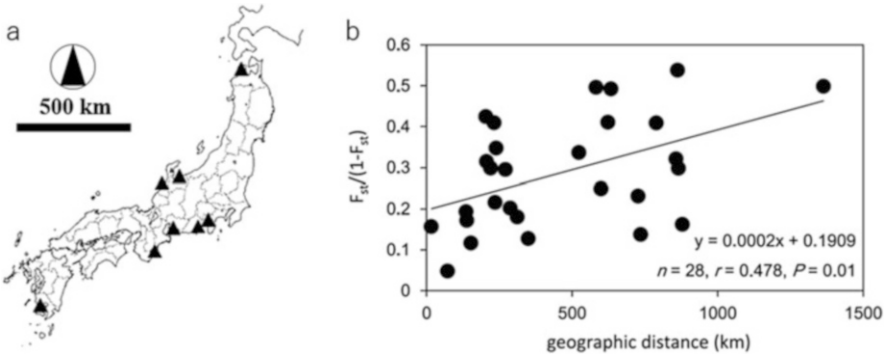

Fig. 14.3 (**a**) Locations of *Cenococcum geophilum* populations in several coastal pine forests in Japan. (**b**) Relationship between genetic differentiation among the populations of *C. geophilum* (*y*-axis) and geographic distance (*x*-axis). The figure was modified from Matsuda et al. (2015)

hand, even though isolates from different geographical regions (USA, Japan and Europe) were clustered together in the phylogenetic tree, another analytical tool (PTP; Zhang et al. 2013) further delimited them into several taxa per major lineage. Based on this stricter, narrower approach, few of the *C. geophilum* taxa harbored samples from different geographical regions or continents. Also, several isolates from the same study sites (e.g. same forest stands) were often delimited into several species and isolates from different geographic regions (e.g. California and Florida, USA) were often interspersed between one another in the trees, suggesting that there are no obvious broad biogeographic inferences that can be made without data from more genes and more isolates. Together the available data indicate that the broad phylogenetic approach used by Obase et al. (2016a) to identify major lineages probably underestimates the total number of cryptic taxa. Also, it is possible that there has been both sympatric and allopatric speciation at nearly the same rate among different continents, regions, and sites for each of the phylogenetic lineages thereby helping to partially explain the high cryptic diversity within *C. geophilum*.

14.6 Future Directions

A series of population genetic studies suggest that cryptic recombination, geographic distance, and physical barriers may structure the spatial genetic patterns in *C. geophilum* at the forest stand and regional scales, similar to what has been observed for other ectomycorrhizal fungi. However, we still have limited knowledge of the ecology of *C. geophilum,* particularly when it comes to reproduction and dispersal. The presence of cryptic species that can only be identified via genetic screening (e.g. DNA sequencing or other similar molecular approaches) makes it even more challenging to understand the broad biogeographical patterns in this

group of fungi. Unfortunately, we also lack information on how environmental factors influence the spatial genetic structure of different lineages in *C. geophilum*. We know that many other soil fungi are strongly influenced by the distance from the equator and mean annual precipitation (Tedersoo et al. 2014) whereas ectomycorrhizal fungi are also clearly affected by the diversity and composition of the host plants.

We assume that because *C. geophilum* is found on the roots of a wide range of ectomycorrhizal host plants in nature and that individual isolates can form ectomycorrhizas on the roots of phylogenetically distinct plants (e.g., pines and oaks), the host does not have a strong influence on the population structure. However, some evidence of host specificity has been documented by inoculation tests using several *C. geophilum* isolates on different host plants (Antibus et al. 1981). Next, although Gonçalves et al. (2007) indicated that soil properties did not influence genotypic differentiation among serpentine sites, we still cannot rule out the possibility that unique environments may select given genotypes (Branco et al. 2015) and therefore contribute to structuring of *C. geophilum* populations.

Although *C. geophilum* is challenging to study due to the unknown aspects of the life cycle, this fungal group has the potential to be a model system for studying ectomycorrhizal fungi because it is so widespread in many habitats from tundra to rain forests. Since *C. geophilum* is culturable and can be found in such widely varying forest types this group would be ideal for studying how genotypic diversity and population genetic patterns are influenced by various kinds of abiotic and biotic factors.

For better understanding of spatial genetic structure over different geographical scales, the meta-analysis of spatial genetic patterns in *C. geophilum* is needed. In the future, it could be helpful to establish common sampling schemes in studies across different sites so that results from different studies and across different biomes could be easily compared to one another.

Another challenge for studying *C. geophilum* is that genetic markers that are used to study one cryptic species do not always work well on the other cryptic species so that individual markers have to be developed for each lineage. Selection of samples based on ITS and GAPDH sequences is a useful first step in any molecular pipeline because both markers are easily amplified from cultures, sclerotia, or ectomycorrhizal roots of *C. geophilum*. Furthermore, the GAPDH locus is phylogenetically informative and has a growing database of identified samples. In the future, it will be best to follow ITS + GAPDH screening with next generation sequencing (NGS) strategies that have been recently developed for population genetics. For example, RAD-seq (randomly amplified DNA sequencing) is a powerful tool that can generate several hundreds to thousands of genetic markers applicable to different samples that contain cryptic species. So far, there have been no population studies that have used NGS sequencing approaches to examine the population biology of *C. geophilum*. Using this type of high-throughput approach in combination with sampling across several global regions (e.g. in areas that have remained unsampled for diversity of *C. geophilum* such as central Asia, Oceania, Africa and South America) would certainly provide a new, comprehensive view of the biogeography of *C. geophilum*.

The use of powerful NGS tools may also potentially provide insights into the unknown ecology of *C. geophilum*. The first matter of concern is the possibility of recombination among individuals of *C. geophilum*. Recent studies of genome sequencing found genes related to recombination, i.e., mating genes, in several ascomycetous fungi for which the mating systems were not previously understood. These studies have showed for example that *Tuber* spp. are heterothallic (Rubini et al. 2011; Belfiori et al. 2013) and also have identified sexual recombination in fungi that were previously considered to only reproduce asexually, such as *Aspergillus* (Pöggeler 2002) and *Ulocladium* spp. (Geng et al. 2014). The recent study found that one isolate of *C. geophilum* had one mating-type gene (MAT1-1-1) that was intact and conserved with close relative *Glonium* species which form fruiting bodies. The presence of genes involving recombination and forming fruit bodies in the genome of *C. geophilum* indicates the possibility that *C. geophilum* retains the ability to have sexual recombination like its close relatives in the genus *Glonium* (Peter et al. 2016). If the presence of other mating type genes (i.e., MAT1-2-1) is found in other isolates of *C. geophilum*, then spatial patterns of *C. geophilum* individuals in relation with the mating types may provide insights about how recombination occurs spatially in forests. This type of data would provide critical information about how populations of *C. geophilum* are structured and whether mating is common, rare, or truly absent in this group of fungi.

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Chapter 15
Biogeography of the Japanese Gourmet Fungus, *Tricholoma matsutake*: A Review of the Distribution and Functional Ecology of Matsutake

Lu-Min Vaario, Xuefei Yang, and Akiyoshi Yamada

15.1 Introduction

Tricholoma matsutake, an ectomycorrhizal (EcM) fungus, is regarded as one of the most desirable mushrooms in the world (Hall et al. 2003). The first research concerning *T. matsutake* was published in Japan over 100 years ago and the field has since grown into a community of researchers in Asia (Ogawa 1978; Yamada et al. 1999; Gong et al. 1999), North America (Hosford et al. 1997; Chapela and Garbelotto 2004) and Europe (Bergius and Danell 2000; Vaario et al. 2010) due to its high value as a non-timber forest product in Japan and the Far East. Recently, global climate change and over-harvesting have raised serious concerns about the resource status and stainability of matsutake populations.

Typically, EcM fungi enhance the nutrient uptake of their host tree and import carbohydrates to the ectomycorrhizosphere through the root–mycelium interface. The ectomycorrhizosphere, which forms a specific interface between the soil and the symbiotic fungi, harbors a large and diverse community of microorganisms that can either inhibit or enhance each other (Smith and Read 2008). The identity of the host-tree and soil characteristics are considered key elements defining the preferred habitat of matsutake and can affect its subsequent productivity. Detailed studies of *T. matsutake* in natural settings led by M. Ogawa during the 1960s and 1970s

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(e.g. The Matsutake Research Association 1964; Ogawa 1978) built the foundation on which modern matsutake research is based (Hosford et al. 1997, see review Wang et al. 2012). Demand for the mushroom as a culinary delicacy has stimulated research that aims to understand the enigmatic role matsutake plays in the forest ecosystem and its highly variable fruiting behavior. Here, we review recent findings from the molecular to ecological scale within the global geographic context, and focus on community structure, biogeography and characterization of the extended shiro and where the mycorrhizae and extraradical mycelium of *T. matsutake* form a whitish mycelium–soil aggregate from which fruiting-bodies develop. The current knowledge base is placed into the context of functional ecology of EcM fungi and forest management.

15.2 Host Diversity of *T. matsutake*

15.2.1 Circumboreal Distribution of T. matsutake *and Related Species*

The taxonomy and phylogeny of matsutake are central to understanding the current distribution of *T. matsutake* and its host associations (Ryman et al. 2000; Ota et al. 2012; Christensen and Heilmann-Clausen 2013). The "Caligata" clade of matsutake mushrooms (Murata et al. 2013b) in the section Caligata (Bon 1991) consists of several *Tricholoma* species associated with conifers, of which the basal member is *T. caligatum* from Europe. According to a phylogeny inferred from retrotransposon elements, the ancestral population of *T. caligatum* shifted host from fagaceous trees to conifers (Murata et al. 2013b). A similar evolutionary shift is also inferred for conifer-associated matsutake in North and Central America, which dispersed through Beringia during the Eocene from a Eurasian ancestor associated with angiosperms (Chapela and Garbelotto 2004). Conifer-associated matsutake also include *T. anatolicum* from the Mediterranean (Intini et al. 2003; Yamada et al. 2010), *T. matsutake* from eastern Asia and central and northern Europe (Kytövuori 1988; Bergius and Danell 2000; Matsushita et al. 2005), and *T. magnivelare* and *Tricholoma* sp. (including *T.* cf. *caligatum* associated with conifers) from North and Central America (Hosford et al. 1997; Amaranthus et al. 2000; Bessette et al. 2013). The occurrence of matsutake in Japan, Korea, China and Fennoscandia suggests that *T. matsutake* is distributed widely throughout Eurasian forests (Yamada 2015), but samples of populations from central Asia and Siberia are currently lacking.

RFLP analyses of the intergenic spacer 1 (IGS1) region of genomic ribosomal RNA gene (rDNA) unfortunately could not resolve the metapopulation structure and dynamics of samples of *T. matsutake* from several locations in Eurasia (Guerin-Laguette et al. 2002; Matsushita et al. 2005). In the analysis of Asian *T. matsutake*, Murata et al. (2008) examined retrotransposon regions in the genome and distinguished local populations of *T. matsutake* in Japan, North Korea, South Korea,

northeast China, and southwest China through Bhutan. In particular, populations at the foot of the Tibetan Plateau and elsewhere in the Far East were highly distinct (Murata et al. 2008; Xu et al. 2010). This suggests isolation and diversification of *T. matsutake* populations during the last ice age (Ray and Adams 2001). One of the main questions to be addressed by future studies concerns the integration and connectivity of *T. matsutake* populations throughout its modern range (Suzuki 2005; Murata et al. 2015a). Although high-resolution genetics can provide evidence of gene flow among populations (Kretzer et al. 2005; Vincenot et al. 2012), it remains difficult to demonstrate the reproductive isolation of any particular one. In an attempt to resolve this issue, monokaryotic cultures of *T. matsutake* populations should be established (Murata et al. 2015a) to determine mating type and interfertility.

Regarding fine population structure, Xu et al. (2008) found a significant positive correlation between genetic distance and geographical distance among populations of *T. matsutake* in southwestern China, which showed significant but low genetic differentiation among populations. Amend et al. (2010) conducted a SNP analysis of *T. matsutake* populations in southwest China that distinguished samples from adjacent watersheds isolated by treeless ridgelines. As a result, they found that high-altitude treeless ridgelines are effective barriers to gene flow, even at distances of less than 65 km. Recently, Zeng and Chen (2015) revealed a clear genetic divergence among *T. matsutake* population from northeastern and southwestern China, two of the main regions producing matsutake for the global market. However, compelling evidence concerning a genetic basis for the host specificity in matsutake is lacking.

15.2.2 Host-Tree Associations of T. matsutake *in Japan*

In Japanese, *matsu-take* means pine mushroom, denoting the well-known association between *T. matsutake* and its main host there—the Japanese red pine (*Pinus densiflora*). Japanese red pine occurs naturally 0–2000 m a.s.l. from Yakushima in the south (30° N) to Hokkaido (42.5° N) in the north (Satake et al. 1989). In Japan, *T. matsutake* can be found in conifer forests from Hokkaido in the north to Kyushu in the south and west (ca. 31° N) (Hamada 1964; Ogawa 1978; Murata and Minamide 1989; Murata et al. 2001; Guerin-Laguette et al. 2002). Matsutake productivity has been monitored in Japan for several decades (Fig. 15.1), and the highest domestic harvests of recent years have come from Japanese red pine forests. In the deep mountainous terrain of Honshu, *Tsuga siebordii* and *T. diversfolia* are the main ectomycorrhizal hosts of *T. matsutake* in temperate and subalpine climates, respectively (Hamada 1964; Ogawa 1976b, 1977a, b; Endo et al. 2015). At the edge of the range of *P. densiflora* in Hokkaido, *P. pumila*, *Picea glehnii*, and *Abies sachalinensis* serve as hosts of *T. matsutake* in alpine, alpine-subalpine, and subalpine climates, respectively (Hamada 1964; Ogawa 1976a, b; Murata and Minamide 1989; Endo et al. 2015). Japanese subalpine forests are quite diverse in terms of

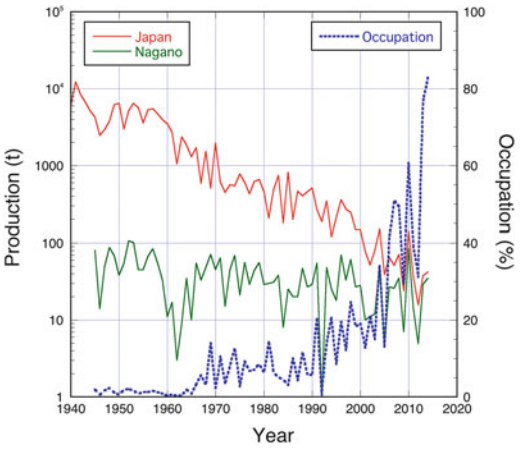

Fig. 15.1 Production of *T. matsuake* in Japan and Nagano Prefecture

conifers, especially on Honshu where pines, firs, spruces, hemlocks, a larch, and a false hemlock can be found. Unfortunately, little is known of their respective roles as host trees for *T. matsutake*, although *Abies veitchii* was recently confirmed as an alternative host (Endo et al. 2015). Given that the association between firs and *T. matsutake* has been confirmed in Japan, a comprehensive survey of host-tree use for populations in China and Fennoscandia should be performed.

15.2.3 Host Associations of T. matsutake *in Other Regions*

In China, *T. matsutake* has been reported in two separate areas: southwest including Yunnan, Tibet, Guizhou, Gangsu, Guangxi and Sichuan provinces, and northeast including Jilin and Heilongjiang provinces (Zang 1990). It is interesting to note that *T. matsutake* populations in China are believed to be naturally associated with both conifers and fagaceous trees (Amend et al. 2010; Yamanaka et al. 2011; Wan et al. 2012), whereas in Japan and northern Europe matsutake appears restricted to the roots of conifers. If a relationship between *T. matsutake* and fagaceous trees (oaks and beeches) is accurate, the evolutionary scenario of host use in this clade must be reconsidered in light of a phylogeny based on retrotransposon data (Murata et al. 2013b). To date, three genera in Fagaceae (i.e., *Quercus*, *Lithocarpus*, and *Pasania*) are listed as EcM hosts of *T. matsutake* in China (Yamanaka et al. 2011). However, these associations should be confirmed with molecular analyses of both partners in conjunction with morphological and ecological observations of EcM and fruiting-body formation in oak-dominated woodlands. Another important point concerning *T. matsutake* populations in China is that an annual mushroom harvest of >1000 tons represents ca. 70% of matsutake imported to Japan (Table 15.1). The Chinese harvest has been 20–50 times larger than that in Japan over recent years. If

Table 15.1 Matsutake import to Japan from abroad in the recent 5 years[a]

	China		USA		Canada		Turkey		Mexico		Morocco		World	
Year	Ton[b]	Yen[c]	Ton	Yen	Ton	Yen	Ton	Yen	Ton	Yen	Ton	Yen	Ton	Yen
2015	497	33	72	3.7	253	10	58	1.7	5	0.3	7	0.3	897	50.3
2014	669	35.3	212	9.7	87	4.2	88	2.1	7	0.4	–	–	1073	54.3
2013	775	39.7	214	7.9	173	6.2	27	0.7	17	0.9	3	0.1	1222	58.4
2012	1132	44.1	79	3.7	54	2.7	111	2.6	7	0.3	33	2.4	1436	56.2
2011	875	42.2	99	4.6	147	6.1	64	1.3	17	0.7	–	–	1215	57.1

The data was extracted from Database of Ministry of Agriculture, Forestry and Fisheries in 2016 (http://www.maff.go.jp/j/tokei/kouhyou/kokusai/index.html)

[a]This list includes *T. matsutake* and other related matsutake mushrooms: China import is mostly *T. matsutake* but included a small amount of *T. bakamatsutake* and potentially *T. fulvocastaneum*, USA and Canada imports are mostly *T. magnivelare*, Turkey and Morocco imports are mostly *T. anatolicum*, and Mexico import is Mexico import is mostly *Tricholoma sp.* (Yamada et al. 2010)

[b]The volume of import is indicated as metric ton

[c]The value means Japanese yen with $\times 10^8$

the host-species identity explains this difference in productivity, matsutake forests could be managed to maximize fruiting through the planting or selection of suitable tree species, controlling tree age and density, and careful harvesting to protect the industry and genetic diversity of the population.

Matsutake was known as *T. nauseosum* in northern Europe until molecular techniques revealed its conspecificity with *T. matsutake* (Bergius and Danell 2000; Matsushita et al. 2005). Unfortunately, studies dealing with its host-species, distribution and productivity there remain sporadic, most likely because matsutake mushrooms are not eaten by north Europeans. During the past 20 years, mapping of harvest data has shown that matsutake can be found at 350–400 localities in Fennoscandia, and the real number may be 10 times higher (The Global Fungal Red List Initiative 2015). In Finland and Sweden, *T. matsutake* has only been found in pine forests of at least 50 years old (Risberg et al. 2004). Among the three major forest tree species in Finland, *T. matsutake* has a confirmed association with *Pinus sylvestris* and *Picea abies* (Vaario et al. 2010), but no symbiotic relationship was found with *Betula pendula*.

15.2.4 Host Specificity

In general, host-plant genotype is believed to determine root colonization, ecological fitness, and metabolic activity of EcM fungi as well as the outcome of competitive interactions between two or more EcM fungi colonizing the same host (Bryla and Koide 1990; Tagu et al. 2005; Courty et al. 2011). In line with natural observations, in vitro trials have shown that matsutake can form root symbioses with conifers such as *Pinus*, *Picea*, *Abies* and *Tsuga* (Yamada et al. 1999, 2014; Gill et al. 2000; Vaario et al. 2010; Endo et al. 2015), as well as form partial associations with other plants (e.g., *Larix kaempferi, Cedrela odorata*, *Prunus* spp., *Betula platyphylla* var. *japonica* and *Populus tremula* × *tremuloides*), but these have not been confirmed in natural settings (Murata et al. 2013a, 2014a, b, 2015b, 2016; Yamada et al. 2014). Although associations based on in vitro trials can help us to understand the genetic basis of EcM specificity, the extent to which results reflect natural phenomena with ecological significance is unclear. By using cloned material of *P. sylvestris*, it has been shown that those individuals containing high concentrations of phenolics and bear thick epidermal cell walls have more limited or no association with matsutake mycelium (Vaario et al. 2015a). Additional studies using genetically-uniform material should be undertaken to understand the factors regulating the compatibility of EcM fungi with their host plants.

15.3 Microbial Diversity in the *T. matsutake* Shiro

In the forest ecosystem, above- and below-ground communities are inextricably linked. Plant species can influence the soil, rhizosphere, and forest-floor microbial community structure through root exudates and leaf litter quality (Grayston et al. 1997; Westover et al. 1997). Similarly, soil microbial activities directly affect plant growth, survival, productivity and can influence plant community composition and ecosystem function (van der Heijden et al. 1998; Zak et al. 2003). The ectomycorrhizosphere, which forms a highly specific interface between the soil and EcM fungi, harbors a large and diverse microbial community capable of self (positive and negative) regulation (Rudnick et al. 2015). A detailed in vitro study of non-EcM microbes in the shiro concluded that the density of fungi and actinomycetes adjacent to actively-growing matsutake mycelium decreased and the overall microflora in the shiro exhibited an annual cycle of deterioration and recovery (Ogawa 1977b). However, in vitro culture methods tend to over-represent the importance of those microbes that lend themselves to artificial culture, and may mislead our understanding of the natural community and its ecology. Recent metagenomic studies emphasize the narrow window through which culture methods view microbial ecology (Amann et al. 1995; Lombard et al. 2011). It should be mentioned that metagenomic analyses are also prone to a systematic bias in the form of primer performance during amplification and the generation of chimeric sequences may similarly over- or underestimate the abundance and importance of certain taxa (Morales and Holben 2011). A summary of recent molecular and culture-based studies is provided in Table 15.2.

15.3.1 Fungal Diversity in the Shiro

A study of seven sampling sites in Japan showed that 96% of mycorrhizal root tips in the shiro belonged to *T. matsutake*, the remaining 4% ascribed to *Rhizopogon* sp., *Russula* sp. and *Tomentellopsis* sp. (Lian et al. 2006). Matsutake usually forms a whitish mycelium–soil aggregate and mycorrhizae in the mineral soil layer. In an analysis of soil microflora above and below the shiro, some EcM fungi (e.g., *Tomentellopsis* sp. and *Tylospora* sp.) above the shiro were identified as potential indicator species, i.e., were significantly and positively correlated with matsutake occurring below them (Vaario et al. 2011). According to an analysis of root tips in the shiro, only a small number of EcM fungi with low abundance were detected, but it should be stressed that the EcM community is dynamic and may recover relatively quickly (Lian et al. 2006). This is consistent with observations of moderately diverse EcM fungi in the shiro (Vaario et al. 2011; Kim et al. 2013). A 3-year fruiting-body survey in southern Finland revealed that only ca. 20% of other macrofungal species fruited during the peak season for *T. matsutake*, with the

Table 15.2 Summary of recent studies of microbial community in *T. matsutake* shiro

	Country	Study location	Sample type	Major host species	Isolation method	Type of analysis	Phylum[a]	Key results[b]	References
Bacterial community	Finland	62°10′N, 22°50′E; 60°18′N, 24°31′E	Soil	*Pinus sylvestris, Picea abies*	Non-culturable	PCR-DGGE-direct DNA sequence	Only Act was studied	37 Act OTUs found in shiro + *Thermomonosporaceae, Nocardia* sp. *Streptomyces* sp. were positively correlated with the presence of *T. matsutake*	Vaario et al. (2011)
	Japan	35°11′N, 135°20′E	Soil	*Pinus densiflora*	Culturable	PCR-RFLP-direct DNA sequence	Pro, Fir, Act	The most frequent bacteria belong to *Stretomyces* sp.	Kataoka et al. (2012)
	China	26°36′N, 102°32′E	Fruiting-body	Pine and oak	Non-culturable	PCR-DGGE-Direct sequencing	Pro, Fir, Act	The dominated bacteria were from Pro and Fim phylum	Li et al. (2014)
	Korea		Soil	NR	Non-culturable	Pyrosequencing	Pro, Aci, Act	More Act in shiro + than Shiro + In and Shiro + Out	Kim et al. (2014)
	China	Yunnan	Soil	Pine and oak	Culturable	PCR-direct sequencing	Pro, Fir, Bac, Act	Pro was the dominated phylum, Act had the lowest percentage (<5%)	Jiang et al. (2015)
Fungal community	Japan	39°56′N, 141°14′E	EcM root tips	*Pinus densiflora*		Morphytyping and PCR-RELP-direct DNA sequence		Matsutake was the dominated species in shiro+, only 4% was other ECM in shiro+	Lian et al. (2006)
	Finland	62°10′N, 22°50′E; 60°18′N, 24°31′E	Soil	*Pinus sylvestris, Picea abies*		PCR-DGGE-direct DNA sequence		Matsutake dominated in shiro soil; *Tomentollopsis* sp. (shiro + abv), *Piloderma sp.* (shiro+) positively correlated with matsutake presenting	Vaario et al. (2011)

	Korea		Soil	NR		Pyrosequencing		Total fungal OTUs was 1.5–2 times lower in Shiro + than Shiro + In, Shiro + Out. 88.57% OTUs in Shiro + accounted for Trichoomataceae	Kim et al. (2013)

[a]*Aci* Acidobacteria, *Act* Actinobacteria, *Bac* Bacteroidetes, *Fir* Firmicutes, *Pro* Proteobacteria
[b]*Shiro+* shiro area, *Shiro + In* inside direction of shiro, *Shiro + Out* outside direction of shiro

majority fruiting thereafter (Vaario et al. 2015c); a phenomemon reflected by fungal diversity and community dynamics in the shiro.

15.3.2 Bacterial Diversity in the Shiro

Ohara and Hamada (1967) investigated the bacterial community inner, within and outer the shiro using dilution plating. They found that *T. matsutake* had antagonistic effects on soil bacteria, which accounts for the rather rare occurrence of actinomycetes and other bacteria in shiro soil. Ohara (1980) isolated *Sarcina* and *Micrococcus* and *Streptomyces* from the shiro, but it should be stressed that an artificial and homogenous culture medium typically supports only a small fraction of the microbes present in the inoculum. Although bacterial diversity appears to be rather low in the shiro (Kataoka et al. 2012), recent molecular analyses have detected Proteobacteria, Firmicutes and Actinobacteria commonly represented in shiro samples from different continents (Vaario et al. 2011; Kataoka et al. 2012; Kim et al. 2014; Li et al. 2014; Jiang et al. 2015).

Species of *Streptomyces* are the most common actinomycetes detected in shiro soil samples screened with traditional culture-plate techniques (Kataoka et al. 2012). PCR-DGGE and direct sequencing revealed that one of these OTUs correlated positively with the presence of matsutake in shiro soil (Vaario et al. 2011). By using barcoded pyrosequencing, Kim et al. (2014) found that the relative abundance of Actinobacteria peaked beneath the fairy ring, agreeing with the earlier results, but Actinobacteria were not detected in fruiting-body samples (Li et al. 2014). Some Actinobacteria, especially *Streptomyces,* are able to facilitate development of mycorrhizae and root nodulation (Schrey et al. 2005; Frey-Klett et al. 2007; Tarkka et al. 2008).

Knowledge concerning the bacterial community and its function in the shiro remains limited and largely outside of the EcM and the process of fruiting-body formation. Recently, a study of soil bacteria during the development of *Tuber melanosporum* fruiting-bodies showed how EcM became significantly enriched with actinobacterial sequences similar to species of *Streptomyces* and *Thermoleophilum* (Antony-Babu et al. 2014). The role played by *Streptomyces* as a plant symbiont has been recently explored in terms of inhibiting the growth of fungal phytopathogens, inducing plant-defence pathways, and even promoting the growth of rhizosphere fungi (Maier et al. 2004; Seipke et al. 2012). These studies have raised the question to what extent do EcM fungi support or encourage the growth of certain bacteria that enhance their symbiosis with the host plant? Compared to the limited fungal diversity in the shiro, bacteria seem to be more diverse. In vitro culture-based studies are required to determine, which taxa inhabiting the EcM and/or fruiting-body participate in nutrient mobilization and other physiological responses of the host plant and fungus.

15.4 Fruiting Pattern of Matsutake in Relation to Climate and Weather

Logistic difficulties of monitoring the variable phenology of a fungus, especially fruiting itself, still limit our understanding of the phenomenon. We must also consider the extent to which phenology is affected by geography (i.e., latitude), how climate varies within the natural distribution area of matsutake, and how the fruiting period is influenced by weather. Herbarium records of European fungi demonstrate a rapid change in phenology in terms of the first fruiting date, last fruiting date, mean fruiting date and duration, all of which are believed to be a response to climate change (Buntgen et al. 2013, 2015; Gange et al. 2007; Kauserud et al. 2010).

Observations of the fruiting phenology of *T. matsutake* date back to the 1940s, when Japanese scholars described the spatial arrangement of fruiting-bodies as a fairy ring with an outward progression of the shiro of 0.1–0.2 m per year (Narimatsu et al. 2015; Ogawa 1978). In Nagano Prefecture, first fruiting date and productivity have been recorded for over 30 years (Furukawa et al. 2016; Fig. 15.2). Similar long-term studies have recently been established in China and Finland (Chen et al. 2011; Yang et al. 2012; Vaario et al. 2015c). Based on field observations, fruiting phenology and production of *T. matsutake* is highly variable among years and across the natural distribution (Table 17.3). In this review, we focus on temperature and precipitation to summarize the main findings of a recently published paper in this area (Furukawa et al. 2016; Table 17.3) with a view towards understanding the fruiting pattern of matsutake in relation to climate and geography.

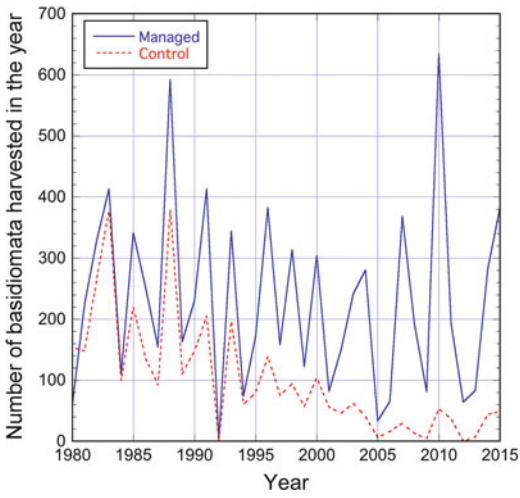

Fig. 15.2 Harvest of *T. matsuake* at Toyooka experimental forest site in Nagano Prefecture (Japan). **Solid line* indicates the harvest in the plot (ca. 0.5 h) which has been managed for sustainable fruiting (e.g., removal of shrubs and litter layer every few years), and the *dotted line* indicates the harvest at the neighboring plot that did not receive such treatment. Redrawn from the data of Furukawa et al. (2016)

15.4.1 Temperature

Matsutake is found in temperate and boreal coniferous forests and mixed woodlands with an annual mean temperature of 4–14 °C, and annual mean precipitation ranging from 600 to 2300 mm (Table 15.3). First fruiting can occur from early summer to late autumn and varies in duration from 15 to 150 days depending on local geographic (i.e., topography and altitude) and climatic factors (Table 17.3). Eleven years of continuous observation from Baoshan (China) revealed a significant delay in the first fruiting date. Comparing similar studies from three countries, the production of *T. matsutake* varies greatly among shiros within a site, among locations and from year to year (Fig. 15.3, Table 15.3). The most productive area occurs in Diqing (China) with an estimated annual harvest of 75–105 kg/ha.

A comparison of climate and weather among sites during the fruiting period in China (Chen et al. 2011) showed that the only factor that significantly differed among sites was maximum temperature. This suggests that the fruiting of *T. matsutake* requires a specific temperature treatment to trigger fruiting, and soil temperatures of 16–16.5 °C at 20–30 cm depth were consistent across sites. In Japan, the fruiting temperature for *T. matsutake* was first determined to be 19 °C at 10 cm depth in a *P. densiflora* forest (Kinugawa 1963). In western Honshu, this temperature was shown to be a good indicator of fruiting (Ogawa 1978). However, in Nagano and Iwate Prefectures, some populations were believed to fruit at lower temperatures (Narimatsu et al. 2015; Endo et al. 2015) because the cool temperate and subalpine forests experience lower soil temperatures. Similarly, soil temperature at first fruiting is much lower based on a 6-year survey in southern Finland (Vaario et al. 2015c). This suggests that some variation, perhaps local adaptation, exists in the fruiting temperature for populations of *T. matsutake*. Some studies have also shown that fruiting could cease soon after soil temperature falls 2–4 °C below that at which it began (Vaario et al. 2015c; Wang et al. 1997). As such, soil temperature may offer a way to remotely monitor fruiting in matsutake and optimize harvesting activity. It is well known that commercially-cultivated saprobic mushrooms such as shiitake (*Lentinula edodes*) vary greatly in terms of the induction temperature for fruiting. Mushroom farmers manipulate this property to create strains suitable for a given location or climate (Hasebe et al. 1998).

Productive areas of *T. matsutake* in Japan are limited to established forests with annual mean temperatures below 13 °C and which expand to a boreal or subalpine climate (Yamada 2015). Higher summer temperatures due to recent global warming will likely have a negative impact on the wild populations of matsutake in these areas (Yamada and Kobayashi 2008; Yamada 2015). Matsutake mycelium cultured on nutrient agar exhibits maximum growth at 20–25 °C but slows to almost zero at 30 °C (Hamada 1953). In the warm temperate forests of Japan, soil temperatures 5–10 cm depth may reach over 25 °C during prolonged hot spells in summer. It remains unclear how soil temperature affects mycelial growth and survival of *T. matsutake* in natural settings. Furthermore, studies from Japanese researchers suggest that a thin litter layer above the shiro could influence soil temperature

Table 15.3 Site information of studies concerning *T. matsutake* fruiting pattern

	China			Japan		Finland
Monitoring site	Chuxiong, Yunnan	Baoshan, Yunnan	Diqing, Yunnan	Toyooka, Nagao[d]	Yokkaichi, Iwate	Nuuksio, Espoo
Location (latitude/longitude)	25°10′N, 99°0′E	25°16′N, 99°18′E	28°23′N, 99°8′E	35°33′N, 137°57′E	39°56′N, 141°14′E	60°18′N, 24°31′E
Elevation (m.s.l)	2450	2350	3300	720–750	360–380	n/a[a]
AMT(°C)	14	12.2	4.7	9.9–11.3–12.2[c]	9.3	4.4–6.7[b]
P(mm)	1140	1200	633.7	1000–1650–2300[c]	1145	596–932[b]
Vegetation	Mixture of *Pinus yunnanensis* and *Castanopsis* spp.	Mixture of *Pinus yunnanensis* and *Castanopsis delavayi*	Mixture of *Pinus densata* and *Quercus semecarpifolia*	*Pinus densiflora*	*Pinus densiflora*	Mixture of *Pinus sylvestris* and *Picea abeis*
# of plots and/or shiros	10	56	10	20–30	5	5
Area	n/a	1 ha	0.1 ha	0.25 ha	n/a	1.35 ha
Observation duration	2009	2000–2011	2009	1982–2014	1994–2011	2008–2013
Years observed	1	11	1	33	18	6
First fruiting day	Jul 14	Jun 7–Jun 19–Jul 19[b]	Jul 25	Aug 29–Oct 18[b]	First 10 days in Sep-first 10 days in Oct	Jul 23–Aug 22[b]
Last fruiting date	Oct 10	Oct 20–Oct 30–Nov 22[b]	Sep 14	Oct 1–Nov 10[b]	n/a	Aug 31–Sep 19[b]
Duration	105	125–136–148[c]	51	15–30[b]	n/a	18–58[b]
Peak of fruiting	Aug–Sep	Aug–Sep	Aug	Oct	Oct	Aug
Multi-year fruiting bodies variation	n/a	233–416–810[b]	n/a	3–231–634[c]	12.5–48.4[b]	7–44–106[c]

(continued)

Table 15.3 (continued)

	China			Japan		Finland
Monitoring site	Chuxiong, Yunnan	Baoshan, Yunnan	Diqing, Yunnan	Toyooka, Nagao[d]	Yokkaichi, Iwate	Nuuksio, Espoo
Productivity (kg/ha)	45–75	30–45[b]	75–105[b]	0.1–25–80[c]	n/a	n/a
Fairy expansion rate	n/a	n/a	n/a	10–20 cm/yr	17 ± 1 cm/yr	n/a
Literature	Chen et al. (2011)	Chen et al. (2011), Yang et al. (2012)	Chen et al. (2011)	Furukawa et al. (2016)	Narimatsu et al. (2015)	Vaario et al. (2015b, c)

[a]No answer
[b]Showing the earliest related date or min value, the latest related date or max value
[c]Showing the earliest related date or min value, the mean related date or value and the latest related date or max value
[d]The values are only the post mid-summer data in this site. Limited natural fruiting occurs prior to the mid-summer season, but this is not recorded in the commercial harvest data

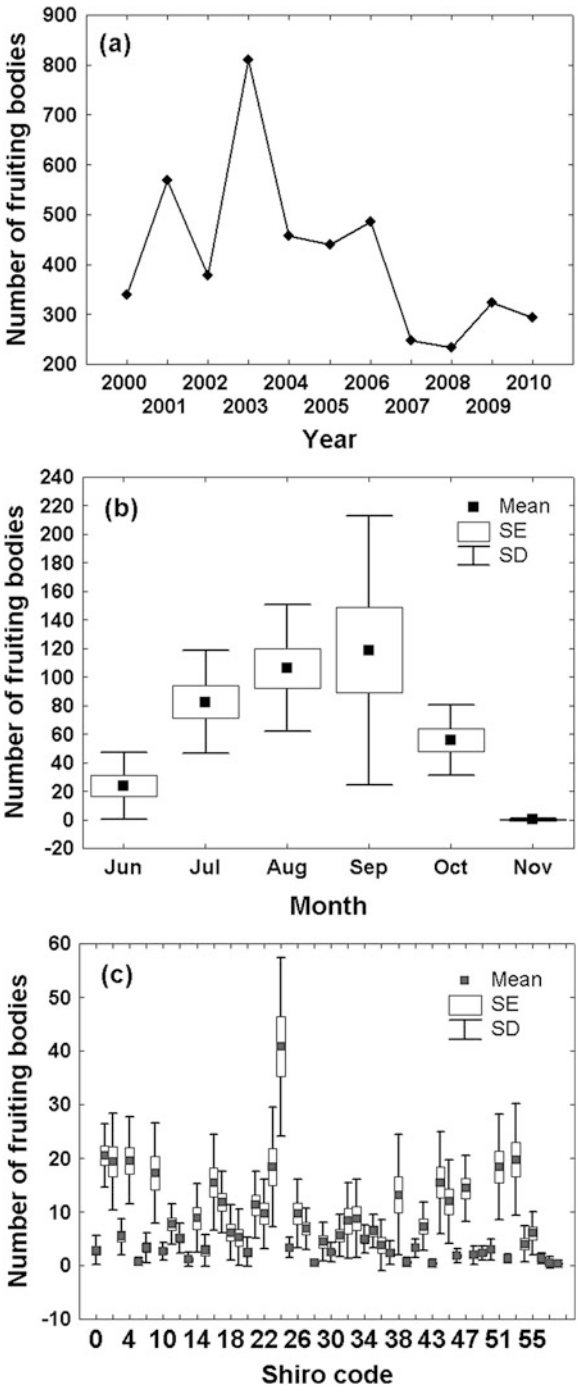

Fig. 15.3 Variation in productivity between years (**a**), months (**b**) and among shiros (**c**) in Baoshan (China). In (**b**) and (**c**), SE is the standard error and SD is the standard deviation from the mean

sufficiently to cause early fruiting (The Matsutake Research Association 1964; Ogawa 1978).

15.4.2 Precipitation

In addition to temperature, precipitation is linked to the productivity of *T. matsutake*. In the prevalent climate in Nagano Prefecture (i.e., Cfa–Dfa boundary of the Köppen climate classification (Peel et al. 2007), *T. matsutake* harvests show a strong and positive correlation with precipitation in August and September prior to fruiting (Furukawa et al. 2016). Precipitation during the fruiting period (i.e., October) does not appear to affect yield. It is worth noting that accumulated precipitation prior to fruiting seems to be negatively related to productivity, i.e., a wet spring-summer typically means a poor matsutake crop in the boreal forest (Vaario et al. 2015c). In contrast, other groups (Furukawa et al. 2016; Yang et al. 2012) observed that abundant rain in August preceded a good matsutake crop in Yunnan and Toyooka, but high rainfall from November/December to May was associated with few fruiting bodies the following season in Yunnan (Yang et al. 2012). Taking into account that the fruiting of *T. matsutake* in Yunnan begins in early June and ends in November (Yang et al. 2012), it seems that the pattern observed in China is inconsistent with that in southern Finland (Vaario et al. 2015c). Furthermore, given that the fruiting phenology of *T. matsutake* differs from other fungi in the shiro (Vaario et al. 2015c), this might reflect the growth of matsutake mycelium in response to soil moisture rather than being tied to temperature (Narimatsu et al. 2015). To understand the relationship between matsutake fruiting pattern and meteorological factors, long-term phenological data from distant and varied locations throughout the range are required.

In addition to variation in climate and geography, two basic issues remain poorly understood but could shed considerable light on fruiting dynamics: (1) the relationship between fruit-body biomass and that of soil mycelia, and (2) the relationship between mycorrhizal biomass and climate. Regarding the first relationship, a recent study in Japan applied a novel method to measure the amount of *T. matsutake* mycelia in a soil sample by quantifying a single-copy DNA element that is uniquely conserved within *T. matsutake* but absent from other fungi present in the shiro (Yamaguchi et al. 2016). Although widely accepted, it has yet to be confirmed that the summer and early autumn is an important period during which matsutake mycelium increases due to an optimal growth temperature. As such, higher precipitation during this time enhances mycelial biomass, which in turn can support a higher biomass of fruiting-bodies (Ogawa 1978). On the other hand, EcM fruiting-body formation exhibits a close relationship with the host plant condition, which is often improved by higher soil moisture and temperature during the growing season, which leads to a richer supply of carbohydrates supplied to the roots where they are used in the formation of fruiting-bodies (Sato et al. 2012). Although experimental evidence is lacking, this provides a mechanistic explanation for why higher

precipitation prior to the fruiting season is associated with higher sporocarp production. Regarding the latter relationship, we still know relatively little about general EcM ecology as few environmental determinants have so far been identified (Smith and Read 2008). In *P. densiflora* forest, annual mean EcM biomass fluctuates significantly, and high precipitation in late autumn is associated with a lower yield the following year (Okada et al. 2011).

15.5 Ecological Strategies of Matsutake

EcM symbiosis is a widespread and important component of the forest soil ecosystem and the fungi involved may occupy one or more positions along the biotrophy–saprotrophy continuum (Taylor and Alexander 2005). The hypothesis that matsutake mushrooms are true EcM mutualists has garnered the attention of many mycologists and mycorrhizologists. Ogawa and coworkers have studied the ecological strategy of matsutake in detail through a soil-sectioning approach and direct observation of shiro structure (Ogawa 1978). Since that pioneering work, research has sought to explain field observations with controlled microcosm experiments in the laboratory. We will now discuss the main findings from recently published studies with the aim of providing a more complete synthesis of the ecological strategy of the fungus.

T. matsutake is a typical EcM fungus in terms of its morphology. Basically, *T. matsutake* shows a typical EcM structure when associated with a compatible host plant, i.e. a Hartig net and mantle (Yamada et al. 1999; Gill et al. 2000). In addition, in vitro inoculation of *T. matsutake* generates a typical EcM structure with a mutualistic effect on the pine host (Guerin-Laguette et al. 2004; Yamada et al. 2006; Murata et al. 2013a). However, in comparison to other EcM fungi such as *Rhizopogon roseolus*, pine seedlings infected with *T. matsutake* may be not a good symbiont for pine seedlings in vitro (Yamada et al. 2010). It is generally accepted that late-stage fungi represent poor inoculum for young seedlings, because hyphae of those fungi have slow growth rates and higher carbon demand (Deacon and Fleming 1992; Cairney and Chambers 1999; Smith and Read 2008).

15.5.1 Functional Diversity and Nutrient Acquisition

A detailed morphological study of *T. matsutake* mycorrhiza recognized four developmental stages of mycorrhizal root tips (Gill et al. 2000). Briefly, whitish ectomycorrhizae gradually turn darker similar to the root cortical cell and finally become black with a thin mantle (Agerer 1987–1998; Yamada et al. 1999). Although data are limited, an in vitro developmental study showed that this sequence can be completed within several months in a granite-based natural soil substrate (Yamada et al. 2006; Kobayashi et al. 2007). Enzyme activities linked with the degradation of organic matter in the shiro (Vaario et al. 2011) have been

identified; *T. matsutake* produces a range of extracellular enzymes including amylases, β-glucosidase, xylosidase and proteinases in vitro (Terashita et al. 1995; Hur et al. 2001; Vaario et al. 2002, 2012; Kusuda et al. 2006, 2008). The growth of *T. matsutake* mycelium in a forest-litter extract containing organic carbon (Vaario et al. 2013) could be explained by relatively high concentrations of hemicellulose occurring in root and leaf litter (Kiikkilä et al. 2011). However, the relative growth of *T. matsutake* and true saprotrophic fungi on this and other organic carbon sources has yet to be studied and compared.

T. matsutake prefers forest sites on soil derived from an acidic parent rock such as granite (Hamada 1964; Ogawa 1978). It has been observed that *T. matsutake* mycelium tightly adheres to the surfaces of small rocks in the shiro. It has been confirmed in vitro how these interfaces enable the fungus to mobilize and absorb many important minerals and trace elements (e.g., Al, Fe, Mn, Zn) directly from the rock fragments. Furthermore, X-ray powder diffraction identified a uniform mineralogical profile containing major phases of quartz, microcline, orthoclase and albite in 14 shiro samples collected in southern Finland (Vaario et al. 2015b). Yet, it remains challenging to draw any firm conclusions concerning a preferred mineralogical profile of the matsutake shiro as a comparison between shiro and non-shiro soil is currently lacking. In relation to this issue, a recent study showed how matsutake mycorrhizae secrete oxalic acid and obtained the soluble phosphoric acid from insoluble aluminum phosphate in the shiro to form the antimicrobial substance as the (oxalate)aluminate complex released into the shiro (Nishino et al. 2016a, b). The extent to which sandy soil over granite bedrock is a prerequisite for *T. matsutake* is an interesting topic for future research.

To date, there are no convincing data that clearly define the relationship of *T. matsutake* with its host plant along a mutualistic-parasitic scale (Yamada 2015). However, evidence is accumulating to suggest that EcM fungi produce degrading enzymes and are able to decompose organic matter (Taylor and Alexander 2005; Cullings and Courty 2009), especially when the carbon supply from the host is experimentally limited (Buée et al. 2005; Mosca et al. 2007). Talbot and colleagues (2008) proposed a hypothetical model of saprotrophic events in the life cycle of EcM fungi when the supply of photosynthate from the host plant is low, or when photosynthate is available but mycelial growth is limited by another resource. A more recent study (Lindahl and Tunlid 2015) proposed that EcM fungi benefit from organic matter decomposition primarily through increased nitrogen mobilization rather than the direct release of metabolic carbon.

15.5.2 Forest Management and T. matsutake *Productivity*

In forest ecosystems, *T. matsutake* can be categorized as a late-stage EcM fungus (Deacon and Fleming 1992), because fruiting occurs in forests where *P. densiflora* dominates the canopy or in climax stands of hemlock (Hamada 1964; Ogawa 1977a, b). In *P. densiflora* forests, it is generally accepted that *T. matsutake* is

more productive when associated with trees that are 40–60 years-old. Forest management measures such as clearing of shrubs and broadleaves and removal of the litter layer is generally thought to prolong the productive period (Ogawa 1978). However, carbon derived from litter seems to have a positive effect on *T. matsutake* fruiting-body formation (Vaario et al. 2013). This suggests that pine root dominance as well as specific and stable physio-chemical properties and soil microbial community is necessary to sustain the shiro over long periods (Suzuki 2005; Yamada 2015). Although the forest management described above has been widely applied in *P. densiflora* forests of Japan, data from other geographic regions are limited, making any comparisons difficult. As the shiro of *T. matsutake* is primarily sustained by the carbon input from the host root system, the mycorrhizal biomass in the forest may be a critical factor for *T. matsutake* mushroom production at the stand level. Therefore, we should seek to develop a theoretical model incorporating mycorrhizal biomass, tree density, tree age and soil chemical and mineralogical properties. It is generally believed in Japan that *T. matsutake* prefer habitats typical of mountain ridges or rocky areas in forests, both of which are well drained, but similar studies from other locations are lacking and prevent more general observations from being made at this time.

Some areas have witnessed a marked decline in matsutake productivity due to various reasons. Unfortunately, in spite of considerable effort, the artificial cultivation of this mushroom remains in its infancy. Outplanting of mycorrhizal seedlings and directly inoculating mature host trees with *T. matsutake* in forest sites has been attempted for a long time in Asia (Ogawa 1978; Guerin-Laguette et al. 2005; Park et al. 2007; Ka et al. 2008; Kobayashi et al. 2015). The only encouraging result in the public domain concerns outplanted mycorrhizal pine seedlings that were grown for at least 2 years following in vitro inoculation (Kobayashi et al. 2015). So far, the majority of data from the in vitro culture of *T. matsutake* with seedlings offer some limited insights into the nutritional and ecological function of *T. matsutake* in association with mature trees. A transcriptome analysis of mycorrhizal root tips and sporocarp samples taken at different stages of development coupled with stable isotope fractionation analysis constitute an ideal approach to clarify the ecophysiology of this species.

15.6 Conclusions

Recent studies have focused on determining the extent to which fungal diversity and its geographical variation play a role in ecosystem processes (Põlme et al. 2013; Tedersoo et al. 2014). *Tricholoma matsutake* is distributed widely in temperate and boreal forests of Eurasia, where it inhabits a diversity of coniferous and fagaceous host tree species in a variety of climates and natural settings. As a late-stage EcM fungus, *T. matsutake* co-exists with several soil microbes in the shiro, and some evidence supports the notion of microbial cooperation in nutrient acquisition and mediation of the host–tree response. In this review, we have seen that the use of

molecular identification and quantification techniques has removed many of the barriers that existed for studying above- and below-ground microbial communities associated with the matsutake shiro. However, systematic surveys over a broad geographic scale are lacking and which prevent general statements from being made about the habitat preferences of this enigmatic and highly sought-after mushroom.

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Chapter 16
Biogeography and Specificity of Ectomycorrhizal Fungi of *Coccoloba uvifera*

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16.1 Introduction

A range of biotic, abiotic and historical variables shape the structure and species richness of ectomycorrhizal (EcM) fungal communities. Host and fungal compatibility (i.e. host preference or specificity) that may vary widely across host taxa, has been increasingly shown to influence the structure and richness of EcM fungal assemblages at various taxonomic levels of plants (Ishida et al. 2007; Morris et al. 2008; Tedersoo et al. 2010b, 2013; Bahram et al. 2012; Põlme et al. 2013). To date, the influence of environment on EcM fungal richness and composition has received relatively limited attention and the biodiversity of several tropical plant groups has remained unknown. Unlike in most other organisms and fungi overall, EcM fungi exhibit greater diversity in temperate compared with tropical and arctic ecosystems (Tedresoo et al. 2012, 2014; Chap. 18). This has been ascribed to historical factors (the lack of earliest evolving Pinaceae hosts in lowland tropical habitats), rapid turnover of organic matter in tropical soils and low relative abundance of hosts.

The genus *Coccoloba* (Polygonaceae) comprises ca. 170 species of shrubs and trees with neotropical distribution (Howard 1960; Chap. 19). *Coccoloba* spp. are probably of South American origin, with greatest richness in northern Amazonia

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and the Atlantic rain forest (Howard 1961; Chap. 20). EcM associations were first described from sea grape (*Coccoloba uvifera* (L.) L.) in Cuba (Kreisel 1970) and thereafter in South American rain forest species (Moyersoen 1993; Bereau et al. 1997). The associated EcM fungi are poorly known, because host plants have remained unsettled in most South American mycological studies (Pegler 1983; Singer et al. 1983; Roy et al. 2016; but see Tedersoo et al. 2010b).

Sea grape is a tropical tree species with a native distribution from southern Florida to eastern Mexico to northeastern Brazil. Sea grape grows in immediate proximity of seashores, representing one of the first colonizers of sandy and rocky coastal areas. These habitats are characterized by high salinity, steady wind, seasonal drought and low soil nutrient availability. EcM symbiosis could potentially enhance plant tolerance of those harsh conditions, particularly of high salinity (Bandou et al. 2006). Séne et al. (2015) conducted a profound study sampling sporocarps and EcM root tips in sea grape communities and reported very limited EcM fungal diversity in Guadeloupe Island. The authors postulated four nonexclusive hypotheses to explain the low EcM fungal diversity: (1) isolation from mainland, (2) overall lower EcM fungal diversity at lower latitudes, (3) environmental filtering due to stressful conditions, (4) recent origin of EcM symbiosis within the Polygonaceae. In spite of profound local sampling effort in a small volcanic island, their study cannot be used to generalize about sea grape EcM communities over a wider geographic context.

In order to test the above hypotheses in a wider geographical and historical context, we sampled six additional sea grape communities around the Caribbean basin and compared these in the biogeographic perspective. We further addressed the potential specificity and origin of EcM fungi of *C. uvifera* by comparing the associated *Tomentella* species—the dominant group of mycobionts—to these from other hosts in North and South America.

16.2 Approaches

We performed root sampling in six study sites in the following locations: USA: Miami (June 2013; 26.0408°N; −80.1145°E), Mexico: Celestún (October 2015; 20.9336°N; −90.3748°E), Costa Rica: Cahuita (June 2013; 9.8590°N; −82.9458°E), Cuba: Cayo Santa Maria (December 2008; 22.6581°N; −79.0413°E), French Guiana: Montabo (November 2013; 4.9436°N; −52.2973°E) and Colombia: Los Naranjos (November 2014; 11.2973°N; −73.8946°E). From each site, roots from ten sea grape individuals were collected (except for the Cuba plot where 14 samples were collected). Randomly selected samples (15 × 15 cm to 10 cm depth) were situated at least 10 m apart.

Roots were cleaned from adhering soil in tap water and morphotyped under a stereomicroscope. EcM morphotypes were distinguished based on colour and roughness of mantle, presence of emanating hyphae and rhizomorphs. At least two EcM root tips from each morphotype per soil sample were stored in CTAB

buffer (1% cetyltrimethylammonium bromide, 100 mM Tris–HCL (pH 8.0), 1.4 M NaCl, 20 mM ethylenediaminetetraacetic acid) for molecular analyses.

DNA was extracted from EcM root tips using Thermo Scientific Phire Plant Direct PCR Kit (Thermo Scentific, Waltham, MA, USA) according to the manufacturer's instructions. In the course of the study, PCR was performed by use of 5× HOT FIREPol Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia). In EcM root tips, fungal rDNA Internal Transcribed Spacer (ITS) region was amplified with a forward primer ITSOF-T (5′-acttggtcatttagaggaagt-3′) in combination with reverse primers LB-W (5′-cttttcatctttccctcacgg-3′) or TW13 (5′-ggtccgtgtttcaagacg-3′). In case of PCR failure, we combined ITSOF-T with universal primers ITS4 (5′-tcctccgcttattgatatgc-3′), or basidiomycete-specific primer ITS4B (5′-caggagacttgtacacggtccag-3′) and LR0B (5′-acccgctgaacttaagc-3′) in order to amplify a shorter fragment of fungal DNA. To improve sequence quality, some root tip extracts were re-amplified with taxon-specific primers (Tedersoo et al. 2008). PCR and sequencing were run following Põlme et al. (2013). Sequences were assembled, checked, trimmed and manually corrected in Sequencher 5.1 software (GeneCodes Corp., Ann Arbor, MI, USA).

Sequences were confirmed to belong to EcM fungal lineages (cf. Tedersoo et al. 2010a; Chap. 6) by use of BLASTn searches against the International Sequence Databases (INSD) or UNITE (Abarenkov et al. 2010a). Sequences were partitioned into operational taxonomic units (OTUs), defined as a group of sequences sharing at least 97% pairwise similarity. Sequences with sufficient length and quality were assigned to UNITE species hypothesis (SHs; Kõljalg et al. 2013) with 3% dissimilarity threshold. We also included the recently published data of Séne et al. (2015) from Guadeluope. This study covered four study plots and had a much higher per-site sampling effort.

Using the PlutoF web platform (Abarenkov et al. 2010b), we downloaded all *Tomentella* sequences originating from South, Central and Northeast America. Identical sequences from the same sampling plots and hosts were removed prior to phylogeny construction. All sequences were aligned using MAFFT software (Katoh and Standley 2013). The alignment was manually adjusted in AliView software (Larsson 2014) and Maximum likelihood analysis was performed in FastTree 2.1 (Price et al. 2010) using default settings, with *Odontia fibrosa* (UDB000284) as an outgroup (Tedersoo et al. 2015).

16.3 Fungal Diversity

Out of 147 EcM root tips subjected to molecular analyses, 131 (89%) yielded good quality sequences. These sequences (including material collected by Séne et al. 2015 from Guadeloupe) were clustered into 42 OTUs (Table 16.1). Altogether 33 of OTUs were accommodated to existing SHs at the 97% sequence similarity cutoff level. Of these SHs, 26 (78.8%) were exclusively associated with sea grape.

Table 16.1 Distribution of fungal species hypothesis and OTUs at 97% cutoff level, associating with *Coccoloba uvifera* individuals in seven sampling areas

EcM lineage	No. of sequences in SH	Species hypothesis or taxon code	Miami, USA	Cahuita, Costa Rica	Los Naranjos, Columbia	Cayo Santa, Maria Cuba	Montabo, French Guiana	Celestún, Mexico	Guadeloupe
/tomentella-thelephora	2	SH491687.07FU[a]	UDB023143						
/tomentella-thelephora	5	SH002563.07FU[a]	UDB023156			UDB004997			
/tomentella-thelephora	7	SH018148.07FU[a]	UDB023168			UDB010534			
/tomentella-thelephora	17	SH009884.07FU		UDB023176		UDB004975			FR682090
/tomentella-thelephora	2	SH494851.07FU[a]		UDB023210					
/tomentella-thelephora	7	SH009960.07FU	UDB023163		UDB023213				KF472143
/tomentella-thelephora	3	SH010081.07FU	UDB023147						
/tomentella-thelephora	4	SH490339.07FU[a]			UDB023211				
/tomentella-thelephora	6	SH489759.07FU[a]			UDB023216				
/tomentella-thelephora		*Tom.* Miami	UDB023145						
/tomentella-thelephora	1	SH494788.07FU[a]		UDB023189					
/tomentella-thelephora	4	SH493262.07FU			UDB023215				
/tomentella-thelephora	1	SH490338.07FU[a]			UDB023208				

/tomentella-thelephora		*Tom.* Costa Rica 1		UDB023171					
/tomentella-thelephora		*Tom.* Costa Rica 2		UDB023174					
/tomentella-thelephora	32	SH009872.07FU[a]	UDB023141			UDB010547			KF472135
/tomentella-thelephora		*Tom.* Cuba				UDB010532			
/tomentella-thelephora	4	SH027506.07FU[a]				UDB010544			
/tomentella-thelephora		*Tom.* Guadeloupe 1							KF472141
/tomentella-thelephora		*Tom.* Guadeloupe 2							KF472148
/tomentella-thelephora	1	SH007321.07FU[a]							KF472158
/boletus		*Boletus* Miami 1	UDB023149						
/boletus		*Boletus* Miami 2	UDB023158						
/boletus	3	SH490763.07FU[a]			UDB023206				
/boletus	1	SH460556.07FU[a]				UDB004996			
/inocybe	18	SH032645.07FU		UDB023179					
/inocybe	1	SH491686.07FU[a]		UDB023188					
/inocybe	1	SH029289.07FU[a]				UDB004995			
/inocybe	2	SH029290.07FU[a]						UDB031216	FR682085
/clavulina	2	SH489802.07FU[a]	UDB023151						
/clavulina	1	SH490342.07FU[a]	UDB023161						
/clavulina	2	SH490812.07FU[a]			UDB023224				
/paxillus-gyrodon	1	SH023513.07FU[a]							KF472137

(continued)

Table 16.1 (continued)

EcM lineage	No. of sequences in SH	Species hypothesis or taxon code	Miami, USA	Cahuita, Costa Rica	Los Naranjos, Columbia	Cayo Santa, Maria Cuba	Montabo, French Guiana	Celestún, Mexico	Guadeloupe
/paxillus-gyrodon	1	SH023512.07FU[a]							KF472152
/pisolithus-scleroderma	40	SH003700.07FU[a]	UDB023152	UDB023172		UDB004993	UDB023202	UDB031196	FR682092
/pisolithus-scleroderma	10	SH003702.07FU[a]	UDB023157	UDB023185	UDB023207	UDB004971			
/sebacina	11	SH016792.07FU				UDB004981			
/sebacina	1	SH494787.07FU[a]			UDB023214				
/ cantharellus	1	SH030040.07FU[a]							FR682089
/ cenococcum	318	SH027498.07FU			UDB023209				KF472151
/russula-lactarius	1	SH004059.07FU[a]							FR682087
/serendipita		*Ser*. Guadeloupe							KF472155
		Total OTU richness	13	9	11	10	1	2	14

[a]Species hypothesis that are exclusively associated with *Coccoloba uvifera*

Only five fungal OTUs found from newly sampled sites overlapped with the Guadeloupe study, whereas eight OTUs remained exclusive to Guadeloupe.

The /tomentella-thelephora was by far most taxon-rich phylogenetic lineage of EcM fungi comprising 21 OTUs. Other sea grape-associating lineages were represented with the following number of OTUs: /boletus (four), /inocybe (four), /clavulina (three), /pisolithus-scleroderma (two), /sebacina (two), /paxillus-gyrodon (two), /russula-lactarius (one), /cantharellus (one), /serendipita (one; questionable mycorrhizal status) and /cenococcum (one). In spite of taxonomical richness of the /tomentella-thelephora lineage, *Scleroderma bermudense* (SH003700.07FU) and *Scleroderma* sp. (SH003702.07FU) were the most common individual taxa that were present in six and four sites out of seven, respectively.

Florida constituted the most OTU-rich site harbouring 13 EcM fungal taxa, followed by Cuba (11 OTUs), Colombia (10) and Costa Rica (9). Interestingly, Mexico and French Guiana sites harboured only two and one EcM fungal OTU, respectively (Fig. 16.1). In comparison, altogether 14 EcM OTUs were identified from root tips from four plots in Guadeloupe, with 4–9 EcM fungal OTUs per plot (Séne et al. 2015), but this can be ascribed to more extensive sampling effort on a local scale. None of the sea grape-associated OTUs overlapped with EcM fungal

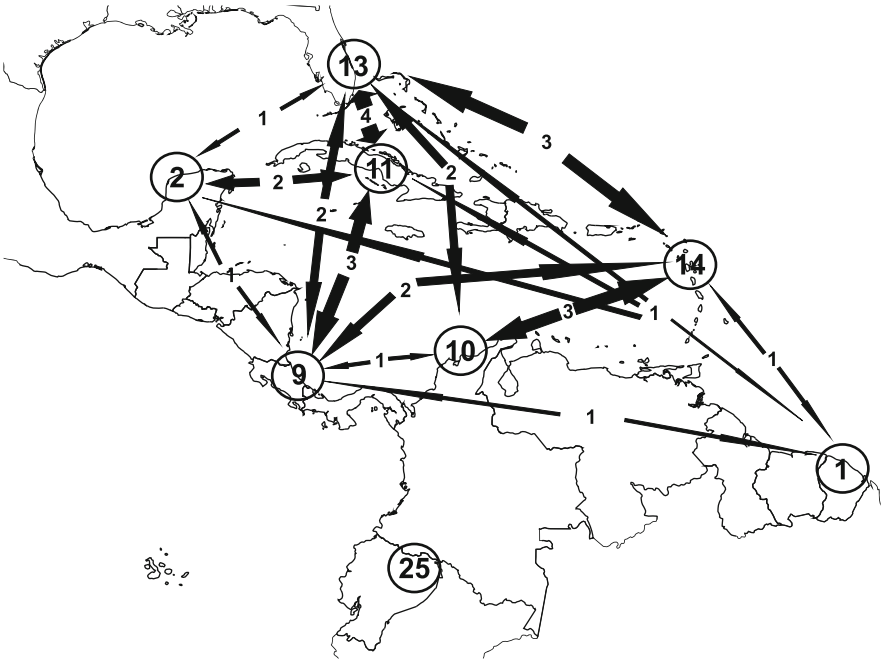

Fig. 16.1 Map of study sites indicating number of fungal OTUs in each sampling area. Shared OTUs between areas are shown with *arrows*. Note that inland *Coccoloba* species from Ecuador (Tedersoo et al. 2010b) do not share any fungal OTUs with *Coccoloba uvifera* communities from the coastal areas

taxa associated with the five sampled *Coccoloba* spp. in Ecuador (Tedersoo et al. 2010b).

The overall OTU richness detected over a broader geographic scale increased nearly three-fold compared to the single study conducted in Guadeloupe (Séne et al. 2015). In addition to formerly reported EcM lineages, we found /sebacina, /clavulina and /boletus lineages from our sampling sites, which were not found in Guadeloupe. Interestingly, several EcM fungal lineages such as /paxillus-gyrodon, /serendipita, /russula-lactarius and /cantharellus were not found outside Guadeloupe. The two Guadeloupean species of *Melanogaster* belonging to the /paxillus-gyrodon lineage are remarkable, because this genus and the entire EcM lineage is not known to associate with hosts of tropical origin. Species of *Cantharellus* fruited in abundance in another *C. uvifera* stand ca. 3 km distant from the Colombian site, but ITS sequencing of these fruit-bodies failed, indicating either primer bias or extensive length of the ITS region (Tedersoo et al. 2016). It is likely that additional sampling effort would have revealed these taxa from the mainland as well and that the true EcM fungal species richness associated with sea grape is considerably higher than the currently reported 42 fungal OTUs (Fig. 16.2). However, the coarse structure of EcM fungal communities was relatively similar to that previously reported in Guadeloupe i.e. /tomentella-therephora being most taxon rich lineage and *Scleroderma bermudense* being the most abundant fungal taxon. Sites in French Guiana and Mexico were extremely species-poor, comprising only one and two fungal OTUs respectively. In French Guiana, *S. bermudense* colonized sea grape root systems in all samples. The French Guiana site was characterized by intense anthropogenic disturbance in addition to a small host tree population. However, the Florida

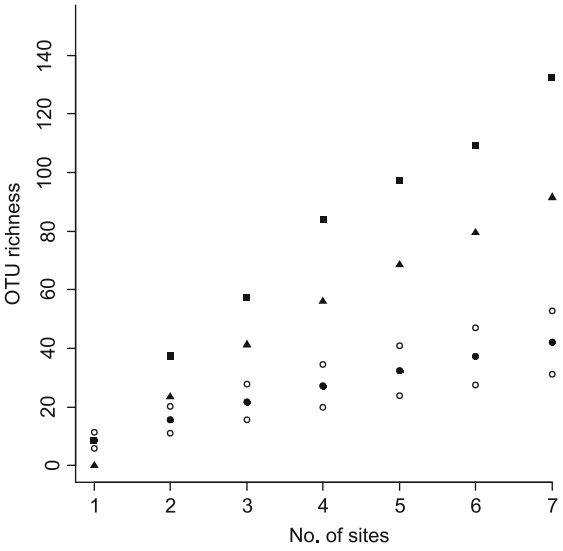

Fig. 16.2 Rarefied OTU accumulation curve of *Coccoloba uvifera* associated EcM fungi found in Caribbean basin. *Closed circles* and *open circles* represent the rarefied curve and its 95% confidence intervals, respectively. *Triangles* and *squares* represent the values of Chao2 and Jacknife2 minimum richness estimators, respectively. The values were calculated based on 999 permutations using EstimateS 9 (Colwell 2013)

site with the highest OTU richness was also characterized by substantial anthropogenic impact but with considerably larger host population.

16.4 Environmental Filtering and Host Specificity

Séne et al. (2015) proposed that the impoverished EcM fungal richness detected in Guadeloupe could partly result due to a founder effect and isolation from mainland. Although our sampling intensity is too low for comprehensive statistical comparison, the mainland and Guadeloupe sea grape communities harboured comparable EcM fungal richness, largely refuting this hypothesis. In spite of phylogenetic and geographical proximity, the absence of shared OTUs with inland *Coccoloba* species from neotropical forest, which harboured higher EcM diversity (Tedersoo et al. 2010b), supports the hypothesis of environmental filtering, also proposed by Séne et al. (2015) as an alternative. This explanation coincides well with the fact that vast majority of the SHs detected were exclusively associated with the sea grape. Similarly, *Pisonia grandis* (Nyctaginaceae) inhabiting small and often guano-rich Indian Ocean and Pacific islands harbours species poor and highly specific EcM assemblage (Suvi et al. 2010; Hayward and Horton 2012). Therefore, putative host specificity in such stressful habitats is most likely bounded with environmental filtering (but see Hayward and Horton 2012). The strong intrageneric ecological specificity in EcM fungi associated with *Coccoloba* contrast to the largely genus-level specific EcM fungi of *Alnus* (Põlme et al. 2013). Interestingly, environmental filtering is likely to have an important role in driving specificity of EcM interactions in both cases (Huggins et al. 2014). Nevertheless, we are unable to confidently disentangle the cause and consequence between the ecological host specificity and environmental filtering, because genetic and physiological compatibility between host and symbiont is likely to evolve mutually in extreme habitats over extended periods of time. Séne et al. (2015) pointed out that EcM origin in *Coccoloba* is relatively recent and this also holds true for *Pisonia* (Chap. 19), possibly explaining low EcM diversity in both groups. The fact that numerous EcM host taxa, with much broader range of mycobionts, have diverged in a comparable time frame (Chap. 19), makes this hypothesis disputable. Taken together, the hypothesis of strong environmental filtering seems the most plausible explanation for the low fungal diversity in *C. uvifera*.

16.5 Biogeography of Thelephoraceae

Using the PlutoF workbench, we were able to recover 832 sequences belonging to the */tomentella-thelephora* lineage originating from eastern North America, northern South America and Central America. After removal of redundant sequences originating from the same host and study site, 525 sequences were subjected to a

phylogenetic analysis (Fig. 16.3). In the large Thelephoraceae phylogram, sea grape-associated sequences clustered together more commonly with sequences originating from Central America and North America rather than with those from South America. This conflicts with the putative South American origin of the genus (Raven and Axelrod 1974; Chap. 20) and previously established patterns in Russulaceae (De Crop et al. 2017) and Sclerodermataceae (Wilson et al. 2012) putatively associating with *Coccoloba* spp. In terms of host identity, Thelephoraceae sequences associated with *C. uvifera* most often clustered together with sequences from *Pinus* spp. and to a lesser extent with those from *Quercus* spp. This suggests potential host shifts from phylogenetically distant host taxa. Previous studies focusing on the mycobionts of introduced plants support that host shifts for EcM fungi may occur between distantly related taxa such as Fagales and Pinales in a very short time frame (Bahram et al. 2013). Currently, the distribution of *Coccoloba* spp. overlaps with that of *Pinus* spp. and Fagales from Central Mexico to Nicaragua and in Cuba (Chap. 20). Furthermore, the fossil record indicates much greater overlap among the geographic range of Pinales, Fagales and *Coccoloba* during the Oligocene and Eocene (Gray 1960; Graham and Jarzen 1969), when *Coccoloba* spp. were distributed up to Central USA in the north, whereas Fagales spp. were more widely distributed in the Caribbean islands. In particular, *C. uvifera* shares its habitat with *Pinus* spp. in coastal sand dunes, although these species do not co-exist in present-day communities, except perhaps in the Bahamas. Nevertheless, the results of phylogenetic analysis should be interpreted with caution, because South America remains relatively under sampled compared to Central America and especially North America, which may slightly overestimate the links between Pinaceae and *C. uvifera* and particularly the genus *Coccoloba* in general.

We also tested, whether the habitats historically influenced by Pinaceae and Fagales (Florida, Costa Rica, Mexico, Cuba) harbour more OTUs of Thelephoraceae shared with northern hosts than the sites far from natural Pinaceae and Fagales habitats (Guadeloupe, Colombia, French Guiana). Contrary to our expectations, there were no differences between the sites with shared and unshared habitats ($n = 34$; $\chi^2 = 0.123$; $P = 0.726$). The wide distribution of EcM fungi of potentially northern temperate origin indicates their effective dispersal and good adaptation to tropical climate.

16.6 Conclusions

Our regional-scale study provides strong evidence that sea grape harbors distinct and relatively species-poor EcM fungal communities throughout its range. These associated fungi are highly distinct from the mycobionts of other *Coccoloba* spp., suggesting that strong environmental filtering due to salinity and perhaps high pH may cause the high observed ecological specificity. Close affinities of *C. uvifera*-associated *Tomentella* spp. with those from the phylogenetically distant Pinaceae

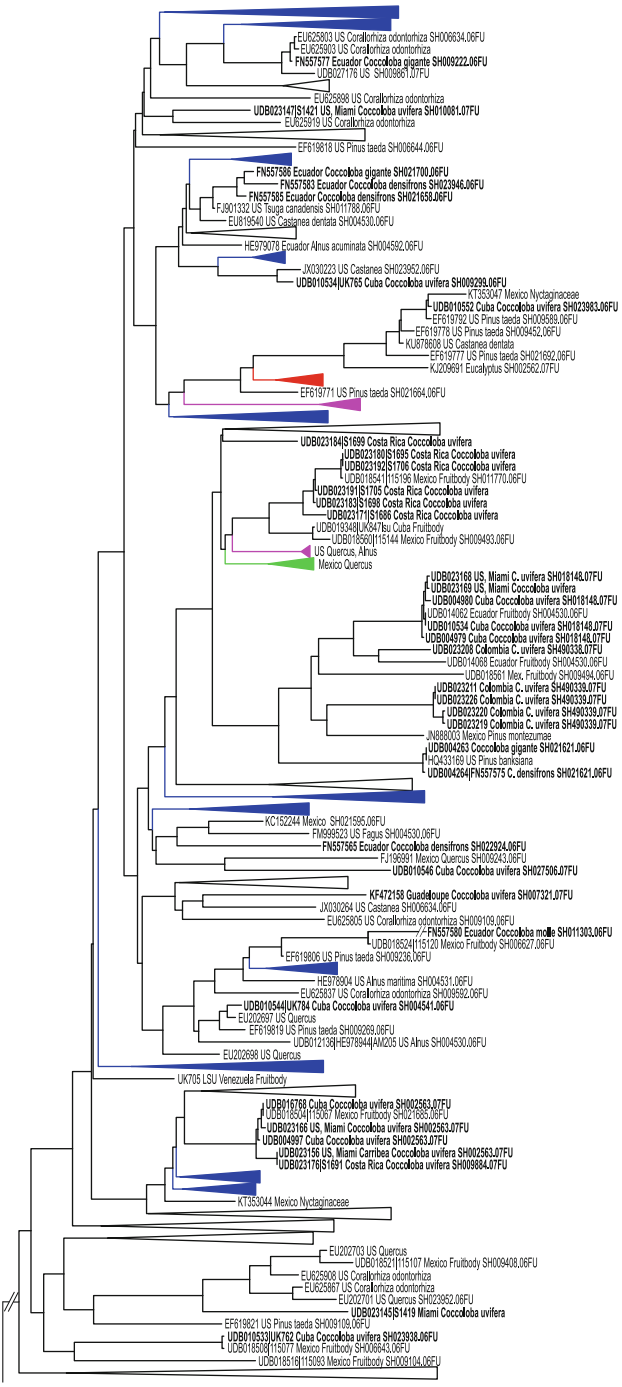

Fig. 16.3 Maximum likelihood phylogram of Thelephoraceae sequences from South, Central and Northeast America. Clades that do not contain *Coccoloba* associated Thelephoraceae sequences have been collapsed. Clades highlighted in *purple*, *red* and *green* represent Northeast, Central and South American groups, respectively. *Blue*, *yellow* and *orange* represent the mixture of North and

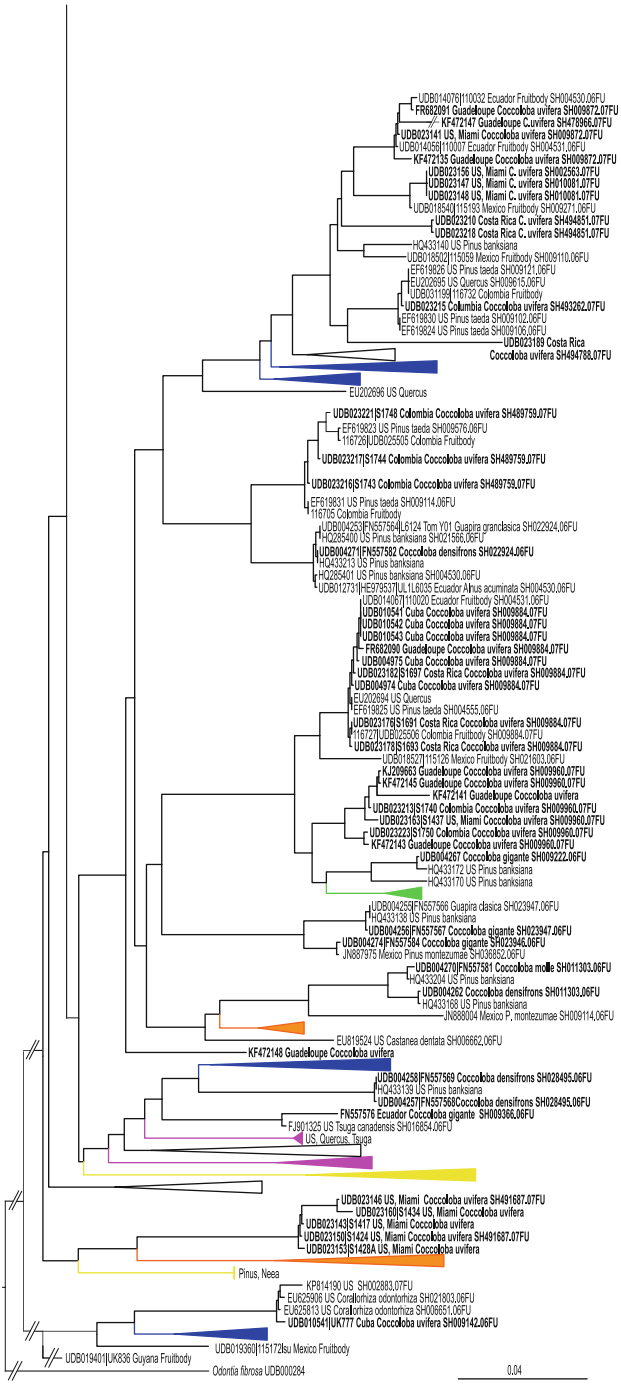

Fig. 16.3 (continued) Central American groups, North and South American groups, and Central and South American groups, respectively. *Transparent clades* represent a mixture of all three regions. *Bold* names indicate Thelephoraceae sequences associating with various *Coccoloba* species. The sequence name includes accession number, country, associating host species and species hypothesis (if available)

and Fagaceae hosts supports multiple host shifts and/or broadening of host range due to more commonly shared habitats in the past. Future phylogeographic studies including more samples from South America are needed to enlighten the evolutionary history of EcM symbiosis in Central and South America.

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Chapter 17
Distribution and Evolution of Mycorrhizal Types and Other Specialised Roots in Australia

Mark C. Brundrett

17.1 Introduction

Australia is a land of contrasts with habitats that extend from temperate and tropical woodlands and rain forests in the north and east to large deserts that occupy most of the interior, as well as temperate forests and shrublands in the southeast and southwest (Fig. 17.1). Each of these bioregions has separate flora and vegetation patterns that have been defined by species turnover or vegetation mapping (Ebach et al. 2015; Keith and Pellow 2015). Vegetation patterns in Australia are also strongly linked to soil properties and water availability (Keith 2011; Jones et al. 2016). In contrast to areas in the northern hemisphere with recent glaciation, which have thin soils that are thousands of years old, most Australian soils consist of very deep weathered profiles that are at least 200 million years (Ma) in age (Twidale and Campbell 2005; Johnson 2009). These deep weathered profiles are referred to as regolith, with only the uppermost 1–2 m classified as soil (Beckmann 1983; Anand and Paine 2002). There have been substantial movements of these materials by wind and water resulting in a relatively flat landscape but few opportunities for rejuvenation to replace nutrients lost due to leaching (Beckmann 1983; Johnson 2009). While the majority of nutrient-absorbing roots are in the surface soil layers, some Australian plants have deep roots, which access groundwater from the regolith at considerable depths (Canadell et al. 1996; Eamus and Froend 2006; Groom and Lamont 2015). Due to long periods of weathering, Australian soils tend to be highly infertile over large areas of the continent with some more fertile soils on younger

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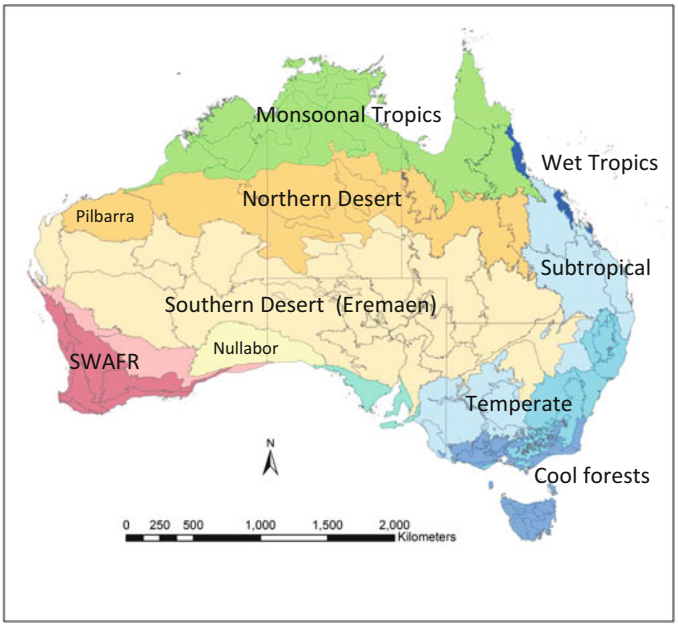

Fig. 17.1 Map of Australia showing IBRA bioregions that represent plant diversity and vegetation structure at a coarse scale (http://www.environment.gov.au/land/nrs/science/ibra). Colours are used to amalgamate these into phytogeographic regions based on plant species turnover (Ebach et al. 2015)

substrates in coastal areas, and soil P levels tend to be lowest in southwestern Australia (Rossel and Bui 2016; Kooyman et al. 2016). Old landscapes are also relatively flat with large areas where drainage flows into ephemeral salt lakes rather than the ocean (Beard 2002; George et al. 2008). Consequently, Australia also has many areas with saline groundwater and ephemeral salt lakes where halophytic plants are dominant (McArthur 2004; Pate and Verboom 2009).

Over the past 65 Ma, a key story has been the gradual desertification of the interior of Australia as the content drifted northwards after separating from Antarctica (White 1986). This process started in the Eocene with cooling temperatures and increasing rainfall seasonality in the centre and southwest of the continent, which became more pronounced in the late Miocene (Macphail 2007). The influence of fire has also dramatically increased during this time with many species having fire-adapted traits becoming more important as fire-intolerant species contracted to coastal areas with higher rainfall (Crisp et al. 2011; Carpenter et al. 2015). It has been suggested that fire had a key role in the increased dominance of angiosperms globally (Bond and Midgely 2012). There is a substantial fossil and pollen history, which shows plant families that include sclerophyllous and fire-adapted species became much more important in Australia, especially in the past 30 Ma, but many of these plant families have much older origin in Gondwana (Hill and Scriven 1995; Crisp et al. 2004; Carpenter et al. 2015; Jordan et al. 2016). Australian arid zones and the monsoonal

tropics also include many examples of relative recent (Tertiary) flora radiation along with many lineages that dispersed into the region from Asia (Crisp et al. 2004; Byrne et al. 2008; Bowman et al. 2009; Crisp and Cook 2013).

The Southwest Australian Floristic Region of Western Australia (SWAFR) is the only global biodiversity hotspot for species richness and endemism in Australia (Hopper 2009). The SWAFR contains about 7000 plant species of which 52% are endemic to that area (Thiele and Prober 2014). By comparison, a similar-sized area in southeastern Australia contained 4810 species, but only 14% were endemic to that region (Thiele and Prober 2014). Smaller biodiversity hotspots for specific groups of organisms have also been recognised in Australia, such as 17 centres for exceptionally high species richness and endemism in Western Australia (Barrett et al. 2007) and 21 centres with high *Acacia* diversity in Australia (González-Orozco et al. 2011). Studies in Australia and elsewhere have shown that species richness is greatest at nutrient-poor sites (Beadle 1954; Specht and Rundel 1990; Cowling et al. 1994; Lambers et al. 2010). For example, shrublands and woodlands in southwestern Australia have more plant species in a 100 m^2 plot compared to soils with higher levels of phosphorus and nitrogen (Gibson et al. 2004; Zemunik et al. 2016). The SWAFR has a large proportion of species that are specialised and adapted to the low phosphorus content of soils that predominate in this highly weathered landscape (Hopper and Gioia 2004; Lambers et al. 2010). Highly infertile soils seem to be linked to higher plant species richness and turnover by reducing the dominance and shading by overstory plants (Specht and Specht 1989).

Relationships between soils, landscapes and climates with plant diversity and vegetation patterns are very complex in Australia. In old landscapes, weathering of regolith and erosion lead to increase in soil complexity, and prolonged soil leaching leads to low soil fertility (Anand and Paine 2002; Verboom and Pate 2013). Ancient landscapes with long periods without major disturbance provide the opportunity for plants to diversify and specialise to local conditions (Hopper 2009; Linder and Verboom 2015). In Australia, vegetation patterns are also strongly driven by land form and hydrology (Fordyce et al. 2007; Keith 2011; Cardillo and Pratt 2013; Reyes et al. 2015). High species richness in the SWAFR is also linked to steep climatic gradients along with longer times since major extinction events (Cook et al. 2015; Jones et al. 2016) and habitat specialisation, especially in rocky outcrops and wetlands (Sander and Wardell-Johnson 2011). Cowling et al. (1994) determined that endemic plants in hotspots tend to be edaphic specialists and these include members of the families Ericaceae, Fabaceae, Proteaceae, Cyperaceae, Myrtaceae, Restionaceae and Asteraceae. Many of these exceptionally diverse families include species with complex or unique root types, as discussed below. It has also been proposed that plants can modify soil profiles and cumulative effects of root exudates have been linked to laterite or clay formation (Pate and Verboom 2009). Impacts of plants on soil structure in ancient landscapes should be expected, but the extent to which this happens requires further investigation.

Climatic stability is also important in ancient landscapes (Mucina and Wardell-Johnson 2011; Cowling et al. 2015). For example, climate is as important as soil chemistry in explaining *Acacia* species richness in Australia (Bui et al. 2014).

Plants in many Australian habitats are adapted to frequent fires, which lead to long-term persistence of species in a location (Orians and Milewski 2007; Carpenter et al. 2015; Groom and Lamont 2015). Thus, vegetation patterns are very complex in areas of high plant diversity in Australia, and these patterns are the product of substrate types, climate, history, fire and vegetation. The majority of explanations for high plant diversity in the SWAFR involve low soil fertility as one of the key mechanisms.

This chapter aims to summarise knowledge about the mycorrhizal associations of Australian plants, which are arranged by plant taxonomic groups and habitat types. Data on the age of plant lineages with newly acquired root types are also presented and linked to changes in soils and landscapes over time. This data is also used to investigate factors linked to exceptionally high root function diversity in some plant families in Australia and possible mechanisms for evolution of new root traits in biodiversity hotspots.

17.2 Mycorrhizal Diversity Comparisons

The following comparisons are based on existing knowledge of Australian plants concerning mycorrhizal associations and other structures that contribute to nutrient uptake by plants. Only key references are provided here, while the rest are available online (Brundrett 2008—mycorrhizas.info/ozplants). The main mycorrhizal types are arbuscular mycorrhiza (AM), ectomycorrhiza (EcM), ericoid mycorrhiza (ErM), orchid mycorrhiza (OM) and nonmycorrhizal roots (NM). A new scheme to classify complex root types is provided in Table 17.1. This scheme extends the terminology used by Brundrett (2009) to include several new categories that designate which mycorrhizal type is most frequent (AM-EcM vs. EcM-AM) or of where a second type of mycorrhiza has been observed but seems to be of limited importance (e.g. EcM (AM)). Note that some of these designations are optional, since it will not always be possible to separate similar categories such as AM-NM from NM-AM due to limited data. The most common category is NM-AM where plants can have AM or NM roots.

Comparisons of the estimated total diversity of plants with mycorrhizal or NM roots on a global scale (Fig. 17.2a) show that Australia has disproportionately large numbers of both EcM and NM species compared to the rest of the world and is a global centre of diversity for these plants (Brundrett 2009). There also is elevated species richness of Ericaceae and Orchidaceae in Australia and about 20% fewer AM host plants compared to global totals. The relative diversity NM-AM of plants, where the presence of mycorrhizas is regulated by habitat, is also very similar at both scales. The NM-AM category of plants is dominated by epiphytes, aquatic plants and halophytes in Australia.

Figure 17.2b provides a comparison of the diversity of plants with different root types in the SWAFR in comparison to a similar-sized area in eastern Australia. Plant families are separated into the root-type categories in Table 17.1 in this graph, except that EcM-AM and AM-EcM were combined for simplicity. The eastern Australian

Table 17.1 Definitions of categories of plants with complex mycorrhizal associations involving mycorrhizal and/or nonmycorrhizal roots

Category	Explanation
AM	Arbuscular mycorrhizas occur in the majority of young healthy primary roots, with many arbuscules in young roots
EcM	Ectomycorrhizas occur on the majority of young healthy primary root, Hartig net well developed
GFC	Glomalean Fungus Colonisation is endophytic activity in non-host without arbuscules (see Brundrett 2009)
NM	Nonmycorrhizal roots where healthy roots remain free of AM or EcM fungus colonisation, but older roots often contain GFC
NM-AM	Species or plant clade (usually a family, genus) reported to have AM in some circumstances but not others depending on soil or habitat conditions with NM reports most common
AM-NM	Similar to AM-NM, but reports of AM much more common (optional category similar to NM-AM)
NM (AM)	Normally NM but some reports of AM, perhaps as misinterpretation of GFC (optional category similar to NM)
AM-facultative	Roots have AM in all cases but colonisation levels low and inconsistent (generally 5–40% of root length)
AM-EcM	AM more important than EcM in mature plants, Hartig net poorly developed (optional category similar to EcM-AM)
EcM (AM)	Occasional reports of AM in otherwise EcM plants, arbuscules rare or absent, AM often most common in young seedlings or plants in disturbed sites
AM (EcM)	Occasional reports of EcM in otherwise AM plants, Hartig net poorly developed or absent (optional category)

region is defined by Thiele and Prober (2014), which includes most of New South Wales and Victoria and is a bit larger than the SWAFR and has greater topographic diversity but lower overall species richness and endemism (Box 17.1). The main groups with higher than expected species richness in the SWAFR are the EcM-AM and NM families with cluster roots, as well as the Ericaceae and carnivorous plants (Fig. 17.2b).

Figure 17.3 includes a series of maps that illustrate general species richness trends for functional or evolutionary groups of plants in Australia. These maps show that EcM, AM and NM plants are widespread across Australia in most habitats. There is a strong trend for lineages of plants that predate the Australia-Antarctica separation such as *Nothofagus* (EcM), gymnosperms (AM) and ferns (AM) to be restricted to higher-rainfall coastal areas, especially temperate rainforests (Fig. 17.3a, b, d). In contrast, most AM families (Fig. 17.3c) and Gondwanan lineages of EcM plants are widespread in most habitats across Australia (Fig. 17.3e), while clades which have diversified primarily after Australia separated from Antarctica are most diverse in higher rainfall areas on infertile soils (Fig. 17.3f). Figure 17.3g–i shows NM plants with cluster roots in the Proteaceae, NM monocots with sand-binding or dauciform roots and genera in the Fabaceae that include species with cluster roots, respectively. The distribution of three of the most specialised categories of NM plants, parasites using haustoria to feed on other plants, cluster roots adapted to low-nutrient soils and

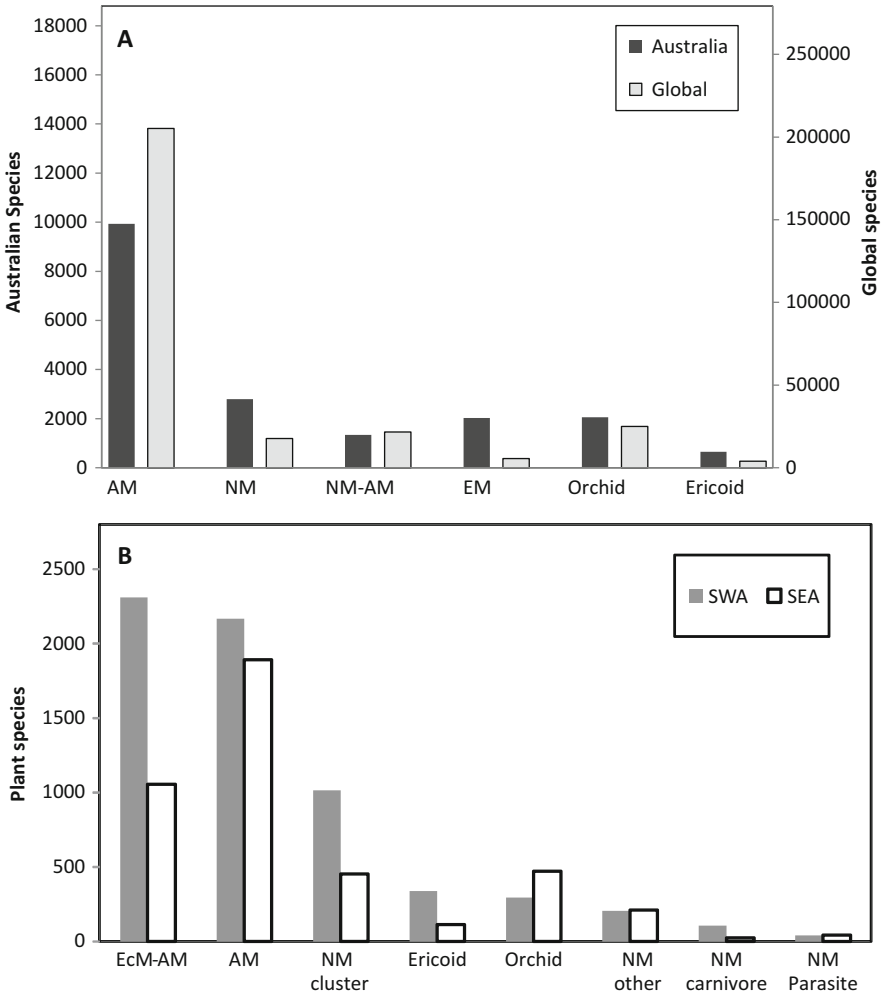

Fig. 17.2 (**a**) The estimated number of plants with different types of mycorrhizas or nonmycorrhizal roots in Australia and globally (EM = estimated number of host plants in a genus where most or all have EM). These data are totals of all taxa that that can were assigned to each category based on a review of the scientific literature (Brundrett 2009). Note there are two separate *y* axis scales. (**b**) Comparison of mycorrhizal and nonmycorrhizal plant diversity in similar-sized areas in eastern Australia (SEA) and the global diversity hotspot in Western Australia (SWA). This graph shows the taxonomic diversity of plants with different root types in each region using data are from Thiele and Prober (2014) and Florabase (florabase.dpaw.wa.gov.au—accessed 4 November 2016). Data are for approx. 10,000 plant species in 50 families

carnivores with insect-trapping leaves, are also shown (Fig. 17.3j–l). Plants in the first two categories are widespread, but carnivorous plants primarily occur in high rainfall areas. Figure 17.3m–o show three types of specialised mycorrhizas in the families Orchidaceae and Ericaceae and the genus *Thysanotus* (Asparagaceae) that

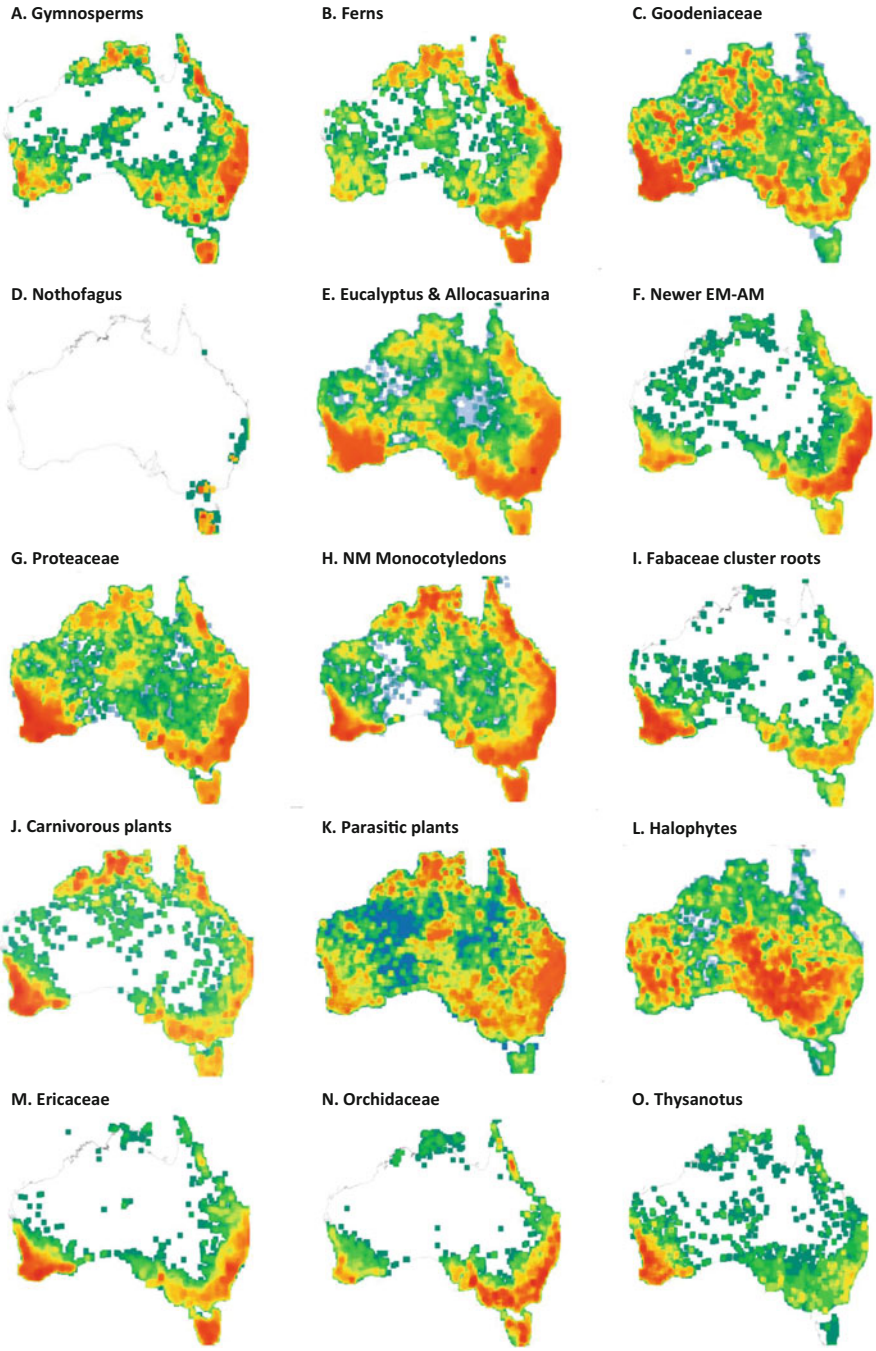

Fig. 17.3 Maps showing the average number of recorded taxa at different locations using data from the Atlas of Living Australia. (**a**) Ancient AM gymnosperms (Podocarpaceae, Araucariaceae and Cycads). (**b**) Ferns and fern allies. (**c**) The Goodeniaceae an example of a modern AM family. (**d**) *Nothofagus* an old EcM genus. (**e**) *Eucalyptus* and *Allocasuarina* two of the most important

predominantly occur in higher-rainfall near-coastal areas. These distribution trends are discussed further in Sect. 17.3 below. Plants with nitrogen-fixing associations are also widespread in most habitats (not mapped).

Figure 17.4 represents a vegetation map that has been modified to show the spatial extent of the root types of dominant plants in Western Australia. This map only shows the status of dominant plants that are used to define vegetation map units, such as trees large shrubs and grasses. This map shows that AM and EcM plants are important in almost every habitat, including some of the harshest landscapes on earth. The widespread occurrence of EcM hosts in relatively arid habitats has other ecological implications, since their associated fungi include truffles with subterranean fruit bodies, which are important food sources for the animals that disperse them (e.g. Claridge and May 1994).

Many Australian plants also have symbiotic N_2-fixing associations. These associations occur in members of the families Fabaceae, Casuarinaceae and Zamiaceae in Australia, but members of these families occur in most plant communities. Nitrogen-fixing nodules are formed by most members of the Fabaceae, which includes the subfamilies Caesalpinioideae and Mimosoideae (acacias), as well as the Faboideae (peas). Some *Allocasuarina* species do not consistently form nodules, and this seems to depend both on host species and soil types (Reddell et al. 1986). Cycads produce upward-facing dichotomously branched "coralloid roots" near the soil surface that host nitrogen-fixing cyanobacteria (Groom and Lamont 2015).

Box 17.1 summarises mycorrhizal and NM plant diversity comparisons between Australia and global averages, between southeastern and southwestern Australia, as well as between Mediterranean climate plant diversity hotspots in Australia and South Africa. In summary, EcM and NM plants (including parasites and carnivores) and nitrogen-fixing plants are especially common Australian habitats and even more so in the SWAFR. Plants with arbuscular mycorrhizal associations are also common in most habitats. Thus, mycorrhizas are important in almost every habitat, including some of the harshest landscapes on earth.

Fig. 17.3 (continued) genera with EcM in Australia. (**f**) Newer genera with AM-EM or AM roots including *Melaleuca, Acacia, Gastrolobium* and many others. (**g**) The Proteaceae a large family with NM cluster roots. (**h**) Major families of NM monocots (Restionaceae, Cyperaceae, Dasypogonaceae). (**i**) New genera in the Fabaceae with NM or AM cluster roots (*Daviesia, Viminaria* and *Kennedia*). (**j**) Carnivorous plants (NM). (**k**) Parasitic plants (NM). (**l**) The largest families with many Halophytes (Chenopodiaceae/Amaranthaceae, Frankeniaceae). (**m**) Ericaceae. (**n**) Orchidaceae. (**o**) The genus *Thysanotus*. These maps were produced by the Species Area Tool using data from all collections and records in the Atlas of Living Australia (www.ala.org.au; accessed 23 November 2016). The Points to Grid function was used with a 0.1 degree scale and 9 cell-moving averages. The highest diversity is shown as *red*, lower diversity as *blue* and a lack of shading reflects the absence of records. These maps show general trends only since recorded diversity is often lower than actual diversity. They are also affected by limited sampling in remote areas in central Australia

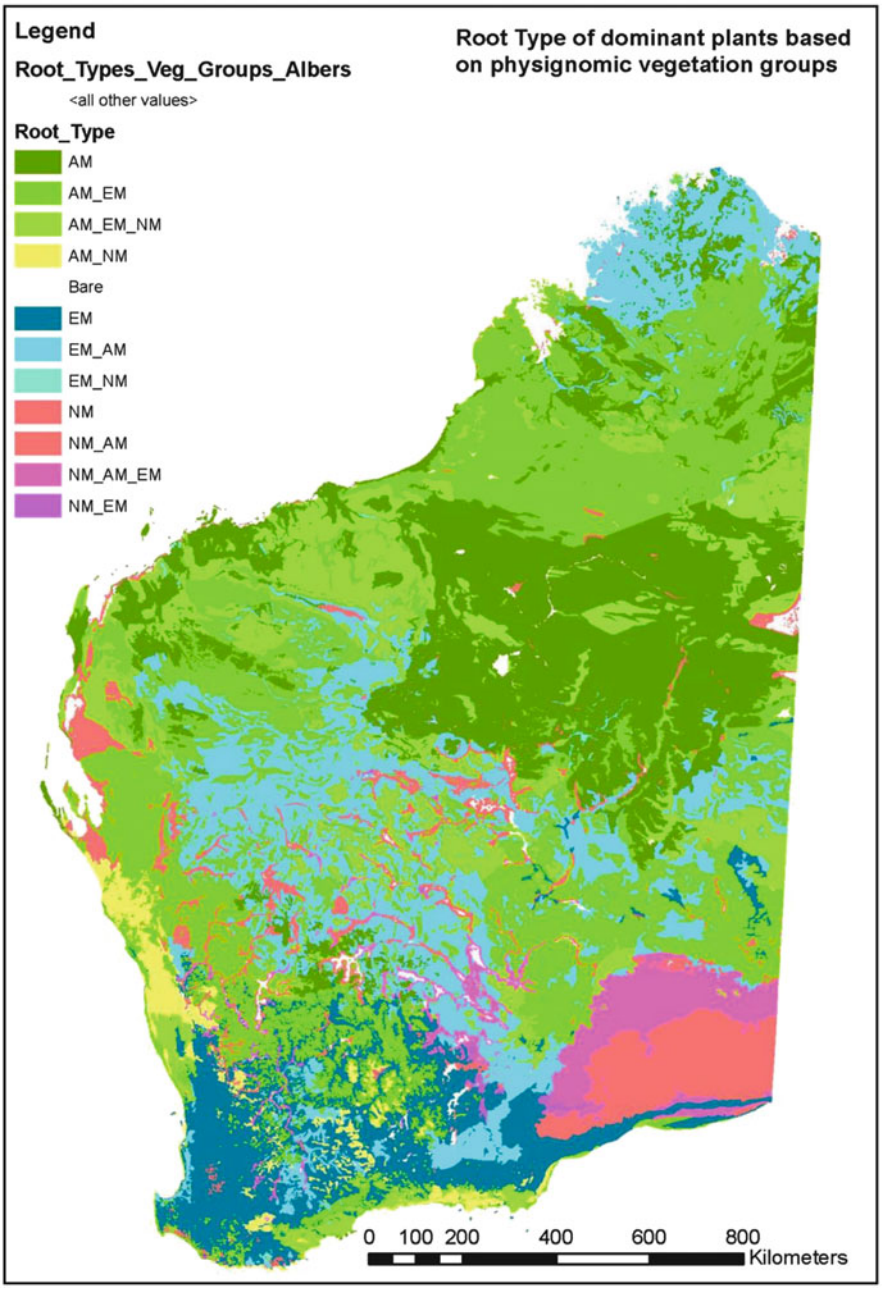

Fig. 17.4 The West Australian vegetation map with major physiognomic vegetation types reclassified to show belowground plant adaptations linked to nutrient uptake. This map provides information on nutrient adaptations of dominant plants in vegetation types, which normally include more than one type. The base map is from Beard et al. (2013). The root status of dominant plants is inferred from existing data and phylogeny (http://mycorrhizas.info/ozplants, Brundrett 2009) (AM = arbuscular mycorrhizas, EM = ectomycorrhizas, NM = nonmycorrhizal)

17.3 Root Evolution in Australia

On a global scale, the mycorrhizal status of the majority of plant families is highly consistent and well understood (Brundrett 2009). However, opinions on the mycorrhizal status of some Australian genera differ as shown in Table 17.2. These contradictory reports arise in part due to the use of different definitions for AM or EcM, especially concerning the use of a Hartig net to define EcM or arbuscules to define AM (Brundrett 2009). In cases were AM have been reported in normally NM plants such as carnivores or sedges, these are designated as NM (AM), since the AM is not present consistently and thus seems to be of limited functional significance. Despite numerous reports, there is no conclusive evidence that NM to AM switching occurs in NM (AM) families such as the Cyperaceae. There are a few well-documented cases, but most reports result from misidentification of endophytic colonisation by AM fungi in older roots of non-host plants (see Chap. 21). Plants with both EcM and AM associations in their roots (EcM-AM) are common in Australia, as illustrated in Fig. 17.5a–d. These dual

Table 17.2 Contradictory information on the ectomycorrhizal status of some Australian plants (see Table 17.1 for abbreviations)

Family	Genera	References for AM-EcM	References for AM only	Notes
Asteraceae	*Angianthus, Podolepis, Waitzia, Helipterum, Helichrysum*	1, 3, 4	8	No Hartig net in most cases (2)
Goodeniaceae	*Dampiera, Goodenia,* etc.	1, 2, 5	6	No Hartig net (2), see Fig. 17.5e, f
Polygalaceae	*Comesperma*	4	6	
Sterculiaceae	*Lasiopetalum, Thomasia*	1, 4	6	
Apiaceae	*Platysace*	1	8	
Proteaceae	*Grevillea* spp.	5, 7	6	AM in NM plant, most reports NM only
Boryaceae	*Borya*	7	8	Unusual EcM definition
Phyllanthaceae	*Phyllanthus*	7	6	
Euphorbiaceae	*Poranthera microphylla*	1, 2,	8	No Hartig net (2), superficial hyphae present (8)
Stylidiaceae	*Stylidium* spp.	1, 3	6	No Hartig net (2)

References: 1, Warcup (1980); 2, Warcup (1985); 3, Kope and Warcup (1986); 4, McGee (1986); 5, Bellgard et al. (1994); 6, Brundrett and Abbott (1991); 7, Reiter et al. (2013); 8, this publication
Plants examined by the author for this table: *Podotheca gnaphalodes, P. chrysantha* and *Asteridea pulverulenta* in the Asteraceae, *Poranthera microphylla* in the Phyllanthaceae and *Platysace filiformis* in the Apiaceae

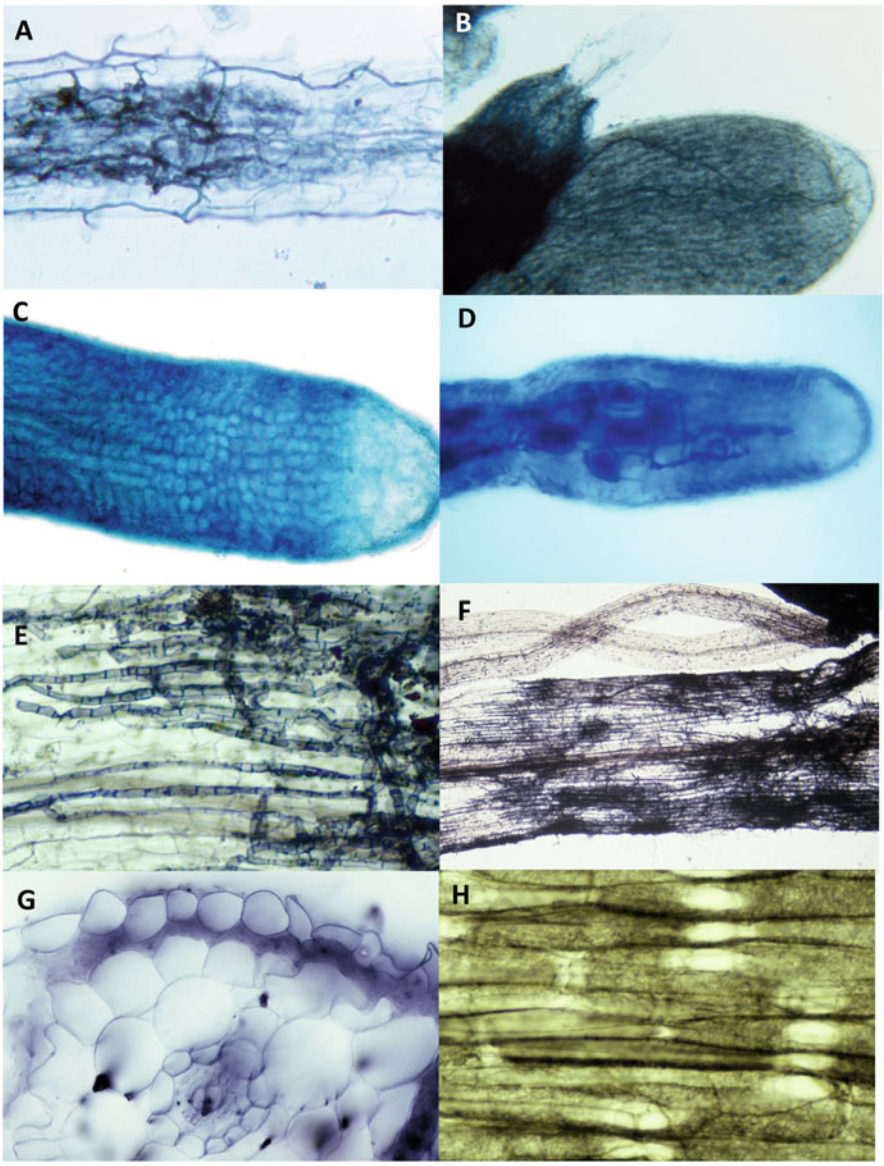

Fig. 17.5 (**a–b**) Examples of mycorrhizal roots from plants with complex or unusual mycorrhizal associations. *Gastrolobium capitatum* in the Fabaceae has both (**a**) arbuscular mycorrhizas (AM) and (**b**) ectomycorrhizas (EcM). (**c–d**) *Melaleuca scalena* in the Myrtaceae. (**c**) EcM only. (**d**) AM and EcM in the same root. (**e–f**) Superficial fungal growth in *Scaevola calliptera* (Goodeniaceae) that is sometimes interpreted as EcM but lacks a Hartig net. (**g–h**) The unique subepidermal mycorrhizal associations of *Thysanotus multiflorus* in cross section (**g**) and viewed in a whole cleared root (**h**)

associations are not always recognised, since investigators often focus on AM or EcM only.

Reports of EcM in plants which normally have AM roots are summarised in Table 17.2. During the preparation of this chapter, a few of these EcM-AM reports by different investigators have been checked, but more sampling is still required. The roots checked belonged to some of the same genera or even some of the same species, as herbaceous plants reported as EcM by Warcup and McGee (1983). In the current study (Table 17.2), none of these plants had EcM roots, as they are currently defined, but *Poranthera microphylla* had patches of superficial fungal growth that may belong to an EcM fungus. In the author's opinion, annual plants such as member so the Asteraceae are unlikely to have EcM roots due to the excessive costs of such associations to very short-lived plants. This is backed up by all subsequent studies of Asteraceae in Australia that only found AM in roots (Brundrett and Abbott 1991; O'Connor et al. 2001; Meers et al. 2010). There are other problems with the EcM records listed in Table 17.2, as some of the fungi used as inoculum are now considered not to be EcM fungi and soil autoclaved for 1 h was used in pot trials, which can be highly toxic to plants (Warcup and McGee 1983).

In contrast to the reports of EcM-AM in Table 17.2 that seem to be incorrect, many Australian plants in the families Fabaceae and Myrtaceae consistently have both AM and EcM in the same root system (Brundrett 2008). For example, roots of *Eucalyptus* seedlings often have dual AM and EcM associations, but EcM becomes increasingly dominant over AM as they mature (Chen et al. 2000). These trends are summarised in Fig. 17.6 which shows how EcM replaces AM as eucalypt seedlings mature in a glasshouse trial. A mycorrhizal succession from AM to EcM also occurs in young eucalypts, especially when they are grown in plantations (Chilvers et al. 1987; Adams et al. 2006). These associations should be classified as EcM (AM) as the EcM is the

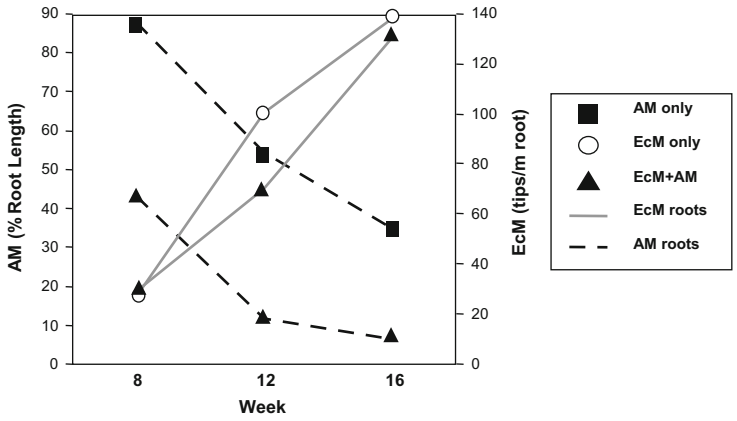

Fig. 17.6 Mycorrhizal succession in *Eucalyptus* roots growing in the glasshouse over their first 16 weeks post emergence. This is a summary of data from Chen et al. (2000) and shows how ectomycorrhizal (EcM) roots gradually replace arbuscular mycorrhizal roots, which occupy a lower proportion of roots over time even in the absence of EcM fungi

primary mechanism for enhanced plant growth in mature *Eucalyptus* species in natural habitats (Brundrett et al. 1996).

Phylogenetic data for clades of Australian plants discussed in this review are summarised in Table 17.3. Molecular clock estimates of lineage ages can vary considerably due to methods, so fossil calibrations of dated phylogenetic trees are very important (Crisp et al. 2014; Thornhill et al. 2015; McLay et al. 2016; Macphail and Thornhill 2016). The majority of clades in Table 17.3 have some fossil calibration. Pollen records and fossil evidence are in close agreement with molecular dating methods for many of these families (Hill 1994; Martin 1994). In this discussion, centres of diversity or biodiversity hotspots refer to areas with higher species richness, endemism and species turnover.

It is intriguing that many of the families and genera with the most complex root strategies have been chosen as phylogenetic case studies for rapid evolution in diversity hotspots with infertile soils (Table 17.3). Driving processes in all cases are the same (increasing aridity, fire frequency, soil infertility, soil complexity, etc.), as explained in the discussion below. Zemunik et al. (2016) found decreasing dominance of host plants by both AM and EcM in roots in older soils in a coastal chronosequence linked primarily to reduced soil fertility. Albornoz et al. (2016) found both AM and EcM in two coastal shrub species (*Acacia rostellifera*, Fabaceae, and *Melaleuca systena*, Myrtaceae). However, EcM roots in their study were not well developed and may not be fully functional. There is a wide diversity of EcM morphotypes, linked to particular fungi, on eucalypt roots with varying degrees of Hartig net and mantle development (Brundrett et al. 1996). A strong positive relationship between Hartig net thickness (regulated by epidermal cell expansion) and mycorrhizal growth responses has been measured in *Eucalyptus* (Burgess et al. 1994). Defining the roles of soil fungi is complex, as they have also been linked to growth responses in cases where mycorrhizal associations do not form (Kariman et al. 2014; Ray et al. 2015).

17.3.1 Arbuscular Mycorrhizal (AM) Plants

Over the past 400 million years, there has been a series of vegetation types in Gondwana, and AM only lineages such as the lycopods, glossopterids, ferns and southern conifers have been dominant for most of that time (White 1986; Hill and Scriven 1995; Jordan et al. 2016). AM conifers in the Podocarpaceae and Araucariaceae and ferns are amongst the oldest AM lineages (Brundrett 2002) and remain important in rainforests in Australia (Fig. 17.3a, b) but were lost from more arid areas in the Late Miocene (Hill 1994; Martin 1994). However, some AM gymnosperms such as *Callitris* species and cycads are still widespread in semiarid areas of Australia. The temperate and tropical rainforests of Australia are dominated by plants with AM and include many plant lineages that have relatively recently dispersed from Asia (Bowman et al. 2009, and see Fig. 21.4).

Plants with AM roots are present in most habitats and are dominant in many habitats in Australia, including temperate and tropical rainforests, grasslands and

Table 17.3 Clades where root strategies have remained the same (**A, D**), switched from AM to EcM-AM (**B**), or from AM to NM (**C**)

Family	Genera or group	Root types	Estimated age of clade	Rapid radiation	References for age of plant clade
A. Arbuscular mycorrhizas (AM)					
Goodeniaceae	Australian family	AM	78 Ma	23 Ma	Jabaily et al. (2014)
Xanthorrhoeaceae	*Xanthorrhoea*	AM	24–35 Ma	10–20 Ma	Crisp et al. (2014)
Poaceae	*Triodia*	AM	65–104 Ma	15 Ma	Crisp and Cook (2013), Jones et al. (2014)
Callitroideae (Cupressaceae)	*Callitris*	AM	>80 Ma		Crisp and Cook (2013)
B. Ectomycorrhizal (EM)					
Casuarinaceae	*Casuarina, Allocasuarina*	AM-EM, AM	63 Ma	25 Ma	Crisp et al. (2004), Li et al. (2015), Larson-Johnson (2016)
Eucalypteae (Myrtaceae)	*Eucalyptus, Corymbia, Angophora*	EM (AM)	65–52 Ma	25 Ma	Thornhill et al. (2015), Berger et al. (2016), Macphail and Thornhill (2016)
Melaleuceae (Myrtaceae)	*Melaleuca*, *Callistemon*	AM, AM-EM, EM-AM	40 Ma	20 Ma	Thornhill et al. (2015), Berger et al. (2016)
Mimosoideae (Fabaceae)	*Acacia*	AM, EM-AM	26 Ma	10–15 Ma	Miller et al. (2013)
Fabaceae	*Gastrolobium*	EM-AM	30 Ma	10–20 Ma	Toon et al. (2014)
Pomaderreae (Rhamnaceae)	Australian genera	EM (AM), AM?	60 Ma	40 Ma	Onstein et al. (2016)
Nothofagaceae (Fagales)	*Nothofagus*	EM	80 Ma		Hill and Dettman (1996), Cook and Crisp (2005)
C. Nonmycorrhizal (NM)					
Proteaceae	*Banksia*	NM cluster	60 Ma	50 Ma	Cardillo and Pratt (2013)
Proteaceae	*Hakea*	NM cluster	18 Ma, 32 Ma[a]	12 Ma, 25 Ma[a]	Lamont et al. (2016), McLay et al. (2016)[a]
Proteaceae	*Grevillea and Hakea*	NM cluster	55 Ma	30 Ma	Mast et al. (2015)

(continued)

Table 17.3 (continued)

Family	Genera or group	Root types	Estimated age of clade	Rapid radiation	References for age of plant clade
Fabaceae	*Daviesia*	NM cluster, AM	40–50 Ma	25 Ma	Toon et al. (2014), Cook et al. (2015)
Amaranthaceae	Australian halophytes	NM	42–26 Ma	11 Ma	Kadereit et al. (2005), Steffen et al. (2015)
Amaranthaceae	*Ptilotus*	NM		25–10 Ma	Hammer et al. (2015)
Schoeneae (Cyperaceae)	Australia	NM, NM cluster	50 Ma	30 Ma	Viljoen et al. (2013), Bouchenak-Khelladi et al. (2014)
Restionaceae, Anarthriaceae	Australia	NM cluster	50 Ma	30 Ma	Bouchenak-Khelladi et al. (2014)
Haemodoraceae	Australia	NM sand-binding	80 Ma	30 Ma	Crisp and Cook (2013), He et al. (2016)
Dasypogonaceae	*Dasypogon, Kingia*	NM	110 Ma		Givnish et al. (2016b)
Droseraceae	*Drosera*	NM carnivore	50 Ma	15 Ma	Yesson and Culham (2006)
Loranthaceae	*Amyema, Nuytsia,* etc.	NM parasite	28–40 Ma		Vidal-Russell and Nickrent (2008a)
D. Others					
Orchidaceae	Australia	Orchid	112 Ma	45 Ma	Givnish et al. (2016a)
Ericaceae	Worldwide	Ericoid	118 Ma	58–8 Ma	Schwery et al. (2015)
Asparagaceae	*Thysanotus*	Subepidermal	Recent clade		Givnish et al. (2016b)

Approximate times are shown for clade origins or arrival in Australia and the start of rapid diversification (if relevant), as millions of years ago (Ma). The second dates are estimates from increased branching in trees. Some root types do not include all species in a clade or cannot be confirmed to include all species due to limited sampling

many shrublands (Hopkins et al. 1996; Brundrett 2008). Many clades of plants with AM roots also include a large diversity of species in Australian diversity hotspots (e.g. Asteraceae, Goodeniaceae, Stylidiaceae, Malvaceae and Lamiaceae). Figure 17.3c shows a map for one widespread Australian family, the Goodeniaceae, but other AM families such as the Asteraceae, Poaceae, Stylidiaceae, Malvaceae and Lamiaceae are equally widespread, except for the Stylidiaceae, which occurs outside of the arid interior of Australia. There are also many species of AM plants in

the Fabaceae and Myrtaceae. Table 17.3 provides some examples of ancient AM or EcM lineages, such as the Xanthorrhoeaceae, Nothofagaceae and Goodeniaceae. I found relatively few recent case studies on evolutionary radiation for AM only families to include in Table 17.3, but more data would be required to confirm that evolution of these clades has been slower overall.

17.3.2 Ectomycorrhizal (EcM) Plants

On a global scale, Australian plant communities have an exceptionally high diversity of EcM hosts (Brundrett 2009; Chap. 19). The majority of plants with EcM are trees or large shrubs, and the most iconic examples in Australia are gum trees (*Eucalyptus* spp.), sheoaks (*Casuarina, Allocasuarina* spp.), paperbarks (*Melaleuca* spp.) and southern beeches (*Nothofagu*s spp.). However, there are also many examples of shrubs with EcM-AM roots and few doubtful reports of EcM in herbaceous or annual plants (see Sect. 17.2). Many Australian plants with EcM also have AM in their roots at the same time (see Fig. 17.5a–d). This is most common in *Melaleuca* and *Acacia* species and seems to be linked to recent mycorrhizal switching. There is a good correlation between AM and EcM-AM switching and plant size, with smaller plants in the same families normally being AM only, with a few exceptions (Fig. 17.7). However, explanations for the evolution of EcM-AM plants from AM plants are complex, and it is not known why it is more common in certain phylogenetic groups within plant families or how this is linked to changes in habitats, soils, climates and host plant physiology (see Chap. 19).

The Antarctic beech family (Nothofagaceae) is a southern hemisphere EcM clade that is closely related to the Fagaceae and Betulaceae, which are EcM families in the northern hemisphere (Veblen et al. 1996; Cook and Crisp 2005). *Nothofagus* fossils and pollen appear in the Late Cretaceous and were a major component of Australian vegetation until the mid-Oligocene (30 Ma) but have declined in importance since then (White 1986; Hill 1994). The distribution of *Nothofagus* is now mostly confined to rainforests in Australia (Fig. 17.3d).

The Myrtaceae is an ancient family that is most diverse in the southern hemisphere and became increasing important in Australia, since the Cretaceous (Hill 1994; Crisp et al. 2011). In the Australian Myrtaceae, EcM associations have been found in 15 genera (Chap. 19). These mostly belong to the clades Eucalypteae (*Eucalyptus, Corymbia, Angophora,* etc.) and the Melaleuceae (*Melaleuca* s. l.). Plants in all of these genera are also capable of forming AM, and many contain AM only species. The Myrtaceae seems to be an AM only family elsewhere. *Melaleuca* is a very large genus and is likely to get even more complex if allied genera such as *Calothamnus* and *Eremaea* are merged into it. However, some genera in the Myrtaceae with EcM and AM roots are is also very complex phylogenetically, including *Agonis, Leptospermum* and *Pericalymma*. Most of the Myrtaceae clades with EcM or EcM-AM roots are fire-adapted species noted for very rapid radiation and increasing dominance in ecosystems after the Cretaceous-Paleogene boundary

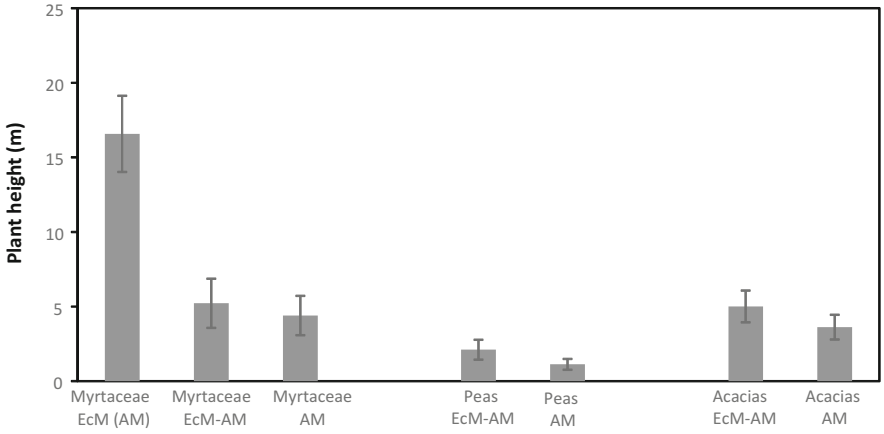

Fig. 17.7 Plant size and mycorrhizal status for AM-EcM plants in the Fabaceae and Myrtaceae where roots have been sampled. These are averages based on typical sizes for plants in each genus where mycorrhizas have been sampled. Data are from two studies where consistent definitions of mycorrhizas were used (Brundrett and Abbott 1991; Brundrett et al. 1995)

(Crisp et al. 2011; Thornhill et al. 2012, 2015). This diversification commenced after aridification began about 50 Ma ago and has accelerated since then (Table 17.3), with peaks of species richness in biodiversity hotspots (Fig. 17.3f). Molecular evidence suggests the Eucalypteae clade is about 65 Ma old, but the fossil evidence is only 53 Ma old (Hill et al. 2016; Macphail and Thornhill 2016). *Eucalyptus* trees are flammable due to essential oil accumulation in leaves, and a feedback between increasing eucalypt dominance and increasing fire frequency during the history of Australia has been suggested (Crisp et al. 2011). The increasing dominance of EcM trees over AM plants has also resulted in the increased importance of hypogeous (truffle-like) fungi in Australian ecosystems (Claridge and May 1994; Abell-Davis et al. 2012). The long-standing interrelationships between truffles and animals that feed on and disperse them confirm that the dominance of EcM trees in Australian woodlands and forests is not a recent event.

Members of the Fabaceae, most of which have nitrogen-fixing nodules on roots, arose in the Late Cretaceous at about 100 Ma (Li et al. 2015). This family has many members with AM only roots as well as EcM hosts and a few NM genera with cluster roots (Chap. 19; Brundrett 2008, 2009). Fifteen genera of Australian Fabaceae have been reported to have EcM roots, but these include several doubtful records, and of the remaining 12 genera, 11 are nested within the genus *Pultenaea* s. l. (including *Mirbelia, Gastrolobium* and *Gompholobium*), with *Platylobium* as an out-group. Allocating root types to genera is complicated by taxonomic issues, since recent phylogenetic studies have shown that most of the genera within the *Pultenaea* clade are not well supported by sequence data. Many of these genera also include species reported to only have AM roots (Brundrett 2008). Within the Australian Fabaceae, the *Bossiaea*, *Daviesia* and *Pultenaea* clades, which includes

most of the plants with complex roots (EcM-AM, AM and NM plants), show exceptionally high rates of recent diversification in Australia, which are even higher in the SWAFR biodiversity hotspot (Crisp et al. 2004; Crisp and Cook 2013). There seems to have been multiple conversions from AM to EcM-AM roots in these clades and possibly some reversions back to AM, but there currently is insufficient sampling of roots of Australian Fabaceae taxa to resolve this.

Most *Acacia* species have AM or EcM-AM roots, with AM being more common. Australia is the global centre of diversity for *Acacia* with over 1000 species, and these vary from large trees such as *A. melanoxylon* which are predominantly EcM to small shrubs, most of which have only AM. The EcM-AM *Acacia* species tend to be a bit larger on average than AM only species (Fig. 17.7). Climate and soil geochemistry are key drivers of *Acacia* species richness and endemism in Australia (Bui et al. 2014). There are 21 centres of diversity for *Acacia* in Australia, of which 10 are within the SWAFR regional hotspot (González-Orozco et al. 2011). Regional endemism in *Acacia* is highest in the SWAFR (Mishler et al. 2014). Phylogenetic analysis of the Mimosoideae by Miller et al. (2013) suggests that *Acacia* diversification was strongly driven by Miocene aridification. *Acacia* plants with EcM are most likely to occur as dominants or codominants in woodlands and shrublands in semiarid regions (Fig. 17.4).

The Casuarinaceae is one of oldest families of plants with N_2-fixing actinorhizal nodules, arising in the Late Cretaceous (~90 Ma) in Gondwana and becoming increasing dominant as the continent became more arid (Hill 1994; Li et al. 2015). This is a characteristic family of Australia which is related to other EcM families in the Fagales (Chap. 19). *Allocasuarina* radiated rapidly in both eastern and western Australia in the past 24 Ma (Crisp et al. 2004). *Casuarina*, *Allocasuarina* and *Gymnostoma* species have complex roots systems with one or more of actinorhizal N_2-fixing nodules, cluster roots, AM and EcM, but the presence of both EcM roots and nodules is inconstant (Reddell et al. 1986). The presence of cluster roots in *Casuarina* depends on phosphorus supply (Reddell et al. 1997). *Gymnostoma* species were widespread in Australia in the Eocene and Oligocene but are now most diverse in New Caledonia, while *Allocasuarina* has become increasingly important in Australia since then (Hill 1994). *Gymnostoma* has AM in beaded roots (Duhoux et al. 2001), while *Allocasuarina* usually has more EcM than AM in its roots (Reddell et al. 1997) and associates with a wide diversity of fungi with hypogeous fruit bodies (Abell-Davis et al. 2012). *Casuarina* seems to be an AM only genus but includes salt-tolerant species that may have NM roots when growing in salt marshes.

The Rhamnaceae has high diversity in Mediterranean ecosystems in Australia, California and South Africa (Onstein et al. 2015) but has only been reported to have EcM roots in Australia (Brundrett 2008). For this family, EcM roots have been well documented in large shrubs, such as species of *Cryptandra, Trymalium* and *Spyridium*, which occur in the understory under eucalypts or in coastal habitats. Sampling of small shrubs in the Rhamnaceae that grow in more arid habitats in Australia is warranted.

17.3.3 Plants with Other Mycorrhizas

Rapid diversification in plants with more specialised mycorrhizas also occurred in the Ericaceae, Orchidaceae and the monocot genus *Thysanotus,* which are very diverse in the SWAFR diversity hotspot. Most *Thysanotus* species are endemic to Western Australia (56 spp.), but seven species have dispersed into Eastern Australia and one species (*T. chinensis*) is widespread in Asia. The genus *Thysanotus* is one of the youngest lineages in the Asparagaceae (McLay and Bayly 2016) and presumably arose in Western Australia after the breakup of Gondwana. Figure 17.5g, h shows the unique subepidermal mycorrhizal association of *Thysanotus* in the Asparagaceae (Lomandroideae). These associations consist of long narrow septate hyphae that grow in a cavity under the epidermal cells of roots (Fig. 17.5e, f). They do not colonise the root cortex but have been shown to promote plant growth (McGee 1988). These associations are morphologically distinct from all other types of mycorrhizas so deserve their own category (Brundrett 2004). *Thysanotus* mycorrhizas require further investigation, especially with regard to the identity of associated fungi.

Western Australian plants in the Ericaceae, which have ericoid mycorrhizas (Chap. 9), are highly diverse in Australia with about 500 species out of a global total of 4426 species (Schwery et al. 2015). There are about 340 Ericaceae species in the SWAFR including over 100 unnamed taxa. The Ericaceae is even more diverse in South Africa where there are over 1000 species (Linder 2003; Schwery et al. 2015). Schwery et al. (2015) identify six major radiation events in the Ericaceae which occurred between 8 and 58 Ma, most of which are associated with the formation of mountain ranges. The age of Australian genera in the Ericaceae tribe Styphelieae is thought to be 60–90 Ma old, but there are no fossils of them this old that can be used to confirm this (Schwery et al. 2015). Extant lineages of the Styphelieae arrived in New Zealand at about 5 Ma, but there is fossil evidence of older lineages that seem to have become extinct there (Puente-Lelièvre et al. 2013).

It seems most likely that the Orchidaceae evolved in what is now Australia prior to the full breakup of Gondwana and rapidly dispersed outwards to new centres of diversity in the humid tropics (Givnish et al. 2016a). The diversity of orchids is more highly constrained by rainfall than most other plant families in Australia (Brundrett 2014). Orchid diversity is similar in southeast and southwest Australia (Fig. 17.2b). The global diversity of orchids is strongly correlated with latitude and peaks near the equator (Linder et al. 2005). The present-day diversity of Australian orchids does not reflect their long southern history, but southern Australia is a diversity hotspot for terrestrial orchid species.

17.3.4 Nonmycorrhizal (NM) Plants

Nonmycorrhizal plants fail to form mycorrhizas, even when inoculum of these fungi are present, but there are also cases of inconsistently mycorrhizal plants, which occur in habits where fungal activity is likely to be suppressed by soil or climatic conditions (Brundrett 2009). Plant species in the second category, which are mycorrhizal in some habitats but not in others, are designated as NM-AM (Table 17.1). Examples of specialised plants that grow in habitats where plant species are less likely to have mycorrhizal roots include hydrophytes, halophytes, xerophytes, epiphytes and alpine and subpolar plants (Brundrett 2009 and see Table 21.3). Aquatic plants and halophytes also include some mycorrhizal species or plants that are intermittently mycorrhizal due to soil conditions that vary seasonally (Brundrett 2009). Australia has a relatively high taxonomic diversity of NM plants on a global scale (Fig. 17.2a), presumably because they are more highly competitive than mycorrhizal plants in low-nutrient soils (Brundrett 2009; Lambers et al. 2010). Many plants that lack mycorrhizas have a replacement strategy for nutrient acquisition such as parasitism, carnivory or cluster roots, and these plants are widespread in Australia (Fig. 17.3g–l). In addition to efficient nutrient acquisition, many Australian native species are efficient at retaining P and other nutrients within the plant, partly through internal recycling and partly due to retention in perennial leaves (Lambers et al. 2010).

Australian NM plants with cluster roots, or a similar root type, include most species in the Proteaceae, many species in the Restionaceae and Cyperaceae, as well as some Fabaceae species (Lamont 1982; Shane and Lambers 2005). Cluster roots have a very large surface area due to closely spaced lateral roots with long root hairs (Shane and Lambers 2005; Lambers et al. 2006). These roots function by increasing the nutrient uptake efficiency of roots and allowing them to tap into relatively insoluble sources of nutrients due to the production of large amounts of root exudates, including organic acids and extracellular enzymes (Shane and Lambers 2005). Cluster roots also occur in a few mycorrhizal plants in the Casuarinaceae, Fabaceae and Myricaceae (Lambers et al. 2006).

Members of the Proteaceae occur in many habitats including rainforests but have reached remarkable rates of diversification in highly infertile soils in South Africa and southwestern Australia (Mast et al. 2015; Reyes et al. 2015). Diversification of NM plants in the Proteaceae is also linked to habitat preferences and climate and pollination syndromes (Reyes et al. 2015; Onstein et al. 2016). Age of the Hakeinae clade (*Hakea* and *Grevillea*), another group where diversity is greatest in the SWAFR, is about 55 Ma and is considered to be one of the most rapidly radiating clades of plants (Mast et al. 2015). Other rapidly radiating clades of cluster-rooted NM plants include *Banksia,* where diversity is ten times higher in SWAFR shrublands on nutrient-poor soils than in forests (Cardillo and Pratt 2013), and the *Dryandra* subgenus within *Banksia*, which are infertile soil specialists, has radiated even more rapidly the SWAFR than other *Banksia* clades (Cardillo and Pratt 2013).

The Proteae clade in the Proteaceae are amongst the most species-rich clades in South Africa (Linder 2003).

In the Australian Fabaceae, NM cluster roots occur in some but not all *Daviesia* and *Kennedia* species. In *Kennedia,* cluster roots may be confined to a single West Australian species (*K. coccinea*), while *Daviesia* species have NM cluster roots in the SWAFR (Brundrett and Abbott 1991) but AM roots in northern Australia (Brundrett et al. 1995). Roots of the majority of species in these genera have not been assessed. Genera which have cluster roots and AM include *Viminaria* in Australia (de Campos et al. 2013) and *Aspalathus* in South Africa (Maseko and Dakora 2013). Over the past 30 Ma, *Daviesia* has been one of the fastest radiating genera in Australia, with speciation about three times higher in WA than in eastern Australia (Crisp et al. 2004; Toon et al. 2014). The presence of NM cluster roots, at least in some species, may help explain why speciation in this group is higher in extremely infertile soils. However, *Daviesia* may have rapid adaptive radiation for a number of reasons that include changes in pollination syndromes (Toon et al. 2014), as well as anomalous secondary growth in roots linked to fire survival (Crisp and Cook 2003). Multiple adaptations to highly infertile soils, fire and new pollinators have evolved in parallel with rapid root-type diversification in this genus. Thus, it is not clear if root adaptations are the most important factor in this explosive clade radiation. More sampling of roots in this genus/family is warranted to compare clades with faster vs. slower rates of diversification. Australian members of the Fabaceae with EcM all seem to have AM as well, but plants with cluster roots and AM seem to be less common and probably represent an intermediate stage in root evolution.

The Poales includes a number of large NM clades in the Cyperaceae, Juncaceae, Restionaceae and other families, which evolved in the Cretaceous (110–65 Ma ago) (Bouchenak-Khelladi et al. 2014). The Cyperaceae are another predominantly NM family of Gondwanan origins that has undergone rapid diversification and repeated dispersal throughout the globe (Viljoen et al. 2013; Spalink et al. 2016). There has been rapid radiation of sedges in dry habitats in Australia (Table 17.3), and they include many edaphic specialised species that are endemic to small areas (Barrett 2013). Sedges in the Cyperaceae often have highly branched and/or swollen lateral roots called dauciform roots that are similar in function to cluster roots (Shane et al. 2006). These occur inconsistently across a number of Cyperaceae genera and presumably are induced by low nutrient supply but occur in the majority of Australian sedges in the tribe Schoeneae (Barrett 2013). The Restionaceae is a southern hemisphere NM family which has shown rapid recent radiation in Australia (Table 17.3) and South Africa (Linder 2003). Families with sand-binding roots, which are NM and have a thick coating of mucilage, are also very diverse in Australia and include members of the Dasypogonaceae, Haemodoraceae, Cyperaceae and Restionaceae (Shane et al. 2011; Smith et al. 2011).

Carnivorous plants have insect traps containing glands with the capacity to digest prey and absorb nutrients from them (Groom and Lamont 2015). The Western Australian biodiversity hotspot is the global centre of diversity for carnivorous plants containing about one fourth of all global species (Brundrett 2009).

Despite their ability to grow in semiarid habitats, Australian carnivorous plants are concentrated in temperate and tropical higher rainfall areas and are most diverse in the SWAFR (~100 spp.). The main radiation of *Drosera* species globally is linked to the establishment of Mediterranean climate zones (Yesson and Culham 2006).

Parasites are plants with haustorial connections to the vascular tissue of a host plant that is used to supply all of their nutrient and water. Hemiparasites are similar but also have leaves and roots so are capable of some photosynthesis and water uptake (Pate 1994; Groom and Lamont 2015). The Loranthaceae include predominantly epiphytic parasites (mistletoes) and three terrestrial species with root haustoria (Vidal-Russell and Nickrent 2008b). This family is Gondwanan and estimated to be over 28 Ma old but is part of the Santalales, an order of parasitic plants which is over 100 Ma old (Vidal-Russell and Nickrent 2008a). With about 90 species out of about 1000, the diversity of parasitic plants in the Loranthaceae is not much greater than expected in Australia (Watson 2011; Vidal-Russell and Nickrent 2008b). With the exception of trees such as *Nuytsia floribunda*, these are epiphytes which are buffered from low soil fertility by their hosts.

NM plant diversity in Australia also includes many halophytes from terrestrial saline soils and salt lakes, as well as marine habitats (mangroves and seagrasses). There have been many origins of new lineages of halophytes, especially in the Caryophyllales, Alismatales and Poales (Flowers et al. 2010; Moray et al. 2015). Plant families that include many halophytes include the Amaranthaceae, Tamaricaceae, Rhizophoraceae, Cyperaceae and Juncaceae (Moray et al. 2015). These families include many NM plants, which apparently are much more likely to become halophytes than plants with mycorrhizal roots. Australian terrestrial halophytes include the Amaranthaceae (now including Chenopodiaceae) and Frankeniaceae. Halophytic NM clades in the Amaranthaceae dispersed to Australia and diversified relatively recently (Kadereit et al. 2005). Samphires in the genus *Sarcocornia* dispersed to Australia as recently as 5 Ma (Steffen et al. 2015). *Ptilotus* is a NM genus in the Amaranthaceae which has also diversified rapidly in arid habitats in the past 25 Ma (Hammer et al. 2015).

17.4 Discussion

This discussion focusses on plant diversity hotspots with low primary productivity due to low rainfall and infertile soils and is less relevant to rainforests and other mesic environments where older clades of plants are normally dominant. Examples of rainforest plants include the EcM trees *Nothofagus*, as well as ferns and gymnosperms with AM roots that have become less important during the past 30 Ma. In the monsoonal climate areas of northern Australia, fire facilitates the expansion of savannah (that includes scattered EcM eucalypts over AM grasses) to replace the AM-dominated rainforest vegetation (Bowman et al. 2009).

In Australia, trees with EcM roots (especially eucalypts, acacias and *Allocasuarina* spp.) are dominant in many relatively productive temperate habitats. Australia also has a long history of AM to EcM replacement within families, especially the Myrtaceae

and Fabaceae, which is linked to increasing aridity, rainfall seasonality, fire and extremely infertile soils. At the same time, there has been a major increase of the importance of families with NM roots, especially cluster roots or dauciform roots in the Proteaceae, Restionaceae and Cyperaceae. There have also been a few new lineages of plants with cluster roots in the Fabaceae. Plants with NM roots that are parasites or carnivores have also been favoured by changes to Australian landforms, soils and climate over the Tertiary era (66 Ma). There has been a major increase in NM halophytes due to salt accumulation in arid landscapes. Members of the Orchidaceae and Ericaceae, which have separate types of mycorrhizas, have also become highly diverse in Australia, but their diversity is more limited by rainfall than plants with other types of mycorrhizas.

On a global scale, Australia includes an exceptionally high number of clades of plants which have switched mycorrhizal types from AM to AM-EcM or from AM to NM roots. These trends are linked to the drying climate and extremely infertile soils in very old landscapes and can be assigned to chronological categories, as explained in Table 17.4. There have been ancient Gondwanan innovations that are consistent within families, as well as more recent switching where root types vary within families. Families with root types that seem to be consistent globally include the Proteaceae and Restionaceae, while other families are only consistent within Australia (e.g. EcM in the Rhamnaceae). There are also regional and species level innovations in families such as the Fabaceae and Myrtaceae linked to the SWAFR biodiversity hotspot.

Plant clades with complex roots can be designated as *Novel and Complex Root* (NCR) *clades*. These have some of the highest rates of increasing diversity (also called explosive diversification or radiation) in Australia. NCR clades include the Proteaceae, the Eucalypteae tribe in the Myrtaceae and the Casuarinaceae, as well as the Bossiaeeae tribe and *Acacia* in the Fabaceae, as shown in Table 17.3. These trends are best illustrated by large genera such as *Acacia* and *Melaleuca* in the Fabaceae and Myrtaceae where high diversity occurs both in AM and EcM-AM plants, sometimes in the same genus. Diversification trends are very complex, and it is not clear if NCR clades with adaptive radiation are more likely to acquire new root traits or reverse is true (rapid diversification leads to more root-type switching). The consistency of these traits in some clades suggests the former is more likely,

Table 17.4 The geographic and taxonomic scale of root evolution in clades where mineral nutrition switching has occurred

Consistency	Order	Family wide (basal)	Genera differ within family	Species differ within genera (NCR)
Global	Fagales, Pinales	Proteaceae, Cyperaceae, Restionaceae	Fabaceae, Myrtaceae	
Continental		Rhamnaceae in Australia	Myrtaceae, Fabaceae	*Acacia, Melaleuca*
Hotspots		Fabaceae, Myrtaceae, etc.	*Thysanotus* (Asparagaceae)	*Daviesia, Kennedia*

but other clades have much more complex trends support the opposite hypothesis (Table 17.4). Other traits also shift quickly in NCR clades (e.g. pollination syndromes and fire responses). Another possibility is that ectomycorrhizal fungi are more capable of colonising roots of predominantly AM plants in Australia than elsewhere. This seems unlikely as most of the EcM fungi in Australia belong to the families and genera that are well represented in other continents (Tedersoo et al. 2010).

Adaptive radiation is a widely used but inconstantly applied concept in the evolution and ecology literature (Givnish 2015; Soulebeau et al. 2015). Soulebeau et al. (2015) define adaptive radiation as "the emergence, in a short period of time, of many new species from a common ancestor accompanied by an ecological and phenotypic diversification of these species in contrasted environments". According to this definition, NCR clades in Australia are undergoing adaptive radiation, but this process can also be called explosive diversification (Givnish 2015). One of the most extreme examples of this is *Banksia* which has NM cluster roots and has ten times higher diversity in SWAFR shrublands on highly infertile soils compared to the rest of Australia (Cardillo and Pratt 2013).

Table 17.4 presents a summary of evolutionary trends in NCR and old lineages of root types, but numbers of these lineages are not fully resolved. There are many more examples of switching from AM to EcM than of switching from AM to NM roots. There seems to be a good correlation between AM to EcM-AM switching and plant size, with smaller plants in the same normally being AM only. It is not known if evolution only occurs in a forward direction or if there have been reversions back to AM only roots from EcM-AM roots. Switching from EcM-AM back to AM again would be expected to be relatively easy since roots should already have most of the required competencies. This question could be answered by sampling more species in large NCR EcM-AM genera such as *Acacia* and *Melaleuca*. Superficial observations suggest that mycorrhizal fungal diversity tends to be low in NCR plants with recently acquired EcM, but this needs to be investigated more thoroughly. NCR clades probably also occur outside of Australia. Some likely examples include EcM of artic plants such as *Polygonum* and *Kobresia* with NM ancestors. There also are clades of EcM plants in tropical habitats that are of relatively recent origin (Chaps. 19 and 21).

The main dates for NCR lineage switching are in the past 30 Ma during the aridification of Australia after separation from Antarctica (Tables 17.3 and 17.4). Diversity hotspots in Australia are engines for plant innovation, not only in roots but also in pollination, seed biology and fire responses. One of the strongest correlations is between nutritional adaptations and fire adaptations such as epicormic resprouting in Australian plants, which tend to occur in the same plant genera. New plant traits in NCR clades also often include animal or bird pollination, persistent soil seed banks and fire-protected canopy-stored seeds. All the Australian plant families in which most or all species have cluster roots seem to be of Gondwanan origin, but several more recently evolved smaller clades of NM or AM plants with cluster roots occur in the SWAFR (Table 17.4). EcM and AM mycorrhizas often coexist in roots but cluster roots are usually fully NM, with a few

exceptions in Fabaceae in species which probably acquired cluster roots recently. It is not yet possible to determine how many times recent AM to NM switching has happened and when these switches occurred. The origins of NM cluster roots seem to be of similar age to the plant lineages with N_2-fixing symbioses, the origin of which is linked to warmer climates and higher CO_2 levels in the Late Cretaceous (Li et al. 2015).

Biodiversity hotspots result from explosive radiations of plant species, which is often linked to new adaptations (Myers et al. 2000). These areas may also be centres of diversity due to low rates of extinction, effective climate refugia and lack of extreme disturbance events such as glaciation and volcanism, as explained in the introduction. It has been assumed that the SWAFR has lower rates of extinction due to higher climate and landform stability over billions of years, but there is also evidence for higher rates of diversification in SWAFR than for the same clades in eastern Australia (Table 17.3). Compared to Australia as a whole, the SWAFR has many smaller hotspots (e.g. 11 out of 21 for *Acacia* in González-Orozco et al. 2011). However, not many NRC clades are confined to WA and their diversity is not necessarily greater than that of other plants in the same habitats.

The Cape Floristic Region (CFR) in South Africa is another Mediterranean climate zone which has experienced very rapid radiation for families such as the Proteaceae and Rhamnaceae (Reyes et al. 2015). One of the most intriguing questions is why has the SWAFR become a centre of diversity for EcM plants when the CFR did not, even though both regions become a diversity centres for NM plants (Box 17.1). Indigenous EcM plants seem to be entirely absent from the Cape region (Allsopp and Stock 1993, Hawley and Dames 2004). Plant families that include species with EcM roots in Australia such as the Myrtaceae, Fabaceae and Rhamnaceae are present but lack EcM roots in South Africa (Box 17.1). However, members of the Fabaceae have evolved EcM roots in some forest or savannah habitats elsewhere in Africa.

Adaptive radiation in NCR plant families arose in parallel with rapid diversification and innovation for many plant families in the SWAFR in diversity hotspot, and it is not clear if root strategy diversity leads to greater rates of phylogenetic diversification or vice versa. Causal relationships are difficult to confirm, since many of NCR clades also include species with new strategies for pollination and fire survival. Several hypotheses can be suggested why key areas of species richness and endemism in Australia are also key areas for root switching. NCR plants are likely to be more diverse in the SWAFR plant diversity hotspot because they are more competitive in highly infertile soils. However, the alternative hypothesis that clades of plants undergoing explosive adaptive radiations in hotspots are more likely to switch root types should also be considered. Another trend seems to be that NCR are more likely to arise in clades that already have diverse roots. This would include new EcM or NM roots in plants with nitrogen-fixing root nodules or plants with three or more roots types such as some members of the Casuarinaceae. Similar evolutionary trends have also occurred outside of Australia but are far less common elsewhere (see Chap. 21).

The ecological benefits of having EcM-AM or NM cluster roots must be substantial since plants in NCR clades are very successful in some habitats. However, these plants tend to be habitat specialists which also have its downside, since families such as the Proteaceae and Fabaceae also include many rare species (florabase.dpaw.wa.go.au). AM plants have not declined substantially in Australia, and there is little evidence that their 400 Ma old AM symbiosis is poorly adapted to current conditions. Australian plants with AM include some of the most adaptable families that occupy habitats ranging from wetlands to deserts. A key difference between Australia and other regions is the number of species that have complex roots with AM-AM, AM and cluster roots or mycorrhizas and N_2-fixing nodules. A few species can support three or more root types at one time, such as *Allocasuarina* and some members of the Fabaceae with AM-EcM-N_2-fixing roots. The cost of supporting two or more root types at once seems to be less important in very infertile soils, which agrees with other evidence that plants are probably not carbon limited in these soils. Other evidence supporting the lack of carbon limitation includes increased nectar production to attract bird and animal pollinators (Orians and Milewski 2007) and the ability of many Australian to support plant and insect parasites as well (e.g. parasitic insect galls and mistletoes are common). Australian plants with complex roots also tend to be highly competitive when they become naturalised in other regions as major weeds. There is much more work to do to sort out the diversity and distribution of plants with different root types in Australia, as well as the diversity of associated fungi.

Most NCR clades diversified most rapidly in the past 30 Ma when drying climates, infertile soils and fire became more important in Australia. There seems to be a strong link between the success of NCR clades and soil properties in the SWAFR plant diversity hotspot, which has extremely old and infertile soils. These soils are also very spatially complex, allowing greater opportunities for edaphic specialisation. Low rates of extinction also important due to landscape stability and refugia from extreme climate events. Reasons for switching mycorrhizal or NM root types can possibly be determined by linking dates for key groups of plants to the climate history of Australia, but soils are also very important. More sampling of roots and their associated fungi in NCR clades are required to investigate these trends.

The recent origin of NCR clades in Australia (and elsewhere) can be considered to be a third wave of mycorrhizal evolution. The first wave of mycorrhizal evolution occurred when plants first colonised land and the AM symbiosis began (Brundrett 2002). The second major wave of mycorrhizal evolution occurred in the Cretaceous when the Orchidaceae, Ericaceae and the first families with EcM or NM roots, parasitic plants carnivorous plants and nitrogen-fixing symbioses originated (Table 17.3). The second wave was more protracted with multiple origins on EcM and NM roots continuing into the Tertiary era. Both the second and third wave of mycorrhizal evolution are linked to climate change as well as increasing habitat and soil diversity, which presumably resulted in a competitive advantage for more specialised plants with new root types.

Despite strong correlation between low soil fertility and increasing dominance by NM plants with cluster roots, the overwhelming root evolution trend in Australia is for codominance of plants with EcM, EcM-AM, AM and NM roots, even in the oldest landscapes and most infertile soils. Trees and shrubs with EcM roots are dominant in many Australian habitats, and there has been a long history of AM to EcM replacement within families such as the Myrtaceae and Fabaceae. The presence of exceptional high species diversity within recent lineages of EcM or NM plants provides strong evidence that the third wave of mycorrhizal evolution in Australia is primarily due to decreasing soil fertility and gradual climate change in the form of increasing aridity. A key process has been the weathering and leaching of regolith/soils in Australia over 200 Ma that has led to very low nutrient availability for plants. Australian ecosystems provide a model for studying the long-term impacts of climate change and soil degradation on plants and their nutrient uptake mechanisms, since it is likely that old impoverished soils and semiarid habitats will become more common globally over time.

Box 17.1 Regional Comparisons of Mycorrhizal Plant Diversity

1. At a continual scale, Australia is:
   a. The global centre of diversity for EcM and EcM-AM plants with about 33% of all species.
   b. A global centre of diversity of NM plants with about 15% of species.
   c. A major centre of diversity and endemism for ericoid plants with about 17% of species.
   d. Overall Orchidaceae taxonomic endemism is lower than expected at their site of origin and is primarily limited by rainfall (over 2000 species). Southern Australia is a global centre for terrestrial orchid diversity.
   e. An important centre for parasitic plants, with about 10% of the global mistletoe diversity and ecosystems where parasitic trees such as *Nuytsia floribunda* and *Santalum* spp. are common.
   f. The centre of carnivorous plant diversity with about 25% of species.
2. Within Australia, there is an even greater concentration of diversity within the SWAFR that includes:
   a. The greatest concentration of NCR lineages with EcM or NM roots.
   b. Several diversity centres for EcM and EcM-AM plants, especially for *Eucalyptus* and *Acacia*.
   c. A high species richness, endemism and turnover for many AM plants in large families such as the Poaceae, Asteraceae, Goodeniaceae, Stylidiaceae, Malvaceae and Lamiaceae.

(continued)

Box 17.1 (continued)

d. A global centre of diversity for NM plants with cluster and dauciform roots, along with South Africa.
e. About 80% of *Thysanotus* species (Asparagaceae—Lomandroideae) with a unique and enigmatic subepidermal mycorrhizal association.

3. Comparing the Cape Floristic Region of South Africa (CFR) to the SWAFR of Western Australia:

   a. Both regions have very high diversity of NM plants with cluster roots or sand-binding roots, especially in the Proteaceae, Restionaceae, but also in the Haemodoraceae and Cyperaceae.
   b. Parasitic and carnivorous plants are common in both regions.
   c. The SWAFR becomes a centre of diversity for EcM plants, but the Cape Floristic Region did not.
   d. The Cape Floristic Region is the global centre of diversity for Ericaceae with ericoid mycorrhizas, but this family consists of 2% of the flora in Australia. It is not clear why plants with ericoid mycorrhizas are very successful in Australia by even more so in South Africa.

4. Comparing other Mediterranean climate regions to the SWAFR:

   a. The Rhamnaceae are important family in all regions but only have EcM roots in Australia.
   b. NM plants are less important Mediterranean ecosystems in Europe or North America, suggesting that soil fertility may be more important than climate.

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Chapter 18
Global Patterns in Local and Dark Diversity, Species Pool Size and Community Completeness in Ectomycorrhizal Fungi

Meelis Pärtel, Martin Zobel, Maarja Öpik, and Leho Tedersoo

18.1 Measures of Diversity

Understanding the distribution of biodiversity is one of the fundamental goals in ecology, biogeography and nature conservation. The term *biodiversity* is usually intuitively used to represent the observed species richness or a measure of species' weighed relative abundance that constitute diversity indices such as Shannon, Simpson and the Hill series (Magurran 2013). While these measures describe essential aspects of local biodiversity, the importance of absent and total diversity has been recently stressed (Pärtel et al. 2011). Biodiversity at larger geographic scales is represented by *gamma diversity* and *species pool*. Gamma diversity covers the number of taxa over large geographic scales, whereas the number of taxa at local scales constitutes *alpha diversity*. The difference between alpha and gamma diversity is reflected by *beta diversity,* a measure of spatial turnover between local habitats within a region (Anderson et al. 2011). The species pool comprises all taxa that are potentially suitable and available for a local site, i.e. of taxa that can reach and inhabit a particular site (for details see Zobel 1992, 2016). Thus, species pool represents the ultimate potential of local biodiversity. *Dark diversity* depicts the absent part of such site-specific species pool and enables to understand the extent to which biodiversity potential has actually realized locally (Pärtel et al. 2011). To express local diversity in relation to its species pool size, a measure of *community completeness* has been introduced. This measure depicts the ratio of local diversity to dark diversity, and it is logarithmically scaled for improved statistical properties (Pärtel et al. 2013). In general, species pool size is expected to be linked to mainly

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large-scale biogeographic processes such as evolution and historical dispersal. In contrast, community completeness is mainly related to local contemporary processes including biotic interactions and local-scale dispersal limitation (Pärtel et al. 2016). Consequently, the effects of local processes on biodiversity are sometimes evident only when the species pool effect is properly accounted for (Fraser et al. 2015).

Theoretical and empirical studies on dark diversity and community completeness have been mostly performed in plant communities (e.g. Riibak et al. 2015; Ronk et al. 2015), but their applicability has remained relatively less understood for animals (but see Lessard et al. 2016) and microbes including fungi (Pärtel et al. 2016). Recently, Pärtel et al. (submitted) demonstrated that species pool size, local diversity, dark diversity and community completeness of the arbuscular mycorrhizal (AM) Glomeromycota display distinct global patterns, emphasizing the importance of historical factors shaping current biodiversity patterns. In ectomycorrhizal (EcM) fungi, taxonomic richness also strongly differs by host families and geographic regions (Tedersoo et al. 2012). This has been ascribed to historical distribution patterns of host plants and phylogenetic signal in determining fungal richness and composition (Põlme et al. 2013; Tedersoo et al. 2013, 2014). The relative proportion of EcM host plants and their richness as well as soil pH were the strongest predictors of EcM fungal richness on a global scale based on multiple regression analyses (Tedersoo et al. 2014). Compared with AM fungi, EcM fungi form more diverse communities that have rarely been exhaustively sampled even when using high-throughput sequencing with hundreds of thousands of sequence reads per study site (Tedersoo et al. 2015). This may generate greater uncertainty and error for calculation of any diversity measure. Following the methods of Pärtel et al. (submitted), we apply the concepts of dark diversity, species pool and community completeness on EcM fungi using a global soil data set and associated metadata (Tedersoo et al. 2014).

In this synthesis, we aimed to detect general richness patterns of EcM fungi from multiple aspects of diversity. We predicted that all the observed metrics—local diversity, dark diversity, species pool size and community completeness—display differences in their global-scale distributions. We hypothesized that local diversity of EcM fungi reflects largely the distribution of species pool, but there are differences when dark diversity and community completeness are considered. In particular: (1) dark diversity of EcM fungi is greatest in regions of relatively low host plant availability because of dispersal limitation (Bahram et al. 2015), and (2) community completeness is lowest in sites with high EcM fungal local diversity, because more intensive competition hampers accumulation of taxa. Finally, we discuss the effects of sampling strategy, sequencing depth (i.e. the number of high-throughput sequencing reads per sample) and taxonomic issues on the perceived diversity patterns of EcM fungi.

18.2 Modelling

We used a global soil fungal data set (Tedersoo et al. 2014) that comprised information from 365 sites and 10,336 operational species-level taxa (OTUs) assigned to EcM fungi and 40,252 OTUs assigned to other fungal taxa. Each site harboured 82.2 EcM fungal OTUs on average, with a range from 0 to 314 taxa. We removed 17 sites with <20 sequences of EcM fungi. As sequencing depth for EcM fungi differed enormously, we calculated local diversity as the extrapolated effective number of species based on Shannon index by using the R package iNEXT (Hsieh et al. 2016). This measure represents the expected local diversity if all taxa have the same abundance (number of sequences) and infinite number of sequences are used. This measure should be less affected by sampling volume than the observed richness (Crist et al. 2003). Dark diversity was calculated based on co-occurrence patterns of OTUs using Beals index following Lewis et al. (2016). We included OTUs of all fungal groups in the calculation of co-occurrence metrics for greater statistical power. Into the dark diversity category, we binned only those taxa that were not recorded from the site but for which the co-occurrence index was larger than the minimum value from sites where the same taxon was present (Münzbergová and Herben 2004). Species pool size was calculated as the sum of local diversity and dark diversity (Pärtel et al. 2011). On average, dark diversity was smaller than local diversity, but since these estimates were generated with different techniques, the absolute numbers are not as informative as the geographical variation and relationships with the environment. Community completeness was calculated as a natural logarithm of the ratio of local diversity to dark diversity (Pärtel et al. 2013).

The global distribution of diversity measures was modelled based on Generalized Additive Models (GAMs) and the spline-over-the-sphere algorithm as implemented in the 'sos.smooth' routine of mgcv package of R with k value 30 (Wood 2003). This technique allows smoothing across geographical coordinates globally without any edges. We used cross-validation by dividing the dataset randomly to 20% bins and predicting values for each bin by using models from the rest. Predicted values were generally well related to the independent observed values (R^2 values for local diversity 0.30, dark diversity 0.72, species pool size 0.51, community completeness 0.53). We used GAMs to relate logarithm-transformed diversity values separately to mean annual temperature (MAT), mean annual precipitation (MAP) and relative basal area of EcM plants. All explanatory variables were correlated with each other ($r = 0.59$ for MAT and MAP, $r = -0.58$ for relative basal area (proportion) of EcM plants compared to both MAT and MAP). For each diversity measure, we report the two strongest relationships based on adjusted R^2 values. In GAM models, we used $k = 5$, which allows nonlinearity but reveals general trends.

18.3 Local and Dark Diversity

GAMs revealed that variation in estimated local diversity of EcM fungi was non-random ($R^2_{adj} = 0.35$, $P < 0.001$), and it was most strongly related to the relative basal area of EcM plants and MAT, which explained 31% and 27% of variation, respectively ($P < 0.001$). The estimated local diversity peaked at +5 °C MAT (Fig. 18.1a) that is in agreement with the residual values (considering sequencing depth) of Tedersoo et al. (2014) and a global metastudy (Tedersoo et al. 2012). EcM fungal local diversity exhibited logarithmic increase in relation to host basal area (Fig. 18.1b). The smoothed map indicates that the local diversity of EcM fungi peaks in temperate ecosystems of the Northern Hemisphere but also regionally in southern South America and New Zealand (Fig. 18.2a).

The co-occurrence-based estimation method recovered high variation in dark diversity patterns among EcM fungi. There was a positive correlation between the estimated local and dark diversity ($r = 0.43$, $P < 0.001$). GAMs revealed that the distribution of dark diversity was strongly structured by spatial distance among sites ($R^2_{adj} = 0.74$; $P < 0.001$). MAP was the strongest individual predictor of dark diversity that explained 50% of variation ($P < 0.001$). Dark diversity peaked at very low MAP values and declined rapidly to 2000 mm annual rainfall and then stabilized (Fig. 18.1c). The relative basal area of EcM host plants explained 37% of variation in dark diversity (Fig.18.1d). In contradiction with our hypothesis, dark diversity of EcM fungi increased exponentially with increasing proportion of EcM plants in the vegetation. This suggests that competitive interactions among EcM fungi at high host relative abundance might in fact drive dark diversity.

Distribution of EcM fungal dark diversity differed from that of the local diversity estimates (Fig. 18.2b). A region centred in East Siberian tundra that covered much of the boreal forest and tundra areas from the Urals to Russian Far East represented the only area of very high dark diversity. Remarkable regions with low values included Eastern North America, New Zealand as well as the entire humid and seasonal rain forest belt. Siberian forest and forest tundra areas are characterized by monodominant ectotrophic coniferous forests, which corroborates the high host relative abundance effect. The northern temperate forests of North America have a stronger deciduous EcM and AM plant component. These hypotheses would require more independent samples and a more regional approach to be tested.

18.4 Species Pool and Community Completeness

Species pool size of EcM fungi was strongly determined by location on a global scale ($R^2_{adj} = 0.55$; $P < 0.001$). MAT was the strongest predictor of species pool, explaining 43% of variation ($P < 0.001$). Similarly to the estimated local diversity, species pool size had a positive unimodal relationship with MAT, but it

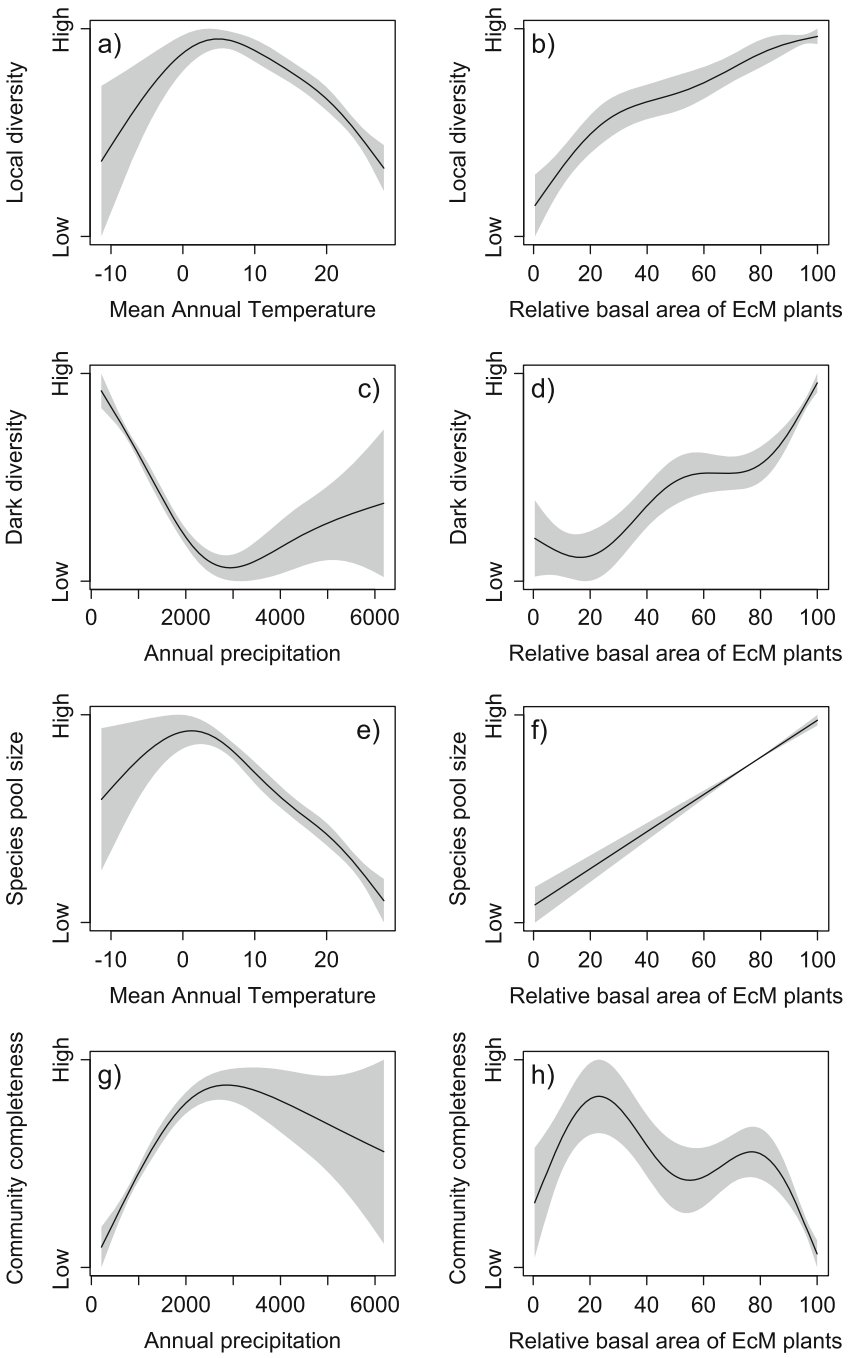

Fig. 18.1 GAM regressions of estimated local diversity (**a**, **b**), dark diversity (**c**, **d**), species pool size (**e**, **f**) and community completeness (**g**, **h**) of EcM fungi to best environmental predictors (based on adjusted R^2 value)

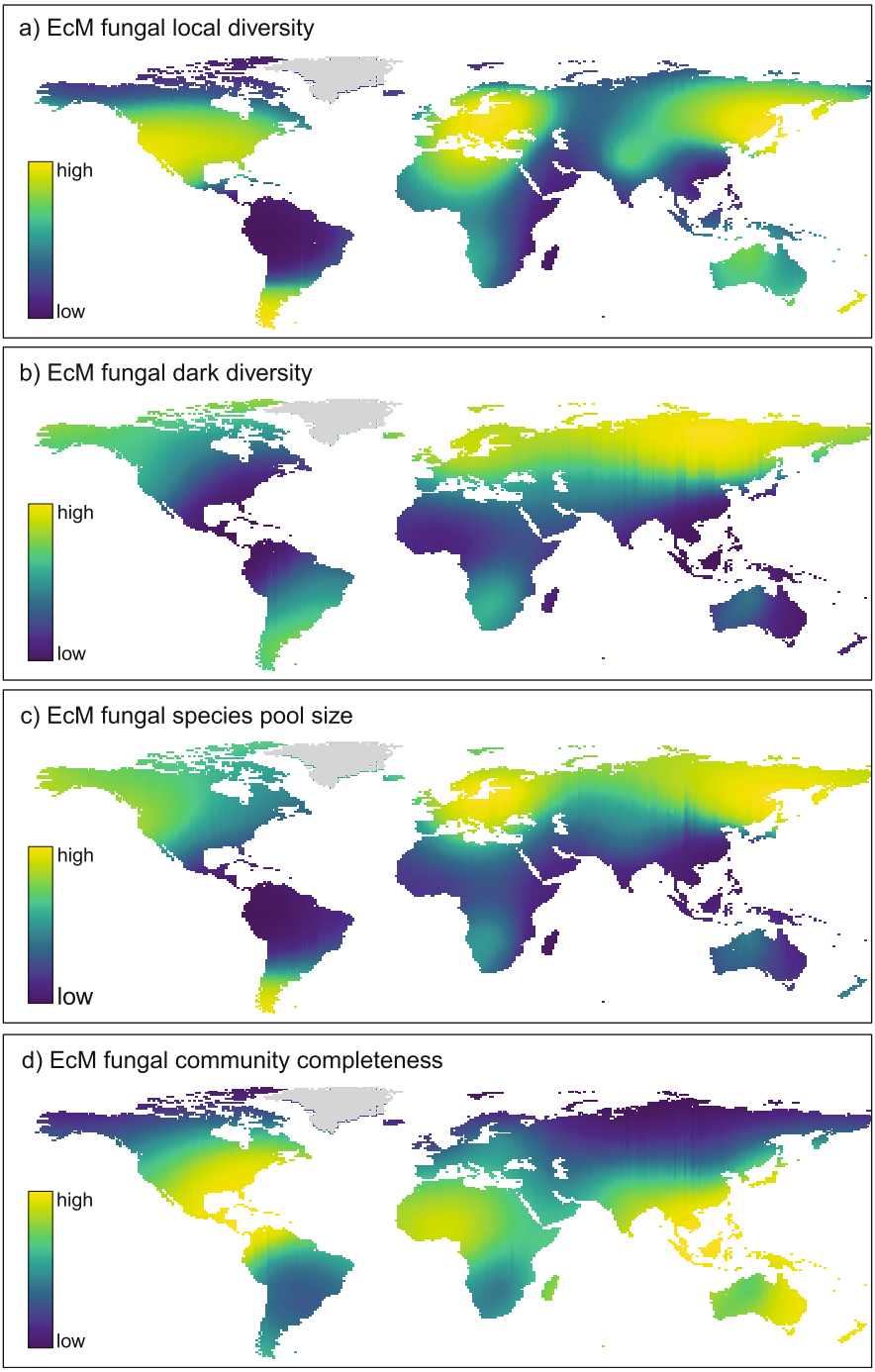

Fig. 18.2 Smoothed maps of (**a**) estimates of local diversity; (**b**) dark diversity; (**c**) species pool size and (**d**) community completeness of EcM fungi

peaked broadly at −5 to +5 °C (Fig. 18.1e). The relative abundance of EcM vegetation had an equally strong positive linear effect on species pool ($R^2 = 0.42$, $P < 0.001$; Fig. 18.1f). Since host availability is strongly related to the carbon resources to EcM fungi, it is likely that the relative abundance of suitable hosts may have promoted diversification of EcM fungi. Some circumstantial evidence for this hypothesis is provided in diversification rate analyses (Chaps. 1 and 13). Similar to the local diversity estimates, the species pool size was greatest in Europe, Russian Far East and southern South America. Both high and low value regions were more pronounced for species pool compared with the estimated local diversity (Fig. 18.2c). This suggests that species pool size might be a good alternative measure in macroecological studies due to its ability to polish stochastic variation resulting from site-specific features and sampling artefacts.

The spatial model explained a high proportion of EcM fungal community completeness on a global scale ($R^2_{adj} = 0.59$; $P < 0.001$). MAP was the best predictor of community completeness that explained 31% of variation in the data set ($P < 0.001$; Fig. 18.1g). Community completeness was lowest at <1000 mm annual rainfall. Furthermore, community completeness had a uni- or bimodal relationship with the relative proportion of EcM plant species, showing higher values at intermediate host availability ($R^2 = 0.16$; $P < 0.001$; Fig. 18.1h). If hosts are widely available, competition among EcM fungi might limit local assemblages, similarly as hinted from the dark diversity analysis (see Sect. 18.3). If hosts are very rare, random drift can exclude some suitable species, or dispersal in between such assemblages can be insufficient. Community completeness had a weak positive correlation with the estimated local diversity ($r = 0.12$) and strong negative correlation with dark diversity ($r = -0.84$), suggesting that dark diversity mainly determines community completeness on a continental scale as it is usually observed for plants (Ronk et al. 2015). As opposed to dark diversity, community completeness was greatest in North and Central America, Indo-Malay and New Zealand (Fig. 18.2d).

EcM fungal species pool size was strongly positively correlated with the local diversity estimates ($r = 0.92$) but less with dark diversity ($r = 0.72$). Note that P-values cannot be provided for correlations between these raw values since these variables are not independent (both local and dark diversity are parts of species pool). However, it is possible to model a trend line between local diversity and species pool size through community completeness. We detected a significant negative relationship between community completeness and species pool size (slope = −0.43; $t = -4.9$; $P < 0.001$). These biodiversity measures are mathematically independent, and the negative relationship between them indicates community saturation (Szava-Kovats et al. 2013). When back-transforming this relationship to untransformed biodiversity measures (local diversity and species pool size), we can see the real trend line of how local diversity and species pool size are related (Fig. 18.3). We found a significant curvature: along with increasing species pool, the proportion of taxa inhabiting local communities decreased. Such a pattern can be explained by the effect of local biotic interactions.

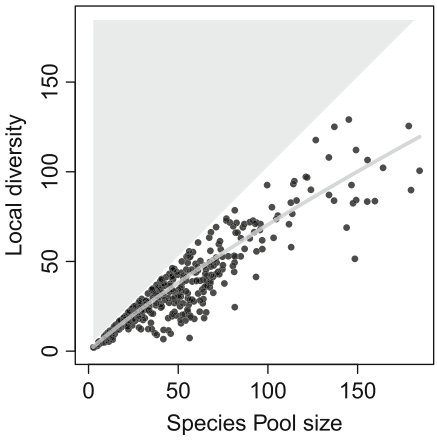

Fig. 18.3 Curved relationship between EcM fungal local diversity and species pool size demonstrating the importance of local processes. The *shaded area* represents impossible values (local diversity cannot exceed species pool size)

18.5 Limitations

This synthesis has several potential limitations related to sampling, technical biases and estimations of diversity measures. Although here, sampling is the most inclusive for fungi so far, and both sampling and sample processing have been highly standardized, the distribution of study sites is aggregated and several important EcM dominated regions such as Greenland, East Siberia, New Caledonia and the Amazon basin remain uncovered. For some of these regions, there are predictions for either relatively low or extremely high EcM fungal diversity, but the real diversity patterns could only be settled by yet more inclusive and even sampling. Differences in sampling intensity may particularly affect estimations of dark diversity, because very rare taxa cannot have many co-occurrences. However, contrary to this logic, dark diversity was estimated to be greatest in East Siberia that had one of the lowest sampling densities in this global dataset.

EcM fungi form diverse and highly spatially aggregated communities in soil. The non-exhaustive sampling of EcM fungal taxa in each site both in terms of a tiny proportion (i.e. hardly representative) of soil volume (2 g of 4 dm^3 soil) and sequencing depth (on average, 1047 EcM fungal sequences per site) certainly underestimates the number of taxa present, probably by a factor of 2–5 (partly based on the same sites analysed in Tedersoo et al. 2014, 2015). Moreover, sampling of soil instead of roots includes spores and other dormant propagules. Spores of several EcM fungal groups may disperse over vast distances and build up a persistent spore bank (Hayward et al. 2015; Chap. 3). If not also part of the active community, these taxa might be a part of the local species pool in case habitat conditions are generally suitable. However, in EcM fungi, this probably overestimates the number of resident species by a few taxa at given sequencing depth as based on fungal taxonomic explorations of non-EcM habitats (Tedersoo et al. 2014).

Because the active EcM fungi are non-exhaustively sampled, raw richness values have limited applications and alternative diversity measures are suggested to estimate local diversity. Rarefactions to the minimum number of sequences from study sites have sometimes been used. However, as discussed in Balint et al. (2016), minimum richness estimates based on high-throughput sequencing data may introduce strong biases, because these estimates rely on the relative abundance of rare species in the data set. The rarest OTUs often represent analytical artefacts and most researches remove these from further analyses. Tedersoo et al. (2010) estimated that in a 454 sequencing data set, roughly half of the global singletons (taxa represented by a single sequence across the whole data set) are artefactual. However, the proportion of artefacts accumulates with increasing sample size and sequencing depth (Dickie 2010), which motivated the removal of all global singletons from the Tedersoo et al. (2014) data set and from the current re-analysis. Hence, in both analyses this must have led to certain level of underestimation of the local diversity, especially in habitats with unique OTU composition, because rare OTUs from highly specific habitats have low probability of being found again. Furthermore, rare OTUs within each sample may represent artefacts of tag switching, i.e. mis-assigned to particular samples because of identifier tag mutations or post-PCR recombination (Carlsen et al. 2012). Here, our estimates of local diversity were based on the effective number of species, i.e. how large is the estimated richness if all taxa have the same abundance and infinite number of sequences has been sampled (Chao et al. 2016). This measure is probably less affected by rare species than, for example, minimum richness estimators. However, the behaviour of effective number of species on high-throughput sequencing data remains to be explored.

Previous works have shown that dark diversity estimation based on species co-occurrences perform better than some alternatives (de Bello et al. 2016; Lewis et al. 2016). A majority of taxa in the current data set were extremely rare (two thirds of all EcM fungal taxa were found only in one or two sites). Therefore, correct estimation of suitable but unoccupied sites for these rare taxa is difficult. It also remains to be explored whether the co-occurrence method is equally applicable to species-poor and species-rich sites. In addition, the dark diversity concept does not cover the species present but undetected due to taxonomic limitations. Taxonomic uncertainty is usually not considered in botanical or zoological surveys, but is more thoroughly addressed in microbiology (Lozupone and Knight 2008) and mycology (Kõljalg et al. 2013) where barcoding is frequently the only way of taxon detection and identification during the past 25 years or more.

The map smoothing exercise also resulted in situations where EcM fungal diversity hotspots were extended to non-EcM habitats such as East Patagonia and North Sahara (Chap. 20). This indicates that the distribution of plant mycorrhizal types and other floristic variables might be useful in the future to improve EcM diversity maps. In addition, more balanced sampling points in non-EcM habitats might improve the global diversity prediction maps.

18.6 Conclusions and Perspectives

Despite the limitations, the application of additional biodiversity measures revealed several novel aspects of EcM fungal biodiversity. Together with estimates of dark diversity, we predicted the potential biodiversity (species pool size) and how much of this has been effectively realised locally (community completeness). The strong concordance of local diversity and species pool size of EcM fungi is much expected, indicating that variation in local diversity is largely defined by evolutionary and historical processes. However, this relationship is significantly non-linear: if species pool size increases, community completeness decreases. This supports the view that competition shapes the composition in local EcM fungal communities (Kennedy 2010), and it could be quantified in local scale analyses. Furthermore, the unexpected increase in dark diversity with host availability also hints on limitations derived from intra-guild biotic interactions.

Taken together, our results are similar to a global grassland study, where regional and local processes acted simultaneously (Fraser et al. 2015). We can assume that species pool size of EcM fungi is more affected by evolution and large-scale dispersal, whereas community completeness is influenced by local biotic filtering and local dispersal. Following this, we can link the exceptional positive latitudinal EcM fungal diversity gradient found for local diversity and species pool size with biogeographic processes. EcM fungal community completeness, in contrast, shows high values close to equator, demonstrating that in tropical ecosystems EcM fungi experience less local competition or that there are better chances for dispersal. The latter option is not supported by fine-scale or continental scale analyses that both indicate relatively stronger dispersal limitation in tropical ecosystems (Geml et al. 2012; Bahram et al. 2013). Future studies on EcM fungal community assembly processes (Chap. 2) in temperate and tropical regions may shed additional light on differences in diversity aspects in these ecosystems.

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Chapter 19
Evolution of Ectomycorrhizal Symbiosis in Plants

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19.1 Introduction

Ectomycorrhizal (EcM) symbiosis has evolved multiple times in plants and fungi (Brundrett 2009; Tedersoo and Smith 2013). Similarly to fungi in general, there is a lot of controversy in understanding the fungal root association of plant species. This can be partly attributed to environmental impact in arbuscular mycorrhizal (AM) associations, where facultatively mycorrhizal plants are common and development of root fungal structures depends on edaphic, floristic and climatic conditions as well as seasonality and ontogeny of plants (Smith and Read 2008). By contrast, the vast majority of EcM plants are obligately mycotrophic, and conflicts in assignment of mycorrhizal status seem to arise more from alternative definitions of the association (Brundrett 2009).

Here we define EcM and EcM-like associations based on the structure, phylogeny and putative function. At least two of these three criteria should be met for considering the associations to be EcM. First, Hartig net and fungal mantle (sheath) are the main structural characteristics of EcM, but these may be incompletely developed or patchy, as often seen in the EcM of herbs and shrubs. EcM associations of the ectendomycorrhiza subtype may additionally exhibit intracellular hyphal development that is characteristic to certain plant-fungal combinations

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(Yu et al. 2001). Second, EcM plants form phylogenetically distinct groups, although reversal to non-EcM habit (unlike in EcM fungi) or switch to mycoheterotrophy may have occurred. Pyroloid and monotropoid subtypes of EcM in Pyroleae and Monotropeae, respectively, are linked to the arbutoid subtype of Arbuteae within Ericaceae and are considered as EcM (Brundrett 2004). Similarly, mycorrhiza of *Pisonia* spp. with transfer cells is considered as a subtype of EcM. Third, EcM associations should also be essentially beneficial to both partners, but Monotropeae, Pterosporeae and perhaps Pyroleae do not fulfil this criterion. The phylogeny criterion clearly places Monotropeae and Pterosporeae among EcM associations but keeps orchids separate as they have evolved exclusively intracellular associations with EcM fungi secondarily and even photosynthetic orchids provide little if any benefits to fungi (Cameron et al. 2008). Furthermore, EcM plants also associate with mutualistic fungi from well-known EcM fungal lineages (Tedersoo and Smith 2013), with no known exceptions. The first EcM plant group that evolved certainly developed EcM associations with 'previously unrecognised' fungi. Theoretically, newly emerging EcM plants may associate with novel fungal groups, but this has not yet happened or these evolving associations have not persisted (see below). From this perspective, superficial root associations of *Entoloma clypeatum* group and Rosaceae (and *Ulmus*) and Helotiales-*Graffenrieda* (Melastomataceae) are to be considered non-EcM.

The oldest known EcM associations involve Pinaceae. The fossil records of unequivocal mycorrhizal structures of Pinaceae date back to the Eocene, although genera of the family evolved in the Late Jurassic or Early Cretaceous (LePage 2003). Since the Late Cretaceous and throughout Tertiary, many extant EcM groups of plants and fungi have evolved (Chaps. 1 and 20), followed by subsequent radiation and dispersal. In the last decade, several plant families have been deeply studied from the phylogenetic and biogeographic perspective that greatly adds to our understanding of the evolution of functional traits as well as biotrophic interactions with fungi, actinomycetes and other organisms (e.g. Werner et al. 2014).

In this synthesis, we critically assess the EcM status of plant genera based on published literature, personal observations as well as phylogenetic evidence. We also propose a number of genera that are potentially EcM based on their phylogenetic position but with no known root-level study. Finally, we discuss the issues in recognition of EcM symbiosis, ongoing evolution and groups with dual mycorrhiza.

19.2 Data Sources

We have compiled global literature about mycorrhizal status of plants for >10 years, also retrieving decades old literature based on the citations in Harley and Harley (1987), Wang and Qiu (2006), Koele et al. (2012) as well as Google Scholar. We carefully evaluated the descriptions of mycorrhizal status of EcM and putatively EcM plants. We also noted the inoculation and synthesis trials and growth benefits of plants. Based on the methodology, sample size and conflicts

with other sources, we determined the reliability of particular studies when interpreting the mycorrhizal status.

Studies on plant phylogeny and molecular dating were searched from Google Scholar by combining names of particular plant genera, families or orders with 'phylogeny' and 'molecular dating' and 'biogeography' as key words. In addition, we searched the Angiosperm Phylogeny Website (www.mobot.org) for additional sources of literature as they often referred to information hidden in supplementary materials and not found by web search engines. We also used the tree file of the most comprehensive vascular plant phylogeny (Zanne et al. 2014). These different sources of phylogenetic information were combined to separate EcM plant genera into monophyletic lineages, allowing reversals to non-EcM habit. We refer to these lineages by taxon names, because in nearly all cases, the lineages fit into particular species, genera, (sub)tribes, (sub)families or orders.

Our consideration of mycorrhizal associations is based on genus level, because members of the same genus usually share the same mycorrhizal status, with multiple notable exceptions in Australian plants (Wang and Qiu 2006; Brundrett 2009). Most economically and ecologically important woody genera have been revised based on molecular phylogenetic tools, which has increased the value of the generic rank. Species-level information is also too sparse for specific conclusions. Plant taxonomy and species richness follows the Plant List (www.theplantlist.org). We follow Werner et al. (2014) and Benson et al. (2004) regarding rhizobial and actinorhizal associations, respectively.

19.3 Evolution of Ectomycorrhizal Habit

Critical evaluation of mycorrhizal and plant phylogenetic literature enabled us to distinguish 30 plant lineages that most probably evolved EcM associations independently (Fig. 19.1; Table 19.1). Searches through plant phylogenies revealed that 335 plant genera can be considered EcM (Table 19.2). Of these groups, 184 (54.9%) plant genera were regarded as ectomycorrhizal based on direct morphological evidence, whereas the remaining 151 genera were considered as belonging to EcM groups based on the monophyly criterion, although only AM has been reported in nine of these genera (see Supporting Information: http://dx.doi.org/10.15156/BIO/587454). The 335 putatively EcM genera were comprised of ca. 8500 species based on the Plant List (except Miller and Seigler 2012 for *Acacia s.str.*). Since <10% of these species exhibit reports on mycorrhizal status, it is highly possible that several genera and multiple species do not function as ectomycorrhizal (see Sect. 19.4.10). We estimate that approx. 6000–7000 species from 250 to 300 genera are truly capable of forming EcM associations.

Phylogenetic analyses revealed 22 potentially EcM genera (comprising 76 species) that represented sister groups to known EcM plant lineages or critical clades

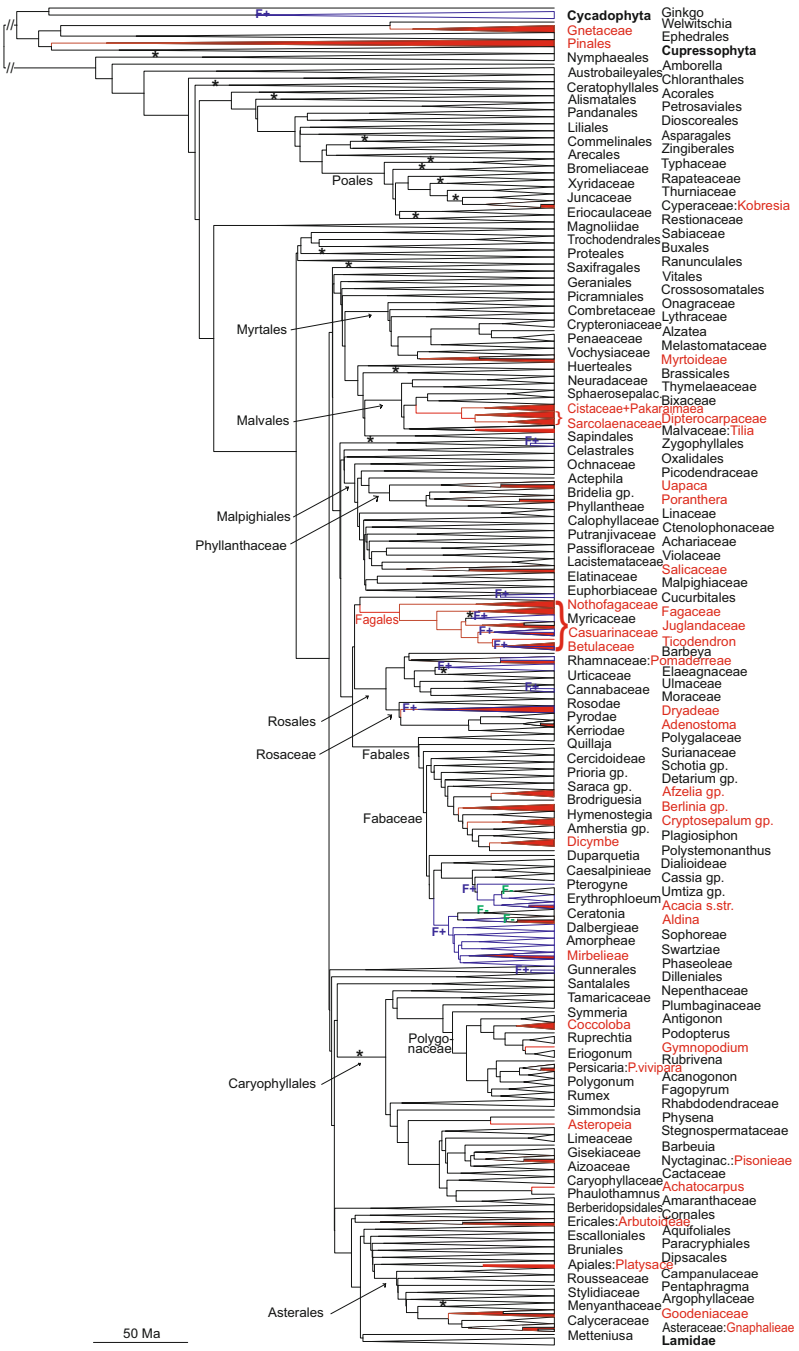

Fig. 19.1 Distribution of 30 ectomycorrhizal plant lineages (red branches and names) in the collapsed dated spermatophyte phylogram of Zanne et al. (2014), with improved taxonomic resolution of Fabaceae from LPWG (2017) and de la Estrella et al. (2017). *Asterisks* indicate

within large EcM groups that had secondarily lost EcM habit in certain occasions (see Sect. 19.7; Fig. 19.2). Thus, the root systems of representative species of these 170 genera nested within or closely affiliated to EcM groups certainly warrant further investigation for better understanding the evolution and distribution of EcM habit.

Taxonomic analysis of EcM plant lineages revealed that EcM habit evolved mostly from AM ancestors, which is consistent with Brundrett (2009). However, EcM symbiosis evolved in at least five occasions from non-mycorrhizal or facultatively AM-dependent ancestors (*Coccoloba*, *Persicaria vivipara*, *Gymnopodium* and Pisonieae within Caryophyllales and *Kobresia* within Poales). The latter finding is in a strong conflict with Maherali et al. (2016) who suggested that only AM habit can be ancestral to EcM habit. However, the authors excluded most of the above-mentioned groups from their final analysis, which must have strongly biased their results.

Of all 30 EcM plant lineages, *Gnetum* and Pinaceae represent gymnosperms, whereas all others belong to angiosperms The Fabaceae family alone includes seven EcM groups. The Myrtoideae represent the most genus-rich and species-rich EcM plant group (Table 19.2). Two additional Australian groups, viz. *Thysanotus* and *Lobelia*, are considered to possess EcM-like root associations, with distinct root anatomy and uncertain mode of nutrition (see Sect. 19.5; Chap. 17).

Integrating the information from community studies of EcM fungi and EcM plant lineages as described here reveals that there are no plant lineage-specific fungal lineages, although certain plant genera may associate with narrow fungal clades. This indicates that the evolution of EcM symbiosis in plants is linked to pre-existing fungal lineages and vice versa. This is a parsimonious scenario that would require critical modification of gene expression in only a single partner to become connected into a mycorrhizal network of a particular type. The lack of unique plant-fungal combinations furthermore indicates that the evolution of the first EcM plant-fungus association was an extremely rare event, which probably occurred and persisted only once or a few times. Unfortunately, there is no information, whether Pinaceae represent the very first EcM plant lineage or whether there was another, now extinct gymnosperm group. Strikingly, all known EcM fungal lineages are much younger than Pinaceae, suggesting that extinct groups of EcM fungi may have primarily associated with plants in the Jurassic period.

Fig. 19.1 (continued) major reversal events to predominantly non-mycorrhizal habit according to Brundrett (2009). To illustrate the evolution of EcM symbiosis in tripartite associations with nitrogen-fixing rhizobia and *Frankia* actinobacteria, these interactions are indicated as branches and clades in blue; F+ and F− (*green*) denote evolution and loss of N fixation, respectively. Data about N fixation are derived from Werner et al. (2014) and searches of literature for minor uncovered Fabaceae groups. Taxa above the order rank are highlighted in bold. The *bar* indicates rough age estimates for higher taxa (Zanne et al. 2014), but these are not refined for Detarioideae and relatively recently derived EcM plant groups

Table 19.1 Plant lineages and their predicted age (Ma)

Lineage	Reliability of EcM habit	Stem age/crown age (reference)	Stem/crown age (Zanne et al. 2014)
Pinaceae	High	325–340/175–198 (Leslie et al. 2012; Lu et al. 2014)	267/238
Gnetum	High	130/26 (Won and Renner 2006)	87/75
Fagales	High	98/94 (Larson-Johnson 2015)	103/82
Pisonieae	High	<37/nd (Tank et al. 2015)[a]	29/26[a]
Achatocarpus	Low	<52/nd (Tank et al. 2015)	12/nd
Coccoloba	High	52/24 (Schuster et al. 2013)	20/14[a]
Gymnopodium	Low	35/nd (Schuster et al. 2013)	15/nd
Persicaria vivipara	High	<28/nd (Schuster et al. 2013)	7/nd
Asteropeiaceae	Medium	nd/nd	34/nd
Acacia	High (mostly AM-EcM or AM)	27/24 (Murphy et al. 2003)	0.9/0.8[a]
Aldina	High	<18/nd (Lavin et al. 2005)	34/nd
Mirbelieae	High (mostly AM-EcM)	52/50 (Toon et al. 2014)	50/45
Afzelia group	High	35/30 (de la Estrella et al. 2017)[a]	7.3/4.1[a]
Berlinia group	High	59/57 (de la Estrella et al. 2017)	15/13[a]
Cryptosepalum group	High	53/34 (de la Estrella et al. 2017)	nd/nd
Dicymbe	High	24/18 (de la Estrella et al. 2017)	nd/nd
Salicaceae	High (mostly EcM-AM)	45/33 (Davis et al. 2005)[a]	46/34[a]
Uapaca	High (mostly EcM-AM)	<<80/nd (Xi et al. 2012)	43/16
Poranthera	Medium	<<80/nd (Xi et al. 2012)	25/19
Pomaderreae	High	55/41 (Onstein et al. 2015)	43/30
Dryadeae	High	75/67 (Chin et al. 2014)	81/73
Adenostoma	Low (AM-EcM)	nd/nd	15/nd
Dipterocarpaceae-Cistaceae	High	33/23 (Wikström et al. 2001)[a]	73/49[a]
Tilia	High	32/17 (Richardson et al. 2015)[a]	14/8[a]
Gnaphalieae	Medium (mostly AM-EcM or AM)	12–16/10–14 (Bergh and Linder 2009)	5.2/4.4

(continued)

Table 19.1 (continued)

Lineage	Reliability of EcM habit	Stem age/crown age (reference)	Stem/crown age (Zanne et al. 2014)
Goodeniaceae	Medium (mostly AM-EcM or AM-NM	78/67 (Jabaily et al. 2014)	54/49
Myrtaceae	High (mostly EcM-AM)	85/72 (Thornhill et al. 2015)	66/63
Platysace	Medium	33/nd (Nicolas 2009)	70/15
Arbutoideae *s.lat*	High	110/102 (Schwery et al. 2015)	51/49
Kobresia	High (mostly EcM-NM)	<10/nd (Starr et al. 2004; Escudero et al. 2012)	5.0/4.5

Reliability indicates the number of studies and proven evidence for EcM formation in particular groups
nd not determined
[a]Values considered underestimates by us (questionable calibration or rate shifts or conflicting fossil evidence; see also Chap. 20)

Divergence times for only a small proportion of EcM fungal lineages have been studied, with oldest groups dating back to the Mid-Cretaceous (Chap. 1). Among the unstudied groups, there are no lineages that could be suspected of being really ancient, although the */cantharellus* and */clavulina* and some pezizalean lineages such as /tuber-helvella (Bonito et al. 2013) may potentially exceed 100 million years.

Given that certain EcM fungi associate with liverworts, it is possible that the necessary genetic mechanisms of establishing mutualism evolved long before the modern EcM anatomy evolved. However, phylogenetic evidence suggests that the association of Aneuraceae spp. (incl. *Aneura* = *Cryptothallus mirabilis*) with EcM *Tulasnella* sp. and ericoid mycorrhizal (ErM) fungi has evolved secondarily and relatively recently (Pressel et al. 2010). Except for a few instances, most of the potentially ancient associations with Endogonales in liverworts, hornworts and other lower plants are unrelated to endogonaceous EcM lineages (Yamamoto et al. 2015), but certainly more sequence data are required from both liverwort thalli, and roots of vascular plants are needed to understand their role in EcM and AM symbioses (Orchard et al. 2017). Taken together, it is more likely that the partially mycoheterotrophic lower plants switched to EcM plant lineages rather than these were ancestrally present in these bryophytes and then evolved to associate with EcM gymnosperms and angiosperms.

Table 19.2 Overview of ectomycorrhizal plant genera arranged by lineages

Taxon	No spp.	References (EcM status)	References (phylogeny)
Pinaceae			
Abies	47	Noack (1889), Sarauw (1903)	Lu et al. (2014)
Cathaya	1	Hu and Wang (1984)	Lu et al. (2014)
Cedrus	3	Sarauw (1903), Noelle (1910)	Lu et al. (2014)
Keteleeria	3	Ge et al. (2012)	Lu et al. (2014)
Larix	11	Sarauw (1903), McDougall (1914)	Lu et al. (2014)
Nothotsuga	1		Lu et al. (2014)
Picea	38	Frank (1885), Noack (1889)	Lu et al. (2014)
Pinus	113	Frank (1885), Noack (1889)	Lu et al. (2014)
Pseudolarix	1	Noelle (1910)	Lu et al. (2014)
Pseudotsuga	4	Noelle (1910), McDougall and Jacobs (1927)	Lu et al. (2014)
Tsuga	9	Noelle (1910), McDougall (1928)	Lu et al. (2014)
Gnetum			
Gnetum	39	Fassi (1957), St. John (1980)	Won and Renner (2006)
Fagales[a]			
Alfaroa (Jug)	8		Larson-Johnson (2015)
Allocasuarina (Cas)	59	McGee (1986), Brundrett and Abbott (1991)	Larson-Johnson (2015)
Alnus (Bet)	37	Frank (1888), Masui (1926)	Larson-Johnson (2015)
Betula (Bet)	98	Frank (1888), Peyronel (1922)	Larson-Johnson (2015)
Carpinus (Bet)	40	McDougall (1914), Doak (1927)	Larson-Johnson (2015)
Carya (Jug)	18	McDougall (1914), Doak (1927)	Larson-Johnson (2015)
Castanea (Fag)	8	Frank (1885), Mangin (1910)	Larson-Johnson (2015)
Castanopsis (Fag)	132	Maeda (1954), Haug et al. (1991)	Larson-Johnson (2015)
Casuarina (Cas)	14	Tandy (1975), Warcup (1980) (AM dominates)	Larson-Johnson (2015)
Chrysolepis (Fag)	2	Trappe (1964), Longway (2015)	Larson-Johnson (2015)
Colombobalanus (Fag)	1		Larson-Johnson (2015)
Corylus (Bet)	17	Frank (1885), Mangin (1910)	Larson-Johnson (2015)
Engelhardia (Jug)	12	Haug et al. (1991), (1994), AM: Sharma et al. (1986)	Larson-Johnson (2015)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Fagus (Fag)	10	Kamienski (1882); Frank (1885)	Larson-Johnson (2015)
Formanodendron (Fag)	1		Larson-Johnson (2015)
Lithocarpus (incl. *Pasania*) (Fag)	334	Asai (1934), Haug et al. (1994)	Larson-Johnson (2015)
Nothofagus (Noth)	34	Frank (1888), Morrison (1956)	Larson-Johnson (2015)
Notholithocarpus (Fag)	1	Kennedy et al. (2003)	Larson-Johnson (2015)
Oreomunnea (Jug)	2	Corrales et al. (2016)	Larson-Johnson (2015)
Ostrya (Bet)	9	Lohman (1926), Doak (1927)	Larson-Johnson (2015)
Ostryopsis (Bet)	2	Bai et al. (2003)	Larson-Johnson (2015)
Quercus (incl. *Cyclobalanopsis*) (Fag)	431	Frank (1885), Noack (1889)	Larson-Johnson (2015)
Ticodendron (Tic)	1	Põlme et al. unpubl.	Larson-Johnson (2015)
Trigonobalanus (Fag)	1		Larson-Johnson (2015)
Pisonieae			
Guapira	79	Moyersoen (1993), Haug et al. (2005)	Cuenoud et al. (2002), Douglas and Manos (2007)
Neea	72	Janos (1980), St. John (1980)	Cuenoud et al. (2002), Douglas and Manos (2007)
Pisonia	20 of 24	Ashford and Allaway (1982), Lodge (1996)	Cuenoud et al. (2002), Douglas and Manos (2007)
Achatocarpus			
Achatocarpus	10	Alvarez-Manjarrez and Garibay-Orijel (2015)	Cuenoud et al. (2002)
Coccoloba			
Coccoloba	172	Kreisel (1970), Moyersoen (1993)	Cuenoud et al. (2002), Schuster et al. (2013)
Gymnopodium			
Gymnopodium	2	Bandala et al. (2011)	Schuster et al. (2013)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Persicaria vivipara			
Persicaria vivipara	2 of 66	Hesselman (1900), Costantin and Magrou (1926)	Cuenoud et al. (2002), Schuster et al. (2013)
Asteropeiaceae			
Asteropeia	8	Ducousso et al. (2008), Tedersoo et al. (2011)	Cuenoud et al. (2002)
Acacia			
Acacia s. stricto	ca 1000	Warcup (1980), McGee (1986)	Murphy et al. (2003)
Aldina			
Aldina	22	Meyer (1991), Moyersoen (1993)	Ramos et al. (2016)
Mirbelieae			
Aenictophyton	1		LPWG (2017)
Almaleea	5		Crisp and Cook (2003)
Aotus	14		Crisp and Cook (2003)
Bossiaea	49	AM: Zemunik et al. (2015)	Zanne et al. (2014)
Brachysema	11	Warcup (1980)	Crisp and Cook (2003)
Callistachys	1		Crisp and Cook (2003)
Chorizema	16	Warcup (1980)	Crisp and Cook (2003)
Daviesia	78	Warcup (1980), Meers et al. (2010), Teste et al. (2017), AM: Bellgard (1991), NM: Brundrett and Abbott (1991)	Crisp and Cook (2003)
Dillwynia	21	Warcup (1980), McGee (1986)	Crisp and Cook (2003)
Erichsenia	1		Crisp and Cook (2003)
Euchilopsis	1		Crisp and Cook (2003)
Eutaxia	7	Warcup (1980)	Crisp and Cook (2003)
Gastrolobium	36	Lamont et al. (1985), Teste et al. (2017)	Crisp and Cook (2003)
Gompholobium	35	Warcup (1980), Kope and Warcup (1986), Brundrett and Abbott (1991), Meers et al. (2010), AM: Zemunik et al. (2015), AM: Bellgard (1991)	Crisp and Cook (2003)
Isotropis	12	AM: Zemunik et al. (2015)	Crisp and Cook (2003)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Jacksonia	37	Warcup (1980), Reddell and Milnes (1992), Zemunik et al. (2015), AM: Brundrett and Abbott (1991)	Crisp and Cook (2003)
Jansonia	1		Crisp and Cook (2003)
Latrobea	5		Crisp and Cook (2003)
Leptosema	6		Crisp and Cook (2003)
Mirbelia	26	Warcup (1980), Bellgard (1991)	Crisp and Cook (2003)
Muelleranthus	1		LPWG (2017)
Oxylobium	17	Warcup (1980), Brundrett and Abbott (1991)	Crisp and Cook (2003)
Phyllota	10		Crisp and Cook (2003)
Platylobium	4	Warcup (1980), Meers et al. (2010)	Crisp and Cook (2003)
Podolobium	1		Crisp and Cook (2003)
Pultenaea	103	Warcup (1980), Warcup (1985)	Crisp and Cook (2003)
Sphaerolobium	18		LPWG (2017)
Stonesiella	1		Crisp and Cook (2003)
Urodon	2		Crisp and Cook (2003)
Viminaria	1	Warcup (1980), Bell and Yasmeen (2010), AM: Brundrett and Abbott (1991), AM: de Campos et al. (2013)	Crisp and Cook (2003)
***Afzelia* group**			
Afzelia	14	Fassi and Fontana (1962), Jenik and Mensah (1967)	de la Estrella et al. (2017)
Intsia	2	Alexander and Högberg (1986), Alexander (1989)	de la Estrella et al. (2017)
***Berlinia* group**			
Anthonotha	30	Peyronel and Fassi (1960), Fassi and Fontana (1962)	de la Estrella et al. (2017)
Aphanocalyx	14	as *Monopetalanthus*: Fassi and Fontana (1962), Newbery et al. (1988)	de la Estrella et al. (2017)
Berlinia	16	Newbery et al. (1988), Alexander (1989)	de la Estrella et al. (2017)
Bikinia	10	as *Monopetalanthus*: Onguene (2000)	de la Estrella et al. (2017)
Brachystegia	34	Peyronel and Fassi (1960), Fassi and Fontana (1962)	de la Estrella et al. (2017)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Didelotia	12	Newbery et al. (1988), Alexander (1989)	de la Estrella et al. (2017)
Englerodendron	2		de la Estrella et al. (2017)
Gilbertiodendron	26	Peyronel and Fassi (1960), Fassi and Fontana (1962)	de la Estrella et al. (2017)
Icuria	1		de la Estrella et al. (2017)
Isoberlinia	5	Alexander and Högberg (1986), Högberg and Piearce (1986)	de la Estrella et al. (2017)
Isomacrolobium	12		de la Estrella et al. (2017)
Julbernardia	11	Peyronel and Fassi (1960), Fassi and Fontana (1962)	de la Estrella et al. (2017)
Librevillea	1		de la Estrella et al. (2017)
Michelsonia	1		Gervais and Bruneau (2002)
Microberlinia	2	Newbery et al. (1988), Alexander (1989)	de la Estrella et al. (2017)
Monopetalanthus	2		Bruneau et al. (2001)
Oddoniodendron	3		de la Estrella et al. (2017)
Pellegriniodendron	1	Alexander (1989), Riviere et al. (2001)	Bruneau et al. (2001)
Pseudomacrolobium	1		LPWG (2017)
Tetraberlinia	7	Newbery et al. (1988), Alexander (1989)	de la Estrella et al. (2017)
Toubaouate	1	Onguene (2000)	de la Estrella et al. (2017)
***Cryptosepalum* group**			
Cryptosepalum	11	Alexander (1989), Rivière (2004)	de la Estrella et al. (2017)
Paramacrolobium	1	Peyronel and Fassi (1960), Fassi and Fontana (1962)	de la Estrella et al. (2017)
Dicymbe			
Dicymbe	15	Henkel et al. (2000, 2002)	de la Estrella et al. (2017)
Salicaceae			
Populus	29	Frank (1885), Stahl (1900)	Davis et al. (2005), Chen et al. (2010)
Salix	475	Frank (1885), Stahl (1900), Hesselman (1900)	Davis et al. (2005), Chen et al. (2010)
Uapaca			
Uapaca	49	Redhead (1974), Högberg (1982)	Wurdack et al. (2004)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Poranthera			
Poranthera	14	Warcup (1980), Kope and Warcup (1986), McGee (1986), Bellgard (1991), Teste et al. (2017), non-EcM: Chap. 17	Wurdack et al. (2004), Vorontsova et al. (2007)
Pomaderreae			
Blackallia	1		Ladiges et al. (2005)
Cryptandra	57	Warcup (1980), Brundrett and Abbott (1991)	Ladiges et al. (2005)
Papistylus	2		Onstein et al. (2015)
Polianthion	4		Onstein et al. (2015)
Pomaderris	70	Ashton (1975), Warcup (1980)	Ladiges et al. (2005)
Serichonus	1		Onstein et al. (2015)
Siegfriedia	1		Ladiges et al. (2005)
Spyridium	40	Warcup (1980), Zemunik et al. (2015), AM: McGee (1986)	Ladiges et al. (2005)
Stenanthemum	14	Zemunik et al. (2015)	Ladiges et al. (2005)
Trymalium	30	Warcup (1980, 1985), Brundrett and Abbott (1991)	Ladiges et al. (2005)
Dryadeae			
Cercocarpus	5	Thomas (1943), Trappe (1964), Williams (1979), AM: Rose (1980)	Potter et al. (2007)
Chamaebatia	2	Trappe (1964)	Potter et al. (2007)
Cowania	1		Potter et al. (2007)
Dryas	9	Hesselman (1900), Jessen (1914)	Potter et al. (2007)
Purshia	6	AM: Williams (1979), AM: Rose (1980)	Potter et al. (2007)
Adenostoma			
Adenostoma	2	Cooper (1922), Allen et al. (1999a,b)	Potter et al. (2007)
Dipterocarpaceae-Cistaceae[b]			
Anisoptera (Dipt)	11	Singh (1966), Chalermpongse (1987)	Dayanandan et al. (1999)
Cistus (Cist)	21	Chevalier et al. (1975), Fontana and Giovannetti (1978)	Guzmán and Vargas (2009)
Cotylelobium (Dipt)	6	Hong (1979), de Alwis and Abeynayake (1980)	Dayanandan et al. (1999)
Crocanthemum (Cist)	20		Guzmán and Vargas (2009)
Dipterocarpus (Dipt)	69	Singh (1966), de Alwis and Abeynayake (1980)	Dayanandan et al. (1999)
Dryobalanops (Dipt)	7	Singh (1966), Hong (1979)	Dayanandan et al. (1999)
Eremolaena (Sarc)	3		Aubriot et al. (2016)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Fumana (Cist)	9	Chevalier et al. (1975), Kovacs and Szigetvari (2002)	Guzmán and Vargas (2009)
Halimium (Cist)	8	Buscardo et al. (2012)	Guzmán and Vargas (2009)
Helianthemum (Cist)	90	Peyronel (1930), Boursnell (1950)	Guzmán and Vargas (2009)
Hopea (Dipt)	102	Van Roosendael and Thorenaar (1924), Singh (1966)	Dayanandan et al. (1999)
Hudsonia (Cist)	3	Malloch and Thorn (1985), Massicotte et al. (2010)	Guzmán and Vargas (2009)
Lechea (Cist)	17	Malloch and Thorn (1985)	Guzmán and Vargas (2009)
Leptolaena (Sarc)	8	Ducousso et al. (2004, 2008)	Aubriot et al. (2016)
Marquesia (Mon)	3	Alexander and Högberg (1986), Högberg and Piearce (1986)	Gunasekara (2004)
Mediusella (Sarc)	2		Aubriot et al. (2016)
Monotes (Mon)	30	Högberg (1982), Alexander and Högberg (1986)	Dayanandan et al. (1999)
Neobalanocarpus (Dipt)	1	Singh (1966), Zainudin (1990)	Dayanandan et al. (1999)
Pakaraimaea (Pak)	1	Moyersoen (2006)	Dayanandan et al. (1999)
Parashorea (Dipt)	14	Noor (1981), Lee (1988)	Gunasekara (2004)
Pentachlaena (Sarc)	3		Aubriot et al. (2016)
Perrierodendron (Sarc)	5		Aubriot et al. (2016)
Pseudomonotes (PsM)	1	Vasco Palacios et al. unpubl.	Morton et al. (1999)
Rhodolaena (Sarc)	7		Aubriot et al. (2016)
Sarcolaena (Sarc)	8	Ducousso et al. (2004, 2008)	Dayanandan et al. (1999), Aubriot et al. (2016)
Schizolaena (Sarc)	22	Ducousso et al. (2004, 2008)	Aubriot et al. (2016)
Shorea (Dipt)	194	de Voogd (1933), Singh (1966)	Dayanandan et al. (1999)
Stemonoporus (Dipt)	15		Dayanandan et al. (1999)
Tuberaria (Cist)	12	Proctor (1960)	Guzmán and Vargas (2009)
Upuna (Dipt)	1		Dayanandan et al. (1999)
Vateria (Dipt)	2	Alexander and Högberg (1986)	Dayanandan et al. (1999)
Vateriopsis (Dipt)	1	Tedersoo et al. (2007a,b)	Gunasekara (2004)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Vatica (Dipt)	65	Singh (1966), Santoso (1988)	Dayanandan et al. (1999)
Xerochlamys (Sarc)	7		Aubriot et al. (2016)
Xyloolaena (Sarc)	5		Aubriot et al. (2016)
Tilia			
Craigia	2		Nyffeler et al. (2005), within Tilia: Zanne et al. (2014)
Tilia	44	McDougall (1914), Peyronel (1922)	Nyffeler et al. (2005)
Gnaphalieae			
Acanthocladium	1		Bayer et al. (2002)
Acomis	4		Bayer et al. (2002)
Actinobole	4		Bayer et al. (2002), Bergh and Linder (2009)
Ammobium	2		Bayer et al. (2002)
Anaphalioides	8		Breitwieser and Ward (2003)
Anemocarpa	3		Bayer et al. (2002)
Angianthus	19	Warcup and McGee (1983), Kope and Warcup (1986), Warcup (1990), AM: Zemunik et al. (2015)	Bayer et al. (2002), Bergh and Linder (2009)
Apalochlamys	1		Bayer et al. (2002)
Argentipallium	6		Bayer et al. (2002), Bergh and Linder (2009)
Argyroglottis	1		Bayer et al. (2002); Bergh and Linder (2009)
Argyrotegium	4		Breitwieser and Ward (2003)
Asteridea	11	Warcup (1990), AM: Chap. 17	Bayer et al. (2002)
Bellida	1		Bayer et al. (2002)
Blennospora	3	Warcup (1990)	Bayer et al. (2002)
Calocephalus	9		Bayer et al. (2002)
Calomeria	2		Bayer et al. (2002)
Cassinia	37		Bayer et al. (2002)
Cephalipterum	1		Bayer et al. (2002)
Cephalosorus	1	Warcup (1990)	Bayer et al. (2002)
Chondropyxis	1	Warcup (1990)	Bayer et al. (2002)
Chrysocephalum	9		Bayer et al. (2002)
Cryptocoryne	2	Warcup and McGee (1983)	Bayer et al. (2002)
Chthonocephalus	7		Bayer et al. (2002)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Coronidium	10		Schmidt-Lebuhn et al. (2015)
Decazesia	1		Bayer et al. (2002), Bergh and Linder (2009)
Dithyrostegia	2		Bayer et al. (2002)
Eriochlamys	4	Warcup (1990)	Bayer et al. (2002)
Erymophyllum	5	Warcup and McGee (1983), Warcup (1990), AM: Teste et al. (2017)	Bayer et al. (2002)
Euchiton	17	AM: Meers et al. (2010)	Breitwieser and Ward (2003)
Ewartiothamnus	1		Breitwieser and Ward (2003)
Feldstonia	1		Bayer et al. (2002)
Fitzwillia	1	Warcup (1990)	Bayer et al. (2002), Bergh and Linder (2009)
Gilberta	1		Bayer et al. (2002)
Gilruthia	1		Bayer et al. (2002)
Gnephosis	17	Warcup (1990), AM: Teste et al. (2017)	Bayer et al. (2002)
Haeckeria	3		Bayer et al. (2002), Bergh and Linder (2009)
Haegiela	1		Zanne et al. (2014)
Haptotrichion	2		Zanne et al. (2014)
Helichrysum (Australian spp.)	30 of 536	Warcup and McGee (1983), McGee (1986), Warcup (1990), AM: Meers et al. (2010)	Bayer et al. (2002), Bergh and Linder (2009)
Hyalochlamys	1		Bayer et al. (2002)
Hyalosperma	9		Bayer et al. (2002)
Ixiolaena	1		Bayer et al. (2002), Bergh and Linder (2009)
Ixodia	1	Warcup (1990)	Bayer et al. (2002)
Lawrencella	2		Bayer et al. (2002)
Leiocarpa	11		Zanne et al. (2014)
Lemooria	1		Bayer et al. (2002)
Leptorhynchos	10	Warcup and McGee (1983), Warcup (1990)	Bayer et al. (2002)
Leucochrysum	7		Bayer et al. (2002), Bergh and Linder (2009)
Leucogenes	4		Breitwieser and Ward (2003)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Leucophyta	1		Bayer et al. (2002)
Millotia	16		Bayer et al. (2002), Bergh and Linder (2009)
Myriocephalus	15		Bayer et al. (2002)
Odixia	2		Bayer et al. (2002)
Ozothamnus	53	AM: Teste et al. (2017)	Bayer et al. (2002), Bergh and Linder (2009)
Parantennaria	1		Zanne et al. (2014)
Pithocarpa	2		Bayer et al. (2002), Bergh and Linder (2009)
Pleuropappus	1		Bayer et al. (2002)
Podolepis	20	Warcup and McGee (1983), McGee (1986), Warcup (1990), Teste et al. (2017)	Bayer et al. (2002), Bergh and Linder (2009)
Podotheca	6	Warcup (1990), AM: Teste et al. (2017), Chap. 17	Bayer et al. (2002)
Pogonolepis	2	Warcup and McGee (1983)	Bayer et al. (2002), Bergh and Linder (2009)
Polycalymma	1		Bayer et al. (2002), Bergh and Linder (2009)
Pterochaeta	1		Bayer et al. (2002)
Pycnosorus	6		Bayer et al. (2002), Bergh and Linder (2009)
Quinetia	1		Bayer et al. (2002), Bergh and Linder (2009)
Quinqueremulus	1		Bayer et al. (2002)
Raoulia	26		Breitwieser and Ward (2003)
Rhodanthe	46	Warcup and McGee (1983), Teste et al. (2017)	Bayer et al. (2002)
Rutidosis	10	Warcup and McGee (1983), Warcup (1990)	Bayer et al. (2002)
Schoenia	6		Zanne et al. (2014)
Siemssenia	1		Zanne et al. (2014)
Siloxerus	4		Bayer et al. (2002), Bergh and Linder (2009)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Sondottia	2	Warcup (1990)	Bayer et al. (2002), Bergh and Linder (2009)
Toxanthes	1	McGee (1986)	Bayer et al. (2002)
Trichanthodium	3	Warcup (1990)	Bayer et al. (2002)
Triptilodiscus	1	Warcup (1990)	Bayer et al. (2002)
Waitzia (Waitzea)	5	Warcup and McGee (1983), Kope and Warcup (1986), Warcup (1990)	Bayer et al. (2002)
Xerochrysum	8		Bayer et al. (2002)
Goodeniaceae			
Anthotium	4		Gustafsson et al. (1996), Jabaily et al. (2014)
Brunonia	1	Warcup (1980, 1985)	Gustafsson et al. (1996), Jabaily et al. (2014)
Calogyne	1	Reddell and Milnes (1992)	Gustafsson et al. (1996), Jabaily et al. (2014)
Coopernookia	6		Gustafsson et al. (1996), Jabaily et al. (2014)
Dampiera	69	McGee (1986), Bellgard (1991), AM: Brundrett and Abbott (1991), AM: Teste et al. (2017)	Gustafsson et al. (1996), Jabaily et al. (2014)
Diaspasis	1		Gustafsson et al. (1996), Jabaily et al. (2014)
Goodenia	ca 200	Warcup (1980, 1985), McGee (1986), AM: Teste et al. (2017)	Gustafsson et al. (1996), Jabaily et al. (2014)
Scaevola	ca 120	Zemunik et al. (2015), Teste et al. (2017), AM: Asai (1934), AM: Peterson et al. (1985), AM: Koske (1988), AM: Brundrett and Abbott (1991)	Gustafsson et al. (1996), Jabaily et al. (2014)
Selliera	ca 5		Gustafsson et al. (1996), Jabaily et al. (2014)
Velleia	29		Gustafsson et al. (1996), Jabaily et al. (2014)
Verreauxia	1		Gustafsson et al. (1996), Jabaily et al. (2014)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Myrtaceae[c]			
Actinodium (Cha)	1		Thornhill et al. (2015)
Agonis (Lept)	6	Brundrett and Abbott (1991)	Thornhill et al. (2015)
Allosyncarpia (Euc)	1	Reddell and Milnes (1992)	Thornhill et al. (2015)
Angophora (Euc)	13	Tandy (1975), Bellgard (1991)	Thornhill et al. (2015)
Arillastrum (Euc)	1	Buyck et al. (2012), Jourand et al. (2014)	Thornhill et al. (2015)
Astartea (Cha)	9		Lam et al. (2002)
Asteromyrtus (Lept)	7	Reddell and Milnes (1992)	Thornhill et al. (2015)
Astus (Cha)	4		Zanne et al. (2014)
Babingtonia (Cha)	2		Lam et al. (2002)
Backhousia (Back)	11	Reddell et al. (1996)	Thornhill et al. (2015)
Baeckea (Cha)	47	McGee (1986), Bellgard (1991)	Thornhill et al. (2015)
Balaustion (Cha)	1		Zanne et al. (2014)
Barongia (Kan)	1		Wilson et al. (2005)
Beaufortia (Mel)	20		Thornhill et al. (2015)
Callistemon (Mel)	37	Warcup (1980)	Thornhill et al. (2015)
Calothamnus (Mel)	44	Zemunik et al. (2015), Teste et al. (2017)	Thornhill et al. (2015)
Calytrix (Cha)	80	Langkamp and Dalling (1982), McGee (1986), Reiter et al. (2013), AM: Brundrett and Abbott (1991), AM: Zemunik et al. (2015), AM: Teste et al. (2017)	Thornhill et al. (2015)
Chamelaucium (Cha)	13		Thornhill et al. (2015)
Choricarpia (Back)	2		Thornhill et al. (2015)
Cloezia (Tri)	6		Thornhill et al. (2015)
Conothamnus (Mel)	1		Thornhill et al. (2015)
Darwinia (Cha)	52	AM: Zemunik et al. (2015)	Thornhill et al. (2015)
Eremaea (Mel)	16	Zemunik et al. (2015), Teste et al. (2017)	Thornhill et al. (2015)
Eucalyptopsis (Euc)	2	L. Tedersoo, unpubl.	Thornhill et al. (2015)
Eucalyptus (incl. *Corymbia*) (Euc)	755	Samuel (1926), Pryor (1956), Trappe (1964), Chilvers and Pryor (1965)	Thornhill et al. (2015)
Euryomyrtus (Cha)	8		Thornhill et al. (2015)
Harmogia (Cha)	1		Thornhill et al. (2015)
Homalocalyx (Cha)	11		Thornhill et al. (2015)
Homalospermum (Lept)	1		O'Brien et al. (2000)
Homoranthus (Cha)	23		Thornhill et al. (2015)
Hypocalymma (Cha)	24	AM: Brundrett and Abbott (1991), AM: Teste et al. (2017)	Thornhill et al. (2015)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Kardomia (Cha)	6		Thornhill et al. (2015)
Kjellbergiodendron (Loph)	1		Thornhill et al. (2015)
Kunzea (Lept)	42	Baylis (1962), Bellgard (1991)	Thornhill et al. (2015)
Lamarchea (Mel)	2		Thornhill et al. (2015)
Leptospermum (Lept)	91	Tandy (1975), Cooper (1976)	Thornhill et al. (2015)
Lophostemon (Loph)	5	Reddell and Milnes (1992), Reddell et al. (1996)	Thornhill et al. (2015)
Lysicarpus (Kan)	1		Thornhill et al. (2015)
Malleostemon (Cha)	6		Lam et al. (2002)
Melaleuca (Mel)	264	Warcup (1980), Alexander and Högberg (1986)	Thornhill et al. (2015)
Micromyrtus (Cha)	50		Thornhill et al. (2015)
Mitrantia (Kan)	1		Wilson et al. (2005)
Neofabricia (Lept)	3		O'Brien et al. (2000)
Ochrosperma (Cha)	6		Thornhill et al. (2015)
Pericalymma (Lept)	4	Brundrett and Abbott (1991)	Thornhill et al. (2015)
Petraeomyrtus (Mel)	1		Thornhill et al. (2015)
Phymatocarpus (Mel)	1		Thornhill et al. (2015)
Pileanthus (Cha)	8	Zemunik et al. (2015)	Thornhill et al. (2015)
Regelia (Mel)	3		Thornhill et al. (2015)
Rinzia (Cha)	12		Lam et al. (2002)
Ristantia (Kan)	3		Wilson et al. (2005)
Sannantha (Cha)	15	Jourand et al. (2014)	Thornhill et al. (2015)
Scholtzia (Cha)	13	AM: Zemunik et al. (2015), AM: Teste et al. (2017)	Lam et al. (2002)
Seorsus (Cha)	4		Zanne et al. (2014)
Sphaerantia (Kan)	2		Thornhill et al. (2015)
Stockwellia (Euc)	2		Thornhill et al. (2015)
Taxandria (Lept)	12		Zanne et al. (2014)
Thaleropia (Tri)	3		Thornhill et al. (2015)
Thryptomene (Cha)	32	Reiter et al. (2013), Teste et al. (2017)	Thornhill et al. (2015)
Triplarina (Cha)	7		Lam et al. (2002)
Tristania (Tri)	1	Tandy (1975), Alexander and Högberg (1986)	Thornhill et al. (2015)
Tristaniopsis (Kan)	42	Perrier et al. (2006), Prin et al. (2012)	Thornhill et al. (2015)
Welchiodendron	1		Wilson et al. (2005)
Verticordia (Cha)	111	AM: Brundrett and Abbott (1991), AM: Teste et al. (2017)	Thornhill et al. (2015)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Whiteodendron (Loph)	1		Wilson et al. (2005)
Xanthomyrtus (Tri)	25		Thornhill et al. (2015)
Xanthostemon (Xan)	49	Richards et al. (2003), AM: Reddell and Milnes (1992)	Thornhill et al. (2015)
Platysace			
Platysace	27	Warcup (1980), Bellgard (1991), Teste et al. (2017), non-EcM: Chap. 17	Nicolas (2009)
Arbutoideae *s.lat*[d]			
Allotropa (Mon)	1	Castellano and Trappe (1985), Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Arbutus (Arb)	10	Dufrenoy (1917), Zak (1974)	Hileman et al. (2001), Kron and Luteyn (2005)
Arctostaphylos (incl. *Arctous*) (Arb)	60	Christoph (1921), Peyronel (1930)	Hileman et al. (2001), Kron and Luteyn (2005)
Cheilotheca (Mon)	2	Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Chimaphila (Pyr)	5	Largent et al. (1980), Massicotte et al. (2008)	Bidartondo (2005), Kron and Luteyn (2005)
Comarostaphylis (Arb)	10	Osmundson et al. (2007), Kühdorf et al. (2015)	Hileman et al. (2001), Kron and Luteyn (2005)
Hemitomes (Mon)	1	Castellano and Trappe (1985), Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Moneses (Pyr)	2	Christoph (1921), Massicotte et al. (2008)	Bidartondo (2005), Kron and Luteyn (2005)
Monotropa (Mon)	1	Kamienski (1882), Frank (1887)	Bidartondo (2005), Kron and Luteyn (2005)
Monotropastrum (Mon)	1	Matsuda and Yamada (2003), Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Monotropsis (Mon)	1	Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Ornithostaphylos (Arb)	1		Hileman et al. (2001), Kron and Luteyn (2005)

(continued)

Table 19.2 (continued)

Taxon	No spp.	References (EcM status)	References (phylogeny)
Orthilia (Pyr)	1	Christoph (1921), Malloch and Malloch (1982)	Bidartondo (2005), Kron and Luteyn (2005)
Pityopus (Mon)	1	Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Pleuricospora (Mon)	1	Castellano and Trappe (1985), Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Pterospora (Pter)	2	Castellano and Trappe (1985), Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Pyrola (Pyr)	30	Kramar (1901), Christoph (1921)	Bidartondo (2005), Kron and Luteyn (2005)
Sarcodes (Pter)	1	Bidartondo (2005)	Bidartondo (2005), Kron and Luteyn (2005)
Xylococcus (Arb)	1	L. Tedersoo, unpubl.	Zanne et al. (2014)
Kobresia			
Kobresia	ca 30 of 58	Fontana (1963), Haselwandter and Read (1980)	Starr et al. (2004)

References to EcM status indicate time of first description and conflicting evidence. The generic and species nomenclature and the number of accepted species follow the Plant List, with specifications from phylogenetic studies in case of unsplit genera (*Acacia s.lat*) or unresolved groups (Goodeniaceae, *Kobresia*, *Pisonia*, *Persicaria*, *Helichrysum*). More references are provided in Supporting Information: http://dx.doi.org/10.15156/BIO/587454

[a]*Bet* Betulaceae, *Cas* Casuarinaceae, *Fag* Fagaceae, *Jug* Juglandaceae, *Noth* Nothofagaceae, *Tic* Ticodendraceae

[b]*Cist* Cistaceae, *Dipt* Dipterocarpoideae, *Mon* Monotoideae, *Pak* Pakaraimaeaceae, *PsM* Pseudomonotoideae, *Sarc* Sarcolaenaceae

[c]*Back* Backhousieae, *Cham* Chamelaucieae, *Kan* Kanieae, *Lept* Leptospermeae, *Loph* Lophostemoneae, *Mel* Melaleuceae, *Tri* Tristanieae, *Xant* Xanthostemoneae

[d]*Arb* Arbuteae, *Euc* Eucalypteae, *Mon* Monotropeae, *Pter* Pterosporeae, *Pyr* Pyroleae

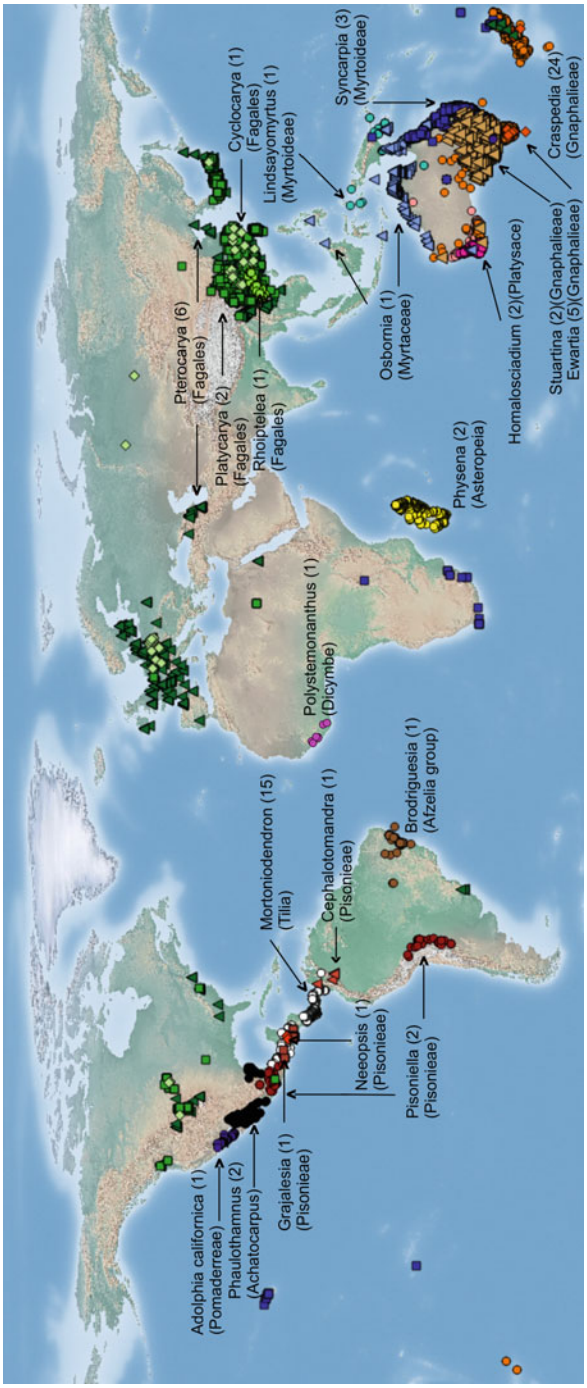

Fig. 19.2 Native and assisted distribution of top 22 most wanted plant genera that have no information about mycorrhizal status but are placed in sister position to known EcM lineages. Colours are grouped according to phylogenetic affinities as indicated in parentheses, and closely related genera are further distinguished by symbols. Numbers in parentheses indicate the number of species. Note that the European and American distribution of the three Juglandaceae genera represents their introduced range. Similarly, Hawaiian and South African distribution of *Syncarpia* indicates its introduced range. All data are based on GBIF (accessed 17.12.2016) records that include coordinates

19.4 Ectomycorrhizal Plant Lineages

19.4.1 Pinales

Pinaceae is the oldest extant ectomycorrhizal plant group that consists of 11 extant genera of trees. The genera *Pinus* and *Picea* were described as ectomycorrhizal in the pioneering study of Frank (1885), but similar root structures were described several decades earlier. Within Pinaceae, only the narrowly endemic genera *Nothotsuga* and *Pseudolarix* remain unconfirmed in terms of EcM habit but are also expected to have EcM. In natural conditions, short roots of Pinaceae are typically fully converted to EcM, but in *Cedrus* EcM colonisation typically remains <50% in native habitats (L. Tedersoo, unpubl.). In contrast to other EcM plant genera, species of *Pinus* exhibit characteristic bifurcately branching root tips. Pinaceae serve as hosts for a wide variety of fungi, but the local diversity in Pinaceae habitats tends to be lower than that in temperate deciduous forests (Tedersoo et al. 2012, 2014) probably because of highly acidic needle litter. Several small and recently evolved EcM fungal lineages are associated only with Pinaceae (Tedersoo and Smith 2013), but this could be due to their preference for acidic soils and paucity of studies of angiosperm roots in conifer forests. It is notable that older fungal lineages tend to have included Pinaceae in their host range relatively recently, indicating that the ancient fungal associations were phylogenetically relatively restricted. The family Pinaceae diverged from other extant gymnosperms roughly 340–320 Ma and radiated to extant genera since 198–175 Ma (average estimates: Leslie et al. 2012; Lu et al. 2014), although one conflicting study indicates only half that age (Crisp and Cook 2011).

19.4.2 Gnetales

The genus ***Gnetum*** is another gymnosperm group that forms EcM. In contrast to Pinaceae, this group represents mostly climbers, from which two species of trees evolved once in Indo-Malay. Similarly to Pinaceae, the root system of *Gnetum* is coarse with thick and conspicuous EcM. However, the EcM anatomy of *Gnetum* is substantially different from those of Pinaceae and any other plant (Brundrett 2009: Fig. 7a). The fungal interface in *Gnetum* occurs above the epidermis and consists of many fingerlike projections (root hairs) in a matrix of hyphae. Epidermal cells in these roots are also exceptionally narrow and densely packed. The level of EcM colonisation varies strongly, but all plants seem to be EcM (L. Tedersoo, unpubl.). *Gnetum* is characterised by extremely low EcM fungal richness that is restricted to a few species of *Scleroderma* in the liana-forming species (Bechem and Alexander 2012). The tree-forming *G. gnemon* exhibits somewhat greater fungal richness with still a prominent role of *Scleroderma* (Tedersoo and Põlme 2012). Although *Gnetum* diverged from the AM *Welwitschia* 130 Ma, modern groups of *Gnetum* radiated since 26 Ma, indicating its recent rather than ancient origin (Won and Renner 2006).

19.4.3 Fagales

The order **Fagales** is likely to be the oldest angiosperm EcM group that is represented by mostly trees and bushes both in the Northern and Southern Hemispheres. Since >80% of genera of Fagales are EcM, it is likely that EcM habit is ancestral in this group (Larson-Johnson 2015). Nothofagaceae represents the earliest diverging branch with current distribution in relicts of Gondwana. Within Betulaceae (incl. Coryloideae), *Alnus* is the only genus to associate with N_2-fixing *Frankia* actinobacteria. The monotypic Central American Ticodendraceae family is closely related to Betulaceae, and it has been proven EcM very recently (S. Põlme et al. unpubl.). The Southern Hemisphere Casuarinaceae family represents a sister group to Betulaceae + Ticodendraceae (Larson-Johnson 2015). Within this group, association with *Frankia* actinobacteria has probably evolved independently. EcM formation is normally present in the genus *Allocasuarina* but more occasional in *Casuarina*. AM symbiosis is always present in their roots, but nodules and EcM may be secondarily lacking, depending on species, plant age and soil properties (Reddell et al. 1986). Two additional Casuarinaceae genera, *Ceuthostoma* and *Gymnostoma*, have probably fully lost their capacity to form EcM (Duhoux et al. 2001), but certainly more information is needed. Fagaceae are certainly the most widely distributed family of Fagales that comprise only EcM-forming genera such as *Quercus* and *Fagus*. Besides the Casuarinaceae family, Juglandaceae represents another group that contains both EcM-forming and non-EcM members. The genera *Engelhardia*, *Oreomunnea*, *Alfaroa* and *Carya* form a monophyletic group that has been proven to associate with EcM fungi. Besides the EcM groups, Juglandaceae comprise at least one non-EcM genus, i.e. *Juglans*. Although sporadic reports on EcM exist, the root systems of *Juglans* have an architecture similar to that of *Fraxinus* with elongated short roots that is not seen in any EcM groups (except *Alnus*). There are several genera of Juglandaceae endemic to East Asia (*Cyclocarya*, *Pterocarya*, *Platycarya*) with no information about their mycorrhizal status. Within Fagales, the actinorhizal family Myricaceae seems to have completely lost the capacity to form EcM (but see the probably incorrect report of Sharma et al. 1986). At the family level, there is no information about the mycorrhizal status of Rhoipteleaceae, a narrow endemic of South China. Given the accumulated information, we hypothesise that Fagales gained the EcM-forming ability once, with multiple consequent losses. Development of actinorhizal symbiosis may be one of the causes for these losses (Myricaceae, Casuarinaceae) and for reduced EcM colonisation (Casuarinaceae, *Alnus*). This does not, however, explain the non-EcM habit of *Juglans* that typically inhabits EcM-dominated forests. It is possible that the strongly allelopathic biocide juglone has evolved to prevent EcM formation in *Juglans* spp., in which this substance is particularly abundant. The evolutionary history of Fagales has been relatively well established compared with other plant groups due to outstanding fossil record and economic importance. The EcM Fagales diverged from Hamamelidaceae some 98 Ma. The extant fagalean families diverged between 94 Ma and 72 Ma. The putatively non-EcM groups

Gymnostoma, Myricaceae, *Juglans* and *Ceuthostoma* diverged from the closest EcM taxa 59 Ma, 56 Ma, 45 Ma and 43 Ma, respectively (Larson-Johnson 2015). Members of Fagales differ strongly in EcM colonisation and the diversity of fungi supported. The actinorhizal genera *Alnus* and these of Casuarinaceae exhibit relatively low level of colonisation, and these groups harbour a limited set of fungi (Põlme et al. 2013), although molecular data are virtually lacking for Casuarinaceae. *Quercus* spp. and Juglandaceae spp. are typically moderately colonised by EcM fungi, whereas most groups in Betulaceae (except *Alnus*), Fagaceae and Nothofagaceae are heavily colonised (>90%). These three families harbour very high diversity of EcM fungi both in Northern and Southern Hemispheres (Tedersoo et al. 2012, 2014). Several EcM fungal lineages are specific to *Nothofagus* spp. or shared with neighbouring plants in Australia (Tedersoo et al. 2010a).

19.4.4 Caryophyllales

Six phylogenetically distinct groups of EcM plants are recognised within the order of Caryophyllales (Cuenoud et al. 2002; Schuster et al. 2013). The Pisonieae tribe, *Achatocarpus* and *Asteropeia* belong to Nyctaginaceae, Achatocarpaceae and Asteropeiaceae families, respectively, whereas *Persicaria vivipara*, *Coccoloba* and *Gymnopodium* belong to Polygonaceae.

Within the **Pisonieae** tribe, trees and shrubs belonging to *Pisonia*, *Neea* and *Guapira* contain EcM species. All species of the two latter genera are always EcM, but not all species of *Pisonia* form EcM (Hayward and Hynson 2014). Most of the EcM *Pisonia* species occur in South and Central America, whereas *P. grandis* inhabits much of the tropical Oceania (Chap. 20). Except for *P. sandwichiensis* in Hawaii, several phylogenetically distant endemic species of *Pisonia* inhabiting the islands of Pacific and Indian oceans are non-ectomycorrhizal and should be transferred to a new genus (Hayward and Hynson 2014). In addition to the genera *Pisonia*, *Neea* and *Guapira*, the monotypic *Pisoniella* belongs to this group based on phylogenetic analyses (Douglas and Manos 2007). On a morphological basis, *Neeopsis*, *Grajalesia* and *Cephalomandra* may also be related to this EcM group (Douglas and Spellenberg 2010), but no phylogenies are available for these small genera. Species and genera of Pisonieae exhibit extremely specific (*P. grandis*: Suvi et al. 2010) or strongly specific (*Neea*, *Guapira*: Tedersoo et al. 2010b) associations with EcM fungi. In all these genera, the EcM colonisation may be very low, and seedlings are not always associated with EcM fungi (L. Tedersoo, pers. obs.). In *P. grandis*, specific transfer cells extending from Hartig net to epidermal cells are characteristic anatomic features of EcM (Ashford and Allaway 1982). No specific age estimates exist for the EcM group, but most probably EcM habit evolved between 35 Ma and 20 Ma (Douglas and Manos 2007; Zanne et al. 2014).

Achatocarpus is a small family of trees in Central and South America. The EcM habit of *Achatocarpus* sp. was convincingly illustrated only recently, and several fungal groups are associated (Alvarez-Manjarrez and Garibay-Orijel 2015; J. Alvarez-Manjarrez, pers. comm.). *Phaulothamnus* constitutes a sister genus to *Achatocarpus*, but there is no information about its mycorrhizal status so far. There are no specific phylogenetic or biogeographic studies involving Achatocarpaceae, but Zanne et al. (2014) estimate this group to date back to <12 Ma.

The Polygonaceae is a predominantly non-mycorrhizal family, but there are some conflicting reports (Andrade et al. 2000) that may be derived from attention to AM colonisation. The South and Central American genus ***Coccoloba*** contains only EcM species that are among the dominant trees in maritime sand dunes or subcanopy trees, bushes or lianas. The EcM roots of *C. uvifera* in sand dunes are relatively much broader and more heavily colonised by fungi compared with scattered *Coccoloba* spp. tree individuals in a rain forest habitat in Ecuador (L. Tedersoo, unpubl.). Fungi associated with *Coccoloba* spp. in both habitats exhibit relatively greater diversity than in Nyctaginaceae but lower diversity compared with South American Dipterocarpaceae and Fabaceae, suggesting certain level of specificity (Tedersoo et al. 2010b). The genus *Coccoloba* diverged from AM ancestors around 52 Ma and radiated 24 Ma (Schuster et al. 2013).

The Central American genus ***Gymnopodium*** was only recently suggested to be EcM, and so far, published molecular and morphological evidence at the root tip scale is lacking (Bandala et al. 2011). *Gymnopodium* forms monodominant stands and supports tens of fungal species that are mostly shared with *Coccoloba* in neighbouring habitats (Bandala et al. 2011). *Gymnopodium* is a relatively young EcM group since its stem age was estimated to date back 35 Ma (Schuster et al. 2013).

In contrast to these three South and Central American EcM groups, ***Persicaria vivipara*** (also known as *Polygonum* and *Bistorta*) represents a perennial herb that is distributed throughout the circumarctic habitat and many glacial refugia in the alpine areas of Europe, Asia and North America. Since there is no recent taxonomic work on the genus *Persicaria* and closely related genus *Polygonum*, it remains unknown whether any other species of this group exhibit EcM habit as there are only a few and unreliable reports as well as some taxonomic confusion. Besides *P. vivipara*, the only reliable report on EcM is derived from *Polygonum weyrichii* in Japan, where all plants exhibited low but consistent colonisation across different habitats (Titus and Tsuyuzaki 2002; Tsuyuzaki et al. 2005) and perhaps *P. paronychia* (both not transferred to *Persicaria*) in dunes of Western North America (Zak 1973). In spite of conflicting reports about the EcM status of *P. vivipara*, we have observed that all individual plants of this species in Estonia and Scandinavia are colonised by EcM fungi, but the level of colonisation usually remains <50% (L. Tedersoo, unpubl.). The root systems and EcM tips of *P. vivipara* are among the finest and shortest among all EcM groups (Massicotte et al. 1998). *P. vivipara* associates with multiple fungi and lacks host specificity relative to other arctic and alpine herbs and shrubs (Botnen et al. 2014). *P. vivipara* seems to be a relatively recently evolved EcM group, with the estimated stem

age < 28 million years (Schuster et al. 2013), but probably much less in case of better taxon sampling (<7 million years; Zanne et al. 2014).

Asteropeiaceae represents a monogeneric family of small trees and bushes that is distributed in Madagascar. The EcM status of *Asteropeia* was first reported in the year 2008 (Ducousso et al. 2004). The roots and EcM tips of *A. micraster* are extremely narrow and difficult to locate without a stereomicroscope. EcM root tips are sparsely distributed along the long root and contribute to ca. 50% of all root tips. Roots of *A. micraster* typically inhabit the fermentation horizon, while other EcM plants spread their roots more commonly in mineral soil in SW Madagascar (Tedersoo et al. 2011; unpubl.). *Asteropeia* associates with a broad range of EcM fungi, most of which are shared with other local EcM plant families (Tedersoo et al. 2011). *Asteropeia* appears to be an ancient group at the base of Caryophyllales, but no age estimates exist for this genus. According to Zanne et al. (2014), the stem age of *Asteropeia* dates to around 34 Ma. *Asteropeia* is sister to *Physena* (Physenaceae), another Malagasy endemic with no known mycorrhiza information (Cuenoud et al. 2002; Ducousso et al. 2008).

19.4.5 Fabales

The order Fabales represents an extremely large and ecologically important group of herbs, shrubs and trees that has several times independently evolved and multiple times subsequently lost the N_2-fixing capacity in association with rhizobial *Proteobacteria* (Werner et al. 2014). In addition to this rhizobial association, the typically obligately AM Fabaceae have evolved EcM habit at least seven times. The large Detarioideae subfamily itself contains four distantly related EcM clades that we term as the *Berlinia* group and the *Afzelia* group, following Bruneau et al. (2008), and *Cryptosepalum* group and *Dicymbe* (monogeneric) following the same logic. The distinctness of these four lineages is sufficiently supported in an inclusive and specifically focused phylogenetic study of de la Estrella et al. (2017) but not in earlier studies with less genes and representative taxa (e.g. Bruneau et al. 2008; Smith et al. 2011). The age for the entire Fabaceae and particularly Detarioideae and *Acacia* is greatly underestimated by Zanne et al. (2014) compared with strictly focused studies of de la Estrella et al. (2017) and Miller et al. (2013).

Acacia s.lat. (Mimosoideae) constitutes a large polyphyletic genus (nearly 1400 species) that has EcM-forming representatives only in the Australian *Phyllodina* group (*Racosperma*), known as ***Acacia s.str.*** (unfortunately not recognised as such in the Plant List). *Acacia s.str.* is the largest EcM genus with ca. 1000 accepted species (Miller and Seigler 2012) that represent small trees, bushes and shrubs, which are typically heavily colonised by rhizobia. Partly due to multiple symbiotic partners, certain species of *Acacia* are among the fastest-growing trees in the world. Most species of *Acacia s.str.* Seem to be facultatively EcM, because very often individual plants lack EcM and the level of colonisation commonly remains <10%. There is a tendency for larger species of *Acacia s.str.* (small or large trees) to have

both EcM and AM, whereas the shrubs in this genus tend to have AM only (Chap. 17). Only about 50 species have been examined for mycorrhizas, of which about half have AM and the rest have both EcM and AM roots (Ducousso and Thoen 1991; M. Brundrett unpubl.). In some cases, EcM roots are poorly developed and may be nonfunctional. The conditions required for EcM fungi are poorly understood, but these are probably related to soil texture and organic matter or paucity of certain micronutrients. The EcM fungal diversity associated with species of *Acacia s.str*. Remains unknown, although only a few species from several EcM fungal genera are found under *Acacia* spp. in Australia and exotic plantations (M. Brundrett, pers. obs.). *Acacia s.str*. Diverged from other Mimosoideae 27–24 Ma and radiated shortly thereafter (Murphy et al. 2003; Miller et al. 2013).

Aldina is a small genus of South American trees that belongs to the subfamily Papilionoideae (papilionoid legumes). Root systems of *Aldina* are heavily mycorrhized (>90%) and support a large number of fungal species that are mostly shared with *Dicymbe* spp. (Smith et al. 2011; L. Tedersoo, unpubl.). The global plant phylogeny suggests that the divergence of *Aldina* from other legumes dates back >34 Ma (Zanne et al. 2014). *Aldina* spp. do not associate with rhizobia. The 'igapó' riparian forests of *Aldina* were the main source of mycological collections of R. Singer in the 1970s and 1980s.

The **Mirbelieae** tribe (sometimes also referred to as Bossiaeeae; papilionoid legumes) represents a group of Australian shrubs and bushes that are most widely distributed in the seasonally dry Mediterranean habitats in P-impoverished soils. Most if not all taxa of the Mirbelieae exhibit root symbiosis with rhizobia. Multiple species have been shown to be EcM, but reports from individual studies are often contradictory. The genera *Pultenaea*, *Gompholobium* and *Mirbelia* are consistently EcM and possess well-developed mantle and Hartig net (Chap. 17). Based on the individual reports of genera, it appears that EcM habit is inherent to the core group of Mirbelieae (Warcup 1980). Published information indicates that EcM habit may have been secondarily lost in certain species and genera. In his pioneering work, J. Warcup inoculated seedlings of Mirbelieae with a number of EcM fungal isolates and demonstrated >tenfold growth benefit of inoculation, although the nature of the control treatment was unspecified. These inoculation trials revealed that at least the tested fungal isolates were not selective among host plant group, allowing us to speculate that some Mirbelieae associate with a broad range of Australian EcM fungi. While there is no information about the colonisation level of Mirbelieae root systems, the EcM structures of most taxa appear poorly developed and only partly matching the morphological EcM definition. The widespread genus *Gastrolobium* is associated with a wide diversity of fungi, many of which form hypogeous fruit bodies that are an important food source for animals (Lamont et al. 1985). The EcM group radiated around 40 Ma (Crisp and Cook 2003; Schrire et al. 2005). Along with species assigned to Mirbelieae, Warcup (1980) reported EcM on *Hardenbergia* and *Kennedia*, but these groups belong to Phaseoleae, and only AM has been found in more recent studies (e.g. Brundrett and Abbott 1991).

When referring these groups as Mirbelieae, Warcup (1980) may have misidentified the plants. Furthermore, some species of *Daviesia* have NM cluster roots, but EcM and/or AM have been reported in others (Table 19.2; Chap. 17).

The ***Afzelia* group** (Detarioideae, caesalpinioid legumes) comprises two closely related genera, *Afzelia* and *Intsia*. Bruneau et al. (2008) identified the South American genus *Brodriguesia* as a well-supported sister taxon to these genera within the *Afzelia* group, but there is no information about the mycorrhizal status of *B. santosii* that is endemic to E Brazil. The roots of *I. bijuga* are heavily colonised by EcM fungi (>70%; L. Tedersoo et al. unpubl.), but we have no such data for *Afzelia* spp. In a few studies, *I. bijuga* associated with a wide array of fungi with no obvious specificity patterns in the Seychelles and Madagascar (Tedersoo et al. 2007b, 2011). Species of the *Afzelia* group do not associate with rhizobia. The age and ancestral distribution of the *Afzelia* group are not known, and the dating of EcM habit would strongly depend on the mycorrhizal status of *Brodriguesia*. The stem age of the entire group (incl. *Brodriguesia*) is 62 Ma (de la Estrella et al. 2017).

The ***Berlinia* group** (Detarioideae) represents at least 20 genera of large dominant trees and subcanopy trees in African miombo woodlands and rain forests. Several rain forest taxa of the *Berlinia* group (e.g. *Gilbertiodendron* and *Microberlinia*) form monodominant stands in the mainly AM matrix. Through extremely recalcitrant litter, these trees seem to control the soil conditions that favour proliferation of their symbionts and suppress seedlings of small-seeded arbuscular mycorrhizal plants. The roots are typically heavily colonised (>50%) by EcM fungi, although there are great differences in mycorrhiza density and root branching among tree genera (L. Tedersoo, unpubl.). Individual species and the *Berlinia* group as a whole establish non-specific associations with EcM fungi (Diedhiou et al. 2010; Tedersoo et al. 2011). Species of the *Berlinia* group do not associate with rhizobia. The *Berlinia* group diverged from other Amherstieae 59 Ma and radiated 57 Ma (de la Estrella et al. 2017).

The ***Cryptosepalum* group** (Detarioideae) consists of *Cryptosepalum* spp. and *Paramacrolobium coeruleum* that represent large and small trees in rain forests and miombo woodlands of Africa. *C. exfoliatum* forms monodominant stands in the dry deciduous forests biome in NE Zambia (L. Tedersoo, pers. obs.). The roots of *C. exfoliatum* are heavily colonised (>50%) by EcM fungi (L. Tedersoo, unpubl.). Fungal symbionts of *Cryptosepalum* spp. and *P. coeruleum* are shared with species belonging to the *Berlinia* group (Diedhiou et al. 2010; Tedersoo et al. 2011). Species of the *Cryptosepalum* group do not associate with rhizobia. The *Cryptosepalum* group diverged from other Detarioideae 53 Ma and radiated 34 Ma (de la Estrella et al. 2017).

Dicymbe (Detarioideae) is a genus of trees that is distributed in South America. Several species of *Dicymbe* form monodominant stands that may be codominated with *Aldina* spp. (Henkel 2003). Roots of *Dicymbe* species are heavily colonised by EcM fungi, and the mycobionts are shared with *Aldina* spp. and *Pakaraimaea* (Smith et al. 2011, 2013). *Dicymbe* spp. do not associate with rhizobia. The genus *Dicymbe* diverged from *Polystemonanthus dinklagei* 24 Ma and radiated

18 Ma (de la Estrella et al. 2017). Treatment of *Dicymbe* as a separate EcM plant lineage is important, because it is the only EcM Detarioideae group in South America and it does not belong to the large African *Berlinia* group that was previously hypothesised to have dispersed to South America. Nonetheless, mycorrhizal status of the West African *P. dinklagei* is not known, and thus it is still possible that EcM ancestors of the *Dicymbe* group evolved in Africa.

19.4.6 Malpighiales

Several families of Malpighiales contain EcM groups. Unfortunately, those in Phyllanthaceae and Euphorbiaceae have not been dated using a taxonomically focused approach.

The core group of **Salicaceae** is the most widely recognised EcM lineage within Malpighiales, consisting of *Populus* and *Salix* (including the monotypic *Chosenia*). The EcM Salicaceae are widely distributed from the arctic tundra to temperate forests, extending into tropical areas in riparian habitats. Species of *Salix* and *Populus* differ greatly in the structure and size of roots and EcM tips as well as the degree of EcM colonisation. All examined species of *Salix* and *Populus* are ectomycorrhizal, although several species include individuals that are non-EcM. Low level of EcM colonisation is characteristic to certain phylogenetic groups as well as individuals inhabiting permanently waterlogged conditions (Lodge 1989; Tedersoo et al. 2013). *Populus* spp. associate with a highly diverse set of fungi, a few of which are genus specific. *Salix* spp. associate with fewer fungal species, and the proportion of *Salix*-specific fungal taxa is greater (Tedersoo et al. 2013). Calibrated phylogenies indicate that EcM Salicaceae diverged from AM groups 45 Ma, whereas *Populus* and *Salix* were separated 33 Ma (Davis et al. 2005). Fossil records, however, suggest that modern Salicaceae *s.str.* Evolved 60–55 Ma (Collinson 1992), which we believe is more likely.

Uapaca (Phyllanthaceae) is a genus of small trees in miombo woodlands and rain forests of Africa and Madagascar. Many rain forest *Uapaca* spp. have stilted roots. Fine roots of *Uapaca* are much broader compared with those of other EcM angiosperms. The broad, brittle, red-brown 'fine' roots are characteristic to all studied species of *Uapaca*. Certain large root clusters are heavily mycorrhizal, whereas others are colonised by AM fungi (L. Tedersoo, pers. obs.). *Uapaca* spp. associate with a diverse community of EcM fungi that is shared with the *Berlinia* group and Dipterocarpaceae in Africa and Asteropeiaceae, Sarcolaenaceae and *Intsia* (*Afzelia* group) in Madagascar (Tedersoo et al. 2011). *Uapaca* diverged from other Phyllanthaceae <50 Ma and diverged at around 16 Ma (Zanne et al. 2014), but these figures are probably underestimates.

Poranthera (Phyllanthaceae) is a genus of small herbs and shrubs that is distributed in Australia and New Zealand. Several independent authors have consistently interpreted *Poranthera* as an EcM genus but with low level of colonisation and some individuals uncolonised. Some West Australian material examined

did not have EcM roots as these are normally defined (Chap. 17). Inoculated fungi displayed 30–40-fold growth benefit to *Poranthera* sp. in sterile soils (Kope and Warcup 1986). However, these experiments need to be repeated, since growth responses of this magnitude are only likely in cases where fungi detoxify sterilised soils and control plants die. There is no information about the natural fungal associations of *Poranthera*, although EcM has been successfully synthesised with fungi from Myrtoideae (Kope and Warcup 1986). There is limited phylogenetic information about *Poranthera*, although the global analysis of Zanne et al. (2014) suggests they would have split from other Euphorbiaceae around 26 Ma and radiated 19 Ma, which we consider realistic.

19.4.7 Rosales

Pomaderreae is a coherent tribe of Rhamnaceae that is mostly represented by small trees and shrubs in Australia and New Zealand. Unlike some other Rhamnaceae, Pomaderreae spp. do not associate with N_2-fixing *Frankia* actinobacteria. *Adolphia californica* forms a sister taxon to the Pomaderreae (Onstein et al. 2015), but nothing is known about its mycorrhizal or actinorhizal status. The root system of *P. apetala* is heavily colonised by EcM fungi (>90%) and associates with a great diversity of mycobionts. The associated fungi display remarkably strong host preference for either *Pomaderris* or *Nothofagus* + *Eucalyptus* (Tedersoo et al. 2008). Molecular studies indicate that Pomaderreae split from other Rhamnaceae 55 Ma and radiated 41 Ma. Phylogenies indicate that the 'Pomaderreae' genera *Alphitonia* and *Granitites* are placed outside this tribe and are most probably AM (Onstein et al. 2015).

Dryadeae (Rosaceae) represents a tribe of small trees (*Cercocarpus*) and shrubs (*Dryas*) that associate with both EcM fungi and *Frankia* actinobacteria. While *Dryas* and *Cercocarpus* are consistently EcM, available information suggest that *Chamaebatia* is associated with at least *Cenococcum* (Trappe 1964), but *Purshia* forms only AM (studies not focused on EcM: Williams 1979; Rose 1980). Information about *Cowania* is lacking completely. Root systems of *Dryas* are moderately colonised by EcM fungi (>50%; L. Tedersoo, unpubl.), but such information is lacking for other groups. Both *Dryas* and *Cercocarpus* appear to associate with a broad diversity of EcM fungi with no evidence for host specificity (McDonald et al. 2010; Botnen et al. 2014). Dryadeae diverged from other Rosaceae tribes 75 Ma and radiated to currently recognised genera 67 Ma (Chin et al. 2014) that is in a good agreement with a global analysis (Zanne et al. 2014).

Adenostoma is a small genus of bushes not associated with *Frankia* actinobacteria in Western North America. *A. fasciculatum* has been reported to form EcM with poorly developed mantle and Hartig net (Cooper 1922; Allen et al. 1999a), but *A. sparsifolium* has only AM (Allen et al. 1999a). Allen et al. (1999b) observed production of EcM fungal fruit bodies in monospecific *Adenostoma* patches far from other EcM vegetation, indicating its performance as a functional

host. Notably, however, *Adenostoma* does not facilitate recruitment of tree seedlings that contrasts with local Arbutoideae (Horton et al. 1999). Taken together, we interpret *Adenostoma* as a facultatively EcM plant genus. We have no information about the root structure, EcM mycobionts or evolutionary history of *Adenostoma*. The global analysis of Zanne et al. (2014) indicated its separation from extant sister groups <15 Ma.

19.4.8 Malvales

The order Malvales contains two EcM plant groups, viz. Dipterocarpaceae-Cistaceae and *Tilia*. Malvales is a relatively young group that dates back to 80–70 Ma based on multiple studies focused on the entire angiosperms (e.g. Wikström et al. 2001; Zanne et al. 2014; Tank et al. 2015). Unfortunately, phylogenetic relationships within Malvales are poorly resolved and the divergence estimates accounting for continental disjunctions are strongly conflicting with clock-based estimates.

We define the EcM **Dipterocarpaceae-Cistaceae group** as a clade that includes all genera of Cistaceae, Pakaraimaeaceae, Dipterocarpaceae *s.lat*. (incl. Monotoideae and Pseudomonotoideae) and Sarcolaenaceae. Close phylogenetic association of Dipterocarpaceae *s.lat*., and in particular the genus *Pakaraimaea* and Cistaceae, has been evident for a long time (Wikström et al. 2001; Ducousso et al. 2004) but considered as an artefact of poor taxon sampling. Strikingly, modern in-depth phylogenetic analyses confirm these early findings (Zanne et al. 2014; Horn et al. 2016, J. Horn, pers. comm.), indicating that the present assumptions about the evolution and biogeography of these groups need to be drastically revised. From the belowground perspective, the monophyly of Dipterocarpaceae-Cistaceae makes sense, because both groups are well known as EcM hosts. Due to great ecological differentiation and the lack of geographic overlap probably within the last 30 My, these subgroups share no fungal species besides *Cenococcum geophilum*. The roots of all examined species of the Dipterocarpaceae subgroup are of average thickness for angiosperms and appear to be heavily colonised by EcM fungi (>70%), except *Monotes* which has relatively lower colonisation level (<30%) and low level of branching. Relatively low branching and low level of colonisation is as also characteristic of Cistaceae (L. Tedersoo, pers. obs., but see Massicotte et al. 2010). Furthermore, Cistaceae exhibit relatively fine roots and EcM tips compared with other EcM groups. In Mediterranean *Cistus* species, the mantle and Hartig net are often poorly developed, but this may be characteristic of pezizalean symbionts that have been frequently studied in this context. Species of the Dipterocarpaceae subgroup associate with multiple mycobionts and display no host specificity in Asia, Africa, Madagascar or South America (Tedersoo et al. 2011; Peay et al. 2015). This also applies to *Pakaraimaea dipterocarpacea* that is endemic to sandy soils of the Guyana shield (Smith et al. 2013). Little is known about the fungal diversity associated with Cistaceae, but sequence data suggests

that Cistaceae associate with a phylogenetically diverse set but species-poor assemblages of EcM fungi (data available in UNITE: www.unite.ut.ee), many of which are Cistaceae specific (e.g. *Hebeloma* spp., *Cortinarius* spp.: Comandini et al. 2006). According to early vascular plant phylogenies, the Dipterocarpaceae-Cistaceae group diverged from other taxa 33 Ma and radiated to families since 23 Ma (average values from Wikström et al. 2001), which are anecdotally low values. Later, the stem and crown age of this group was pushed back to 73 and 49 Ma, respectively (Zanne et al. 2014). Given the slow evolution and continental disjunctions in these woody plants, the age of Dipterocarpaceae *s.lat*., Cistaceae and *Pakaraimaea* is almost certainly underestimated (Moyersoen 2006; see also Chap. 20).

Tilia is a small genus of bee-pollinated trees that also includes *Craigia* nested therein. The Central American *Mortoniodendron* spp. form a sister group to *Tilia* and *Craigia* (Nyffeler et al. 2005), but there is no information about the mycorrhizal status of this genus. Roots of *Tilia* are heavily colonised by EcM fungi (>90%), and fungal richness tends to be among the highest of all EcM plants (Tedersoo et al. 2014, unpubl.), although no *Tilia*-specific EcM fungal species are known. In contrast to most other EcM trees, litter of *Tilia* species is nutrient rich and degrades rapidly. Richardson et al. (2015) estimate the stem age and crown age for *Tilia* + *Craigia* at 32 and 17 Ma, respectively, but these are certainly underestimates based on the fossil record (Chap. 20).

19.4.9 Asterales

Gnaphalieae (Asteraceae) is a tribe of herbaceous plants (as Inuleae; Warcup and McGee 1983; Warcup 1990) that is comprised of a large number of genera, some of which have been reported as EcM but many others are probably fully non-EcM. Apart from the image of *Podolepis* by Warcup and McGee (1983), the majority of reported associations lack a Hartig net, and the occurrence of a mantle is inconsistent and may require the presence of companion EcM plants. The same genera, or even species, of Asteraceae examined in Australia were reported to be EcM and AM or AM only in different studies (Table 17.2). It seems most likely that all Asteraceae are predominantly AM plants and the role of EcM-like associations on their roots requires further study. Only the crown group of this tribe with Australian distribution comprises EcM members (clades D-X; cf. Bayer et al. 2002). The taxonomy of Gnaphalieae is poorly resolved, with many currently recognised genera being polyphyletic (Bayer et al. 2002). Especially the genus *Helichrysum* stands out in terms of polyphyly as certain species belong to the EcM clade, whereas others belong to the neighbouring non-EcM clades (Smissen et al. 2004). Certain species have distributed from Australia to neighbouring islands, but to our knowledge, the mycorrhizal status of the EcM core group of Gnaphalieae has not been addressed outside Australia. Likewise, there is no information about the natural mycobionts of Gnaphalieae. Warcup (1980) also described the genus

Isoetopsis as EcM, but this genus is closely related to *Aster* (Bayer and Cross 2002), and the report is almost certainly incorrect. The EcM status of most genera and vast majority of species remains poorly understood, but the groups that may have EcM evolved in the time frame of 10–16 Ma (Bergh and Linder 2009).

Goodeniaceae represents another Australian-centred family of herbs and shrubs that are reported as EcM or without EcM, sometimes in the same species. The root system of Goodeniaceae has typically low level of superficial fungal colonisation along with AM, and the roots generally lack a Hartig net (see Fig. 17.5); yet, inoculation with EcM fungi was reported to provide plants 10–100x growth benefits in sterile soils (Warcup 1985), but these experimental results have been questioned (Chap. 17). Information about natural mycobionts of Goodeniaceae is lacking, but EcM-like associations were synthesised using fungi from Myrtoideae (Warcup 1985). Many Goodeniaceae spp. are halophytes or hydrophytes, and these are very unlikely to be ectomycorrhizal. Molecular dating studies suggest that Goodeniaceae is an ancient group that separated from its sister groups 78 Ma and radiated 67 Ma (Jabaily et al. 2014). However, the Cretaceous origin of Goodeniaceae is probably overestimated (Zanne et al. 2014 report around 55 Ma for stem age).

19.4.10 Myrtales

The order Myrtales contains probably a single EcM group—the subsection of **Myrtoideae** that bear dry seeds. The Myrtoideae subfamily has complex mycorrhizal relationships, especially in Australia. Altogether 95 species of Australian Myrtoideae have been assessed for mycorrhizas: 35% with EcM, 36% with AM and EcM and 29% with AM only (M. Brundrett, unpubl.). To illustrate the present knowledge about Myrtoideae mycorrhizal status from a phylogenetic perspective, we mapped the confirmed lineages on a dated tree (Fig. 19.3). Species within many genera of Myrtoideae differ greatly in their consistency of EcM status, level of EcM colonisation and root morphology, which requires further investigation (Brundrett 2009). It is also common for them to have both AM and EcM in their roots. Despite conflicting evidence or a lack of information about the mycorrhizal status of many Myrtoideae genera, there are well-resolved EcM clades, which are phylogenetically centred around *Eucalyptus*, *Leptospermum* and *Melaleuca*. In the crown group of Myrtoideae, the Myrteae, Syzygeae and Metrosidereae tribes have probably secondarily switched to arbuscular mycorrhizal habit (Thornhill et al. 2015), although conflicting and probably incorrect reports on EcM of *Campomanesia* and *Ugni* exist from South America and that of *Syzygium kuranda* from Australia. Evidence that the Myrtoideae gained many of their EcM symbionts from *Nothofagus* in the Late Cretaceous is provided by low specificity of fungi between eucalypts and southern beeches (Tedersoo et al. 2008). General observations suggest that large trees such as *Eucalyptus s.lat*. Host many EcM fungi, whereas bushes and shrubs support relatively low fungal diversity based on fruit-body

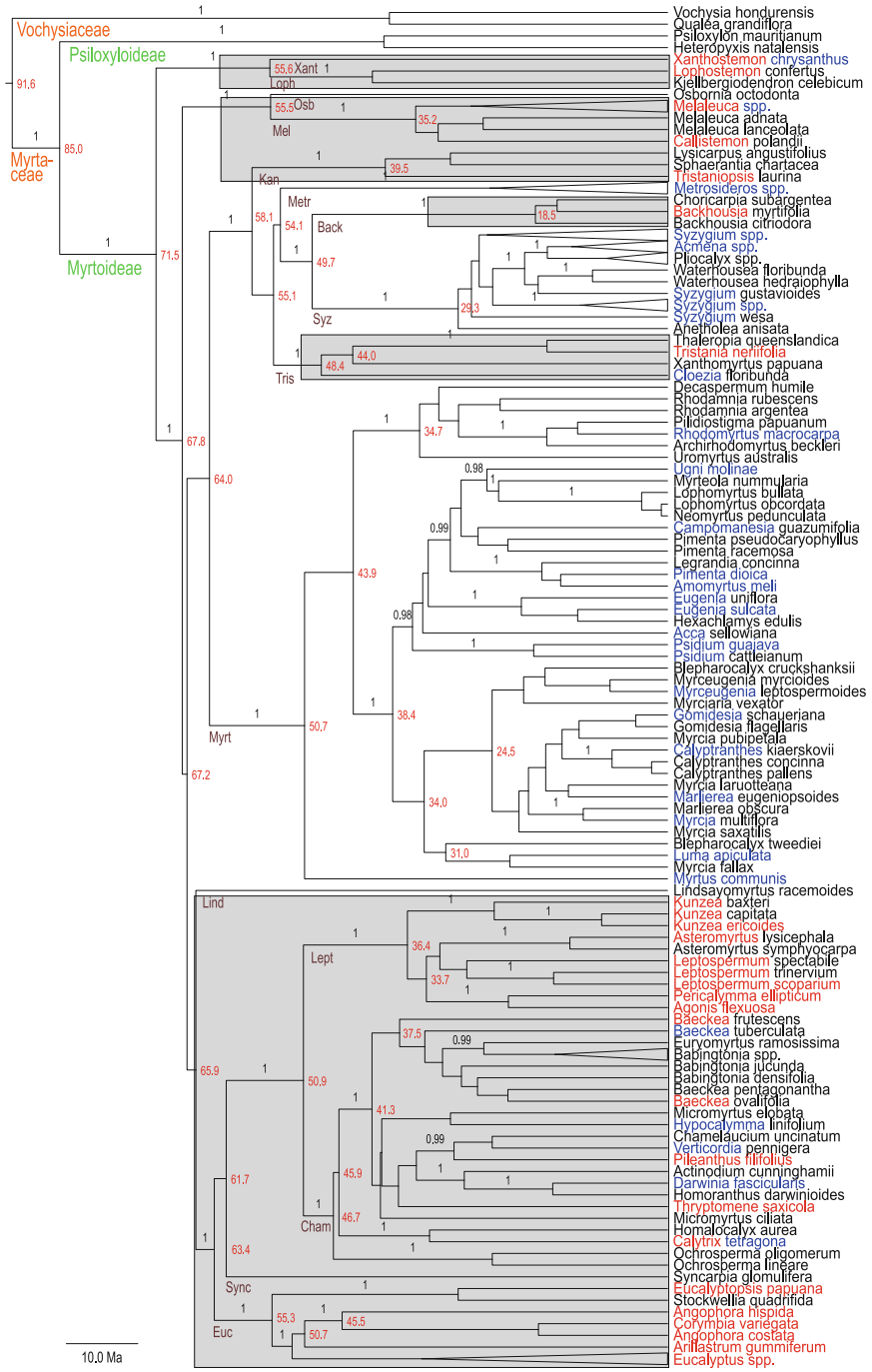

Fig. 19.3 Distribution of mycorrhizal types in the phylogeny of Myrtoideae (Myrtaceae). The backbone tree is adapted from Thornhill et al. (2015). Numbers at nodes and above branches

records (Chap. 17). Interestingly, Myrtoideae are able to associate with indigenous fungi of the Seychelles, Madagascar and continental Africa (Tedersoo et al. 2007b, 2011; Buyck 2008), but not with those of Europe (Pennington et al. 2011). The EcM Myrtoideae diverged from other groups around 85 Ma and radiated 72 Ma. The AM groups evolved probably secondarily between 25 Ma and 65 Ma (Thornhill et al. 2015).

19.4.11 Apiales

Platysace (Apiaceae) represents a single EcM genus in Apiales. The Australian endemic perennial herb genus *Platysace* forms a sister group to *Homalosciadium*, another Australian genus (Nicolas 2009), for which there is no available information about mycorrhiza status. Phylogenies suggest that *Platysace* and *Homalosciadium* diverged ca. 35 Ma, but these were separated from other subfamilies of Apiaceae some 78–84 Ma (Nicolas 2009) that are probably overestimates. *Platysace* spp. have been reported to form a well-developed mantle and Hartig net, and individuals exhibit consistent colonisation (Bellgard 1991; Zemunik et al. 2015), but other plants in the same genera have been shown to be AM only (Table 19.2). The occurrence of EcM in this group requires further investigation since the rest of this family seems to be consistently AM. There is no available information about the root structure, EcM colonisation and associated mycobionts.

19.4.12 Ericales

Ericaceae is one of the largest plant families on earth that is particularly well-known for ericoid mycorrhiza (ErM; Chap. 9). At the base of Ericaceae, however, the AM Enkianthoideae forms a successive sister group to the large ErM and relatively small EcM lineage (Schwery et al. 2015). The ErM lineage comprises subfamilies Cassiopoideae, Harrimanelloideae, Ericoideae, Styphelioideae and Vaccinioideae (Chap. 9), whereas the monophyletic EcM group contains Arbutoideae, Pyroloideae and Monotropoideae (tribes Monotropeae and Pterosporeae: Kron

Fig. 19.3 (continued) indicate divergence times and Bayesian posterior probabilities, respectively. Names highlighted in *red* and *green* denote EcM habit and non-EcM habit, respectively (highlights covering genus name only indicate that different species were assessed for mycorrhiza status). Clades believed to be dominated by EcM habit are shaded. Abbreviated names under major branches indicate tribe names: *Back* Backhousieae, *Cham* Chamelaucieae, *Euc* Eucalypteae, *Kan* Kanieae, *Lept* Leptospermeae, *Lind* Lindsayomyrteae, *Loph* Lophostemoneae, *Mel* Melaleuceae, *Metr* Metrosidereae, *Myrt* Myrteae, *Osb* Osbornieae, *Sync* Syncarpieae, *Syz* Syzygieae, *Tris* Tristanieae, *Xant* Xanthostemoneae

and Luteyn 2005) that we refer to collectively as ***Arbutoideae s.lat***. Of these individual subfamilies, Monotropoideae comprises fully non-photosynthetic, so-called mycoheterotrophic plants that form monotropoid subtype of EcM with usually a thick mantle, intensive intracellular root colonisation of hyphae and extensive digestion of hyphal coils (Smith and Read 2008). Most of the Pyroloideae form pyroloid subtype of EcM that has low to moderate intracellular colonisation and usually lacks a mantle (Smith and Read 2008), but mantle development is a function of plant species, fungal species and habitat (L. Tedersoo unpubl.). Nearly all species of *Pyrola*, *Orthilia* and *Moneses* are partially mycoheterotrophic, whereas *Chimaphila* appears to gain little if any carbon from forest trees via EcM fungi (Tedersoo et al. 2007a; Hynson et al. 2012). Only members of the Arbutoideae subfamily appear fully autotrophic, although they form arbutoid mycorrhiza with intracellular colonisation in addition to a Hartig net and a poorly or fully developed mantle (Smith and Read 2008). Since the fungi and many anatomical features of these specific mycorrhiza types are shared with typical EcM, we continue to consider these as specific subtypes of EcM. While Arbutoideae and *Pyrola*, *Orthilia* and *Chimaphila* from Pyroloideae associate with an extremely wide range of EcM fungi, *Moneses* (Pyroloideae) and members of the entire Monotropoideae display substantial selectivity for specific fungal groups that are often unrelated (Bidartondo et al. 2015). *Moneses* associates with *Amphinema* and *Tylospora* species (Hynson et al. 2015; L. Tedersoo, unpubl.). Both the ErM and EcM groups diverged from the putatively AM ancestor 110 Ma and radiated further since 102–103 Ma (Schwery et al. 2015). Besides this most recent and comprehensive study, other age estimates for Ericaceae and mycorrhizal groups therein are 1.3–3 times more recent, but these conflict with the fossil record.

19.4.13 Cyperales

The genus ***Kobresia*** is a perennial genus of sedges, part of which are EcM in arctic and alpine habitats of the Northern Hemisphere. We consider ectomycorrhizal only the 'uniseriate' group (cf. Starr et al. 2004), which is monophyletic within the paraphyletic *Carex* and contains proven EcM plant species (e.g. *K. myosuroides*, syn. *K. bellardii*). *Kobresia* species outside this core clade are probably non-EcM. However, not all individuals (or perhaps populations) of *K. myosuroides* are EcM, suggesting the facultative nature of EcM mutualism at least in some habitats. *Kobresia* is the dominant plant group in the Tibetan Plateau and other Central Asian lowlands, where the EcM habit is consistently reported in several species. The EcM colonisation of individual plants is relatively low, and EcM roots are arranged as unbranched pinnate terminal roots (resembling the structure of *Alnus* spp.) branching off the main feeder root (L. Tedersoo, unpubl.). *Kobresia* spp. associate with multiple fungal partners that are not specific to this genus (Gao and Yang 2010). The diversity appears to be, however, relatively low (Tedersoo et al. 2012), but this may result from the tundra and grassland habitat, where EcM plant

relative abundance is low compared to forest habitats. Phylogenetic analyses suggest that *Kobresia* is a relatively recently evolved EcM group as the large paraphyletic genus *Carex* dates back 21 Ma (Escudero et al. 2012), which pushes the divergence date of EcM *Kobresia* to <10 Ma (Starr et al. 2004) or <5 Ma (Zanne et al. 2014). The EcM roots of *Kobresia* seem be derived from dauciform roots, which are swollen lateral roots produced by many members of the Cyperaceae (Chap. 21).

19.5 Groups Forming EcM-Like Associations

We have taken a precautionary approach to assigning EcM status to taxa where such evidence is poor or conflicting and descriptions are lacking or open to multiple ways of interpretation. We briefly discuss these taxonomic groups below.

Multiple groups of orchids form the orchid type of endomycorrhiza with typical EcM fungi that colonise root cells, but these associations are morphologically and functionally distinct from EcM (Dearnaley et al. 2012). Certain thalloid liverworts of Aneuraceae family also establish symbiosis with EcM fungi inside the cells of their belowground and aboveground tissues (Bidartondo and Duckett 2010). In both cases, associations with EcM fungi have evolved secondarily, and EcM fungi and their tree hosts are to a greater or lesser extent exploited by orchids and liverworts as the fungal hyphae are digested inside the root cells, indicating mixotrophic and mycoheterotrophic interactions. Notably, these associations are distributed only in the mycorrhizosphere of EcM plants and never distant from EcM vegetation. There is no evidence that orchids and thalloid liverworts can sustain EcM fungi in the absence of other EcM vegetation (Cameron et al. 2008). As obviously non-mutualistic for the exploited fungi, we do not consider these interactions here. Evolution and biogeography of these mixotrophic and mycoheterotrophic plants has been comprehensively addressed in Merckx (2013).

Lobelia is a paraphyletic cosmopolitan genus of contrasting life forms that evolved in Neotropics 55 Ma and spread further to Africa and Australia (17 Ma; Antonelli 2009). The Australian annual *Lobelia* spp. have been demonstrated to form a strange form of root symbiosis with both EcM and AM fungi, but perennial species had only AM (Fraser 1931; Warcup 1988). The mycorrhizal isolates displayed 1–100x growth benefits to plants (Warcup 1988), suggesting functional and beneficial associations to their hosts. However, field samples of other *Lobelia* spp. only had AM in their roots (Brundrett and Abbott 1991). Given the strange seedling development belowground (Fraser 1931), we speculate that some *Lobelia* species may display mixotrophic lifestyle briefly as seedlings, but their mycorrhizas do not conform to the definition of EcM and require further study.

Thysanotus (Laxmanniaceae, Asparagales) is a genus of monocot herbs endemic to Australia, except two species distributed west to East Asia and Indo-Malay (Sirisena 2010). The Australian species have been reported to form ‘thysanotoid’ mycorrhizal associations with both AM and EcM fungi (Chap. 17). Aseptic

synthesis experiments recovered up to twofold growth benefits that were evident in the presence of another mycorrhizal plant (McGee 1988). These experiments suggest that *Thysanotus* spp. may exhibit mixotrophic associations with EcM or endophytic fungi. Given the association with EcM fungi and formation of EcM-like sheath but not Hartig net, the Australian *Lobelia* and *Thysanotus* warrant further mycological, ecological and physiological research to resolve their mycorrhizal status.

19.6 Some Remarkable Examples of Incorrect Reports

The topic of diagnosis and misdiagnosis of mycorrhizal associations is discussed in detail elsewhere (Brundrett 2009), so we present only a few striking cases that have taken root or become influential in mycorrhizal ecology. Both false-positive and false-negative reports about the EcM status of plants are common. False-negative observations are at least partly related to the fact that only AM colonisation has been assessed or insufficient fresh/living material has been studied. False-positive EcM reports may be derived for multiple reasons:

(1) The authors consider any hyphal network on the root surface as a mantle that is indicative of EcM (work of J. Warcup and his followers, early and middle twentieth-century researchers).
(2) Consideration of a weft of dark septate endophytic (DSE) hyphae as poorly developed EcM of *Cenococcum* (work of T. Dominik and his students).
(3) Careless tracing of roots leading to sample contamination (e.g. EcM reports in ferns, work of early researchers).
(4) Misidentification of plant species (suspected in some reports of J. Warcup).
(5) Careless suggestion of mycorrhiza type based on fruiting habits of fungi without clear belowground evidence (work of B. Peyronel, R. Singer, D. Pegler and that of many other mycologists; summarised in Trappe 1962).
(6) Influence from former publications and wishful thinking.
(7) A general tendency to exaggerate the significance of observed fungal structures in an attempt to publish a 'more interesting' story.

Some of these incorrect or incomplete reports have been widely accepted and further cited by other authors without critical reassessment (e.g. Daft et al. 1985; Wang and Qiu 2006; Smith and Read 2008; Phillips et al. 2013; Fisher et al. 2016; Maherali et al. 2016; Lin et al. 2017). In particular, Maherali et al. (2016) assessed the evolution of gains and losses of EcM associations in plants based on mapping mycorrhizal status to phylograms of Zanne et al. (2014). In contrast to this review, they considered *Calliandra*, *Gleditsia*, *Lonchocarpus*, *Robinia* and *Senegalia* (all Fabaceae), *Cerasus* and *Padus* (both Rosaceae), *Graffenrieda* (Melastomataceae) and *Ceratopetalum* (Cunoniaceae) as ectomycorrhizal, representing nine additional EcM lineages. For most of these genera, there is ample evidence for the occurrence of only AM in the literature (members of Fabaceae and Rosaceae), or the described

structures cannot be considered EcM (*Ceratopetalum*, *Graffenrieda*). Furthermore, Maherali et al. (2016) ignored altogether 11 EcM plant lineages as described here, although many of these are well established.

In Europe, there are multiple reports of EcM occurrence in Rosaceae, especially in the fruit tree genera *Malus*, *Pyrus* and *Prunus* as well as closely related *Crataegus*, *Padus* and *Sorbus*. These reports are particularly evident in the East European and Russian literature published in the 1950s and 1960s. The same authors describe these plants as EcM or non-EcM in their different studies but provide no illustrative evidence. Most commonly, *Cenococcum* has been reported as a putative symbiont, suggesting that dense colonisation of DSE may have resulted in incorrect assignment of the EcM status. Furthermore, fine roots of Rosaceae exhibit swollen tips; if these become old and turn brown, it is tempting for an inexperienced eye to suspect EcM association. That could be, however, easily checked by examining the squashed root tip under a stereomicroscope. Another example comes from *Juniperus communis* that is known to be AM, but there are several EcM reports that probably represent misidentification of roots. For example, Reinsvold and Reeves (1986) described a tuberculate EcM of '*J. osteosperma*' that is clearly donated by a neighbouring *Pinus* individual. Notably, the roots of pines may distribute >30 m from the trunk even when mature trees are <5 m high. There are several records of EcM in the nitrogen-fixing *Elaeagnus angustifolia* (Elaeagnaceae) in Russia, although reports of the same species and other *Elaeagnus* species from Europe and North America have revealed only AM (see Daft et al. 1985).

In North America, Grand (1971) reported tuberculoid EcM from *Photinia* (Rosaceae), but their images remind us of suilloid mycorrhiza of *Pinus*. More recent reports suggest AM or NM habit for *Photinia* spp. Several physiological experiments have been performed based on inoculation of *Ulmus americana* with EcM fungi. It is anecdotal, because *Ulmus* spp., incl. *U. americana*, are non-EcM and form AM based on multiple reports and authors' personal observations. Morphological studies of these roots by Brundrett et al. (1990) and others have clearly shown that they consistently have AM associations and also have structural features that would make EcM formation unlikely or impossible (suberised epidermis and exodermis). Certain companies (established by former EcM researchers) also promote universal EcM inoculum that supposedly benefits the growth of all trees, including AM trees and *Alnus*.

Of Asian records, *Elaeocarpus* (Elaeocarpaceae) has been reported and illustrated to be an EcM genus in Taiwan (Haug et al. 1994), but multiple previous and subsequent studies indicate only AM colonisation for members of this genus. *Pimelodendron* (often misspelled *Pimeleodendron*) is a small euphorbiaceous genus of trees that is distributed in the Sunda Islands and New Guinea. *P. amboinicum* was reported as EcM two decades ago in New Guinea (Verbeken and Walleyn 1999), but these records remain unconfirmed. More recent stable isotope analyses of EcM and AM plant leaves place *P. griffithianum* deeply into the AM category (Tanaka-Oda et al. 2015). Based on original studies (Tian et al. 2003 and their earlier research), *Robinia pseudoacacia* has been misinterpreted as

EcM by Wang and Qiu (2006). The original descriptions by Bratek et al. (1996) indicated either AM or some intracellular colonisation of *Mattirolomyces terfezioides*, which is not an EcM fungus.

In South America, many authors have carelessly claimed that certain plant species host putatively EcM fungi. Oft-cited examples include *Allophylus* (Sapindaceae), *Pradosia* (syn. *Glycoxylon*; Sapotaceae), *Haematoxylum* (syn. *Haematoxylon*; Fabaceae), *Swartzia* (Fabaceae) and *Inga* (Fabaceae). Later it appeared that not all these fungi were in fact ectomycorrhizal (*Gyrodon rompelii*, *Phlebopus* spp.); *Aldina*, Pisonieae and *Coccoloba* represented local hosts (Meyer 1991; Moyersoen 1993). Other commonly cited South American EcM associations were reported by Thomazini (1974) who claimed that *Campomanesia* (Myrtoideae) and *Bauhinia* (Fabaceae) form EcM in Brazil. Furthermore, Frioni et al. (1999) reported EcM associations in *Gleditsia*, *Senegalia* (as *Acacia bonariensis*), *Calliandra*, *Prosopis* and *Lonchocarpus*. However, multiple more recent studies have been unable to confirm these findings, reporting only AM. *Graffenrieda* (Melastomataceae) has been described to possess a specific type of ectendomycorrhiza (Haug et al. 2004). Given its phylogenetic position, poorly developed mantle-like structure and association with typical root endophytic/ fungi related to *Rhizoscyphus ericae*, we interpret this as somewhat differentiated root endophytic interaction rather resembling ericoid mycorrhiza.

In Africa, Högberg and Piearce (1986) suggested EcM habit for *Faurea* (Proteaceae) and *Pericopsis* (Fabaceae), which are commonly cited as examples of African EcM plants. However, several other studies as well as the first author's observations suggest that these trees are not EcM in Africa or elsewhere. Recently, Bechem et al. (2014) conducted an extensive survey of mycorrhizal status in plants of Cameroon, reporting EcM habit for *Angylocalyx*, *Baikiaea*, *Baphia*, *Calpocalyx*, *Dialium* and *Hymenostegia* (all Fabaceae), *Antidesma* (Phyllanthaceae), *Leptonychia* (Malvaceae) and *Soyauxia* (Peridiscaceae) in addition to known EcM members of the *Berlinia* group and *Uapaca*. Roughly half of these findings are not supported by previous studies at genus level, but others lack independent evidence.

In Australia, the floristic distribution of EcM habit is particularly complicated, because commonly accepted EcM plants such as shrubs in the Myrtoideae other than eucalypts may have poorly developed mycorrhiza structures. The pioneering work of Warcup (1980) can be regarded as the most confusing, because he was the primary describer of EcM in multiple plant groups, but he also followed a relaxed criterion for EcM by considering plants with a hyphal weft on a root surface as mycorrhizal. Because he rarely provided illustrations and did not describe the methods used in synthesis trials, his findings have been heavily criticised (Brundrett and Abbott 1991; Brundrett 2009). Nonetheless, subsequent evidence has confirmed some of his striking findings, whereas others appear very unlikely in the context of plant phylogeny and subsequent studies (mycorrhizas.info/ozplants). Therefore, we consider the genera *Lasiopetalum* (Sterculiaceae), *Thomasia* (Sterculiaceae), *Pimelea* (Thymelaeaceae), *Opercularia* (Rubiaceae) and *Isoetopsis* (Asteraceae) as insufficiently supported for EcM habit. Based on updated

phylogenetic and mycorrhizal information, we also consider doubtful and unlikely the EcM status of the following Australian genera: *Ceratopetalum* (Cunoniaceae), *Astroloma* (Ericaceae), *Comesperma* (Polygalaceae), *Erythrophleum* (Fabaceae) and *Stylidium* (Stylidiaceae, Asterales). The latter genus represents a group of perennial herbs in Australia that is reported as EcM with poor mantle and Hartig net development by Warcup and his students. Interestingly, several species of *Stylidium* are reported to be protocarnivorous, but this is not supported by substantial evidence. Other doubtful examples of EcM in Australian plants, where more recent studies have only found AM, are listed in Table 17.2. Most discrepancies between earlier and more recent studies of Australian plants result because the Hartig net was used to define EcM in recent studies but not in the past.

Multiple putatively incorrect false-positive reports of EcM have propagated themselves across studies and along research projects. Some of these may represent intermediate steps in the AM to EcM evolutionary continuum but in many cases can be more easily explained as the results of misidentification of fungal structures. We acknowledge that there certainly are cases where a continuum of AM to EcM host plants occurs in the same family or genus, and these are worthy of further study. Some of the worst cases of misidentification warrant published corrections for research articles or PhD theses. However, designation of EcM is complex and the status of some plants cannot be fully resolved by us at this time. This complexity arises because evolution of the EcM symbiosis is an ongoing process that is initiated at the level of plant individuals and populations.

19.7 Losses and ‘Facultative’ EcM Associations

Several EcM plant groups stand out as possessing poorly developed mycorrhizal structures and/or inconsistent root colonisation. Furthermore, some groups comprise multiple species with non-EcM populations (Fagales, Myrtoideae, Dryadeae, *Acacia s.str.*, Mirbelieae, Goodeniaceae, Gnaphalieae), which indicates secondary losses of EcM habit. Maherali et al. (2016) found more losses of EcM habit than gains. Although this is probably true, their analysis was based on incorrectly assigned mycorrhizal types and exclusion of many EcM taxa. Our review suggests that there are two floristic features characteristic of such facultative EcM habit and loss of it: herbaceous or shrubby life form and nitrogen-fixing strategy. It is remarkable that EcM evolution—both gains and losses—is closely related to the nitrogen-fixing habit as seen in Fagales, Fabaceae, Rhamnaceae and Rosaceae that altogether comprise nine EcM groups. Furthermore, there are reports about non-EcM habit for nearly all nitrogen-fixing EcM plants. *Frankia*-associating Myricaceae, some members of Casuarinaceae and perhaps some Dryadeae such as *Purshia* have lost EcM associations. Similarly, certain *Acacia* spp. and Mirbelieae spp. associated with rhizobia seem to have lost EcM capacity completely. A deeper look into the Fabaceae phylogeny (Werner et al. 2014; de la Estrella et al. 2017) indicates that the *Berlinia* group, *Cryptosepalum* group,

Dicymbe and *Afzelia* group evolved EcM associations before the two major Fabaceae groups evolved rhizobial symbiosis. By contrast, the genus *Aldina* evolved EcM associations after the nitrogen-fixing trait was lost in its papilionoid ancestors. The Fabaceae phylogeny also suggests that plants either evolved associations with rhizobia first and then evolved EcM associations with subsequent losses of these EcM associations in some groups (*Acacia s.str.*, Mirbeliae). Such losses of EcM are not seen in non-nodulating lineages of Fabaceae (*Berlinia* group, *Afzelia* group, *Cryptosepalum* group, *Dicymbe, Aldina*; Fig. 19.1). In Fagales, however, the genus *Alnus* and the whole Casuarinaceae evolved actinorhizal associations when ectomycorrhizal (Larson-Johnson 2015). EcM habit was lost in certain Casuarinaceae, and it was reduced in *Alnus* as compared to the sister taxa. Within the Rhamnaceae family, EcM habit in Australian Pomaderreae and actinorhizal state in the Chilean Colletiae and NW American *Ceanothus* is phylogenetically unrelated (Onstein et al. 2015). In Rosaceae, Dryadeae exhibit both EcM and actinorhizal associations, whereas *Adenostoma fasciculatum* hosts only EcM fungi. Thus, it remains unclear whether the EcM habit or actinorhizal association evolved first in Dryadeae, but it is probable that EcM evolved first considering the pathways in *Adenostoma* and *Alnus*. Construction of dated phylogenies of *Frankia* and evolutionary history of symbiosis-related genes in plants may provide an answer to this question.

Root-associated actinobacteria and rhizobia have the potential to render EcM habit redundant for plants, because much of the nutritional benefit of EcM symbiosis is related to nitrogen acquisition. Actinorhizal plants have usually established their niche in early successional habitats that have poorly developed soils with limited nitrogen and little carbon but ample mineral phosphorus supply, except its poor availability at extreme pH values. High phosphorus demand by nitrogen-fixing microbes usually requires assistance of mycorrhizal fungi, probably depending on soil properties and other mycorrhizal benefits. If EcM fungi become too costly for maintenance in terms of carbon energy or phosphorus trade, plants may simply avoid such associations and exploit AM fungi. Except for Myricaceae and *Daviesia* (Mirbelieae), most rhizobial and actinorhizal plants have high dependency on mycorrhiza.

Low level of EcM formation in non-actinorhizal plants is characteristic to arctic and alpine habitats on the one hand (*Persicaria vivipara*, *Kobresia*) and the summer dry Mediterranean biome (Cistaceae in Europe, many plant groups in Australian semidry habitats) on the other hand. Both habitats suffer from severe seasonal drying of soil and paucity of nutrients. The vegetation in these ecosystems is dominated by herbs and shrubs, which may provide insufficient energy to sustain EcM mycobionts. If there are no large EcM trees maintaining the EcM mycelium network, EcM associations may be non-beneficial to plants in AM-dominated communities. Apart from herbs and shrubs, slowly growing trees may also display reduced EcM colonisation in heavily drought-stressed conditions (Lodge 1989; Swaty et al. 2004), further reinforcing the hypothesis of low carbon availability. EcM fungi may not be efficient enough in organic-poor substrates that are derived from low rates of leaf litter accumulation or frequent fires. Over time, Mediterranean

and arctic plants may have evolved low colonisation and mycorrhiza biomass to optimise between benefits and costs of EcM mycobionts.

Similarly to seasonally very dry habitats, wetland plants tend to have reduced EcM colonisation. Since EcM fungi have high oxygen demand due to active metabolism, anoxic environments are not optimal for EcM growth. This has been shown experimentally for *Salix*, *Melaleuca* and *Casuarina* species which have both EcM and EM roots and grow in wet habitats but primarily form AM roots when soil is waterlogged (Lodge 1989; Watson et al. 1990; Khan 1993).

Arctic and alpine habitats are dominated by herbs and shrubs, for many of which there are conflicting reports about the mycorrhizal status. Dwarf *Betula* and *Salix* as well as *Dryas*, *Bistorta vivipara* and certain *Kobresia* species are nearly always EcM. In addition to these well-established EcM groups, individuals of *Potentilla* spp., *Saxifraga* spp., *Cassiope tetragona* and *Pedicularis* spp. are strikingly commonly reported as EcM by independent researchers in different regions, although most studies treat these as NM or AM (Table 19.3). Arctic species of *Potentilla* (Rosaceae) have been reported as EcM in four studies but only AM or NM in 15 studies. *Saxifraga oppositifolia* (Saxifragaceae) has been considered EcM in three studies but NM or AM in 13 studies. Kohn and Stasovski (1990) reported

Table 19.3 Conflicting reports for EcM and non-EcM status in selected arctic and alpine plants

Genus	References
Pedicularis (Lamiales: Orobanchaceae)	Stutz (1972), Kohn and Stasovski (1990), Väre et al. (1992), AM: Dominik et al. (1954), AM: Mikeladze (1960), NM: Katenin (1972), NM: Baikalova and Onipchenko (1988), NM: Treu et al. (1996), AM/NM: Clemmensen and Hansen (1998), NM: Onipchenko and Zobel (2000), NM: Cripps and Eddington (2005), NM: Cázares et al. (2005), AM: Li and Guan (2008)
Cassiope (Ericales: Ericaceae)	Stutz (1972), Miller and Laursen (1978), Miller (1982), Kohn and Stasovski (1990), ErM: Bledsoe et al. (1990), ErM: Väre et al. (1992), ErM: Michelsen et al. (1996), ErM: Clemmensen and Hansen (1998), ErM: Treu et al. (1996), ErM: Cázares et al. (2005)
Saxifraga (Saxifragales: Saxifragaceae)	Stutz (1972), Read and Haselwandter (1981), Kohn and Stasovski (1990), AM: Stahl (1900), AM: Costantin and Magrou (1926), NM: Daubenmire (1941), AM: Thomas (1943), AM: Nespiak (1953), AM: Katenin (1972), NM: Baikalova and Onipchenko (1988), NM: Väre et al. (1992), NM: Treu et al. (1996), AM/NM: Clemmensen and Hansen (1998); NM: Ruotsalainen et al. (2004), NM: Cázares et al. (2005), non-EcM: L. Tedersoo, unpubl.
Potentilla (Rosales: Rosaceae)	Thomas (1943), Bledsoe et al. (1990), Clemmensen and Hansen (1998), Cázares et al. (2005), AM: Schlicht (1889), AM: Jessen (1914), AM: Klecka and Vukolov (1935), NM: Daubenmire (1941), AM: Nespiak (1953), AM: Mikeladze (1960), AM: Read and Haselwandter (1981), AM and NM: Lesica and Antibus (1986), AM: Baikalova and Onipchenko (1988), NM: Kohn and Stasovski (1990), AM: Väre et al. (1997), AM: Onipchenko and Zobel (2000), AM: Kovacs and Szigetvari (2002), NM: Cripps and Eddington (2005), non-EcM: L. Tedersoo, unpubl.

EcM colonisation in 75% of *S. oppositifolia* individuals but none of *S. tricuspidata* individuals in the Canadian Arctic. In *Cassiope tetragona*, EcM root tips in addition to intracellular colonisation have been recovered in four studies, while six studies report only ErM. In Ellesmere Island, 44% of *C. tetragona* individuals were considered EcM (Kohn and Stasovski 1990). In the hemiparasitic *Pedicularis capitata*, EcM was reported in two out of eight individuals, but *P. hirsuta* was non-mycorrhizal (Kohn and Stasovski 1990). Across all studies, EcM has been reported in *Pedicularis* spp. three times but AM or NM associations ten times. Many other arctic plant genera have been reported as EcM only once or twice (e.g. *Silene*, *Campanula*, *Homogyne*; Read and Haselwandter 1981), but these are likely to be incorrect. In all these four above-mentioned arctic/alpine EcM groups, the EcM habit has been described for one or a few closely related species. If not systemically incorrect, these results suggest either a recent evolutionary shift to EcM strategy or facultative EcM habit for a group of species. It is possible that in *C. tetragona* and *S. oppositifolia*, EcM trait is characteristic of populations and has not become a common trait for a species. Therefore, also population-level analyses are urgently needed to shed further light into the ongoing EcM evolution and adaptive EcM to non-EcM balance in plants. From this perspective, some of the orphan EcM reports may actually represent recent evolutionary trends that cannot be captured in other congeneric species or populations of the same species. The alternative explanation of a highly facultative nature of EcM habit is also likely, because both local and regional processes (soil moisture, pH, limiting nutrients, neighbouring plants, climate) may affect the potential benefits of EcM habit and thus associations with EcM fungi. Nonetheless, in the era of molecular identification technologies, we urge that the authors confirm their unconventional findings of EcM habit with molecular tools or at least voucher the material for such possibility. We also strongly recommend that such novel findings be illustrated for a possibility of alternative interpretation (e.g. Haug et al. 2004).

Besides nitrogen-fixing bacteria, many EcM plants exhibit dual root colonisation with AM fungi. This seems to be a relic of the ancestral AM habit in vascular plants (Cazares and Smith 1996), but it certainly represents an adaptation for nutrition early in ontogeny or at low availability of EcM inoculum. In Salicaceae, much of the EcM colonisation level is phylogenetically determined (Tedersoo et al. 2013), but it depends on soil moisture (as above; Lodge 1989) and nutrient demand (van der Heijden 2001) at the individual and species levels. This indicates that dual mycorrhizal symbiosis may secure the plant host with sufficient nutrients and plants can optimise among the mycorrhiza types or even among fungal individuals (AM fungi: Werner and Kiers 2015) to maximise nutritional benefits. In natural conditions, most dual mycorrhizal plants in Fagaceae, Salicaceae and Myrtoideae become more dominated by EcM fungi at the sapling stage (Dominik 1956; Chen et al. 2000; Egerton-Warburton and Allen 2001), which can be explained by improved carbon availability and accumulation of recalcitrant litter with nutrients in the organic form that favours EcM symbionts over AM mutualists.

19.8 Conclusions

Our study took a critical view on the EcM status of plants and assigned 335 putatively EcM genera with roughly 8500 species into 30 phylogenetically well-delimited lineages. Because of multiple reversals to AM-only habit in several species-rich Australian EcM groups, we believe that around 250–300 genera and 6000–7000 species can be considered consistently ectomycorrhizal, but there is an urgent need for additional analyses especially in Australia and Central America. Based on phylogenetic evidence, the multiple losses of EcM habit in favour to AM (or NM in Myricaceae) and decline in EcM colonisation are related to the evolution of symbiotic nitrogen fixation and reduction of trees and bushes to shrubs and herbs, that is, a common adaptation to harsh Mediterranean and arctic/alpine climate. We also point to multiple potentially erroneous reports, many of which have propagated themselves in the literature, in a hope to better inform subsequent ecological and mycorrhizal studies.

Refining our knowledge about the mycorrhizal status of both fungi and plants will strongly improve our understanding about the evolution of EcM symbiosis. Furthermore, it will have strong implications on our understanding of ecosystem functioning on landscape and global scales due to differential nutritional balance that potentially affects all guilds of soil organisms (Phillips et al. 2013; Averill et al. 2014; Soudzilovskaia et al. 2015; Fisher et al. 2016). Mistakes in mycorrhizal type assignments in modelling studies of ecosystem function may severely bias our understanding of the ecosystem processes and biodiversity. For example, a number of meta-analysis and regional studies of mycorrhizal importance or functioning have included many misallocations of host plants in their datasets, so their results are in doubt. We recommend that an agreed list of EcM hosts be developed as an essential resource for future mycorrhizal and ecological studies. This would be based on the comprehensive summary we have provided here, with resampling and/or reassessing taxa where required.

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Chapter 20
Global Biogeography and Invasions of Ectomycorrhizal Plants: Past, Present and Future

Leho Tedersoo

20.1 Introduction

The vast majority of terrestrial plants obtain mineral nutrients via mycorrhizal fungi. Arbuscular mycorrhizal (AM), ectomycorrhizal (EcM) and ericoid mycorrhizal (ErM) fungi deliver differential benefits to their host plants because of their contrasting ability to access soil organic nutrient pools and water. These mycorrhizal effects are well reflected in the distribution of plant species on a landscape scale in boreal and temperate forests of the Northern Hemisphere (Read et al. 2004). Although strongly confounded by historical processes and past climatic fluctuations, specific patterns of distribution of mycorrhiza types are also evident on a global scale (Read 1991; Allen et al. 1995; Brundrett 2009). Both landscape-scale and regional-scale differences in the distribution of mycorrhiza types strongly affect the basic soil processes such as decomposition and N and P cycling (Phillips and Fahey 2006; Phillips et al. 2013; Brzostek et al. 2015; Soudzilovskaia et al. 2015). However, EcM habit may play relatively different roles compared with AM symbiosis in different ecosystems (Mayor et al. 2015) The dominant groups of EcM plants produce more slowly decomposing litter, although multiple exceptions exist (Cornelissen 1996; Koele et al. 2012). Since a vast majority of plants are arbuscular mycorrhizal, information about the global distribution of EcM plants would make a great leap forward in understanding the distribution of soil processes that favour EcM symbiosis (Soudzilovskaia et al. 2015).

EcM plants have evolved multiple times and persisted in at least 30 occasions, differing greatly in the estimated time of divergence as well as richness of species and genera (Chap. 19). EcM plant groups may have marked differences in their habitat preferences, life form, local dominance and floristic traits including

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palatability, nutrient concentration and decomposability (Cornelissen 1996; Brundrett 2009; Koele et al. 2012). However, the historical and extant distribution patterns in relation to climate change and occurrence of suitable soil properties have remained poorly understood for individual EcM plant lineages and for this entire functional group taken together (Dickie et al. 2014b).

Besides natural dispersal, human introductions have greatly shaped the distribution patterns of plants. Since there are very few important crops among EcM plants, most EcM tree species have been planted in exotic habitats as forestry plantations for timber, to reduce erosion and for ornamental purpose (Richardson 1997). In favourable habitats, many of these plants became naturalised and sometimes invasive (Richardson and Rejmanek 2004). Since forest plantations and perhaps invaded stands will continue to provide an important source for timber, understanding the invasion ecology of these exotic EcM trees becomes increasingly more important for both economic and conservation purposes.

In this synthesis, I first review the available information about the calibrated phylogenies and fossil record to identify the area of origin for the 30 EcM plant groups. By using databased observations and sporadic distribution maps, the current geographic range of these EcM groups is mapped and discussed in the historical biogeographic and EcM biodiversity context. In addition, I provide an overview of invasive EcM plants by addressing their invasion history and underlying mechanisms. Finally, these data are discussed in an overall synthesis about the future biogeographic scenarios considering climate change, emerging pathogens and human impact.

20.2 Sources

Digitising the biological collections and availability of these data in specific repositories has greatly improved our understanding about the distribution and function of living organisms (Graham et al. 2004). The Global Biodiversity Information Facility (GBIF; www.gbif.org) was used to compile geographically recorded information about observations and specimens of EcM plant genera that were determined as such and separated into 30 monophyletic EcM plant groups (Chap. 19). Fossil records, clearly erroneous records (i.e. placed in ocean and latitude or longitude with zero values) and duplicate records were removed. Collections represented by geocode of countries' midpoint, certain botanical gardens, dendrological parks or exotic plantations were ignored. The nonnative range represented by >1 non-redundant records were considered for interpreting the nonnative distribution of plant groups. Since floristic information about Russia and former soviet states of Central Asia was disproportionately poorly available, I used the maps of the Interative Agricultural Ecological Atlas of Russia and Neighboring Countries (www.agroatlas.ru). I also utilised the Atlas of North European Vascular Plants (http://linnaeus.nrm.se/flora/) and Missouri Botanical Gardens (www.mobot.org/MOBOT/Research/APweb/) and information from

Kubitzki and Bayer (2003) to supplement and confirm the GBIF information. These data w compared with published biogeographic and phylogentic studies for consistency. The GBIF data were imported to a global base map in QGIS 2.16.3 (OSGeo, Switzerland) for producing distribution maps and generating an overall EcM plant lineage diversity heat map.

Basic information about the exotic distribution of EcM plants was obtained from the GBIF data set. I further studied relevant literature, local checklists and data bases about native and endemic plants, exotic plants and invasive plants. This information was supplemented by regional checklists of invasive plants (Henderson 2007; Howell 2008; Richardson and Rejmánek 2011; Simberloff and Rejmanek 2011; Rejmanek and Richardson 2013; http://www.europe-aliens.org/; http://www.arc.agric.za; http://www.nzflora.info/; www.invasiveplantatlas.org/; http://www.hear.org/gcw/) that are not always specifically referred to. The detailed overview about the native and introduced range of EcM plant genera is given in Supporting Information (http://dx.doi.org/10.15156/BIO/587454).

Plant taxonomy follows the Plant List (www.theplantlist.org). Terminology and interpretation of geological time follows Gradstein et al. (2012). The main geological, climatic and evolutionary events in EcM symbiosis are illustrated in Fig. 20.1.

20.3 Distribution of EcM Plant Lineages

20.3.1 Pinales and Boreal Forests

All **Pinaceae** are EcM trees that are widely distributed in the Northern Hemisphere (Fig. 20.2a). The large and well-known genera *Pinus, Picea, Abies* and *Larix* are distributed in all northern continents, whereas *Keteleeria, Cathaya, Nothotsuga* and *Pseudolarix* have a very narrow extant range in E Asia (Wang and Ran 2014). Although Pinaceae have no paleological records from the Southern hemisphere, *Pinus merkusii* has dispersed south of equator (until 2.10°S) in the Barisan range of Sumatra during the Pleistocene. Tropical Pinaceae (except *Pinus*) are confined to montane habitats with relatively cool climate, which may have prevented their migration across a few hundred kilometres of lowland ecosystems to South America or further east in Malesia along with many other plant groups. The genera *Abies, Picea, Pseudotsuga, Larix, Tsuga* and *Pinus* exhibit disjunct distribution in Europe, E Asia and N America. Fossil records and phylogeographic studies indicate that early ancestors of *Pinus* migrated across the Beringian land bridge from E Asia to N America, whereas other genera dispersed in the opposite direction (reviewed in Wang and Ran 2014).

South China is inferred to be the ancestral area for the Pinaceae as a whole and centre of paleoendemism and neoendemism for most extant genera (Wang and Ran 2014). Multiple reconstructions of Pinaceae evolution are conflicting in establishing the age of divergence, but most studies converge to the estimates of

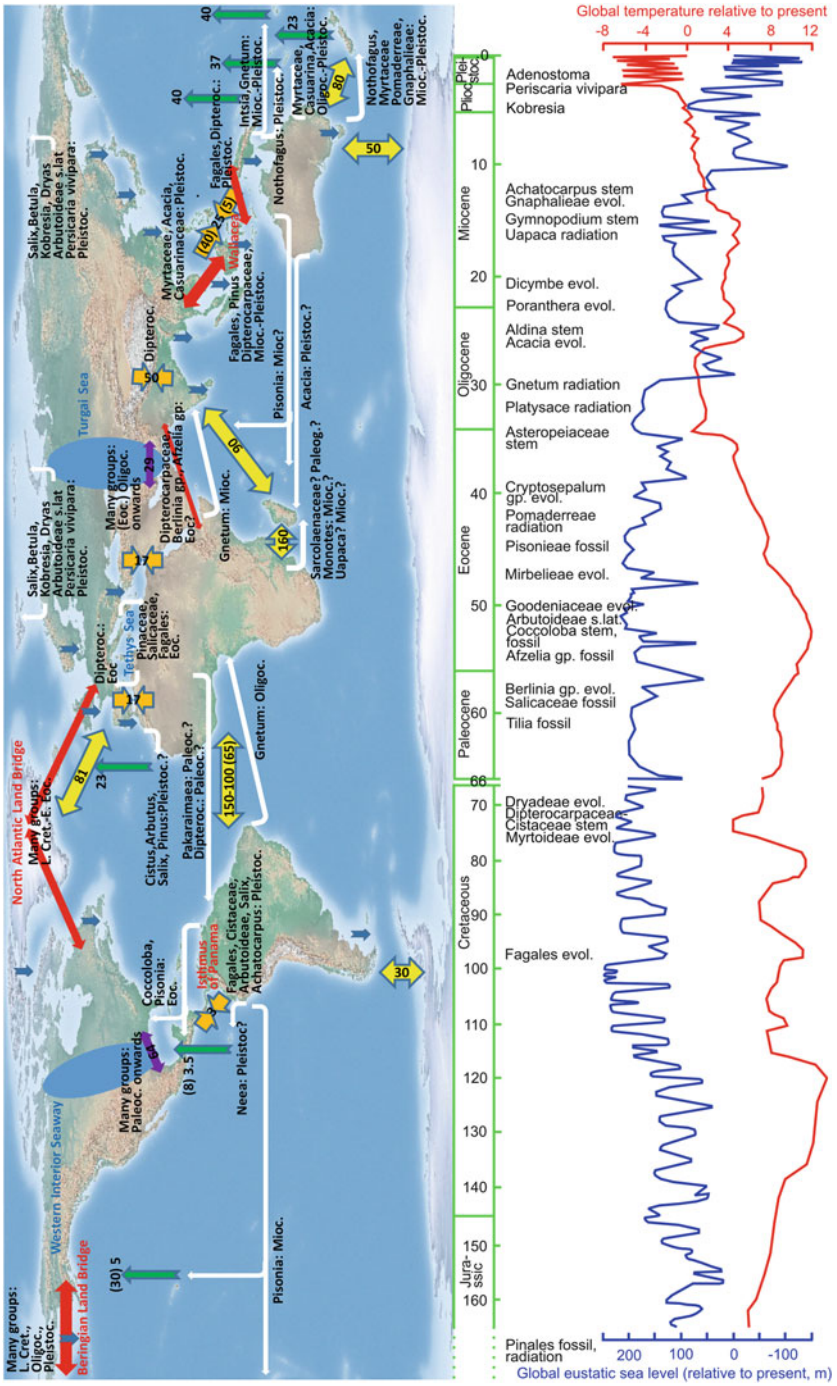

Fig. 20.1 Schematic overview of the main historical biogeographic events in the distribution and evolution of ectomycorrhizal symbiosis in plants. (**a**) The main continental disruptions (*yellow arrows*), historical land bridges (*red arrows*), land bridges across interior seaways (*violet arrows*),

Pinaceae radiation into the currently circumscribed genera from the Early to Late Jurassic (Leslie et al. 2012; Lu et al. 2014) but with apparent paucity of unambiguous fossil material from that period. Since the Late Jurassic to Eocene, fossil Pinaceae have been mostly found in contemporaneous montane temperate and subtropical habitats in Asia and NW America (LePage 2003; Taggart and Cross 2009). At high latitudes, Pinaceae were sporadically present in montane habitats. Surprisingly, this group was very poorly represented in Alaska and NE Siberia in the Late Cretaceaeous and Paleocene (Herman 2013). By contrast, ancestors of most extant Pinaceae genera were present and among the dominant taxa in mountains of the Canadian Arctic (75–78°N paleolatitude) and Svalbard from the Mid-Cretaceous (ca. 120 Ma) to Mid-Eocene (Richter and LePage 2005; Harland et al. 2007), with Taxodiaceae dominating in lowland habitats. Mainly the ancestors of *Pseudolarix*, *Picea* and *Keteleeria* became dominant in high latitudes of Asia and North America after the Late Eocene climate cooling and established the vast temperate and boreal coniferous forests biomes in lowland habitats (Taggart and Cross 2009). In the Cretaceous, Pinaceae were nearly absent from the Central and S European archipelago, but multiple genera of Pinaceae migrated to Europe from North America over the North Atlantic land bridge in the Paleocene and Eocene or from Asia after the recession of Turgai Strait in the Oligocene (Manchester 1999; Donoghue and Smith 2004).

Multiple genera of Pinaceae dispersed to Borneo across the emerging land connections in the Early Oligocene and especially in the Mid-Miocene, with savanna-inhabiting *Pinus* spp. crossing the equator in the Greater Sunda Islands (Morley 2000). Similar pine savannas were widely distributed in N Africa south to 20°N since the Early Oligocene. Climate change eliminated all this Pinaceae-dominated vegetation from much of N Africa and Malesia by the Late Miocene (Morley 2000). The Early Pleistocene glacial cycles probably extirpated *Tsuga* and *Pseudolarix* from Europe (Manchester 1999; Svenning 2003). Besides the 11 extant genera, four extinct Pinaceae genera from the Cretaceous era are known from the Northern Hemisphere. Most of the described paleospecies are related to *Pinus* and *Pseudolarix* (*Pityostrobus* and *Obirastrobus*) and *Keteleeria-Cedrus* group (*Pseudoaraucaria*), further substantiating the importance of these groups in the early history (Smith et al. 2016).

Due to their rapid growth, good-quality timber production and ornamental properties, many species of Pinaceae are widely planted in their native habitats as

Fig. 20.1 (continued) continental collisions (*orange arrows*), emergence of islands (*green upward arrows*), Pleistocene land connections at low sea levels (*downward blue arrows*) and the main long-distance dispersal events/pathways in EcM plant groups (*white arrows*). Numbers indicate initiation of the event in Ma (and end if given in an interval). (**b**) Fluctuations of global temperature (*red line*; compiled and redrawn and from Zachos et al. 2001 and Price et al. 2013) and eustatic sea level (*blue line*; compiled and redrawn from Haq 2009, 2014) from the Mid-Jurassic to present. Note differences in scale for the Mesozoic and Tertiary. The scale (Ma) follows Gradstein et al. (2012). The most relevant evolutionary events in ectomycorrhizal plant lineages are indicated on the time scale

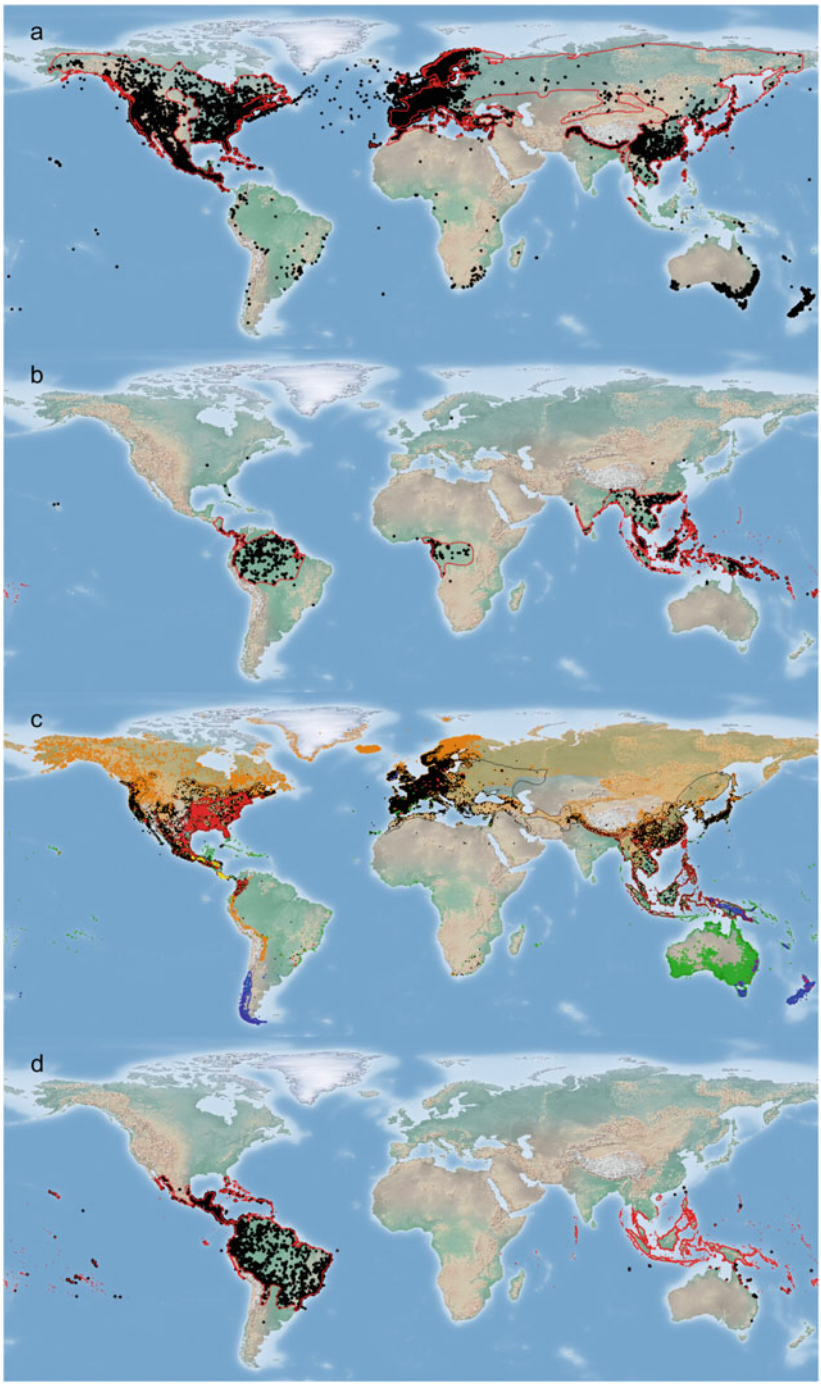

Fig. 20.2 Native range of (**a**) Pinaceae, (**b**) *Gnetum*, (**c**) Fagales, (**d**) Pisonieae, as indicated by *red border lines*. GBIF records are indicated as *dots*. For Fagales; *blue*, Nothofagaceae; *green*, Casuarinaceae; *red*, Juglandaceae; *black*, Fagaceae; *orange* (and *orange shading*), Betulaceae;

well as forest plantations outside their natural range. A handful of species of the genus *Pinus* are massively planted in the tropical dry forest, savanna and grassland biomes in both hemispheres. *Pinus* spp. fail to associate with local EcM fungi and require co-introduced symbionts for establishment in exotic plantations and outside (Hayward et al. 2015b). In seasonal tropical and southern temperate ecosystems, different species of Pinaceae have become invasive. Tropical species of *Pinus* (especially *P. radiata, P. caribaea, P. merkusii* and *P. kesiya*) have become highly invasive in seasonal grassland-dominated tropical ecosystems of Central S America and S Africa, somewhat less so in India, Madagascar and Australia (Richardson 2000). Certain species of *Pinus* and *Pseudotsuga menziesii* have become invasive in the southern temperate forest habitats in S Chile, W Argentina and New Zealand. Many exotic species of Pinaceae have also become invasive in northern temperate forests. For example, the North American *Picea sitchensis, Pinus strobus* and *Pinus contorta* are regarded as unwanted alien pests in several Central and W European countries (http://www.europe-aliens.org/). The invasion of Pinaceae in grasslands and shrublands is particularly alarming, because open habitats are transformed into forest. While the initial effects of establishing *Pinus* spp. are context-dependent, adult trees strongly modify the soil towards greater acidity, deeper humus layer, lower moisture content and lower decomposition rates compared with deciduous trees and shrubs (Richardon 2000; Augusto et al. 2015). The acidic and allelopathic litter of *Pinus* spp. prevents the establishment of understorey that further loops back to greater acidity and recalcitrance of humus (Richardson 2000). Build-up of deep litter layer together with high flammability of the trunk and leaves transforms the invaded ecosystems to greater susceptibility to heavy fires.

20.3.2 Gnetales

Based on fossil record, gnetophytes represented one of the prominent gymnosperm groups in warm temperate and tropical habitats from the Triassic to Cretaceous, with massive extinctions in Paleocene (Morley 2000). Besides the monotypic *Welwitchia*, ***Gnetum*** is the only extant plant genus in this gymnosperm lineage. The recently diverged EcM genus *Gnetum* is distributed in rainforest habitats worldwide (Fig. 20.2b). Modern species of *Gnetum* diverged in South and Central America in the Oligocene (Won and Renner 2006). Via birds or rafting, *Gnetum* dispersed to Central Africa and became established in the rainforests in the Late Oligocene. Species of *Gnetum* probably dispersed through the African continent to East Africa and subsequently established in Indo-Malay in the Early Miocene, followed by waves of speciation, including development of tree forms

Fig. 20.2 (continued) yellow, Ticodendraceae. Note that Betulaceae is overlaid by Fagaceae that is overlaid by Juglandaceae, leaving an illusion about their overall dominance in Europe and North America, respectively

(*G. gnemon* and *G. costatum*) in the Mid-Miocene. Most of the current species are narrow endemics in Malesia and New Guinea that represent the main centres of neoendemism (Won and Renner 2006). *G. gnemon* has probably more recently dispersed to Micronesia, Solomons, Vanuatu, New Caledonia, Fiji and Samoa. Humans may have distributed this fruit species to smaller islands and further in Polynesia including Hawaii (Won and Renner 2006). *G. africanum* may have been similarly distributed by humans, because its leaves represent one of the most popular crops in Central Africa. Besides the recent range expansion, species of *Gnetum* must have become extinct from E Africa and coastal S America. *Gnetum* species become easily naturalised near gardens, but none are known to be invasive.

20.3.3 Fagales and Temperate Forests

Fagales is considered as a single EcM lineage that has multiple reversals to AM or non-mycorrhizal state (Chap. 19). EcM families within Fagales display contrasting patterns of distribution on a global scale (Fig. 20.2c) that results from vicariance, long-distance dispersal and large-scale extinction events. Fagales have an excellent fossil record, because these wind pollinated trees are prolific pollen producers. The early Fagales were collectively assigned to the 'Normapolles group' (>60 fossil genera) that represented one of the most widespread and diverse pollen types from the Mid-Cretaceous (95 Ma) to Paleocene in tropical and warm temperate Europe and NE America (Friis et al. 2006; Larson-Johnson 2015). These land masses were connected over the North Atlantic land bridge and were collectively termed as the 'Normapolles paleobotanical province'. Although the Normapolles group dominated in Europe and NE America, it was also present in NW America, E Asia and N Africa at relatively low abundance and low diversity (Friis et al. 2006). Following the closure of the Turgai Strait and Western Continental Seaway, vegetation in eastern and western parts of modern continents became homogenised (Donoghue and Smith 2004; Friis et al. 2006), except Europe lost much of its biodiversity perhaps due to its fragmentation into multiple islands (Morley 2000). Although the distribution of extant families (except Ticodendraceae) is centred to S China, it is unlikely to be the ancestral area for Fagales given the early distribution of Normapolles group and paucity of Cretaceous fossils.

The highly disjunct distribution of Nothofagaceae and Casuarinaceae in the Southern Hemisphere relative to other extant fagalean families is paradoxal, suggesting long-distance dispersal over vast oceans or migration across land. Early biogeographers believed that the ancestors of modern *Nothofagus* and several other now extinct early fagalean groups migrated from SE Asia to Australia (Whitmore 1981; Truswell 1993; Veblen et al. 1996) over an ocean barrier >3000 km wide (not considering the mysterious Argoland: Hall 2009; Metcalfe 2012). Raven and Axelrod (1974) proposed that Fagales migrated from Europe to Australia through Africa, but this would have required an even longer trip across the Indian Ocean. Because Nothofagaceae and Casuarinaceae emerged earliest in

Patagonia (Poole 2002; Barreda et al. 2012), it can be speculated that early Fagales may have dispersed from North America across the Proto-Caribbean islands to South America and Patagonia (Morley 2000), which was a widely used migration route in the Late Cretaceous (Wilf et al. 2013). There is, however, no fossil evidence supporting this or any alternative migration route in the equatorial zone 100–80 Ma (Muller 1981; Friis et al. 2006). The modern Nothofagaceae and Casuarinaceae thus probably evolved in the Patagonia-Antarctic Peninsula that were broadly connected to Australia (so-called 'Weddellian floristic province') in the Late Cretaceous and Paleocene.

At present, the Nothofagaceae family has a highly disjunct distribution in the Southern Hemisphere including E Australia, New Zealand, W Patagonia, New Caledonia and New Guinea, where it inhabits humid temperate forest ecosystems (Veblen et al. 1996). Dating back to Mid-Cretaceous, the Nothofagaceae family was the first extant group that diverged from the rest of the fagalean complex (Larson-Johnson 2015). Nothofagaceae diverged rapidly in the Weddellian province and became disjunctly distributed after its break-up 50–30 Ma (Hill 2004). *Nothofagus* was present in New Guinea until the Late Eocene but became re-established from N Australia in the Late (or Mid-) Miocene (Morley 2000). New Zealand lost much of its flora and fauna during a period of nearly complete submergence in the Oligocene, and it was re-colonised by *Nothofagus* from Australia in multiple events in the Miocene and Pleistocene (Wallis and Trewick 2009). New Caledonia was completely submerged in the Eocene, and it persistently emerged above water in the Late Eocene (37 Ma; Grandcolas et al. 2008). It was colonised by *Nothofagus* from Australia, New Guinea or New Zealand ca. 2 Ma, followed by rapid sympatric and allopatric speciation (Hope 1996; Cook and Crisp 2005). It is notable that early biogeographers considered long-distance dispersal of *Nothofagus* nearly impossible, which used to be a cornerstone for vicariance biogeography (Veblen et al. 1996). This paradigm also motivated research in co-biogeography of associated EcM fungi and pathogens (Horak 1983; Pirozynski 1983).

The Late Cretaceous forests of the Weddellian province were dominated by various AM gymnosperms with abundant tree ferns and ferns in the understorey (Hill 2004; Poole and Cantrill 2006; Wallis and Trewick 2009). Signs of the major angiosperm groups, Proteaceae and early members of Fagales emerged in the fossil record around 94 Ma (Poole 2002; Hill 2004; Wilf et al. 2013). Within the next 10 million years, angiosperms became an important component of the canopy and *Nothofagus* radiated into multiple recognisable paleospecies in New Guinea, Australia, Antarctica, New Zealand and Patagonia (Hill 2004). In the Eocene, E Australia and N Australia were dominated by warm tropical rainforests, where angiosperms including *Nothofagus* became increasingly abundant. The reign of *Nothofagus* continued in the Oligocene, when the global climate cooled, but remained humid. With successive drying since the Mid-Miocene, the Australian *Nothofagus* rainforests became replaced by sclerophyll forests dominated by *Eucalyptus* and Casuarinaceae. In the Pliocene, the once extensive forests of diverse *Nothofagus* became depauperate and fragmented. The abundance of Poaceae and

Asteraceae promptly increased in Central Australia that was accompanied with the extension of dry sclerophyll forests and shrublands at the expense of rainforest (Hill 2004). Although fire was a prominent feature in Central Australian vegetation since the Late Cretaceous (Crisp et al. 2011), rapid spread of ecosystems with regular wildfire further reduced rainforest habitat as its vegetation was maladapted to burning (Hill 2004). Similar trends in vegetation were evident in Patagonia until the Miocene. In Eocene, *Nothofagus* was dispersed north up until 5°N in W South America (Jaramillo et al. 2006), and it occupied both E Patagonia and the Falklands (Markgraf et al. 1996). By the Mid-Oligocene (30 Ma), South America became fully separated from Antarctica. In the Mid-Miocene (15–12 Ma), both the Andean uplift was initiated and the cold Circum-Antarctic current was established (Palazzesi et al. 2014). The latter event resulted in successive glaciation of the isolated Antarctic and extinction of much of its biota, with *Nothofagus* persisting until the Late Pliocene or Early Pleistocene in the Antarctic Peninsula and coastal habitats (Poole and Cantrill 2006). Active glacier formation caused overall cooling and aridification in the Southern Hemisphere and in the rest of the world. In Patagonia and New Zealand, *Nothofagus* forests contracted to roughly their present-day margins in the Mid-Miocene (Markgraf et al. 1996; Wallis and Trewick 2009). In the Southern Hemisphere, Pleistocene glaciations wreaked havoc especially on the vegetation of Patagonia. Fossil evidence suggests that forests disappeared south of 43°S in glacial maxima and temperate *Nothofagus* forests survived in the western side of Cordilleras in a narrow strip in Central Chile. The current northern distribution margin of *Nothofagus* at 43°S is limited by moisture availability (Veblen et al. 1996).

Casuarinaceae is another family with Southern Hemisphere distribution, with *Allocasuarina* restricted to Australia. As an exception, the beach crest tree *Casuarina equisetifolia* has dispersed from Australia via Malesia westward until Bangladesh and northward to the Philippines by floating seeds or birds. It has also dispersed east to New Caledonia, Fiji, French Polynesia and Samoa (Parrotta 1993). *Casuarina grandis* and *C. oligodon* are endemic to New Guinea, whereas *C. junghuhniana* is endemic to eastern Sunda Islands and *C. collina* to New Caledonia. Casuarinaceae first emerged in Patagonia in the Late Cretaceous (Barreda et al. 2012) and subsequently in Australia and New Zealand in the Early Paleocene (Muller 1981; Macphail 2007) and became common in the sclerophyll vegetation after climate drying, especially in Central Australia (Hill 2004). At present, SW Australia has the greatest richness of *Casuarina* and *Allocasuarina* species. Historically, Casuarinaceae had long-distance dispersed to S Africa. In Africa and S America, Causarinaceae persisted until the Mid-Miocene (Campbell and Holden 1984; Coetzee and Muller 1984), but in New Zealand until the Pleistocene (Lee et al. 2016). Apart from Australia and New Zealand, other continents may have been colonised only by non-mycorrhizal members of the family, i.e. the genus *Gymnostoma*, which cannot be reliably differentiated based on pollen morphology.

The Betulaceae family is distributed widely across the tundra, boreal and temperate zones of the Northern Hemisphere, reaching perhaps greatest ecological

importance in wet tundra habitats. Several genera such as *Carpinus* and *Alnus* extend to S Central America by dispersal along the mountain chains. Furthermore, *Alnus* has spread across the Isthmus of Panama that opened 2.8 Ma (O'Dea et al. 2016), with current distribution in the high montane rainforest and mixed paramo habitats south to NW Argentina. In NW Colombia, *Alnus* emerged in the Early Pleistocene and became one of the dominant trees by the Mid-Pleistocene (van der Hammen 1974). There are also doubtful Pleistocene records of *Alnus* in Suriname (van der Hammen 1974), but this is unlikely given the separation by vast lowland tropical forests and potential for contamination by eastward blown pollen (Muller 1981). The earliest fossils of both *Alnus* and *Betula* date back to the end of Mid-Cretaceous in E Asia, Late Cretaceous in N America and Paleocene in Europe (Muller 1981). *Alnus* colonised Borneo in the Oligocene (Chen et al. 1999), but became regionally extinct more recently.

The Fagaceae family has a wide distribution in the Northern Hemisphere from cool temperate to tropical biomes. SE Asian species of *Lithocarpus* and *Castanopsis* successfully colonised Malesia in multiple waves since the Late Eocene (Cannon and Manos 2003) and reached New Guinea probably in the Pleistocene. In Central America, the *Quercus* group migrated via the Cordilleras southward. *Quercus humboldtiana* crossed the Isthmus of Panama in the Mid-Pleistocene and became a dominant tree in montane rainforests of NW Colombia in the Late Pleistocene (van der Hammen 1974). The rare NW Colombian endemic *Colombobalanus excelsa* probably also originates from Central America, where it became extinct in the Pleistocene. Different forms of Fagaceae first emerged in E Asia in the end of Mid-Cretaceous (90–85 Ma) and appeared in N America and Europe some 10 Ma later (Song et al. 2004). Based on fossil record, the principal genera of Fagaceae became abundant in subtropical and warm temperate habitats in the Late Cretaceous reaching up to 80°N paleolatitude in the Eocene (Jahren 2007). These genera diversified and adapted to cooler climate in the Oligocene (Axelrod 1983). The genus *Fagus* appeared in Puerto Rico in the Late Oligocene (Graham and Jarzen 1969), although Fagales do not occur in the Caribbeans (except W Cuba) at present.

The EcM group of Juglandaceae (Engelhardioideae and *Carya*) has a more narrow Northern Hemisphere distribution compared with Betulaceae and Fagaceae, and it is naturally absent from Europe and West Asia at present. The genus *Englehardia* has the widest distribution spanning from W Himalayas to E China and Taiwan. *Engelhardia* has followed the route of *Lithocarpus* in colonising much of Malesia in several waves and reaching New Guinea probably in the Pleistocene. In Central and NE America, species of *Carya* represent important canopy trees in temperate and subtropical forests. The neotropical genus *Alfaroa* extends from Central America to NW Colombia, but its history of colonisation is unknown. The *Oreomunnea*+*Alfaroa* clade was present in W USA in the Late Cretaceous, in SE USA in the Late Cretaceous (Muller 1981), in Panama since the Late Eocene (Graham 1985) and in Puerto Rico in the Late Oligocene (now extinct; Graham and Jarzen 1969). The Engelhardioideae has been recorded in Europe from the Mid-Paleocene to Late Eocene (Muller 1981; Manchester 1999). Similarly, *Carya*

had a broad Northern Hemisphere distribution including Europe from the Late Paleocene to Early Pleistocene (Muller 1981; Svenning 2003).

Ticodendron incognitum from the monotypic Ticodendraceae family has a fragmented range from S Mexico to Panama, which are obviously remnants of once much wider distribution. Other members of this family were distributed in North America and Europe in the Early Eocene (Manchester 2011).

The Northern Hemisphere genera of Fagales have experienced multiple dispersal events across the North Atlantic land bridge >30 Ma (*Ticodendron*, *Carya*, *Betula*, *Castanea*, *Quercus*) and the Beringian land bridge more recently (*Carya*, *Alnus*, *Betula, Fagus*) (Axelrod 1983; Donoghue and Smith 2004; Manchester 2011). While *Quercus* probably originated in N America, other extant Fagaceae genera as well as Juglandaceae and Betulaceae seem to have Asian origin (Donoghue and Smith 2004). These patterns are well reflected in extant species richness (Manos and Stanford 2001). Europe had nearly all northern fagalean genera present in the Oligocene, but many of these became extinct in the Pleistocene (Svenning 2003).

Individual species of Fagales have been widely introduced to nonnative habitats. European settlers extensively planted species of Fagaceae and Betulaceae in the Americas, S Africa, Australia and New Zealand as ornamental trees. More recently, inoculated *Quercus* spp. have been planted as hosts for edible truffles, whereas *Carya*, *Castanea* and *Corylus* have been introduced as sources of edible nuts and sometimes additionally as hosts for truffles. The current broad range of *Castanea sativa* probably reflects Roman time introductions from glacial refugia in the Balkans and Apennines northward and westward (Mattioni et al. 2013) and more recently all over the world. Species of *Alnus* have been planted to control erosion and reduce N limitation. The European species *Alnus glutinosa* has become invasive in New Zealand, S Africa and certain parts of Patagonia, occupying mostly the riparian habitats. *Betula pendula* has become invasive in SE Australia and New Zealand. Of the Casuarinaceae family, *C. equisetifolia* has been widely planted to coastal habitats to prevent erosion and for ornamental value since the early 1800s (Parrotta 1993; Potgieter et al. 2014). However, erosion has been often intensified instead, because the allelopathic litter prevents growth of more fine-rooted and more strongly mycotrophic herbs. *C. equisetifolia* has become invasive in most of the tropical sites where it is planted, including coastal areas of Africa, NE America, SE America, E Asia and oceanic islands (Potgieter et al. 2014).

20.3.4 Caryophyllales

The EcM **Pisonieae** (Nyctaginaceae) are distributed in the New World from S Florida and NW Mexico to N Argentina inhabiting semidry to wet habitats in subtropical and tropical biomes (Fig. 20.2d). The EcM Pisonieae are of S American origin (Raven and Axelrod 1974), with a centre of richness in Amazonia (Douglas and Spellenberg 2010). Multiple species have successfully dispersed to the

Caribbean islands. *Pisonia floribunda* is endemic to the Galapagos archipelago, but its mycorrhizal status is not known. The EcM *P. sandwicensis* is endemic to Hawaii (see Hayward and Hynson 2014). By sticky seeds attached to birds, the guanophilic EcM *P. grandis* has been highly successful in westward dispersal to Samoa, French Polynesia, Australian coast, Micronesia, E Asian coast, Maldives as well as coral islands around Rodrigues and Seychelles and near E African coast (St. John 1951). Apart from other Pisonieae, *P. grandis* is a keystone species that forms monodominant stands, and it has been used to revegetate some devastated coral islands (Burger 2005). The fossil records indicate that *P. grandis* has been a characteristic component of the Pacific vegetation for tens of millions of years, since its fossils have been found since the Early Miocene (Leopold 1969). In the American continent, fossil Pisonieae are known from a few records in SE USA from the Mid-Eocene to Miocene (Hueber et al. 1991). The Mid-Eocene fossils attributed to *Pisonia* sp. clearly predate the divergence time estimates for the entire group (<37 Ma; Douglas and Spellenberg 2010; Zanne et al. 2014). There is no evidence for invasive behaviour in EcM Pisonieae.

The genus ***Achatocarpus*** has a disjunct distribution in Central America, NW S America and Central S America (Fig. 20.3a). It is possible that this disjunt pattern is artefactual, given the uneven coverage of GBIF records and the presence of *A. praecox* and *A. nigricans* in both S American habitats and further extension of *A. nigricans* to NE Mexico. Central Mexico harbours the highest number of *Achatocarpus* species, potentially representing the area of origin for this genus. However, the only reliable fossils are described from the Mid-Miocene deposits in Central Patagonia >1000 km south of present southern range limit (Palazzesi et al. 2014). *Achatocarpus* is dated to the Mid-Miocene (Zanne et al. 2014). There is no information about introducing *Achatocarpus* spp. out of their native range.

Coccoloba has a native range from S Florida and NW Mexico to N Argentina and a habitat from rainforests to semideserts (Fig. 20.3b). Most *Coccoloba* species are small trees in rainforest habitats, but this genus includes also climbers, shrubs and emergent trees (Howard 1961). The sea grape (*C. uvifera*) is one of the best known species from this group due to its edible fruits and monodominant coastal forests or thickets in the sandy shores from Florida to Caribbean islands to NE Brazil. The taxonomy and phylogeny of *Coccoloba* spp. is poorly understood, and there are multiple undescribed species in Amazonia. Although *Coccoloba* spp. have successfully colonised the Caribbean islands, they have not managed to establish in the Pacific islands or Africa by natural means. The fossil record of *Coccoloba* is scant, with pollen and wood from the Eocene (sub)tropical flora in E Central USA and SE USA (Gray 1960; Graham 1964) and Panama (Graham 1985). Given its greatest diversity hotspots in Venezuela and the Atlantic rainforests of Brazil (Howard 1961), it can be speculated that *Coccoloba* evolved in Amazonia in the Early Eocene (Raven and Axelrod 1974; Schuster et al. 2013). Of *Coccoloba* species, only *C. uvifera* is widely planted in sea shores to stabilise soil and provide shelter. *C. uvifera* has been planted in the coast of West Africa, Canaries, Mascarenes, Philippines, the Hawaiian Archipelago and several other Pacific

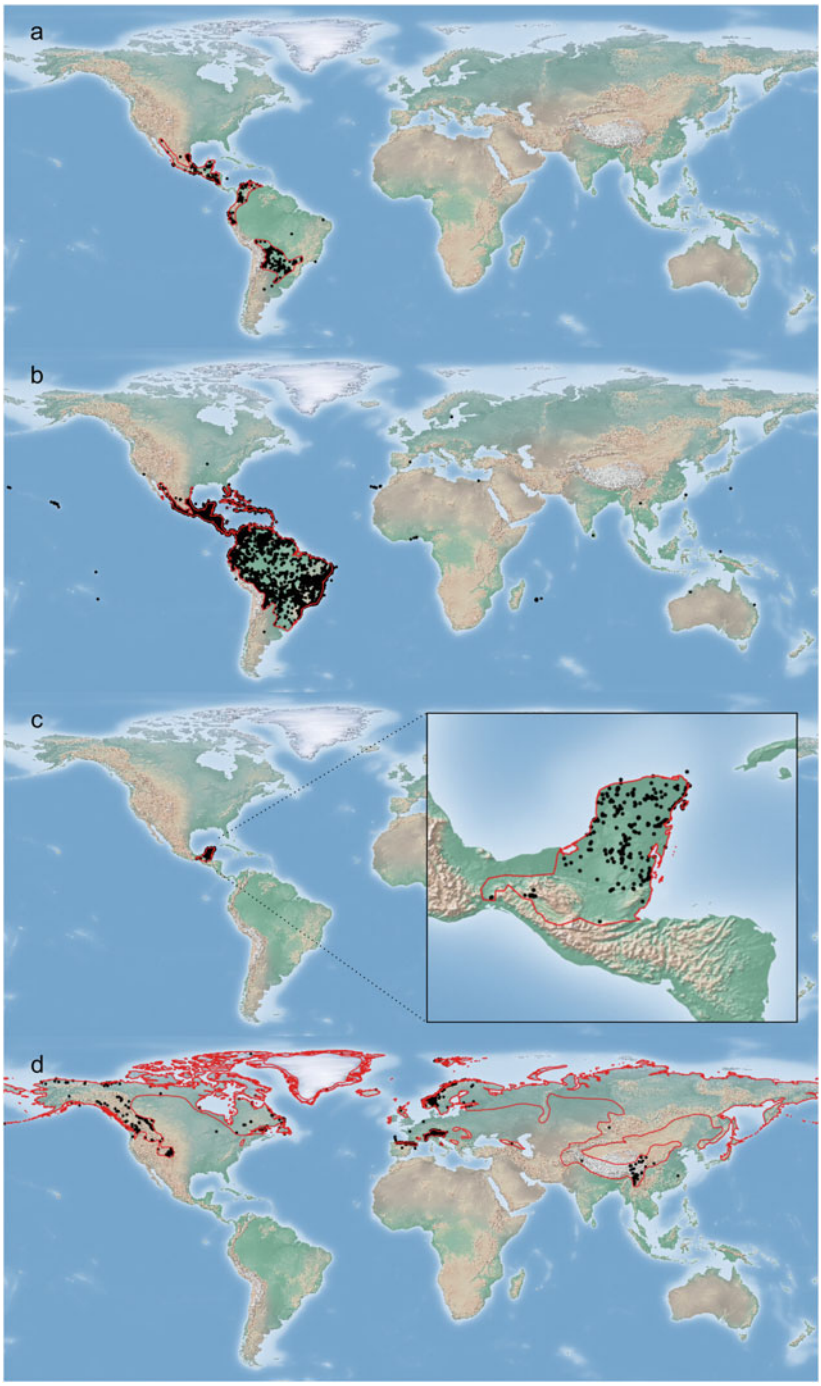

Fig. 20.3 Native range of (**a**) *Achatocarpus*, (**b**) *Coccoloba*, (**c**) *Gymnopodium*, (**d**) *Persicaria vivipara*, as indicated by *red border lines*. GBIF records are indicated as *black dots*

islands, where it has naturalised and regarded as potentially invasive (http://www.hear.org/gcw/).

The two accepted species of genus ***Gymnopodium*** are distributed mainly in the dry tropical forests of S Mexico, particularly in the Yucatan Peninsula (Fig. 20.3c). *Gymnopodium floribundum* forms monodominant thickets that provide a good source for edible mushrooms (Bandala et al. 2011). There is virtually no information about the ecology and biogeographic history of *Gymnopodium*. *Gymnopodium* probably evolved in S Mexico, which may have taken place in the period from Oligocene to Miocene based on calibrated phylogenies (Schuster et al. 2013; Zanne et al. 2014). *G. floribundum* has been cultivated for bee-keeping since the Mayan civilisation (Mejia and Echazarreta 1999) and may have been planted outside its native range for that purpose.

Persicaria vivipara (syn. *Bistorta vivipara, Polygonum viviparum*) is a perennial forb with a wide distribution in the tundra and subarctic forest zone in the Northern Hemisphere (Fig. 20.3d). *P. vivipara* is also widely distributed in the Tibetan plateau, the northern Cordilleras and surrounding highlands. During the glacial maxima, *P. vivipara* migrated south to areas currently covered by warm temperate forests. After glacial retreat, *P. vivipara* survived in multiple mountainous refugia in Europe, Caucasia, NE Asia and N America. In the Arctic zone, this species inhabits the northernmost islands, indicating both its relatively good dispersal ability by birds and relative tolerance to cold and short vegetation season compared with other arctic EcM plants. The polyploid habit may contribute to its wide ecological amplitude (Marr et al. 2013). There are no pre-Pleistocene fossils of *P. vivipara* and no direct phylogenetic treatment of *P. vivipara* and related species. *P. vivipara* is thought to have evolved in Asia in the Pleistocene, because the ancestral diploid forms and morphologically most similar species (e.g. *P. paleacea*) occur in the Tibetan Plateau and E Asia (Li et al. 2003; Marr et al. 2013). *P. vivipara* is widely planted in alpinaria, but there is no evidence for naturalisation due to climate. I believe that *P. vivipara* has extremely high invasion potential in the subantarctic islands because of its ecological versatility and suitable climate. Since mature individuals of *P. vivipara* are not always colonised by EcM fungi (Chap. 19), this species may not require EcM symbionts for completing its life cycle.

Asteropeiaceae is a monogeneric family endemic to Madagascar (Fig. 20.4a). *Asteropeia* spp. have adaptively radiated in semidry shrublands, dry tropical forests and rainforest habitats. The highest diversity of this genus occurs in the eastern rainforest habitat (Birkinshaw et al. 2004). Given its relatively recent radiation from another Malagasy endemic, Physalaceae and other Caryophyllales in the Late Eocene (34 Ma; Zanne et al. 2014), its primary evolution in Madagascar is most likely. Nothing is known about the phylogeography and fossil history of Asteropeiaceae, although this small group may have been easily overlooked. The paucity of fossil record and poor phylogeographic treatment haunts historical biogeographic interpretation of all EcM groups within Caryophyllales.

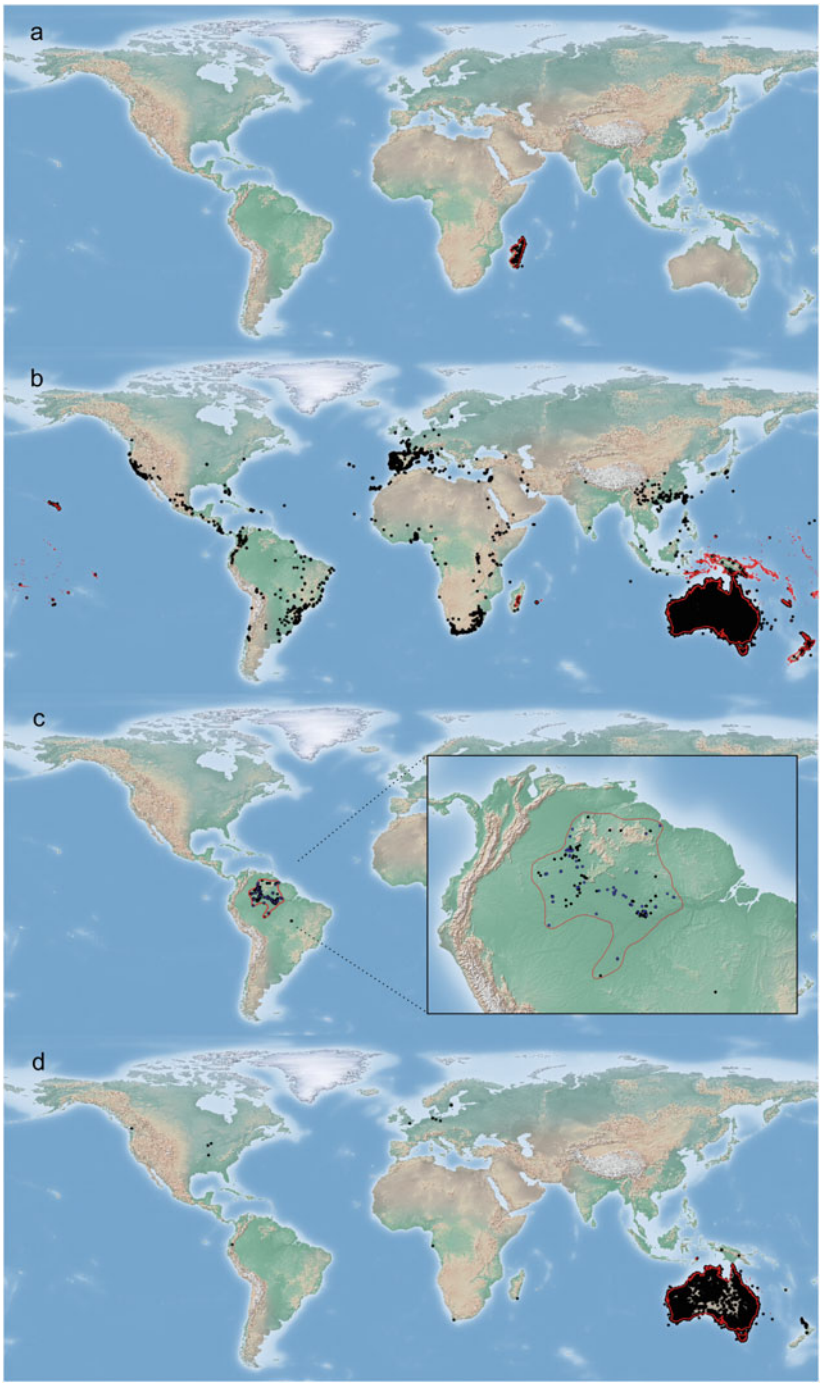

Fig. 20.4 Native range of (**a**) Asteropeiaceae, (**b**) *Acacia s.str.*, (**c**) *Aldina*, (**d**) Mirbelieae, as indicated by *red border lines*. GBIF records are indicated as *black dots*. For *Aldina*, *blue dots* represent additional records from ter Steege et al. (2013)

20.3.5 *Fabales*

Acacia s.str. (Faboideae) includes only the phyllodinoid group (*Racosperma*) that is distributed throughout Australia (Fig. 20.4b). Multiple species of *Acacia s.str*. are distributed outside Australia: *A. simsii, A. crassicarpa, A. aulacocarpa, A. leptocarpa* and *A. auriculiformis* in New Guinea; *A. mangium* in New Guinea and the Moluccas; *A. solandri* in New Guinea and New Hebrides; *A. oraria* in Flores and Timor; *A. spirorbis* in New Caledonia; *A. simplex* in New Caledonia, Fiji, Tonga and Samoa; *A. auriculiformis* in the Kai (Kei) islands of Indonesia; *A. wetarensis* in Wetar*; A. mathuataensis* and *A. richii* in Fiji; *A. confusa* in Taiwan and the Philippines*; A. koa* and *A. kauaiensis* in Hawaii; *A. heterophylla* in the Mascarenes and *A. xiphoclada* in Madagascar (Pedley 1975). Population genetics analyses indicate that *A. heterophylla* in Reunion originates from *A. koa* in Hawaii rather than species in Australia, which represents one of the most extreme long-distance dispersal events (Le Roux et al. 2014). The seeds of *Acacia* are able to germinate after many years of exposure in salt water, which may account for its efficient dispersal (Macphail and Hill 2001). Although *Acacia* is thought to have evolved in humid tropics, SW Australia represents the area for greatest paleoendemism and neoendemism (Mishler et al. 2014). Semidesert areas of Australia that receive only 200–400 mm annual precipitation also harbour high species richness of *Acacia*, but their mycorrhizal status in deserts remains unknown. Similarly, the mycorrhiza status for *Acacia* is unknown in their native habitats outside Australia (except *A. simplex* in New Caledonia that is EcM; Jourand et al. 2014). Many of the Australian *Acacia* spp. may have secondarily reverted to AM-only root symbiosis, but this warrants more detailed research (Chaps. 17 and 19). *Acacia* was estimated to have evolved in the Mid-Oligocene (Murphy et al. 2003), which is agreement with earliest fossils in W Australia and S Australia from the Late Oligocene (Macphail and Hill 2001). According to fossil pollen, *Acacia* was also present throughout New Zealand since the Early Miocene and became extinct during the last glaciations (Macphail and Hill 2001). In contrast to other EcM legumes, *Acacia* spp. have been widely planted outside their native range in wet tropical, semidesert and Mediterranean biomes because of their extremely rapid growth and improvement of soil nitrogen level via rhizobial root association. Altogether 21 species of *Acacia* (especially *A. mangium, A. mearnsii, A. melanoxylon, A. dealbata, A. saligna, A. auriculiformis, A. cyclops and A. longifolia*) have been recorded as invasive especially in the Mediterranean and seasonally dry habitats in South Africa, Madagascar, Central South America, California, East Asia, North Africa and the Iberian peninsula (Richardson et al. 2011). In several cases, *Acacia* species nearly completely replace the indigenous flora due to rapid growth and high flammability. EcM fungi have been rarely found in invasive *Acacia*, suggesting that at least some species may be capable of rapid growth and invasion without EcM symbionts (Ducousso 1990).

The tree genus ***Aldina*** (Papilionoideae, papilionoid legumes) is endemic to Amazonia (Fig. 20.4c). *Aldina* spp. often co-occur with *Dicymbe* spp. in the

white-sand podzols in the Guyana shield. *Aldina* spp. sometimes dominate the plant communities in heavily flooded areas (igapó) along the northern tributaries of the Amazon. In the banks of more nutrient-rich rivers from E Andes, *Aldina* is found more rarely. There is no traceable fossil record of *Aldina* due to paucity of studies in Amazonia. The distribution and calibrated phylogenies suggest that *Aldina* probably evolved in N Amazonia in the Late Oligocene (Lavin et al. 2005; Zanne et al. 2014). *Aldina* spp. are not known to have been planted outside their native range, except in botanical gardens.

The **Mirbelieae** (also referred to as Bossiaeeae) tribe is distributed throughout Australia, with a single species *Gompholobium subulatum* extending to the Wetar island of Indonesia (Fig. 20.4d). Mirbelieae is most diverse in SW Australia and SE Australia with a seasonally dry climate (Crisp et al. 2004), which also potentially represent the ancestral area for this tribe. Mirbelieae probably evolved in the Mid-Eocene (Toon et al. 2014; Zanne et al. 2014). There is no pre-Pleistocene fossil record of Mirbelieae. Multiple species of Mirbelieae have been introduced to New Zealand, where certain members of *Brachysema*, *Callistachys*, *Chorizema*, *Dillwynia*, *Eutaxia*, *Pultenaea* and *Viminaria* have become naturalised (Howell 2008; http://www.nzflora.info/; multiple GBIF records).

Within the ***Afzelia* group** (Detarioideae), the genera *Afzelia* and *Intsia* have strongly contrasting means of dispersal and patterns of distribution. *Afzelia* is widely distributed in the rainforests and miombo woodland belt of Central Africa that receives above 600–800 mm annual rainfall (Fig. 20.5a). The *Afzelia* group and *Brodriguesia* diverged from other Detarioideae in the Early Paleocene. The *Afzelia* group diverged from the closely related *Brodriguesia* in the Late Eocene, whereas *Afzelia* radiated further in the Mid-Oligocene according to dated phylogenies (de la Estrella et al. 2017). The African rainforest species are ancestral, with further distribution to miombo woodlands (*A. africana, A. quanzensis*) and dispersal to SE Asia (as *Afzelia* and *Intsia*; de la Estrella et al. 2017; but see Donkpegan et al. 2017). The woodland species of *Afzelia* evolved 7–10 Ma (Donkpegan et al. 2017) that coincided with severe drying of the African interior (Senut et al. 2009). The genus *Intsia* that is nested within *Afzelia* (de la Estrella et al. 2017) is distributed from coastal rainforests of Madagascar and Seychelles to Indo-Malay, Malesia, New Guinea, NE Australia, New Caledonia and Fiji until Samoa in the east (Fig. 20.4c) due to efficient means of dispersal by water. There are no records of mycorrhizal habit for the sister species to the *Afzelia* group, *Brodriguesia santosii* that is a narrow endemic to NE Brazil (Chap. 19) and probably originates from Africa (Schrire et al. 2005). There is ample evidence that *Afzelia* or *Intsia* spp. (as *Pahudioxylon*) were also distributed in North Africa from the Early Eocene to Miocene (Damblon et al. 1998), although these reports were disregarded by de la Estrella et al. (2017). The *Afzelia* group was present in NW India and possibly throughout the Indo-Malay in the Miocene and Pliocene (Mehrotra et al. 2005), followed by subsequent extinction in much of the Asian range. Both *Afzelia* and *Intsia* species are planted in their native habitats for restoration of woodlands and indigenous forests, respectively, but the species have difficulties in establishment.

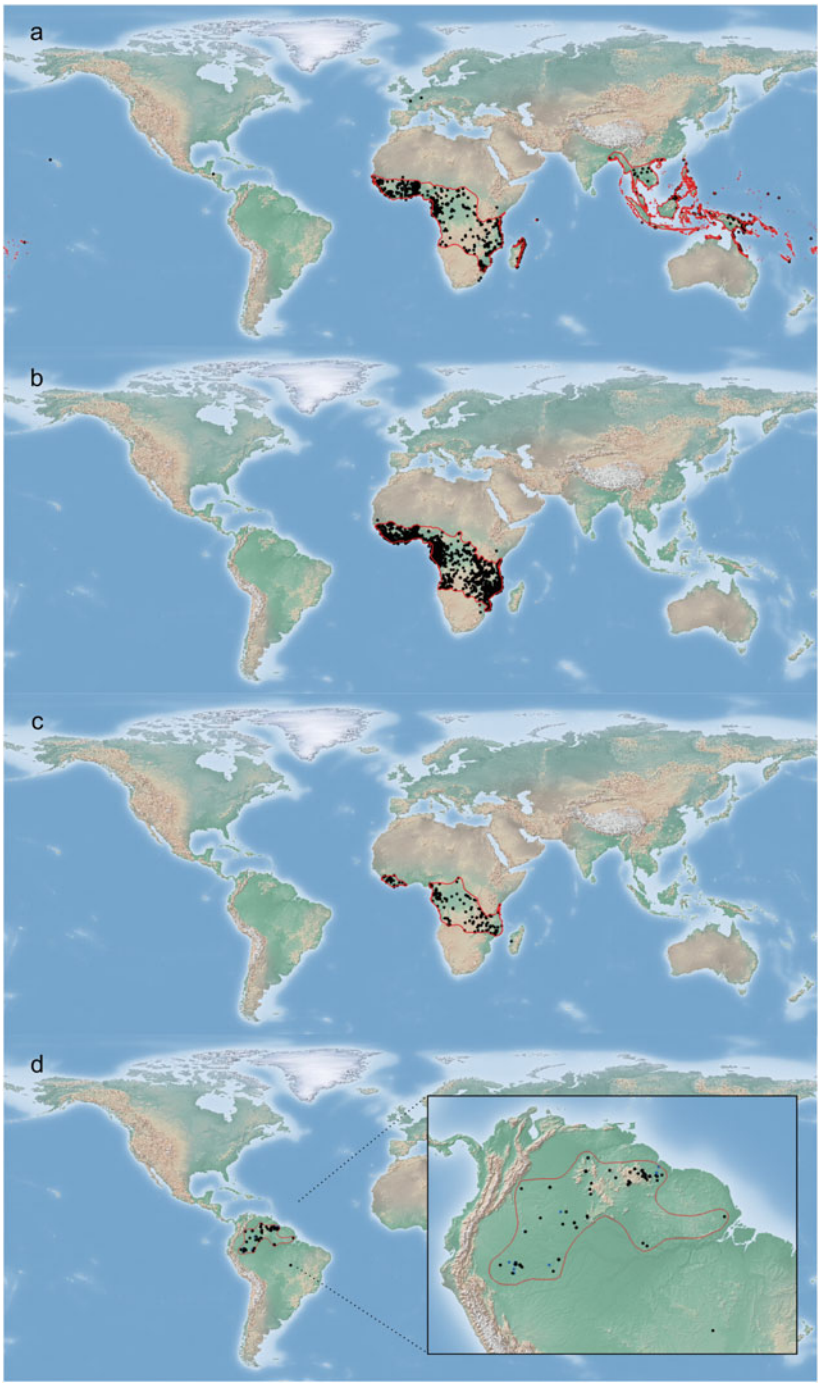

Fig. 20.5 Native range of (**a**) *Afzelia* group, (**b**) *Berlinia* group, (**c**) *Cryptosepalum* group, (**d**) *Dicymbe*, as indicated by *red border lines*. GBIF records are indicated as *black dots*. For *Dicymbe*, *blue dots* represent additional records from ter Steege et al. (2013)

The ***Berlinia* group** is distributed in tropical and subtropical Africa covering the lowland rainforest and miombo woodland habitats (Fig. 20.5b). Many species represent the emergent upper canopy trees, and several species may establish monodominant patches of vegetation (e.g. *Gilbertiodendron dewevrei;* Torti et al. 2001). Species of the *Berlinia* group contribute even more to the vegetation of the miombo woodland belt, commonly accounting for >90% to total basal area (L. Tedersoo, personal observation). In habitats receiving <600 mm annual rainfall, these EcM plants are replaced by other legume trees and bushes that associate with AM fungi and commonly with rhizobia (Burgess et al. 2004; Werner et al. 2014). Throughout their range, the *Berlinia* group is relatively rare in volcanic soils, mangrove habitats and at elevation >1500 m. The *Berlinia* group evolved in rainforest habitats of Central Africa in the Late Paleocene, with later evolution of woodland-inhabiting taxa (de la Estrella et al. 2017). Fossil evidence shows that the *Berlinia* group was also distributed in North Africa from the Late Paleocene to Mid-Miocene and became extinct due to climate cooling and drying (Pan et al. 2010). The *Berlinia* group (*Julbernardia*) occurred in NW India and possibly throughout the Indo-Malay in the Miocene and Pliocene (Mehrotra et al. 2005), with complete extinction from Asia thereafter.

The ***Cryptosepalum* group** is distributed exclusively in Africa, with a geographic disjunction in the Dahomey gap due to the lack of suitable habitats (Fig. 20.5c). *Cryptosepalum* spp. are distributed in lowland rainforests of West Africa and Central Africa and in the dry deciduous forests and moist miombo woodlands of S Central Africa (*C. exfoliatum*). *Paramacrolobium coeruleum* is a sister species to *Cryptosepalum* spp., with a wide distribution in rainforest habitats from Liberia to Tanzania. The *Cryptosepalum* group diverged from other Detarioideae in the Early Eocene and diverged in the Early Oligocene (de la Estrella et al. 2017). Given the phylogeny and age, it is most likely that the rainforest habitat is ancestral in the *Cryptosepalum* group. The only record of fossil *Cryptosepalum* is dated to the Pliocene in Central Ethiopia (Jolly-Saad et al. 2012), >500 km northeast from nearest present-day habitats of EcM Fabaceae. Earlier fossils of wood and pollen of this group cannot be reliably distinguished from that of related Detarioideae.

Dicymbe is small genus of trees that are distributed in lowland rainforests of N Amazonia (Fig. 20.5d). *Dicymbe* species are particularly common on highly weathered white-sand soils originating from the Guyana shield (ter Steege et al. 2013). Especially in Guiana shield, but also in SE Colombia, *Dicymbe* spp. form monodominant stands, which may include also *Aldina* spp. as codominants (Henkel 2003). *Dicymbe uaiparuensis* has the broadest distribution from the Colombian and Peruvian Amazon to the Amapa state of Brazil north of the Amazon. Molecular phylogenies establish separation of *Dicymbe* from the monotypic *Polystemonanthus* in the Early Miocene and futher radiation in the Mid-Miocene (de la Estrella et al. 2017). Because the mycorrhizal associations of the West African *P. dinklagei* remain unknown, there is no information whether the EcM habit of *Dicymbe* evolved in Amazonia or it roots back to Africa similarly to other Detarioideae. Given the shared habitat of *Dicymbe* with the phylogenetically older

South American *Aldina* and Pakaraimaeaceae in poor sandy soils, I consider both scenarios equally likely. This question can be answered by addressing the mycorrhizal status of *P. dinklagei*. There is no evidence for planting species of *Dicymbe*, the *Afzelia* and *Berlina* groups outside of their native range.

20.3.6 Malpighiales

The EcM **Salicaceae** (*Populus* and *Salix*) are widely distributed from the arctic tundra to temperate forests, extending into tropical areas in riparian habitats (Fig. 20.6a). *Populus* and especially *Salix* spp. have exploited riparian corridors for migrating south to Central Argentina (*S. humboldtiana*), S Africa (*Salix* spp.), Kenya (*P. ilicifolia*) and Indo-Malay (*S. tetrasperma*). Throughout Sub-Saharan Africa, endemic *Salix* spp. have disconnected ranges (Burtt Davy 1922). Due to efficient wind dispersal, endemic species of *Salix* exist in Madagascar (*S. madagascariensis*), Macaronesia (*S. canariensis*), the Caribbeans, the Aleutes and many subarctic islands. East Asia harbours the highest diversity of *Salix* and *Populus*, but the ancestral distribution of these genera is not known (Chen et al. 2010). Based on calibrated phylogenies, Salicaceae evolved in the Mid-Eocene and diverged in the Late Eocene (Davis et al. 2005; Zanne et al. 2014). However, the first fossil records ascribed to *Populus* appeared first in N America in the Late Paleocene and subsequently in E Asia and Europe in the Late Eocene (Collinson 1992). The modern *Salix* emerged somewhat later, but also first in USA and E Asia in the Late Eocene and in Europe since the Mid-Oligocene (Collinson 1992). *Salix* has been present in Puerto Rico since the Oligocene (Graham and Jarzen 1969). There is no evidence for the presence of Salicaceae in Southern Hemisphere in the pre-Pleistocene era. Species of *Populus* and *Salix* have been widely planted within and outside their native range to secure rapid pulp and bioenergy production, to control erosion in river banks and for bioremediation. Furthermore, certain *Salix* (silver-leaved, round and weeping forms) and *Populus* species (pyramidal forms) are highly valued ornamental trees. Especially, the riparian *Salix* species have become invasive in Patagonia, New Zealand, Australia and S Africa (Kuzovkina et al. 2008). Multiple species are invasive in other continents within the general Salicaceae range.

Uapaca (Phyllanthaceae) is a genus of small trees in miombo woodlands and rainforests of Africa and Madagascar (Fig. 20.6b). Its occurrence is relatively more restricted by low annual rainfall compared with the *Berlinia* and *Afzelia* groups of legumes. In rainforests, *Uapaca guineensis* and *U. heudelotii,* which form stilted roots, are particularly common in swampy habitats and stream banks. These species accumulate a thick litter layer between the 'root cage', where EcM fungi proliferate and any seedlings rarely become established (L. Tedersoo, pers. obs.). Since genus-level phylogenies are lacking and there is no information about *Uapaca* in the pre-Pleistocene fossil record, it remains unknown whether *Uapaca* originates from Africa or Madagascar and whether rainforest or woodland biomes were ancestral,

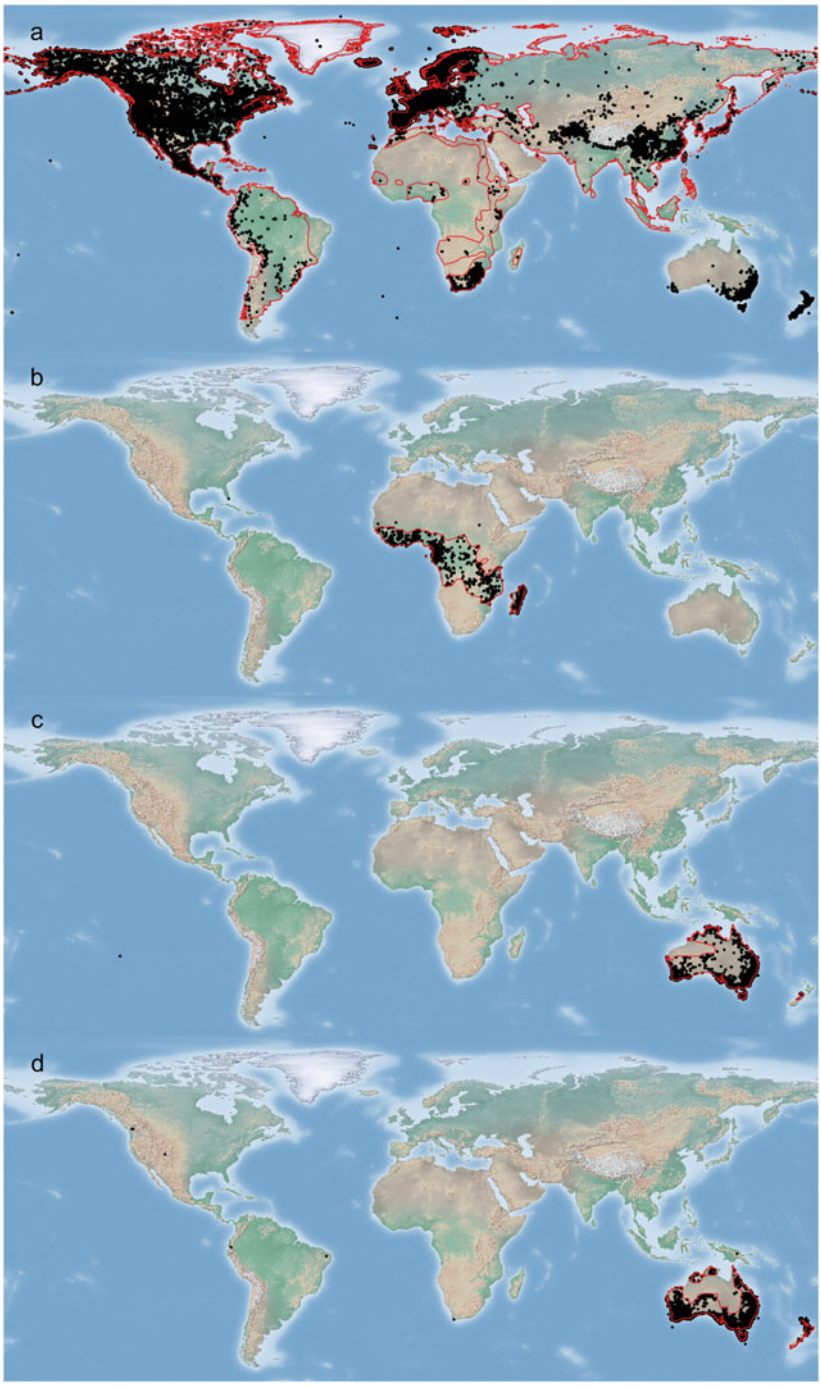

Fig. 20.6 Native range of (**a**) Salicaceae, (**b**) *Uapaca*, (**c**) *Poranthera*, (**d**) Pomaderreae, as indicated by *red border lines*. GBIF records are indicated as *black dots*

although Central African rainforests are the most likely ancestral habitats. The best indirect molecular estimates so far suggest that *Uapaca* diverged from the closest extant relatives in the Mid-Eocene and radiated to known species in the Mid-Miocene (Zanne et al. 2014). There is no evidence for planting *Uapaca* spp. outside their native range, although fruits of certain species (*U. kirkiana*) are an important local food source.

Poranthera (Phyllanthaceae) is a genus of perennial or annual microphyllous herbs and shrubs with almost entirely Australian distribution (Fig. 20.6c). Notably, the group is very rare or absent in the dry W Central Australia. Two herbaceous species, *P. microphylla* and *P.* (*Oreoporanthera*) *alpina*, have dispersed to New Zealand (probably in the Pleistocene). While *P. microphylla* has a disjunct range, *P. alpina* is endemic to northern part of the South Island inhabiting alpine ecosystems (Vorontsova et al. 2007). SW Australia and SE Australia constitute centres of *Poranthera* diversity, suggesting that the seasonally dry Australian woodlands represent the ancestral habitat. Calibrated phylogenies suggest that *Poranthera* diverged and radiated in the Late Oligocene and Early Miocene, respectively (Zanne et al. 2014).

20.3.7 Rosales

Pomaderreae (Rhamnaceae) is a tribe of shrubs and small trees with predominately Australian distribution (Fig. 20.6d). Pomaderreae are common in the humid parts of Australia but very rare or absent in the desert habitats. Seven species of *Pomaderris* are distributed in New Zealand, being either endemic or exhibiting a disjunct distribution pattern with Australia (Ladiges et al. 2005). SW Australia harbours the greatest number of Pomaderreae species. Fossil evidence suggests the presence of *Pomaderris* in New Zealand in the Late Oligocene (Ladiges et al. 2005), but there are no other fossil evidence for Pomaderreae before the Pleistocene. This is suggestive of multiple long-distance dispersal and extinction events in New Zealand (Ladiges et al. 2005). The EcM Pomaderreae diverged from the Californian *Adolphia californica* of unknown mycorrhizal and nodulation status in the Early Eocene (Onstein et al. 2015). I speculate that Pomaderreae probably evolved EcM in seasonally dry Australian woodlands in the Eocene. Of Australian Pomaderreae, *Cryptandra amara* has become naturalised in New Zealand based on several GBIF records.

Dryadeae represents one of the two groups of EcM Rosaceae that is comprised of five genera of miniature shrubs (*Dryas*) to small trees (*Cercocarpus*). *Cercocarpus, Chamaebatia* and the potentially EcM *Purshia* and *Cowania* are distributed from S Mexico to NW USA. Conversely, *Dryas* is more widely distributed throughout the Holarctic tundra belt and has multiple relictual habitats since the last glacial maximum in montane grassland and alpine ecosystems in Europe, Asia and North America (Fig. 20.7a). All five genera have their centre of biodiversity in NW America that probably represents the ancestral area for Dryadeae. Pollen

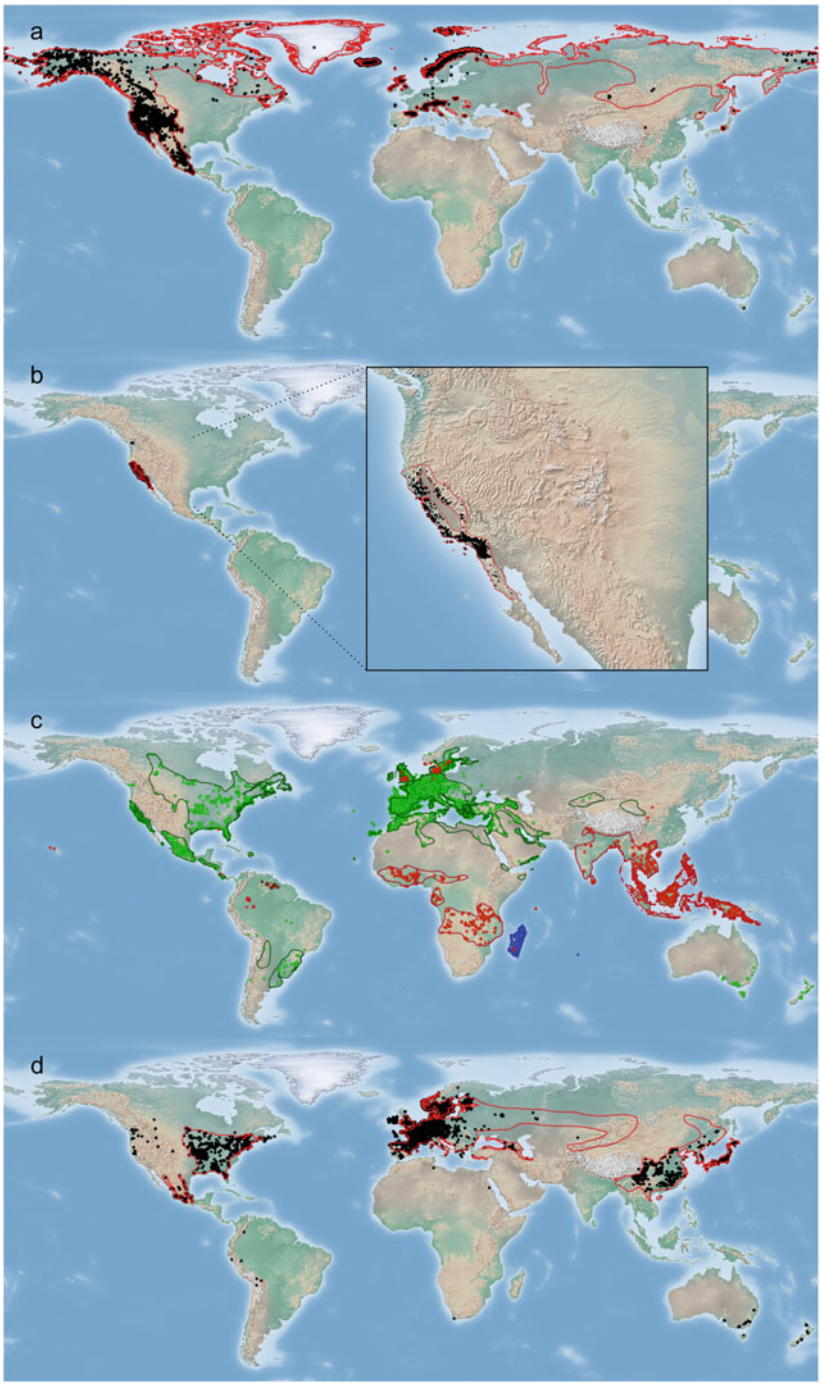

Fig. 20.7 Native range of (**a**) Dryadeae, (**b**) *Adenostoma*, (**c**) Dipterocarpaceae-Cistaceae, (**d**) *Tilia*, as indicated by *red border lines*. GBIF records (and records of *Pseudomonotes* from ter Steege et al. 2013 and Tedersoo et al. 2014a) are indicated as *dots*. In

of *Cercocarpus* has been found from Colorado, USA, in the Late Eocene (DeVore and Pigg 2007). *Dryas* has ample fossil pollen records, and it has served as one of the indicators for migration of vegetation during the glacial cycles (Skrede et al. 2006). Dryadeae had probably a narrow range in NW America until the Late Pliocene, when *Dryas* became widely distributed in Greenland (Bennike and Böcher 1990). *Dryas* spread further to Eurasia probably in the Late Pliocene or Pleistocene, taking advantage of glacial cycles and wind for dispersal. Although *Dryas* is widely grown in alpinaria due to lovely flowers and conspicuous leaves and fruits, there is no evidence for its naturalisation due to slow growth and unsuitable climate.

The two species of ***Adenostoma*** represent bushes that exhibit a narrow distribution in California and NW Mexico (Fig. 20.7b). Such a restricted distribution and potential evolution not earlier than the Late Miocene or Pliocene (Zanne et al. 2014) suggests that the genus evolved in that region during successive climate drying. *Adenostoma* emerged in fossil record in the Late Pleistocene, being also restricted to California (DeVore and Pigg 2007). There are no reports of naturalisation of *Adenostoma* outside its native range, although it is cultivated in botanical gardens. Given its association with *Frankia* actinobacteria, facultative mycotrophy, allelopathic litter and monodominant habit, I believe that *Adenostoma* has a potential to become a hazardous invader in Mediterranean habitats.

20.3.8 Malvales

The **Dipterocarpaceae–Cistaceae** group includes Dipterocarpaceae *s.lat*., Sarcolaenaceae, Cistaceae and Pakaraimaeaceae families (Chap. 19). The extant distribution of trees and shrubs belonging to Sarcolaenaceae covers Madagascar, except the driest southwestern section (Fig. 20.7c). *Pakaraimaea dipterocarpacea*, which is more closely related to Cistaceae than Dipterocarpaceae (Horn et al. 2016), is a narrow endemic in dry forest and savanna habitats in SE Venezuela. The South American dipterocarpaceous tree species *Pseudomonotes tropenbosii* has three disconnected populations in rainforest habitats of SE Colombia. Small trees and bushes of *Monotes* spp. are characteristic of African miombo woodlands, except *M. madagascariensis* occurring in dry savannas in S Central Madagascar. Emergent tree species of *Marquesia* inhabit restricted areas in rainforests of Gabon or moist miombo woodlands in the Copperbelt region of Zambia and D.R. Congo. Natural distribution of *Vateriopsis seychellarum* is restricted to a single mountain gorge in Mahé Island of the Seychelles. Multiple other genera of Dipterocarpaceae *s.str*. are widely distributed in India and SE Asia, extending further to Hainan,

Fig. 20.7 (continued) Dipterocarpaceae-Cistaceae: *red*, Dipterocarpaceae *s.lat*.; *blue*, Sarcolaenaceae; *green*, Cistaceae; *brown*, Pakaraimaeaceae

Malesia and New Guinea. Cistaceae represent perennial shrubs and small bushes that are widely distributed in the Mediterranean and grassland habitats of Europe and North America (Fig. 20.7c). In North Africa and Arabic peninsula, Cistaceae species also grow in relatively moist desert habitats, being perhaps the most drought tolerant of all EcM plants. From the ancestral habitat in the Mediterranean basin, multiple Cistaceae species have spread to colonise other regions mainly in the Northern Hemisphere (Guzman and Vargas 2009). The low shrub genus *Helianthemum* is distributed in Scandinavia, northern Sahara, Middle East, Central Asia (*H. songaricum*), southern Arabic peninsula (*H. citrinum*), W Pakistan, the Somali peninsula and Socotra island (all *H. lippii*). Several distinct species of *Cistus* occur in the Canaries, whereas *C. gorgoneum* is endemic to Cape Verde. Cistaceae have probably dispersed over the Atlantic to North America in two independent events in the Mid-Miocene (*Lechea*) and Late Miocene (*Crocanthemum,* erected from *Helianthemum* to denote American species; Guzman and Vargas 2009). These events may be pushed back considering the early unaccounted fossil record and potentially slowed evolution. Species of *Crocanthemum*, *Hudsonia* (nested in *Crocanthemum*) and *Lechea* colonise the savanna and Mediterranean habitats from S Canada south to Costa Rica. *Crocanthemum rosmarinifolium* (syn. *Halimium domingense*) has dispersed from SE USA to the Hispaniola Island (Dominican Republic and Haiti). *Crocanthemum* has spread further southeast from Central America to South America, with an extant disjunct distribution of *C. brasiliensis* in SE Brazil and Uruguay. The few GBIF records elsewhere in South America require verification. The lack of Cistaceae in E Asia could be due to unsuitable Mediterranean climate at present as well as historically (no fossil records), because alkaline soils suitable for Cistaceae are common both in SE China and NE China.

Historical biogeography of the Dipterocapaceae–Cistaceae complex is enigmatic given the multiple potential vicariance vs. long-distance dispersal events and great difficulties in obtaining well-supported phylogenies with reasonable dating (Ducousso et al. 2004; Moyersoen 2006) The present distribution of Cistaceae and Dipterocarpaceae subgroups has no overlap, but both groups co-occurred both in North Africa and Europe in the Eocene (Morley 2000; Arrington and Kubitzki 2003). North Africa probably represents the ancestral region for this extended group. Morley (2000) speculated that the common ancestors of Dipterocarpaceae *s.lat* inhabited seasonally dry climate in Africa, which is in agreement with the Mediterranean origin of Cistaceae (Guzman and Vargas 2009). Since both the extant Sarcolaenaceae and Cistaceae are mostly represented by small bushes and the African *Monotes* are small trees and bushes, it is likely that the ancestors of Cistaceae-Dipterocarpaceae were small woody plants in seasonal habitats rather than tropical trees. However, this scenario contradicts with the prevailing humid tropical climate in North Africa and South European archipelago from the Late Cretaceous to Late Eocene (Morley 2000) and massive extinctions of dipterocarps after the Oligocene climate cooling trend (see next paragraph).

Although all extant species of Sarcolaenaceae are endemic to Madagascar, paleobotanical records indicate its much wider distribution in S Africa until the Mid-Miocene, suggesting continental African origin of this family (Raven and

Axelrod 1974; Goldblatt 1997). The presence of *Vateriopsis seychellarum* in the Seychelles is probably a relic of historical connections between the broken continental plates of Madagascar, India and Mahé (Briggs 2003). *Monotes madagascariensis* must have crossed the Mozambique strait to reach Madagascar, but the timing of this event is not known. Thus, it is possible that the ancestors of the non-monophyletic *Pseudomonotes* and *Pakaraimaea* migrated over the much narrower Atlantic from W Africa to South America independently in the Eocene given the calibrated dating of these groups (Zanne et al. 2014). Alternatively, molecular dating analyses strongly underestimate the age of Dipterocarpaceae due to the slowed rate of evolution of these large trees. This would push back the dates of divergence and dispersal, although the Mid-Cretaceous dates of divergence (Moyersoen 2006) are unlikely and unsupported by the fossil record. Based on fossil evidence, the ancestors of modern Asian dipterocarps probably spread from Africa to Asia over the Arabic Peninsula or by rafting on the Indian Plate in the Late Eocene (Ashton 1988; Feng et al. 2013). Dipterocarps shortly arrived in E China where they co-occurred with other EcM trees from Pinaceae, Fagaceae and Juglandaceae families already in the Late Eocene (Feng et al. 2013). In Malesia, the earliest dipterocarps are known since the Oligocene in Borneo (Muller 1981). During the Pleistocene glacial maxima, probably another wave of dipterocarps arrived from SE Asia and further migrated to New Guinea and the Philippines across the narrow seas (Morley 2000). In spite of their remarkable age, there is no fossil evidence for South American dipterocarps, but this is probably related to poor paleobotanical sampling in lowland rainforests of the Northern Amazon, including the podzolic habitats. It is most plausible that the ancient distribution of South American dipterocarps was much wider, because it is unlikely to survive across multiple geological epochs in such narrow ranges (Morley 2000). Similarly, the distribution of African dipterocarps used to be much wider given their presence in vast areas of Africa, Central Europe, East Asia and North America (probably crossing over the North Atlantic land bridge; Raven and Axelrod 1974; Boureau et al. 1983) in the Late Eocene. According to an enormous decline of Dipterocarpaceae in the fossil record, climate cooling and drying in the Terminal Eocene must have extirpated much of the dipterocarpaceous vegetation outside its present range. The Cistaceae subgroup is uncommon in the fossil record, with earliest records in the Baltics and Central Europe since the Late Eocene (Arrington and Kubitzki 2003). Cistaceae probably became more common in the Mid-Miocene, when the Mediterranean biome evolved (Palamarev 1989).

Although Dipterocarpaceae are widely planted in botanical gardens and arboretums world-wide and used for ecological restoration of native forests, there is little evidence for their naturalisation or invasiveness outside their native range. Both natural forms and cultivars of Cistaceae have been planted extensively outside their native range as ornamental plants due to their abundant bright flowers and long flowering time. Certain species of Cistaceae (especially *C. creticus*) have become widely naturalised in SE Australia, New Zealand, Madeira and the Central Andes (GBIF records). Their mycorrhizal status in the introduced range is not known.

The tree genus ***Tilia*** has disjunct Holarctic distribution in temperate forests in Europe, W Asia, E Asia and NE America (Fig. 20.7d). *Craigia*, endemic to South China but historically distributed throughout the Northern Hemisphere, represents a taxon nested within *Tilia* (Zanne et al. 2014). Although *Tilia* is phylogenetically dated to the Oligocene or Miocene (Zanne et al. 2014; Richardson et al. 2015), the oldest authentic fossils are derived from Europe and E Asia in the Early Paleocene (Muller 1981; Song et al. 2004) and from SE USA and Canadian High Arctic in the Mid-Eocene (Axelrod 1989; Ya and Ren 1996; Richter and LePage 2005), suggesting that the calculated divergence times are strongly underestimated as explained for dipterocarps. Fossil records indicate that *Tilia* once had a broader distribution in the Northern Hemisphere including Svalbard, NW America and Beringia (Ya and Ren 1996). According to a non-specific analysis of Zanne et al. (2014), *Tilia americana* represents the oldest extant lineage of the *Tilia*+*Craigia* group. This casts some doubt into the suggested evolution in SE China in the Late Cretaceous (Ya and Ren 1996), although E Asia has the greatest taxonomic richness of *Tilia*. Notably, the closest sister genus *Mortoniodendron* with unknown mycorrhizal status is exclusively distributed in Central America (including fossils dating back to the Late Eocene; Graham 1985), which further hints to the potential North American origin of *Tilia*. *Tilia* species represent a very important component in urban vegetation in the temperate and boreal zone due to their relatively high tolerance of pollution and salt, wind resistance and rapid litter decomposition. Therefore, European species of *Tilia* have been widely planted in North America and in the Southern Hemisphere. *Tilia cordata* has become locally invasive in deciduous forests of North America, whereas *T. cordata, T. tomentosa* and *T. americana* have become naturalised in New Zealand and SE Australia (GBIF records).

20.3.9 Asterales

The Australian group of **Gnaphalieae** arrived from South Africa in a single long-distance dispersal event some 16 Ma (Bergh and Linder 2009). The EcM habit evolved in the Australian group that has centres of endemism in N Australia and SE Australia (Schmidt-Lebuhn et al. 2015; Fig. 20.8a). Certain genera have further dispersed to New Guinea (*Euchiton*, *Anaphalioides*), New Caledonia (*Ozothamnus*) and New Zealand (*Euchiton*, *Anaphalioides*, *Ewartiothamnus*, *Helichrysum*, *Leucogenes*, *Raoulia*, *Ozothamnus, Argyrotegium*) including the Chatham Islands, Auckland Islands, Campbell Island and Antipodes Islands (*Anaphalioides bellidioides*). *Euchiton* spp. have further dispersed to E China, Taiwan, Japan, Polynesia, Java, Timor, Sulawesi, Philippines and potentially to several other islands in the Malesian archipelago. It is notable that not all species in these groups are EcM (Chap. 19), and there are no records of mycorrhizal status of any Gnaphalieae in their natural or introduced range outside Australia. Pollen of the unassigned Asteraceae was first recorded in Australia in the Mid-Miocene

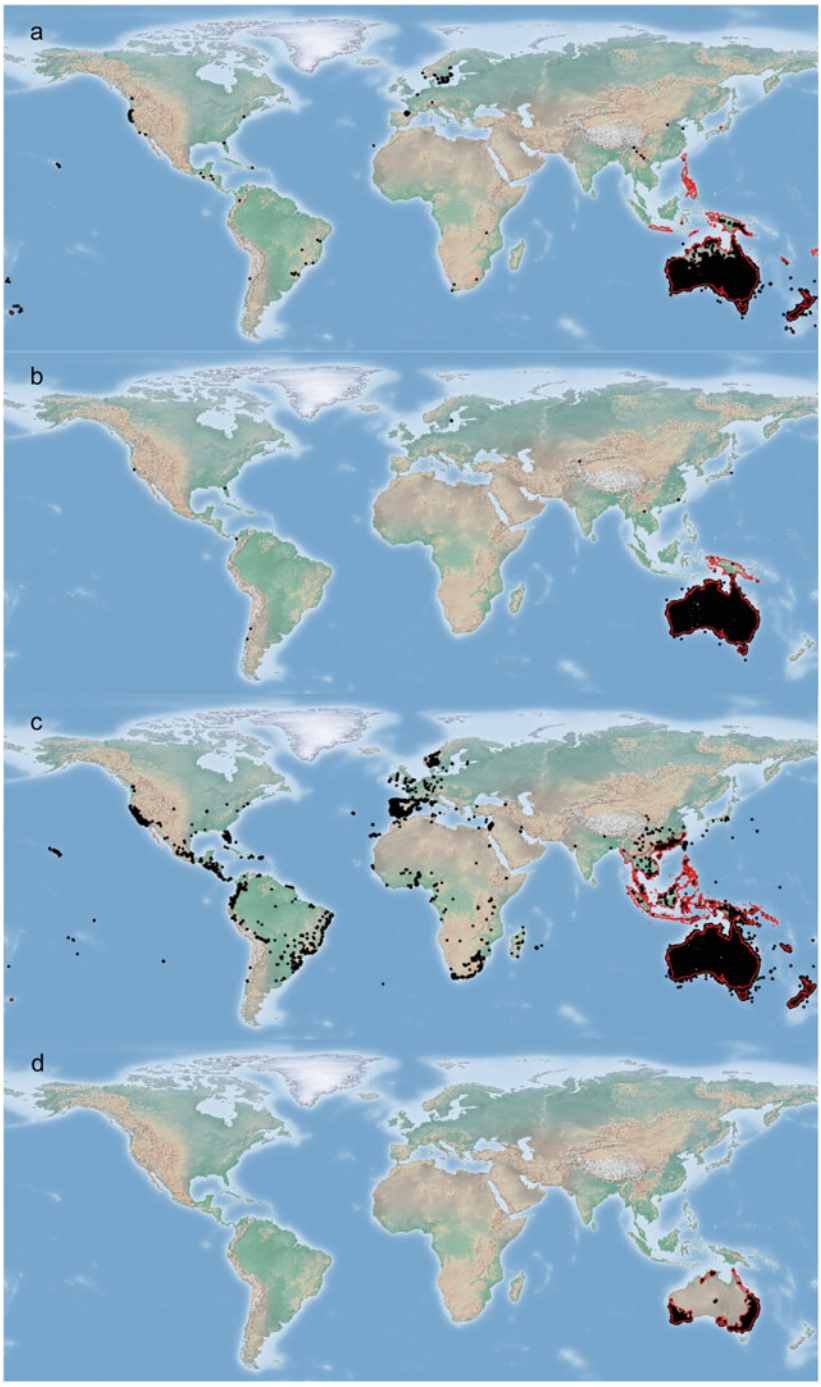

Fig. 20.8 Native range of (**a**) Gnaphalieae, (**b**) Goodeniaceae excluding *Scaevola+Selliera*, (**c**) Myrtoideae, (**d**) *Platysace*, as indicated by *red border lines*. GBIF records are indicated as *black dots*

(Macphail 2007). Gnaphalieae became abundant in the fossil record in Pliocene, when Central Australia became a dry grassland and shrubland ecosystem (Macphail 1997; Hill 2004). Multiple species of Gnaphalieae have been imported as ornamental plants to multiple regions. These species have occasionally escaped from gardens and became naturalised based on GBIF records. *Leptorhynchos squamatus* became naturalised in N Spain, whereas *Euchiton sphaericus* naturalised in Hawaii and West coast of USA. *Xerochrysum bracteatum* has become widely naturalised in SE Brazil, Central American highlands, S Scandinavia and Central Europe, mostly as a pioneer in wastelands. *Rhodanthe chlorocephala* has become naturalised in Scandinavia.

Goodeniaceae is another Australia-centred family within Asterales (Fig. 20.8b). Within this group, the genus *Scaevola* is particularly widely distributed in Australasia and Oceania, extending to Hawaii, Tahiti and New Zealand. Certain species also inhabit Socotra (*S. socotraensis*) and Cuba (*S. wrightii*). Furthermore, *S. plumieri* is a pantropical coastal species that has seeds adjusted to dispersal by water (Howarth et al. 2003). There is no evidence for EcM habit in *Scaevola* species outside Australia, and the few studies have reported only AM (Supporting Information: http://dx.doi.org/10.15156/BIO/587454). Several species of *Goodenia* are distributed in S New Guinea, whereas *Goodenia konigsbergeri* is endemic to Java (Jabaily et al. 2014). The salt marsh species *Selliera radicans* has dispersed from Australia to New Zealand and Central Chile probably in the Pleistocene. Unfortunately, there is no information about its mycorrhizal status, but given its halophilic habit it is very unlikely to be EcM. SW Australia is the main centre of radiation and endemism for Goodeniaceae and apparently to all genera therein (Jabaily et al. 2014). Although the divergence of Goodeniaceae is dated to the Late Cretaceous or Paleocene (Jabaily et al. 2014; Zanne et al. 2014), the first paleobotanical evidence is derived from the Mid-Oligocene in W Central Africa and from the Late Oligocene onwards in New Zealand and Patagonia. Goodeniaceae pollen emerged in Australia in the Mid-Miocene (Macphail 2007). It is likely that Goodeniaceae were present in Australia much earlier given the presence of all extant genera in Australia. Most probably the early forms of non-Australian Goodeniaceae became extinct and were replaced by groups of Australian origin in the Late Miocene and Pliocene (Jabaily et al. 2014). I believe that many of the early extra-Australian pollen records are misassigned, and the age of Goodeniaceae is seriously overestimated perhaps due to an increase in the rate of evolution. There are no reports of naturalisation of Goodeniaceae, although the weedy coastal species may have a high invasion potential.

20.3.10 Myrtales

The EcM **Myrtoideae** (Myrtaceae) have an Australian-centred distribution pattern (Fig. 20.8c). Several taxa have more recently dispersed to New Zealand (*Leptospermum, Kunzea*), New Caledonia (*Arillastrum*, *Tristaniopsis, Melaleuca*,

Baeckea, Sannantha, Cloezia), New Guinea (*Eucalyptus, Eucalyptopsis, Thaleropia, Welchiodendron*), New Britain (*Eucalyptus deglupta*), Borneo (*Whiteodendron*), Mindanao (Philippines; *Eucalyptus, Tristaniopsis*) and Timor (*Eucalyptus urophylla*) (Ladiges et al. 2003; Thornhill et al. 2015). *Xanthomyrtus* and *Xanthostemon* inhabit New Caledonia, New Guinea, Sulawesi, Borneo and the Philippines. *Kjellbergiodendron* with unknown mycorrhizal status is endemic to Sulawesi (Thornhill et al. 2015). Furthermore, *Tristaniopsis*, *Leptospermum* and *Melaleuca* have successfully dispersed from Australia to New Guinea, Malesia and Indo-China (Thompson 1989; Brown et al. 2001). While *Melaleuca* is characteristic of riparian swamps and wetlands, and *Tristaniopsis* of swamp forests of Java and Sumatra, *Leptospermum* spp. commonly dominate high elevation sites near the tree line. The Tasmanian *L scoparium* is also widespread in New Zealand. A few species of *Asteromyrtus* inhabit the Moluccas and New Guinea (Thornhill et al. 2015). *Baeckea fruticosa* has perhaps the most outlying range that covers a vast area from Indo-Malay to SE China. The westward distribution of *Baeckea*, *Tristaniopsis*, *Leptospermum* and *Melaleuca* has been underestimated in EcM literature so far.

Fossil record indicates that the order Myrtales and Myrtaceae therein evolved in tropical Africa and S America in the Mid-Cretaceous and dispersed to other continents by the Late Cretaceous (Berger et al. 2016). The authors suggested that the ancestors of Myrtoideae *s.lat*. and the sister group Psiloxyloideae evolved in Africa, followed by long-distance dispersal to Antarctica or Australia in the Late Cretaceous, accompanied with an unexplained increase in diversification rate (Berger et al. 2016). It is, however, more plausible that the ancestral, putatively AM Myrtoideae and Psiloxyloideae were more widely distributed in E Gondwana (fossil evidence from Central Africa and Colombia: Thornhill and Macphail 2012) and the early Myrtoideae migrated to the Weddellian province across land via South America. Diversification of the Myrtoideae group started in the Latest Cretaceous (Berger et al. 2016) probably after evolving the EcM habit. The location of EcM evolution is less clear due to the paucity of fossil record for the earliest diverging lineages that leave a 10–15 My gap in time (Hill et al. 2017). Notably, the earliest diverging EcM are restricted to N Australia and New Guinea, with potentially more recent dispersal to New Caledonia and Malesia (Morley 2000). There is no evidence for their historical occurrence in South America, New Zealand or Antarctica. If the pollen species *Myrtaceidites parvus*/*M. parvus-mesonesus* is to be considered belonging to the early EcM Myrtoideae rather than any parallel group, it becomes more likely that EcM habit evolved in Australia since this pollen is found first in Australia in the Latest Cretaceous and subsequently in Patagonia and New Zealand since the Paleocene (Macphail and Thornhill 2017). This pollen group went extinct in Patagonia and New Zealand by the (Mid?)-Miocene, but it persisted in Australia until the Pleistocene (Macphail and Thornhill 2017).

Eucalypteae are certainly the best known tribe of the EcM Myrtoideae. Abundant Eucalypteae fossils have been found in Patagonia from the Late Paleocene to Mid-Miocene (Hermsen et al. 2012; Hill et al. 2017) and in the Falklands from the Oligocene to Pliocene (Macphail and Cantrill 2006). There are discontinuous records from New Zealand since the Late Paleocene to Pleistocene (Thornhill and

Macphail 2012; Lee et al. 2016; Hill et al. 2017). In the Mid-Miocene, some of the tropical EcM Myrtoideae found their way to Malesia and SE Asia (Morley 2000), with another wave of species arriving in the Pleistocene. Since the Late Cretaceous, Myrtoideae have co-evolved with fire particularly in Australia (Macphail and Thornhill 2017), and many Eucalypteae species require high temperature to release the seeds. Development of sprouting habit may have facilitated eucalypts to shift to the expanding savanna and shrubland ecosystems since the Late Miocene (Crisp et al. 2011).

Myrtoideae include several clades that have reverted to AM-only habit (Chap. 19), suggesting that the early Australian Myrtoideae were dual mycorrhizal and perhaps only facultatively EcM. The AM groups Myrteae, Metrosidereae and Syzygieae have successfully dispersed and established in rainforest and savanna habitats of S America, Africa, Pacific Islands and Indo-Malay (Thornhill et al. 2015). The relatively greater success of AM Myrtoideae is at least partly due to more efficient dispersal of fleshy fruits by birds (Thornhill et al. 2015), but it may be additionally related to the lack of suitable EcM mycobionts.

Eucalyptus spp. are the most widely planted trees in exotic habitats at tropical and subtropical latitudes between 27°S and 27°N. In village and urban areas, eucalypts serve for ornamental and shade purposes. Eucalypts contribute to nearly one-half of all forest plantations in tropical and subtropical ecosystems due to rapid production of high-quality timber (Harcharik 2000; Rejmanek and Richardson 2011). Eucalypts were first imported to S Africa, India and Europe in the end of the eighteenth century, with massive plantations being established since 1850s. India alone has >5 million ha eucalypt plantations, whereas eucalypt plantations cover >6% of total land area in Portugal (Harcharik 2000). Especially in seasonally dry savanna and shrubland ecosystems in Brazil, S Africa, Iberian Peninsula and California, multiple *Eucalyptus* spp. have become highly invasive (Rejmanek and Richardson 2011). Eucalypts may grow their root systems up to 40 m deep, which provides a competitive advantage over other vegetation in accessing deep water sources (Dell et al. 1983). Eucalypts are often the only trees that are able to withstand long dry season and become established in dry grassland and shrubland ecosystems. Eucalypts also promote the flammability of vegetation by emitting volatiles and accumulating litter. In addition, the thick and strongly allelopathic litter further retards growth of native plants. By the same mechanisms, paperbarks (*Melaleuca* spp.; Melaleuceae tribe) have become highly invasive in wetlands of tropical and subtropical ecosystems that is particularly severe in E Madagascar and SE USA (e.g. Dray et al. 2006).

20.3.11 Apiales

Platysace (Apiaceae) is a genus of Australian perennial herbs and shrubs. *Platysace* is strictly endemic to Australia, with no species distributed to neighbouring islands including Tasmania (Fig. 20.8d). Within Australia, *Platysace* species are common

in moist and moderately dry southwestern and southeastern parts of the continent. Only a few species are distributed in the moist tropical North Australia. SW Australia represents a diversity hotspot for *Platysace* and its sister genus *Homalosciadium* with no mycorrhizal records. These genera diverged in the Early Oligocene (Nicolas 2009). There are no fossil records of *Platysace* and no records of its naturalisation in the rare cases of planting in botanical gardens.

20.3.12 Ericales

Arbutoideae *s.lat.* (incl. Arbutoideae *s.str.*, Pyroloideae, Monotropoideae; Chap. 19) is an EcM group that is widely distributed in the Northern Hemisphere (Fig. 20.9a). The fully autotrophic Arbutoideae *s.str.* is distributed in tundra (except Greenland and Arctic islands), boreal and temperate forest and Mediterranean ecosystems of the Northern Hemisphere, with *Arbutus phillyreaefolia* and *Arctostaphylos elliptica* extending beyond the Isthmus of Panama and reaching

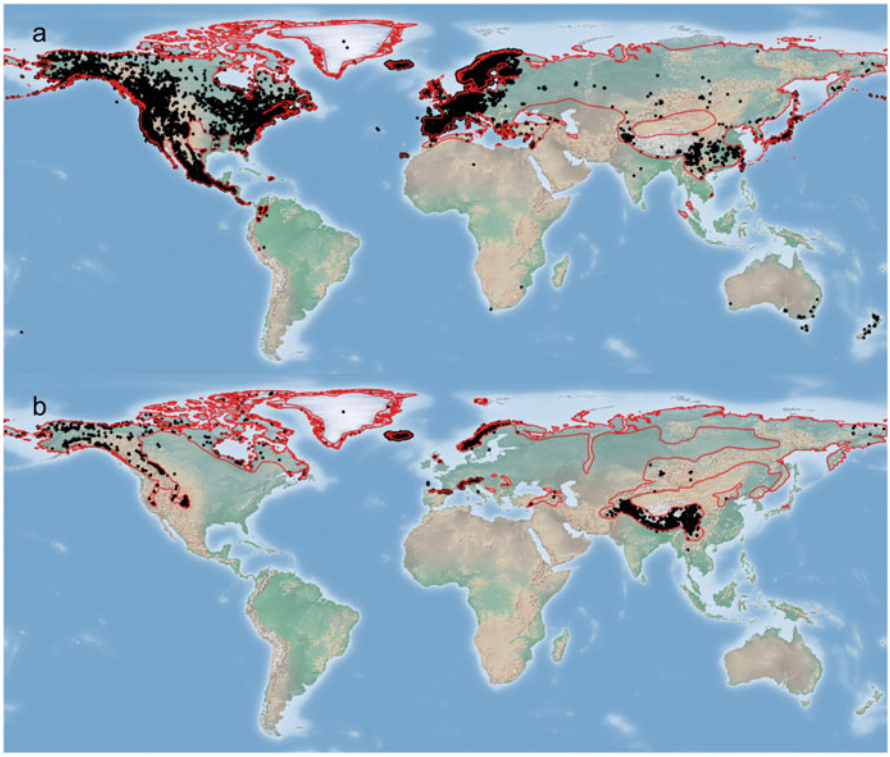

Fig. 20.9 Native range of (**a**) Arbutoideae *s.lat.*, (**b**) *Kobresia* as indicated by *red border lines*. GBIF records are indicated as *black dots*

the Peruvian Andes. *Arbutus canariensis* has dispersed to the Canaries probably from NW Africa. The partly mycoheterotrophic Pyroloideae subfamily is distributed more widely in the Northern Hemisphere (incl. Arctic islands) and subtropical forests compared with Arbutoideae *s.str*., but members of Pyroloideae are by far less common in Mediterranean ecosystems. The genus *Chimaphila* is distributed in habitats dominated by Pinaceae or Fagaceae along the Cordilleras south to W Panama and Hispaniola Island, whereas *Pyrola* has a disjunct subrange, coinciding with that of *Pinus merkusii*, in N Sumatra. The fully mycoheterotrophic Monotropeae and Pterosporeae tribes follow the distribution of other EcM trees and shrubs, and they are distributed from warm boreal forests to subtropical forests and montane rainforests (Kron and Luteyn 2005). *Monotropa uniflora* is distributed from temperate North America to Panama and the Andes in NW Colombia. *Monotropastrum* and *Cheilotheca* are distributed in E Asia, with disjunct ranges in S Vietnam, Malay Peninsula and N Sumatra. The overall phylogenetic diversity and richness of all subfamilies peak in NW America, which is potentially the ancestral area for Arbutoideae *s.lat*. and *s.str*. (Kron and Luteyn 2005). By contrast, the earliest records of *Arbutus* in Europe are derived from the Early Oligocene, with high relative abundance in the Mediterranean habitats since the Mid-Miocene (Palamarev 1989). *Arbutus* fossils have been recovered from Central and W USA since the Mid-Miocene (Axelrod 1989; Mahall et al. 2010). Calibrated phylogenies suggest that the entire group dates back to the Mid-Cretaceous (Schwery et al. 2015) or Early Eocene (Zanne et al. 2014), the latter date being more likely. The European *Arbutus unedo* has been widely planted in North America and in the Southern Hemisphere due to its ornamental value and edible fruits. Although *A. unedo* is not regarded as invasive, it has become locally naturalised in California, SE Australia and New Zealand.

20.3.13 Cyperales

The genus ***Kobresia*** comprises multiple EcM species of perennial herbs (sedges) that are the only known ectomycorrhizal monocots so far. The EcM group of *Kobresia* is widely distributed in Arctic and alpine habitats of the Northern Hemisphere (Fig. 20.9b). The high Alpine habitats in Europe and Japan represent interglacial refugia. *K. myosuroides* (syn. *K. bellardii*) and *K. simpliuscula* have circum-Arctic distribution and further extend to the refugial habitats in Europe and North America. The diversity of *Kobresia* is much greater in West Asia and particularly in the Tibetan Plateau that harbours tens of species. In Tibet and surrounding highlands, *Kobresia* spp. often dominate the herbaceous vegetation at altitudes 3500–5000 (6000) m above sea level, indicating their great ecological importance in this region (e.g. Miehe et al. 2011). *Kobresia* spp. often constitute the main forage plants for ungulates in Asian highlands. Pollen of *Kobresia* spp. is abundant in the deposits of Pleistocene glacial maxima, but unequivocal pre-Pleistocene records are not known. Differentiation of pollen from closely

related *Carex* species is also problematic. Taken together, it is likely that the EcM Kobresia evolved in the Tibetan plateau or nearby highlands in the Late Miocene or Pliocene (Starr et al. 2004; Zanne et al. 2014). There is no evidence for naturalisation or invasion of *Kobresia* spp., although many species may have a potential for this in Subantarctic islands and Antarctic Peninsula.

20.4 Overall Patterns of Natural Distribution

The combined sources of information revealed that the range of EcM plant groups may differ from regional (*Gymnopodium* in Central Mexico, *Adenostoma* in California, Asteropeiaceae in Madagascar) to nearly global (Fagales, Salicaceae; Supporting Information: http://dx.doi.org/10.15156/BIO/587454). In terms of species richness, EcM plant lineages range three orders of magnitude, from 1–2 (*Gymnopodium, Adenostoma, Persicaria*) to >1000 spp. (Myrtoideae, *Acacia;* Chap. 19). There are groups endemic to each continent except Europe, Asia (and Eurasia) and Antarctica. Multiple lineages are exclusive to Australia. Both the coastal areas of southwestern, eastern and northern Australia, New Guinea and montane regions of Europe and Mexico represent major global hotspots of EcM plant richness at the group level (Fig. 20.10). The high group-level richness of montane regions is a matter of scale, because I did not take into account landscape-scale habitat differences, although subtropical, temperate and alpine species do not co-exist in natural communities. Although there is no direct proof, the group-level richness seems to be strongly correlated with the overall species richness of EcM plants, (1) because individual plant groups tend to exhibit diversity hotspots in group-rich areas and (2) due to sampling effect. The overall species richness of EcM plants peaks in SW Australia and SE Australia that display greatest richness of *Acacia*, Myrtoideae and Gnaphalieae, the most diverse EcM groups (Chap. 19). Other relatively species-rich regions include SE Asia (incl. S China) and N Mexico, where Fagales, Pinaceae and Dipterocarpaceae–Cistaceae are highly diverse among multiple other groups. The EcM plant richness patterns are only partly consistent with the overall global hotspots of plant diversity (Kreft and Jetz 2007). The relative richness of EcM plants to all plants is clearly the highest in Australia, because Australia harbours only moderate total plant richness on a global scale. Except for SE Asia, the hyperdiverse rainforests support medium level diversity of EcM plants and therefore, relative proportion of EcM plants in terms of richness is lowest in South America including Patagonia. Recognising the global hotspots of EcM plant species richness clearly requires a more specific approach and use of regional grid-based data sets (Menzel et al. 2016).

In the Southern Hemisphere, Australia is strikingly more diverse in terms of EcM plant groups compared with New Zealand, Africa and especially Patagonia. In spite of relatively high EcM fungal diversity (Chap. 18), Patagonia supports only *Nothofagus* spp. and *Salix humboldtiana*, with *Alnus acuminata, Pisonia zapallo, Coccoloba* spp. and *Crocanthemum brasiliensis* occurring only in the northern

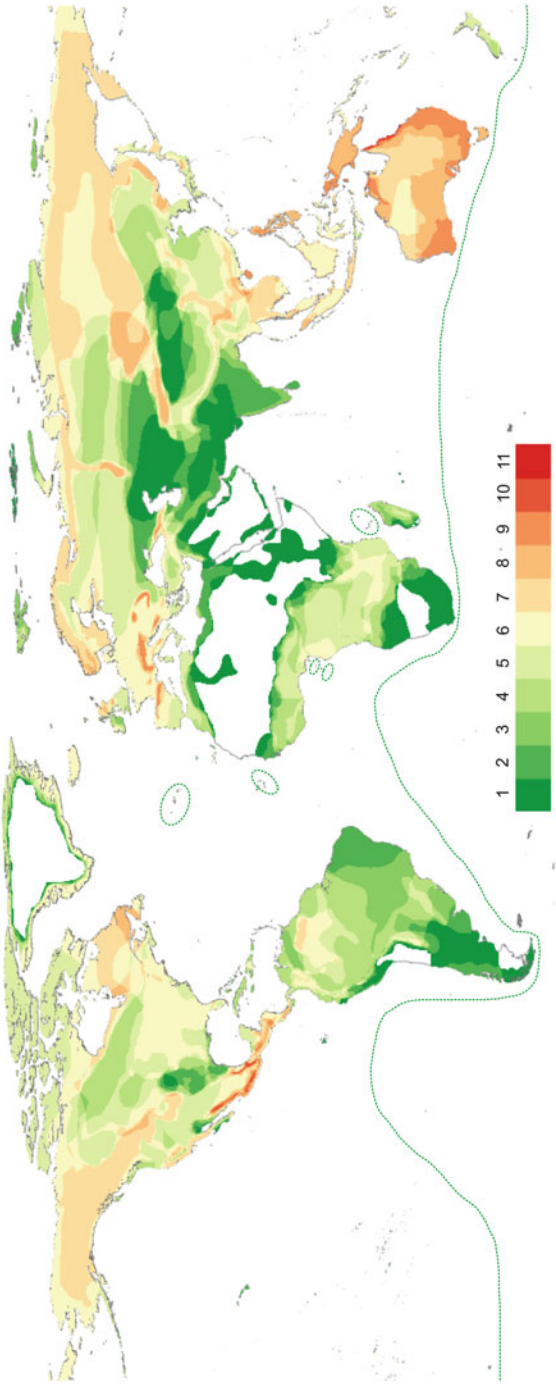

Fig. 20.10 Heat map indicating lineage richness of native EcM plants that ranges from 0 to 11 groups. White land surface indicates no native EcM plants. *Green lines* border islands with no native EcM plants

margin of Argentina. There is ample evidence that multiple EcM groups became extinct due to unfavourable climate in Patagonia and South Africa in the Mid-Miocene and Pleistocene. New Zealand, the Mascarenes and New Caledonia have been fully or nearly fully submerged that wiped out the archaic vegetation and opened the reappearing land to colonisation by long-distance dispersal and rapid speciation. Many Australian EcM plant lineages are younger than the separation of Gondwanan land masses (Chap. 19), suggesting that the greater size of Australia and/or its climate may have been more favourable for the evolution and persistence of EcM plant lineages as also observed for EcM fungi (Tedersoo et al. 2010). Nonetheless, the connection between Antarctic Peninsula and Patagonia provided an extremely important migration pathway for the Weddellian province, and it simultaneously served to secure temperate climate in the far southern latitudes until the Mid-Miocene.

In the Northern Hemisphere, temperate and boreal forest and tundra biomes of Europe, Asia and North America share nearly all EcM plant lineages among continents. In terms of species richness, E Asia has greater diversity than North America that in turn exceeds Europe. This is undoubtedly related to the general impoverishment of N American and particularly European flora in the Mid-Miocene and Pleistocene, with a gradual loss of genera of Pinaceae and Fagales. S China has distinctly the greatest number of species and genera of most EcM plant lineages, which corroborates the general floristic patterns in the Northern Hemisphere (Qian and Ricklefs 2000; Donoghue and Smith 2004). Roughly similar proportions of EcM plant species in the flora of northern continents suggests that both mycorrhizal types were equally vulnerable to habitat loss related to glacial cycles, although Dickie et al. (2014b) argued that AM trees became more massively extinct in Europe. Reanalysis of the original data (Svenning 2003) with updated mycorrhizal type assignments (Chap. 19) indicated that tolerance to drought and cold enabled tree species to survive, with no residual effect of mycorrhizal type per se. Probably due to greater climatic tolerance, EcM trees may have persisted longer than AM trees in the forefront of glaciation in N America (Lankau et al. 2015).

Temperate and subtropical ecosystems of the northern continents differ greatly in their EcM connections to tropical habitats. Subtropical and Mediterranean habitats of N America are most unique in terms of EcM plant lineages. S Florida has strong influence from S American vegetation by the presence of *Pisonia* and *Coccoloba*. Together with *Acanthocarpus*, these groups extend to subtropical NE Mexico. California harbours a narrow endemic *Adenostoma* lineage and relatively high phylogenetic diversity of Dryadeae and Arbutoideae. S European and N African flora have very limited impact from Central Africa at present (but see Sect. 20.3.8), whereas subtropical E Asia is strongly influenced by EcM vegetation of SE Asia and Australia: the current distribution of Dipterocarpaceae reaches Hainan and S Yunnan, *Pisonia grandis* (Pisonieae) is distributed in smaller islands off the Asian coast at $<22^{\circ}$N, *Baeckea fruticosa* (Myrtoideae) is widely distributed in S China, and multiple species of Gnaphalieae inhabit Japan and Taiwan.

There are striking differences in EcM and overall plant richness between arctic ecosystems in the Northern and Southern Hemispheres. Partly due to greater land

mass and less isolation, Arctic ecosystems harbour an order of magnitude more plant species than the Subantarctic habitats. Subantarctic tundra habitats lack EcM vegetation, and flora of the very limited alpine zone of Australia and New Zealand includes very few EcM plant species (*Nothofagus gunnii*, *Poranthera* and certain myrtaceous shrubs). There are two alternative but non-exclusive explanations for the lack of EcM vegetation in the Southern Hemisphere tundra habitats. Although Antarctica was covered with temperate forests until the Mid-Miocene (stunted *Nothofagus* until the Late Pliocene), the last cold-adapted putative EcM plant groups may have become extinct during the successive glaciation of Antarctica in the Pleistocene. Alternatively, truly cold-adapted EcM groups (such as *Persicaria vivipara* and *Kobresia* in the Northern Hemisphere) may have never existed in the Antarctic realm, but this is unlikely given the well-established niche of EcM plants in Arctic habitats, similar of which must have existed in the Interior Antarctica since the Early Oligocene (Poole and Cantrill 2006). The climatic conditions that nowadays prevail in Subantarctic islands are comparable to these in Iceland, Svalbard and the Aleutes, except Subantarctic islands receive more radiation (more distant from the pole) but less warm air due to the cold Circum-Antarctic current. Nearly all Subantarctic islands are of volcanic origin, relatively young and small in size, which may have hampered dispersal of seeds and mycorrhizal inoculum from distant land masses. Both in Arctic and Antarctic islands, glacial cycles wiped out the pre-Pleistocene vegetation (e.g. in the Falklands: Macphail and Cantrill 2006), but Subantarctic islands had much poorer opportunities for re-migration due to the lack of vegetation in Antarctica and remoteness of other continents. The most cold-tolerant Australian EcM plants *Nothofagus, Eucalyptus* and *Poranthera* are not good dispersers. *Leptospermum* and certain species of Gnaphalieae have successfully inhabited small temperate islands off the main islands of New Zealand but no further than 1000 km distance.

20.5 Historical Distribution

20.5.1 Overall Patterns

Many of the EcM plant groups such as Pinaceae, Fagales and Myrtoideae have extensive pollen and macrofossil record, whereas many others have none due to their limited pollen production, lack of distinct characteristics for identification, rarity or neglectance (presence near the detection limit). Therefore, several EcM groups are absent from paleobotanical data sets (Muller 1981). The fossil record and current biogeographic disjunctions indicate that the historical distribution of EcM plants differs greatly from the present patterns. In most EcM groups, there is evidence for dispersal, range expansion and range contraction in relation to climate change and dramatic shifts in the sea level.

In many EcM groups, several-fold differences are common in divergence time estimates among studies (Chap. 19) and in calibrated phylogenies vs. fossil record. Although both approaches have their pros and cons, such striking differences indicate the overall poor reliability and ample uncertainty in our understanding of critical evolutionary events in EcM plants. Nonetheless, both fossil record and phylogenies strongly agree that Pinaceae represent the oldest EcM group that dates back to at least the Mid-Jurassic (Leslie et al. 2012; Lu et al. 2014; Zanne et al. 2014). Pinaceae have been present only in the Northern Hemisphere throughout the geological history and therefore their influence on Southern Hemisphere EcM plant and fungal groups is indirect and mediated through the more widely distributed Fagales and Dipterocarpaceae–Cistaceae that probably evolved EcM in the habitats involving pinaceous and fagalean taxa, respectively (see Sect. 20.3). Fagales, Dipterocarpaceae–Cistaceae and Myrtoideae evolved in the Late Cretaceous and became widely distributed either in warm tropical or temperate/subtropical habitats. These groups and Pinaceae probably had a great direct influence on the evolution of multiple EcM plant and fungal lineages due to their monodominant habit and transformation of soil conditions to highly organic that is favourable for EcM symbiosis in general. Myrtoideae probably had a substantial role in Australian EcM plant and fungal evolution, because they colonised the drier and more seasonal interior of the continent, while *Nothofagus* were in continuous decline since the Miocene (Hill 2004). The well-known Arctic EcM groups *Kobresia* and *Persicaria vivipara* probably evolved EcM habit in high plateaus of Central Asia (see Sect. 20.3), potentially in the rhizosphere of dwarf Betulaceae, Salicaceae or Pinaceae. The evolution of South American EcM groups Pisonieae and *Coccoloba* is poorly understood, but it could be related to the habitat of ancestral Dipterocarpaceae, *Pakaraimaea* or *Dicymbe* given the S American rather than N American affinities of their mycobionts (see Sect. 20.5.5).

20.5.2 Mesozoic

The Jurassic and Cretaceous climates were relatively moist and warm and supported extensive tropical forests and temperate forests dominated by multiple groups of gymnosperms and ferns, many of which have become fully extinct or nearly so (e.g. ginkgophytes; Morley 2000). Pinaceae evolved in the Mid-Jurassic, but their distribution remained sporadic and mainly restricted to subtropical mountains in Central and E Asia and NW America in the Mesozoic (LePage 2003; Taggart and Cross 2009). Since the Mid-Cretaceous (100 Ma), angiosperms became more frequent in the pollen record worldwide (Morley 2000) and the early Fagales (*Normapolles*) established dominance in the interconnected NE America and Europe (Friis et al. 2006).

In the Mid-Cretaceous, S America broke off and slowly rotated away from Africa, with formation of the Atlantic Ocean. Due to close proximity and multiple oceanic islands, floristic interchange must have been common in the Late

Cretaceous and Early Tertiary relative to that at present. In the Late Cretaceous, Myrtoideae probably evolved in Australia, Dryadeae in North America and Dipterocarpaceae–Cistaceae in North Africa.

The Terminal Cretaceous (TC) impact caused some 40–50% loss in plant generic (palynomorph) richness, with much greater reduction at the species level (Morley 2000). There is no evidence for a strong decline in EcM vegetation based on fossils of the surviving lineages (but see Friis et al. 2006 for *Normapolles*), but we have no information about the mycorrhizal status of groups that went extinct. The losses of TC impact took ca 10 Ma (entire Paleocene) for plants to reach similar level of diversity (Morley 2000).

20.5.3 Paleocene and Eocene

In the Paleocene, *Tilia*, Salicaceae, the *Berlinia* group and Goodeniaceae (questionable) evolved, followed by EcM evolution in the *Afzelia* group, *Coccoloba*, Arbutoideae *s.lat*., Mirbelieae, Pisonieae, Pomaderreae and Asteropeiaceae in the Eocene. In Africa, the *Berlinia* group and *Afzelia* group of caesalpinioid legumes became rapidly common throughout the African continent along with Dipterocarpaceae. In the Paleocene or Early Eocene, ancestors of *Pseudomonotes, Pakaraimaea* (Dipterocarpaceae–Cistaceae) and *Dicymbe* (*Berlinia* group) probably dispersed from W Africa to S America over the narrow Atlantic, which is more likely than vicariance events given the Early Cretaceous initiation of separation of the continents.

From the Late Cretaceous to Late Eocene, Fagales became increasingly common in the canopy of Europe and NE America including the high latitudes, whereas Pinaceae had a relatively greater role in mountain habitats of NW America and Asia (Morley 2000; LePage 2003; Friis et al. 2006). In the Southern Hemisphere, *Nothofagus* became more abundant in the Early Tertiary. Relative increase in the proportion of EcM vegetation worldwide was certainly not a linear process given short-term but influential climatic fluctuations. Although there is no firm evidence, it is likely that EcM plants established monodominant patches of vegetation in the predominately AM matrix, because all early evolved EcM groups have such a capacity (all groups that evolved before the Late Paleocene; cf. Fig. 20.1).

In the Early Paleocene, the Western Interior Seaway between NW America and NE America receded, allowing migration of biota including Fagales, Pinaceae and Salicaceae initially over the southern land bridge in Texas (Donoghue and Smith 2004). In the Paleocene and Eocene, many EcM plant groups including Pinaceae, Fagales, Salicaceae and Arbutoideae dispersed over the North Atlantic land bridge. In the Early Eocene, Australia broke off from Antarctica+South America and drifted northward, causing vicariance patterns in all Nothofagaceae, Casuarinaceae and Myrtoideae.

The Eocene was an epoch for the ‘Great North African Interchange’ for EcM symbiosis. Species of the *Berlinia* group (ancestors of modern *Isoberlinia*) and

Dipterocarpaceae–Cistaceae co-existed with several fagalean families as well as with Pinaceae and Salicaceae in N African and S European rainforests (Morley 2000; see Sect. 20.3), potentially facilitating host shifts and broadening of host range in multiple fungal species.

In the end of Eocene, the global climate cooled abruptly to roughly present level, which was unprecedented considering the prevalence of hot climate in the preceding 100 My (Fig. 20.1). This resulted in massive loss of rainforests from the present temperate latitudes including coastal Antarctica and severe decline in most AM gymnosperms but also thermophilic groups of EcM Fagales, Fabales and Dipterocarpaceae (Morley 2000; Friis et al. 2006).

20.5.4 Oligocene and Miocene

After the Terminal Eocene cooling, EcM vegetation thrived both in temperate and subtropical forests. In the Mid-Oligocene, the narrow Turgai Strait became closed in West Asia, allowing massive biotic exchange between E Asia and S Europe that included many EcM plant groups. This event rendered European and W Asian climate more continental and, along with the Terminal Eocene cooling, resulted in development of northern boreal forests dominated by Pinaceae and temperate woodlands dominated by modern genera of Fagales and Pinaceae (Tiffney and Manchester 2001; Taggart and Cross 2009). The Beringian land bridge became again available as a dispersal corridor that was used by multiple temperate and subtropical plants. By contrast, the North Atlandic land bridge between NE America and Europe became submerged.

In the Mid-Oligocene, Patagonia lost its land connection with Antarctica, generating a vicariance event for *Nothofagus*, Myrtoideae and Casuarinaceae. Probably in SW Australia, the EcM genus *Platysace* evolved in the Early Oligocene. *Acacia* evolved most probably in N Australian moist forests in the Late Oligocene (Miller et al. 2013). In South American rainforests, both *Aldina* and *Gnetum* evolved in the Oligocene. The Oligocene climate drying trends continued in the Early Miocene, when *Gymnopodium* and *Achatocarpus* evolved in Central America. *Poranthera*, *Amperea* and Gnaphalieae evolved in Australia. In the Early Miocene, the uplift of New Zealand was initiated, which opened its remaining depauperate vegetation and vacant niches for immigrants mainly from Australia, but perhaps also from New Caledonia and Antarctica (Wallis and Trewick 2009). Although *Nothofagus*, *Eucalyptus*, *Acacia*, Casuarinaceae, Pomaderreae and perhaps some other Australian groups became rapidly established in New Zealand, these woodland taxa (apart from *Nothofagus*) became extinct again in the Late Miocene or Pliocene due to increasing humidity, loss of fire-dependent savanna ecosystems and orogeny that greatly reduced the area of nutrient-poor soils (McGlone et al. 2016).

By the Mid-Miocene, Patagonia had rafted to sufficient distance from the Antarctic Peninsula to enable formation of the very cold Circum-Antarctic current that severely intensified climate cooling and drying in all Southern Hemisphere

land masses and strongly expanded glaciation in the Interior Antarctica that was initiated in the Oligocene (Zachos et al. 2001). These events resulted in decline of global temperature and drop of sea level to 100 m below the current level, both representing the minimum values of the previous 100 My. Intensive glaciation and drying were catastrophic to Antarctic vegetation that lost much of its forest cover and became a steppe and tundra habitat in glacier forefronts. The Southern Hemisphere rainforests became fragmented and replaced by sclerophyll vegetation, savannas and shrublands. These changes were less severely mirrored in the Northern Hemisphere, where temperate and boreal forests dominated by Fagales and Pinaceae became increasingly dominant over vast areas. In addition, the summer-dry Mediterranean ecosystems evolved in North America and Europe, comprising the characteristic EcM *Cercocarpus* (only NW America), *Quercus*, *Pinus*, Cistaceae and *Arbutus* (Axelrod 1989; Palamarev 1989). Across the Americas, the Cordilleran uplift took place in the Mid-Miocene, re-directing the Amazon and generating rain shadow habitats (Unruh 1991; Gregory-Wodzicki 2000; Hoorn et al. 2010).

The tropical biomes were also strongly affected by climate cooling and drying, resulting in unprecedented contraction of rainforests and evolution of savanna and tropical grassland habitats that were subjected to wildfires. As floristic novelties, semideserts and deserts evolved in the heart of N Africa and Central Asia and in rain shadow areas of the Americas (Morley 2000; Senut et al. 2009). Substantial changes in temperature and precipitation resulted in adaptive radiation of multiple 'woodland' EcM plant groups such as Myrtoideae, *Acacia*, Cistaceae, *Berlinia* and *Afzelia* groups, *Uapaca* and perhaps Asteropeiaceae.

The Mid-Miocene drop in sea level enabled members of Dipterocarpaceae, Fagales and Pinaceae to successfully colonise Borneo, Sumatra and Java islands (Morley 2000) but not New Guinea. New Guinea was re-colonised by *Nothofagus* in the Miocene and probably received a number of Myrtoideae and Casuarinaceae species from Australia. In spite of the reduction of southern temperate *Nothofagus* forests and development of non-EcM desert habitats, EcM vegetation certainly gained the greatest relative importance due to the development of completely EcM-dominated seasonal woodland ecosystems in Africa and Australia as well as increasing importance of boreal and temperate forests and Mediterranean ecosystems.

20.5.5 Pliocene and Pleistocene and Recent Migrations

In the Pliocene, the Late Miocene climatic drying trends proceeded. In the dry Central Asian highlands, probably, the ancestors of *Kobresia* and *Persicaria vivipara* evolved EcM, whereas, the EcM genus *Adenostoma* evolved in California. In the Late Pliocene, the Isthmus of Panama formed that disrupted circulation of warm oceanic water and resulted in climate cooling especially in the temperate and polar habitats.

Multiple plant and animal groups migrated over the Isthmus of Panama in a process termed 'the Great American Interchange' (Stehli and Webb 2013). Although most plants migrated from south to north, the EcM vegetation 'behaved like animals'–*Alnus*, *Quercus*, *Colombobalanus*, *Alfaroa* (all Fagales), *Arbutus*, *Arctostaphylos*, *Monotropa* (all Arbutoideae s.lat), *Salix*, *Crocanthemum* and *Achatocarpus* all migrated from Central America to S America. The S American EcM groups Pisonieae and *Coccoloba* had reached N America in the Eocene, whereas *Aldina*, *Dicymbe, Pseudomonotes* and *Pakaraimaea* probably never made their way to NW Colombia. This great imbalance is certainly caused by the fact that the latter S American tropical EcM plants are specialists of very poor soils, and such edaphic conditions are not met in NW Colombia and E Panama. Conversely, the N American and Central American taxa are of temperate or subtropical origin that migrated successfully along the mountain ranges. Since these groups were long present in high plateaus of Mexico, they may have had an evolutionary competitive advantage over S American high elevation plants that had much less time (since the Mid-Miocene) to adapt to upper montane conditions. Both S American and N American plants migrated along with their EcM root symbionts, providing an opportunity for broadening of host range. However, present evidence indicates that this has happened to a very limited extent, because Pisonieae and *Coccoloba* associate with S American clades and Fagales and Arbutoideae associate with N American clades of the same fungal lineages (Wilson et al. 2012; Tedersoo et al. 2014b; De Crop et al. 2017). There is evidence for the additional association with N American fungi in *Coccoloba uvifera*, but this could be related to its unusual habitat in coastal coral sands (Chap. 16).

The Pleistocene epoch is renowned for its unprecedented glacial cycles and the rise of humans as ecosystem engineers through mastering fire. The multiple glacial cycles caused mean annual temperature shifts of >10 °C in arctic and temperate latitudes in both hemispheres (Tzedakis et al. 2012). Although cooling was less severe in tropical habitats, decline in land and ocean temperature and increase in land area reduced annual precipitation. This resulted in severe contraction of lowland rainforests that were largely replaced by savannas at the glacial maxima (Ray and Adams 2001). This was most severe in Africa and North Australia that lost nearly all rainforest cover except fragments in small refugia including riparian and coastal mangrove habitats (Hill 2004; Plana 2004). The Australian rainforest decline was somewhat mitigated by opening of the Arafura corridor that, together with New Guinea, must have provided a refugium for Australian rainforest plants (Hill 2004). Climate drying affected Malesian vegetation the least because of its highly heterogeneous landscape (Kershaw et al. 2011).

Throughout the world, populations of plant species migrated towards the equator and down the elevational gradient with declining temperature and advancing glaciers. Of temperate regions, Europe, New Zealand and Patagonia were certainly hit the hardest by glacial maxima because of limited possibilities for latitudinal migration but also relatively small land area. In Europe, the number of surviving tree species was an order of magnitude less than in E Asia, with NW America and NE America being intermediate (Qian and Ricklefs 2000). Although there were

multiple glacial refugia in coastal areas much north of the glacial front, these contributed to the survival of populations of certain species but did not replace lower latitude refugia for preserving species. Interestingly, recolonisation of flora was more efficient from refugia in lower latitudes, perhaps due to their greater size and head start as these areas were released earlier from glacial influence (Bennett et al. 1991). In the Pleistocene glacial cycles, *Kobresia, Dryas* and *Persicaria vivipara* probably used the mountain tundra corridors to reach the Arctic habitats, adding to dwarf *Salix* and *Betula* that were probably common in the Arctic Circle previously.

A substantial proportion of water was locked in glaciers during the Pleistocene maxima, which resulted in the drop of sea level by up to 130 m below the current level and 30 m below the Mid-Miocene climate minimum (Fig. 20.1). These events caused merging or increase in size of many continental and a few volcanic islands (New Zealand, Mascarenes, Kerguelen, New Caledonia, Fiji, some Galapagos Islands, some Vanuatu Islands and especially the Seychelles) and connected multiple islands to continents (Japanese Islands+Sakhalin, Tasmania, Sri Lanka, the Falklands, the British Islands, most Arctic islands). Emerging land connections facilitated migration of biota and enhanced allopatric speciation after the connections were lost. More importantly, the drop in sea level closed several shallow seas and straits and connected nearby large land masses. Of these, the Beringian land bridge and Canadian Arctic land bridge were too cold to allow dispersal of any non-Arctic species.

The Wallacea land bridge represents the best known pathway for migration for plants and animals during the Pleistocene glacial maxima (Sniderman and Jordan 2011). More specifically, this land bridge connected SE Asia to the Philippines, Borneo, Sumatra, Java and smaller islands from Bali to Alor but also the Lesser Sundas and Andaman Islands. However, there was probably no SE Asian land connection to Sulawesi (which was formed after the collision of eastern and western fragments in the Late Miocene; Hall 2009), Wetar, Timor and the Moluccas. Australia was broadly connected over the Arafura Corridor to New Guinea that had direct land connection to New Britain and New Ireland but not to the Solomons in the east. This land mass was further connected to the Halmahera and Seram Islands but not to Sulawesi in the west (Hall et al. 2012). The magnitude of sea floor opening and migration of biota were much greater in the Pleistocene than in the Miocene, but the migration was highly asymmetric, with nine times more SE Asian plants becoming established in the eastern islands (Sniderman and Jordan 2011). This 'Great Wallacean Interchange' was similarly intense but more symmetric for EcM plants, characterised by eastward migration of Dipterocarpaceae, Pinaceae, Fagales and the *Afzelia* group one the one hand and northward and westward migration of multiple Myrtoideae groups, Casuarinaceae, *Acacia*, Goodeniaceae and Gnaphalieae on the other hand. While *Pinus* reached only the Philippines and Central Sumatra, other Asian groups dispersed to Sulawesi and New Guinea in multiple events. Notably, none of the SE Asian taxa (except *Intsia*) reached Australia, or they became soon extinct due to unsuited climate or soils. Although several groups of early diverging rainforest Myrtoideae were long present in New Guinea, it is likely that most groups

such as *Baeckea*, *Tristaniopsis*, *Leptospermum* and *Melaleuca* migrated from Australia via New Guinea to the Sunda Islands and SE Asia in the Pleistocene due to the paucity in the earlier fossil record. *Casuarina equisetifolia* may have used the same route to reach Bangladesh, but it may have equally likely used dispersal by sea as for reaching the Pacific Islands. The same dispersal options have been used by Goodeniaceae, Gnaphalieae and *Acacia s.str*. The Great Wallacean Interchange resulted in exchange of mycobionts after >60 My separation (excluding the less intense Mid-Miocene contact). There is ample evidence that multiple fungal taxa from the /laccaria, /descolea, /hysterangiales and /boletus lineages (cf. Tedersoo and Smith 2013) shifted their historical hosts and migrated over the Wallacea (Horak 1983; Pirozynski 1983; Hosaka et al. 2008; Chap. 13), but the prevailing directionality and timing remains to be established.

20.6 Invasions

Since the emergence of agriculture, humans have intentionally distributed plants by carrying seeds and sometimes rhizomes of staple plants when migrating and trading (Davis and Landis 2011). For millennia, this concerned vegetables, cereal crops and trees with highly nutritious edible fruits that are all AM or non-mycorrhizal, except *Castanea sativa* and *Carya* spp. that are EcM. The crop plants but also weeds associated with ploughing and disturbance were distributed to vast distances already in the Early Bronze Age, becoming naturalised and locally invasive several millennia ago (Davis and Landis 2011). In the Ancient Greek and Roman colonies, trees (including the EcM *Arbutus*, *Quercus* and *Tilia*) were systematically planted for ornamental purpose. Large-scale planting of forest trees with a focus on many EcM plants was undertaken after the deforestation of land in the industrial era in late eighteenth century. Since then, land owners became careful about selecting proper tree species and varieties (seed lots) to ensure good revenues. Simultaneously, European countries established colonies in other continents and encouraged intercontinental trading of exotic plants as seeds but also potted seedlings in both directions. In the Linnean era, the influence of religion weakened and there was a trend of growing naturalism among the wealthy. In the late eighteenth century, many exotic trees were first planted in parks and arboretums. Successful establishment and growth motivated the selection of high-quality timber species in large-scale forestry trials in European countries and their overseas colonies. Since the mid-nineteenth century, large-scale forest plantations with exotic trees were initiated all over the world (Simberloff et al. 2010). Most plantations failed due to insufficient knowledge about the species' ecological requirements including root symbioses. While AM trees and most rhizobial and actinorrhizal plants obtained their root symbionts from the existing indigenous soil microbial pool (Chaia et al. 2010; Rodriguez-Echeverria 2010), many EcM plants were more demanding and especially pines failed to establish without compatible EcM inoculum (Mikola 1969; Richardson et al. 2000; Rejmanek and Richardson 2011). A single

co-introduced EcM fungal species may have often resolved the problem (Hayward et al. 2015a). By contrast, the introduced angiosperms became associated with local fungi to some extent but depending on system (Tedersoo et al. 2007; Hayward et al. 2015b; Bogar et al. 2015). At least some of the Australian eucalypts arrived as potted plants that were already colonised by EcM fungi, which were further co-introduced to Africa, Europe and Americas by the mid-nineteenth century (Harcharik 2000). In a suitable climate, many EcM plant species became soon naturalised and several taxa became invasive (Simberloff et al. 2010). The introduced and invasive range of EcM plant genera is indicated in Supplementary Information (http://dx.doi.org/10.15156/BIO/587454).

EcM plants form a disproportionately high share of all invasive trees, and many species are regarded as the most severe invaders (Richardson 1997; Richardson et al. 2000; Richardson and Rejmanek 2004). Among the 90 most hazardous invasive plants (Traveset and Richardson 2014), 21% are EcM, whereas EcM plants contribute 1.7–2.4% to global higher plant richness (Chaps. 19 and 21). Many EcM plant lineages such as Pinaceae, Fagales, *Tilia*, Salicaceae, Dipterocarpaceae–Cistaceae, Arbutoideae *s.lat.*, Myrtoideae, *Berlinia* and *Afzelia* groups, *Aldina*, *Gymnopodium,* Pisonieae (*Pisonia grandis*), *Coccoloba* (*C. uvifera*), Dryadeae (*Cercocarpus ledifolius*), *Adenostoma, Acacia* and *Kobresia* (in herb category) commonly form monodominant patches of vegetation in their native range (Torti et al. 2001; L. Tedersoo, personal observation). Members of most of these groups (except *Kobresia*, *Tilia* and the *Afzelia* group) produce slowly decomposing litter that accumulates under the canopy either due to slow decomposition rate (Cornelissen 1996) or species-specific edaphic conditions unfavourable for decomposition. In humid conditions, the thick litter and humus layers are heavily colonised by EcM roots and fungi that have improved capacities to take up organic forms of N and P compared with AM systems (Read et al. 2004). It is an evolutionarily viable strategy to promote its own growth via facilitation of its symbionts through positive soil feedback (Newbery et al. 1997). The thick and commonly allelopathic litter often prevents germination and establishment of other small-seeded AM and non-mycorrhizal native plants. Furthermore, high loads of dry litter and dry biomass promote flammability. Burning kills competing plants and their seeds (Kull 2004) but specifically triggers seed germination or sprouting from buttresses in species of *Pinus*, *Acacia* and *Eucalyptus*. Several ecosystems invaded by easily flammable plants such as Madagascar, New Caledonia and New Zealand are almost completely 'naïve' to fire (Kull 2004; Bond et al. 2005). Invasions of these EcM woody plants are particularly striking in dry and wet grasslands and shrublands of S America, New Zealand, South Africa and highlands of oceanic islands, where invading trees introduce multidimensional habitat alteration in terms of shelter, decomposing wood, altered water regime, nutrient cycles and fire intensity that transform grassy biomes to forests (Rundel et al. 2014). In these biomes, species of *Pinus*, *Acacia* and *Eucalyptus* commonly form mixed communities with sparse understorey comprising of native plants or invasive AM shrubs (such as *Lantana camara*), ferns and climbers (e.g. S Brazil and Madeira; L. Tedersoo, personal observation). These 'alien communites' may comprise

dominant members and co-introduced root symbionts originating from two or three continents and the native habitat. Competitive interactions and evolution of biotic association networks in these spontaneous communities remain poorly understood, but warrant urgent research because of their rapid spread and unique dynamics.

Tropical and subtropical island ecosystems harbour the highest proportion of exotic and invasive species (Richardson and Pyšek 2012). Distant volcanic islands have several vacant ecological niches due to high elevational range and relatively low native plant richness due to low migration rates. Madeira, Hawaii and New Zealand perhaps suffer most from localised invasion of multiple EcM (and AM) plants because of massive introduction of temperate and subtropical plants and their high rates of naturalisation.

The invasive EcM plants and their co-invasive mycobionts (cf. Dickie et al. 2010) provide a synergistic effect termed as invasional meltdown, where the interacting organisms promote each other's fitness or ecological harm (Simberloff and Von Holle 1999). While EcM plants typically depress indigenous plants by direct competition and indirectly by increased incidence and severity of fires, EcM fungi may outcompete native fungi or alter soil nutrient cycling (Chapela et al. 2001; Schwartz et al. 2006). Direct interaction between co-introduced EcM fungi and native non-host plants is probably of minor importance (but see Streiblova et al. 2012). In association with invasive hosts, EcM fungi certainly reduce nutrient uptake of native AM plants due to more efficient uptake mechanisms especially in organic-rich soils (Read et al. 2004). Deep-rooted, well-nourished EcM trees such as *Eucalyptus* and *Acacia* may exhibit high rates of transpiration that deplete local ground water sources that further suppress native vegetation (Calder et al. 1997). The alien EcM fungi may further shift to the root systems of native EcM trees (Jairus et al. 2011; Wolfe and Pringle 2012) and possibly outcompete indigenous mycobionts, although no examples are known. Furthermore, habitats with higher EcM plant and EcM fungal dominance exhibit lower overall fungal richness (reanalysis of Tedersoo et al. 2014a data set), suggesting that increasing abundance of invasive EcM fungi may reduce local fungal diversity.

20.7 Speculations About Future Anthropogenic Biogeography

20.7.1 Climate Warming and Drying Due to Rising CO_2 Levels

For the next few decades and centuries, there are multiple conflicting climate change scenarios that depend on the included parameters, parameter values and consideration of their interactive effects. Most climate models agree that given the continuation of current trends in rising CO_2 and methane emissions, the global temperature will rise by 2–4 °C, low and mid-latitudes will receive generally less

rainfall, the sea level will rise by 1–2 m and annual climatic patterns become more variable by the end of the twenty-first century (Stocker et al. 2013). Hence, the global temperature will soon surpass the maxima unexperienced since the Late Eocene.

Changing climate is expected to promote invasions of plants and exotic pests and pathogens, because populations of native plant species become increasingly more stressed (Thuiller et al. 2007; Bradley et al. 2010; Garrett et al. 2014). Even if the changing climate may not be suited for species that are already invasive, it may trigger invasive behaviour amongst the many introduced species that have locally naturalised. Invasive EcM plants tend to be habitat generalists with proven capacity of adaptation in spite of their low genetic diversity in the invasive range. Increasing fire frequency and enhanced nutrition via co-introduced or native EcM fungi may further promote their invasive potential.

Elevated concentration of CO_2, warming and drying have both direct and indirect effects on vegetation and mycorrhizal associations (Johnson et al. 2013). Elevated CO_2 facilitates photosynthesis and respiration, resulting in greater demand for mineral nutrients and carbon allocation belowground. Under elevated CO_2, EcM trees allocate relatively more carbon to their fungi belowground compared with AM trees to mine for calcium from basalt in a weathering experiment (Quirk et al. 2014). Consistent with this, a global meta-analysis indicated that EcM plants experience greater growth benefits than AM plants in nutrient-poor soils, but the response to elevated CO_2 is similar in fertile soils (Terrer et al. 2016). These results indicate altered competitive balance and predict greater dominance of EcM symbiosis from temperate to arctic ecosystems. Nitrogen pollution may slightly ameliorate this, because labile N compounds are easily accessible to AM plants (Read et al. 2004). Altered temperature and soil moisture may have more context-dependent and species-specific effects.

Warming in particular will have marked effects on communities in interglacial refugia such as alpine habitats colonised by EcM *Dryas, Kobresia* and *Persicaria vivipara*. The present alpine and arctic habitats will be taken over by temperate and boreal coniferous forests (Millar and Stephenson 2015) that would result in greater overall EcM domination. The arctic vegetation would persist in the northern parts of continents and areas currently covered by glaciers such as Greenland, N Canada and Arctic islands.

In warm temperate habitats that are predicted to receive similar amount or less precipitation, shrublands and savannas will expand (Millar and Stephenson 2015), reducing the proportion of native EcM vegetation but increasing the invasive EcM *Acacia* and *Eucalyptus* at the expense of native AM plants. Much of the EcM-dominated Mediterranean habitats and subtropical lowland forests are expected to shift to AM-dominated deserts, semideserts and shrublands, with potentially increasing role of *Acacia* and perhaps Cistaceae in relatively moist habitats. Besides climate drying, soil salinisation due to receding saline lakes (Central Asia, North America) and mobilisation of deep ground water (SW Australia, Central Asia) promote desertification in subtropical ecosystems (Dregne and Chou 1992). The montane subtropical forests will be probably relatively little

affected by climate change, except losing the most moisture demanding AM plants that are expected to be replaced by modern dry tropical forest trees that are also mostly AM. In all parts of the Southern Hemisphere, *Nothofagus* species already indicate signs of decline, suggesting that the warming and drying climate but also extremely cold spells are relatively unfavourable for this group now and in future (Veblen et al. 1996). *Nothofagus* spp. become mostly replaced by the co-occurring AM trees, except additionally by the EcM Myrtoideae in Australia.

Depending on region, dry tropical forests and woodlands will gain or lose rainfall, either shifting to shrublands and dry grasslands or involving more evergreen components, respectively. Similarly, tropical rainforests would turn into savannas and dry forests or retain their structure in case of extra rainfall as predicted for coastal habitats and rain catching lower and upper montane forests (Morley 2000). Since tropical savannas and dry forests of Africa, Central America and Australia are notably more dominated by EcM vegetation compared with flanking rainforests (this is largely comparable in SE Asia), the relative proportion of EcM vegetation will probably increase in the tropical belt. The native dry tropical vegetation will receive a considerable supplement from the local pool of invasive plants, in particular savanna-inhabiting species of *Pinus, Acacia* and *Eucalyptus*. Because of maladaptation to disturbance, tropical forests will certainly suffer most from the interacting direct human influence (Morley 2000; Cochrane 2011). Fragmentation and agricultural activities induce longer dry spells, which in turn facilitate hotter and more destructive fires. Fires will hit S America worst, because it has experienced relatively less burning so far (Cochrane 2011). Since tropical rainforest plants tend to exhibit the smallest range (Rapoport's rule), many AM and EcM species will become extinct because of their inability to migrate across the anthropogenic landscape (Urban 2015) and adaptation to specific soil conditions. I believe that of EcM plants, this may especially concern members of the *Berlinia* group in Central Africa, dipterocarps in Sri Lanka, all Malagasy EcM plants and *Coccoloba* spp. and *Pseudomonotes tropenbosii* in S America. Species distribution/niche modelling would greatly improve our understanding about the potential effects of climate change on native and invasive plants at the level of alpha taxonomy (Hui et al. 2014).

20.7.2 Mutualists and Pathogens

The loss of natural mutualistic symbionts and accumulation of pathogens pose a threat to native plants (Tylianakis et al. 2008). Given the wide distribution and functional redundancy of mycorrhizal fungi and nitrogen fixing bacteria and their relatively low levels of specificity, loss of certain root symbiotic microbial species is probably a negligible concern from the plant perspective. The loss of pollinators represents a much greater problem for plants and agriculture, because many tropical plants are pollinated by a few species of insects or birds (Kearns et al. 1998). Extinction of local bee pollinators in America is partly driven by the invasion of

hybrid 'killer bees' (Africanised honey bees, *Apis mellifera scutellata*), extensive use of pesticides for genetically modified crops, climate shifts and spread of parasites (Kearns et al. 1998). Most EcM plants are pollinated by wind or bees or more rarely with birds, bats, wasps and/or other insects, but species-specific associations are not known.

The rise and dispersal of fungal and oomycete pathogens, viruses and insect pests may play an increasingly important role in determining the distribution of both native and invasive plants, with a potential to re-format the scenarios of vegetation shifts as based on examples from recent decades (Desprez-Loustau et al. 2007; Garrett et al. 2014). Due to anthropogenic introduction of the pathogen *Cryphonectria parasitica* from Asia, NE American deciduous forests have nearly lost *Castanea dentata,* one of the dominant trees that now survives vegetatively in the understorey (Desprez-Loustau and Rizzo 2011). The newly emerged hybrid pathogen *Phytophthora alni* threatens *Alnus* stands in Central Europe, whereas *Phytophthora ramorum*, the causal agent of 'sudden oak death' syndrome, devastates *Quercus* forests in NW America and W Europe (Rizzo 2011). The global trade of timber has caused inadvertent introduction of several insect pests since the early 1800s. For example, the Caucasian beech scale insect *Cryptococcus fagisuga* infests *Fagus americana,* paving a way for further attacks by a potentially alien fungus *Neonectria faginata* and collectively causing massive dieback of stands; the wood wasp *Sirex noctilio* degrades NW American *Pinus* forests (Liebhold and McCullough 2011). Desprez-Loustau et al. (2007) indicate two basic mechanisms of pathogen (and pest) invasion: first, pathogens may find naïve alternative hosts amongst taxa that are genetically of functionally similar to their native hosts and become serious pathogens (e.g. *C. parasitica* case); second, distant populations of the same species or closely related allopatric species may cross and become highly virulent to their native hosts or switch to other hosts that may be physiologically or phylogenetically unrelated (Olson and Stenlid 2002; *P. alni* case). Due to climate change and growing global trade, outbreaks of invasive pathogens and pests and their damage are exponentially increasing, a trend likely to continue in the future (Garrett et al. 2014).

It is theoretically possible to recruit specific biocontrol agents and genetically modified pathogen clones for eradication of invasive pathogens, insects or plants, but this is unlikely due to poor previous results, high costs and great risks. The function and distribution of biocontrol organisms is largely uncontrollable in natural conditions and there is a serious risk of their persistence and potential host shifts. Moreover, these biocontrol agents could accidentally disperse to the native range of the target hosts, potentially causing catastrophic damage (consider the ecological importance of Pinaceae, *Eucalyptus* and *Acacia* in their native range!). In addition, the evolving national and international laws would not permit such actions on a large scale without the consent of potentially affected countries. The scenario of bioterrorism targeting EcM plants is unlikely because of the relatively low immediate damage compared with humans, domestic animals and crops (Elferink and van der Weijden 2011).

20.7.3 Synergistic and Overall Effects

Since the two last centuries, mankind has had much greater effect than natural processes on the distribution of plants and animals, either directly or indirectly through altered climate and introduced biota. Anthropogenic impacts have been increasing exponentially due to globalisation, growth and more luxurious resource consumption of the human population, the associated pollution and their interactions.

The future biogeography of biota including EcM plants will be determined by a function of climate change, trade-off between economic benefits and conservation, invasion ecology and natural dispersal. According to an optimistic scenario, around 7–10% of species will become actually or effectively (no mating partners) extinct by the twenty-first century, with a greater threat to tropical and island species with small ranges (Urban 2015). The alien plants with wide ecological amplitude will continuously invade natural habitats. Many of these are already accepted as part of the 'local' community and cultural heritage (Dickie et al. 2014a). Eradication measures would be overly costly and inefficient considering the political borders and conflict of interest. Probably all countries will increasingly suffer from the spread of exotic pathogens that devastate crops and native plants but also invasive EcM trees.

Decades ago, many biodiversity hotspots were identified under strong human influence and were declared as severely degraded and threatened, with urgent needs for conservation measures (Myers et al. 2000 and references therein). However, the degradation status has improved in none of these in spite of the alarm. Instead, Beaumont et al. (2011) indicate that especially tropical biodiversity hotspots are being subjected to intensive climate change that may severely reduce the surviving biota and increase the extinction dept because of plants' inability to cross anthropogenic dispersal barriers (e.g. fields, plantations, settlements) to reach climatically suitable habitats. This will be exacerbated by the adverse effects of increasing drought events and rising sea level on human population that may require to recruit the remaining fragments of native vegetation for agriculture and re-settlement (India, SE Asia, Pacific islands). Increasing globalisation and homogenisation of human population is being followed by globalisation of biota including vegetation as well as the associated mutualistic and pathogenic microorganisms (Hulme 2009). Local communities will become impoverished, globally homogenised and structurally altered compared with any pre-Anthropocene mass extinction event. From economic perspectives, habitat transformation by invasive EcM plants may be beneficial due to increased timber revenues and honey production (Myrtoideae and *Acacia* produce excellent nectar) and sentimental value but also long-term soil carbon storage (Averill and Hawkes 2016). Over a longer perspective, populations of invasive species will continue both integration into the biotic interaction networks and rapid adaptive evolution in exotic habitats, with sympatric and allopatric speciation taking place in the frame of 10^5–10^6 years (Pennington et al. 2010).

The next glacial maximum is estimated to occur in approx. 10,000–11,000 years. However, the amplitude of insolation and orbitual configuration of the Earth will strongly interact with the disruption of polar ocean currents, unprecedented atmospheric CO_2 level, methane and dust concentrations, and mankind's economic interests that, taken together, may delay or ameliorate the forthcoming glaciation (Tzedakis et al. 2012). Depending on our ability to control carbon cycling, this may take our planet to a new geological era with greenhouse climate and high sea levels (Archer and Ganopolski 2005) with hardly predictable effects on vegetation and associated soil biota.

20.8 Conclusions

Ectomycorrhizal vegetation is globally more widely distributed than previously anticipated, with phylogenetic richness hotspots in SE and SW Australia and montane regions of Mexico and Europe. Humans have intentionally distributed EcM trees into habitats where EcM symbiosis was previously very rare or virtually absent (S Africa, Central S America, volcanic islands). Particularly in tropical grassland and shrubland habitats, exotic EcM plants have become invasive and transformed the communities to species-poor woodlands with greatly altered water, carbon and nutrient cycling and fire regime. These trends will be certainly accentuated in the near future, but over the forthcoming centuries, human economic and conservationist interests, climate change, invasion ecology and natural dispersal mechanisms will collectively determine the fate and distribution of vegetation including EcM plants.

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Chapter 21
Global Diversity and Importance of Mycorrhizal and Nonmycorrhizal Plants

Mark C. Brundrett

21.1 Introduction

Morphological features, host plants and fungal associates for different types of mycorrhizas are summarised in Table 21.1. Mycorrhizal associations are classified according to the way in which the fungi interact with the host plant root, in particular, the structure of fungal hyphae that form a symbiotic interface with host cells (Brundrett 2004). There are five distinct types of mycorrhizal associations, but only the two most abundant associations, arbuscular mycorrhizas (AM) and ectomycorrhizas (EcM), occur in multiple plant families. Orchid and ericoid mycorrhizas are confined to genera within the Orchidaceae and Ericaceae families, respectively. The sub-epidermal associations of *Thysanotus* species are restricted to a single genus in the family Asparagaceae (Chap. 17).

Mycorrhizal association types are usually consistent within plant species, genera and families, but there are exceptions to this rule as discussed below. Families of plants with multiple root types can be designated as families with both AM and NM species, such as many Australian plants in the families Fabaceae and Myrtaceae, which have both AM and EcM (Chaps. 17 and 19). The designation of plants with nonmycorrhizal (NM) or inconstantly mycorrhizal (NM-AM) roots can also be difficult.

Objectives of this chapter are to discuss issues with the identification of mycorrhizal plants and provide updated information on the global importance of mycorrhizas, as well as regional case studies where mycorrhizal plant diversity or dominance has been determined. The lists of plants provided here are updated from Brundrett (2009) to reflect changes in plant phylogeny and newer databases of plant diversity following the

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Table 21.1 Structural definitions, roles, host plants and associated fungi for different types of mycorrhizas (after Brundrett 2004)

Category	Definition	Main role	Hosts plants	Fungal symbionts
Arbuscular mycorrhizas (AM)	Associations formed within roots that usually have arbuscules and often have vesicles	Nutrient acquisition for plant (P, K, N, etc.)	Most families of vascular plants and some bryophytes	Glomerales (formerly Glomeromycota is now part of the Mucoromycota)
Ectomycorrhizas (EcM)	Associations with a hyphal mantle enclosing short lateral roots and a Hartig net of labyrinthine hyphae that penetrate between root cells	Nutrient acquisition for plant (N, P, etc.)	Certain families or genera of flowering plants and some gymnosperms (some host AM also; Chap. 19)	Most are higher fungi (some ascomycetes and many basidiomycetes; Chap. 6)
Orchid mycorrhizas	Associations where coils of hyphae (pelotons) penetrate within cells in a root or stem in the plant family Orchidaceae	Nutrient acquisition for plant (N, P, etc.)	Orchidaceae	Mostly basidiomycetes in *Rhizoctonia* alliance, also EcM fungi in some cases (Chap. 8)
Ericoid mycorrhizas	Coils of hyphae within very thin roots (hair roots) of the Ericaceae	Nutrient acquisition for plant	Ericaceae	Most are Ascomycetes (Chap. 9)
Thysanotus (subepidermal) mycorrhizas	Hyphae in cavities under epidermal cells, only known from a monocot genus	Expected to be nutrient uptake	*Thysanotus* spp. (Laxmaniaceae)—all but one species is Australian	Unknown (Chap. 17)

latest family classifications (Angiosperm Phylogeny Group 2016) and using revised plant species totals for families (Christenhusz and Byng 2016). Values for the diversity of EcM plants presented here include monotopoid and arbutoid mycorrhizas which are now recognised as variants of EcM (Brundrett 2004). Values for the diversity of mycorrrhizal hosts also include mycoheterotrophic variants of their associations.

21.2 Defining Mycorrhizal and Nonmycorrhizal Plants

Current knowledge about the diversity and ecology of plants with NM, NM-AM or facultatively AM roots is summarised in Tables 21.2 and 21.3. Our knowledge of these plants is substantially limited due to problems with consistency of identification of mycorrhizas in roots, especially in cases where only hyphae and vesicles are present (Brundrett 2009). These issues are summarised in Box 21.1.

Table 21.2 List of all plant known families with nonmycorrhizal (NM) or nonmycorrhizal and arbuscular mycorrhizal (NM-AM) roots

Order	Family	Habit	Ecology	NM-AM	NM
Nymphaeales	Nymphaeaceae	Herbs	H	70	
Ceratophyllales	Ceratophyllaceae	Herbs	H		4
Laurales	Lauraceae (Cassytha only)	Climber	P		19
Piperales	Hydnoraceae (now in Aristolochiaceae)	Herbs	P		7
Piperales	Piperaceae	Woody, herbs	E	3700	
Acorales	Acoraceae	Herbs	H	2	
Alismatales	Alismataceae	Herbs	H	115	
Alismatales	Aponogetonaceae	Herbs	H	56	
Alismatales	Araceaea (some only)	Herbs	H, E	1300	
Alismatales	Butomaceae	Herbs	H	1	
Alismatales	Cymodoceaceae	Herbs	M		17
Alismatales	Hydrocharitaceae	Herbs	M, H		135
Alismatales	Juncaginaceae	Herbs	H	34	
Alismatales	Posidoniaceae	Herbs	M		9
Alismatales	Potamogetonaceae	Herbs	H	110	
Alismatales	Ruppiaceae	Herbs	M, H		8
Alismatales	Zosteraceae	Herbs	M		22
Pandanales	Cyclanthaceae	Herbs	E	230	
unplaced	Dasypogonaceae	Herbs	SB		16
Commelinales	Commelinaceae	Herbs	R (E)		731
Commelinales	Haemodoraceae	Herbs	SB		102
Commelinales	Pontederiaceae	Herbs	H	34	
Poales	Bromeliaceae	Herbs	E	3475	
Poales	Cyperaceae	Sedges	RD, SB, A	5500	
Poales	Hydatellaceae	Herbs	H		12
Poales	Juncaceae	Rushes	H, RD, R		464
Poales	Restionaceae (includes Centrolepidaceae)	Herbs	AT, SR		572
Poales	Typhaceae	Herbs	H		51
Poales	Xyridaceae	Herbs	H		399
Proteales	Nelumbonaceae	Herbs	H		3
Proteales	Proteaceae	Woody	RC		1660
Fabales	Fabaceae (*Lupinus, Daviesia* only)	Shrubs, herbs	CR (a few)		700
Ranunculales	Papaveraceae	Herbs	R	775	
Caryophyllales	Aizoaceae (includes Mesembranthaeae)	Herbs, woody	X, S		1900
Cucurbitales	Apodanthaceae	Herbs	P		10

(continued)

Table 21.2 (continued)

Order	Family	Habit	Ecology	NM-AM	NM
Caryophyllales	Amaranthaceae (includes Chenopodiaceae)	Herbs, shrubs	S, R		2040
Caryophyllales	Caryophyllaceae	Herbs	R, AA		2625
Malvales	Cytinaceae	Internal	P		10
Caryophyllales	Droseraceae	Herbs	C		180
Caryophyllales	Drosophyllaceae	Herb	C		1
Caryophyllales	Frankeniaceae	Shrubs	S		90
Caryophyllales	Molluginaceae	Herbs	X, R		80
Caryophyllales	Nepenthaceae	Climbers	C		150
Caryophyllales	Nyctaginaceae	Woody	Other		400
Caryophyllales	Phytolaccaceae	Woody, herbs	R		33
Caryophyllales	Plumbaginaceae	Herbs, woody	X, R, S	725	
Caryophyllales	Polygonaceae	Most herbs	R		1200
Caryophyllales	Portulacaceae (s.s)	Woody, herbs	X	115	
Caryophyllales	Tamaricaceae	Woody	D, S	78	
Santalales	Olacaceae (parasites only)	Woody	P		59
Santalales	Balanophoraceae	Herbs	P		39
Santalales	Opiliaceae	Woody	P		36
Santalales	Loranthaceae	Mistletoes	P		1039
Santalales	Misodendraceae	Mistletoes	P		8
Santalales	Santalaceae s.l.	Woody	P		1097
Saxifragales	Cynomoriaceae	Herbs	P		2
Saxifragales	Crassulaceae	Herbs, shrubs	D	?	1400
Saxifragales	Haloragaceae (aquatics only)	Herbs	H	50	
Saxifragales	Saxifragaceae	Herbs	AA, X	640	
Zygophyllales	Zygophyllaceae	Herbs, woody	X, S	285	
Fagales	Myricaceae	Woody	RC		57
Malpighiales	Erythroxylaceae	Woody	Other	242	
Malpighiales	Podostemaceae	Herbs	H	300	
Malpighiales	Quiinaceae (Ochnaceae s.l.)	Woody	Other		50
Malphigiales	Rafflesiaceae	Internal	P		25
Malpighiales	Rhizophoraceae	Woody	M	147	
Oxalidales	Cephalotaceae	Herb	C		1
Rosales	Urticaceae	Herbs, woody	R	2625	
Brassicales	Brassicaceae	Herbs	AA, D, R		3628

(continued)

Table 21.2 (continued)

Order	Family	Habit	Ecology	NM-AM	NM
Brassicales	Capparaceae	Shrubs, herbs	R, S		450
Brassicales	Cleomaceae	Herbs, shrubs	X, S		346
Brassicales	Limnanthaceae	Herbs	H		8
Brassicales	Resedaceae	Herbs, shrubs	DR		107
Cornales	Loasaceae	Herbs, shrubs	R		308
Ericales	Roridulaceae	Shrubs	C		
Ericales	Mitrastemonaceae	Internal	P		2
Ericales	Sarraceniaceae	Herbs	C		34
Boraginales	Lennoaceae	Herbs	P		4
Boraginales	Hydrophyllaceae (Boraginaceae s.l.)	Herbs, woody	D	300	
Lamiales	Avicenniaceae (Acanthaceae s.l.)	Trees	M		8
Lamiales	Byblidaceae	Herbs	C		8
Lamiales	Callitrichaceae (Plantaginaceae s. l.)	Herbs	M	75	
Lamiales	Hippuridaceae (Plantaginaceae s. l.)	Herbs	H		3
Lamiales	Lentibulariaceae	Herbs	C		316
Lamiales	Orobanchaceae (Scrophulariaceaea s.l.)	Herbs	P		1957
Solanales	Convolvulaceae (Cuscuta only)	Climbers	P		172
Asterales	Menyanthaceae	Herbs	H	60	
Total				21,044	24,814

Based on Brundrett (2009) with updated species allocation and numbers following Christenhusz and Byng (2016), Nickrent (1997-onwards) and The plant list 1.1 (www.theplantlist.org)
SB Sand-binding roots, *H* Hydrophytes (aquatic), *M* Marine hydrophytes, *AA* Arctic or alpine, *CR* Cluster (proteoid) Roots, *RD* Dauciform Roots, *P* Parasitic, *R* Disturbed habitats, *S* Saline soils, *E* Epiphytic, *X* Arid habitats, *C* Carnivorous

Table 21.3 Global diversity of different ecological categories of plants with nonmycorrhizal (NM) roots or predominantly NM roots (updated from Brundrett 2009)

Root trait category	Families	Species	Notes
Cluster and dauciform roots	6	7853	Dauciform roots occur in some sedges and rushes
Carnivores	7	689	Sundews, bladderworts and pitcher plants (occasional AM in some)
Parasites and hemiparasites	15	4244	Some hemiparasites have AM
Epiphytes	4	11,155	Most epiphyte families also include AM plants
Arctic and Alpine	1	640	Most belong to families with many AM plants
Aquatic	29	2236	Plants with species growing partly of fully submerged (also many AM plants in the same families)
Marine	3	48	Seagrasses, mangroves, etc.
Halophytes	4	701	Samphires and other salt-tolerant species, some may have AM depending on soil conditions
Arid	10	2265	Many succulent plants are AM
Disturbance opportunists	13	14,909	Short-lived weedy plants in disturbed habitats
Total	95	46,737	Includes many NM-AM plants which sometimes have AM

Families are listed in Table 21.2 and all species are allocated to the most important category

Box 21.1 Mycorrhizal Diagnosis Issues

1. Arbuscular mycorrhizal (AM) roots can be misdiagnosed as nonmycorrhizal (NM) if arbuscules are not seen due to poor sample preparation or root quality (arbuscules are digested in older roots).
2. AM or NM roots with superficial hyphal growth are sometimes diagnosed as ectomycorrhizal (EcM) despite the lack of a Hartig net (Chap. 19). Growth of hyphae on non-host roots is common and can lead to growth responses in sterilised soils (Chap. 17). Some plants have both AM and EcM roots, but this is uncommon or rarely reported in most ecosystems.
3. NM plants are defined as plants that fail to form mycorrhizas when inoculum of these fungi are present, so they have roots that are highly resistant to fungal colonisation (Tester et al. 1987; Giovannetti and Sbrana 1988; Schreiner and Koide 1993; Brundrett 2009). These families are listed in Table 21.2.
4. NM roots become less resistant to fungal colonisation with age, and many NM plants will contain vesicles and hyphae of AM fungi along with

(continued)

saprophytic and endophytic organisms (Brundrett 2006). This endophytic activity by mycorrhizal fungi has been referred to as Glomalean Fungus Colonisation (GFC) and is normally asymptomatic (Brundrett 2006). GFC also occurs in other subterranean plant organs such as rhizome scales and seeds.

5. NM plants with GFC are often misdiagnosed as AM (Brundrett 2009). These roots often contain hyphae and vesicles but not arbuscules. Arbuscules are the defining feature of AM, but are not always used for diagnosis, since they are missing in old AM roots.
6. Plants with roots that can be mycorrhizal or not depending on soil or habitat conditions are known as NM-AM (Table 17.1). These include members of the Cyperaceae, Chenopodiaceae and other NM-AM families listed in Table 21.3. They often grow in the same habitats as NM plants.
7. The mycorrhizal status of many species in NM-AM families such as the Cyperaceae, Papaveraceae and Chenopodiaceae cannot be resolved with existing data (Brundrett 2009). Most species in these families have NM roots (often with GFC), but there are also plants in these families that are considered to have AM (see Sect. 21.3).
8. Some plant families include both fully AM and fully NM species. These are also referred to as AM-NM families in Table 21.2. A few plant species have both AM and NM in healthy primary roots at the same time, because AM fungi only grow in the finest lateral roots, which are attached to coarser NM roots (e.g. *Sanguinaria canadense*—Brundrett and Kendrick 1988). This seems to be rare and has been linked to the patterns of accumulation of fungistatic chemicals in roots.
9. A comparison of published lists of mycorrhizal plants suggests that about 5% of taxa have been misdiagnosed (Brundrett 2009). This error rate has little impact on estimated numbers of host and non-host plants, but there is a tendency for errors to accumulate in lists of mycorrhizal plants. Many NM plant families are misclassified in Wang and Qiu (2006), who do not attempt to resolve conflicting information within families. Their list includes about 100 families that are incorrectly diagnosed relative to lists of NM plants produced by Tester et al. (1987) and Brundrett (2009). There are also many errors in the list of EcM taxa in Wang and Qiu (2006) and Smith and Read (2008), for the same reason.
10. Resolving apparent misidentifications requires more consistent diagnosis of roots with sparse fungal colonisation by the rigorous application of definitions of AM and EcM associations (Brundrett 2009). In many cases, errors can be detected by comparing results to other studies that include plants in the same families, since mycorrhizal status of plants is usually consistent within families (but see Chap. 19).

As explained in Box 21.1, several categories of inconsistently or weakly mycorrhizal plants can be recognised based on patterns of root colonisation by mycorrhizal fungi (see also Table 17.1). However, distinguishing these NM-AM plants from NM plants is difficult since it is very rare of any NM plant to have roots that are consistently free of mycorrhizal fungi (Brundrett 2006; Toju et al. 2014). Most NM-AM plants have inconsistent associations where the degree of AM formation is limited by habitat conditions that cause mycorrhizal fungus activity to be inhibited. The main categories of NM-AM plants are hydrophytes, halophytes, xerophytes and epiphytes, as well as alpine and arctic plants (Table 21.3). Some aquatic plants and halophytes have roots that are mycorrhizal at times but not at other times due to soil conditions that vary seasonally or spatially. Both NM-AM and fully NM plants also tend to be more common in colder arctic and alpine habitats (Brundrett 2009; Newsham et al. 2009). Roots of aquatic plants are often NM or NM-AM, but some fully submerged plants have AM roots (Brundrett 1991, 2009). Marine seagrasses are fully NM but have endophytes in their roots (Vohník et al. 2015). Weedy plants also tend to be NM (Miller 2005; Brundrett 2009; Betekhtina and Veselkin 2011). Daehler (1998) summarised the taxonomic distribution of the worst weeds in agricultural habitats, and his list includes 15 NM or NM-AM families and only 2 AM families in the top 17. However, weeds that invade natural areas include a more even mixture of mycorrhizal and NM plants (Daehler 1998).

Most NM plants have a replacement strategy for nutrient acquisition (Table 21.3). With only rare exceptions, plants lose the capacity to form mycorrhizas if these are no longer required for nutrient uptake, as in the case of parasitic and carnivorous plants (Brundrett 2009; but see Chap. 19). Table 21.3 provides estimates of the overall number of species of plants in these categories. NM plants with specialised means of nutrition also include cluster-rooted species and sedges with dauciform roots that excrete organic acids to "mine" soil for immobile forms of soil phosphorus (Shane and Lambers 2005; Lambers et al. 2006). These root systems tend to have high production costs, but plants with NM roots seem to be more competitive in extremely infertile soils (Lambers et al. 2006). Delaux et al. (2014) found that some of the symbiosis specific genes in mycorrhizal plants were missing in NM plants such as *Lupinus* sp. Their data suggest that once plants evolve another nutrient uptake adaptation strategy such as cluster roots, the ability to form AM is lost and will not be readily reacquired. Some of these genes are now known to be ancestral in land plants (Wang et al. 2010b) and their presence in NM plants is worthy of further study.

The category of facultative AM was first originally applied to plants, which consistently had low levels of colonisation (Janos 1980; Brundrett 1991, 2009), but other authors use this term to refer to NM-AM plants. Figure 21.1 shows that samples need to be taken throughout the year to resolve differences in mycorrhizal colonisation between species. This graph of seasonal AM levels in Canadian deciduous forest shows that there is a continuum of mycorrhizal colonisation intensity and that these levels are fairly consistent within species with perennial roots throughout the year. There seems to be a threshold of 40% of root length colonised by AM that separates plants with high or low root colonisation levels, but

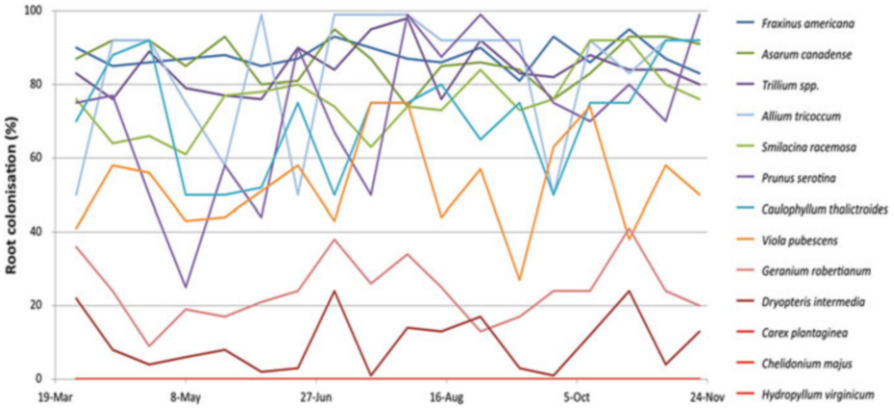

Fig. 21.1 Seasonal variations in arbuscular mycorrhizal colonisation for Canadian deciduous forest plants. Data are root length colonised (RLC) from 735 root samples taken every 2 weeks times throughout the year, except when soil was frozen (from Brundrett and Kendrick 1988). Three geophyte species with short-lived roots are omitted for clarity

this requires further investigation. In reality, most mycorrhizal studies do not include sufficient sampling within a species over time or space to allow facultative mycorrhizas to be recognised or to allow meaningful comparison of colonisation intensity between species or habitats. Consequently, plants with facultative AM will be designated only as AM plants in most mycorrhizal studies. There do not seem to be any facultative EcM plants, as few if any EcM host plants have sparse or intermittent root colonisation as adults, unless they are growing in extremely inhospitable or highly disturbed substrates. Some possible exceptions to this rule (listed in Chap. 19) include EcM-AM plants that form AM, when conditions are not favourable for EcM (facultative with respect to EcM but not to AM). A few EcM hosts also form NM roots when they are submerged in water (Khan 1993).

21.3 Resolving Conflicting Mycorrhizal Information

It has long been recognised that a definition of mycorrhizas based on morphology is required to identify associations consistently (Harley and Harley 1987; Brundrett 2004, 2009). Errors in published data most often result from diagnosis problems, especially when trying to distinguish endophytic activity of mycorrhizal fungi from mycorrhizal associations. A protocol to address common diagnosis problems was published (Brundrett 2009), but it has not been widely adopted, so it is still common for mycorrhizal studies to lack a clear definition of mycorrhiza types (note to journal editors and reviewers). The most common errors are listed in Box 21.1, and some specific examples are provided in Table 21.4.

Further research is required to resolve the status of some families of plants reported to have both AM and NM roots, which are called NM-AM families

Table 21.4 Case studies showing examples of endophytic activity by glomalean fungi (GFC) in NM or NM-AM plants

Plant (family)	Habitat	Status	Evidence	References
NM-AM families				
Chenopodiaceae (3 sp.), Cyperaceae (1 sp.)	Desert spring ephemerals	GFC	No arbuscules, limited colonisation	Shi et al. (2006)
Ceratocarpus arenarius (Chenopodiaceae)	Desert annual	GFC	No arbuscules or P increase but growth responses and 15% colonisation	Zhang et al. (2012)
Chenopodium quinoa (Amaranthaceae)	Alpine	GFC	Endophytes common	Urcelay et al. (2011)
Stelleria media (Amaranthaceae)	Glasshouse study	GFC	AM fungi cause growth reduction	Veiga et al. (2013)
Cyperaceae (3 genera, 5 sp.)	Tropical ultramaphic soils	GFC	Some hyphae but few or no arbucules	Lagrange et al. (2013)
Amaranthaceae, Brassicaceae (12 sp.)	Temperate	GFC in 7 sp.	Colonisation (<5%) requires a companion plant, no arbuscules	Hirrel et al. (1978)
Carnivorous plants				
Drosera (2 sp.)	Tropical	GFC or NM-AM?	Low colonisation with few arbuscules	Harikumar (2013)
Drosera rotundifolia	Temperate	GFC?	Many endophytes present including AM and EcM fungi	Quilliam and Jones (2010)
Halophytes				
Mangrove vegetation (10 sp.)	Tropical	NM-AM or GFC	AMF hyphae and spores, arbuscules rare	Wang et al. (2010a, b)
Mangrove vegetation (17 sp.)	Tropical	NM-AM, NM	AMF in most species (1 sp. NM)	D'Souza and Rodrigues (2013)
Seasonally dry saline habitats (12 spp.)	Mediterranean	AM (4), GFC (9)	Asteraceae AM, Amaranthaceae, Caryophyllaceae and Pumbaginaceae NM	Sonjak et al. (2009)
Hydrophytes				
Aquatic and wetland plants (20 spp.)	Tropical	NM (5), GFC (12), AM (3)	Hyphae and vesicles in most, arbuscles in 3 spp. only	Radhika and Rodrigues (2007)
Aquatic (8 sp.) and wetland plants (50 sp.)	Tropical	NM (37), AM (21)	Most species had limited or no AM	Seerangan and Thangavelu (2014)
Hydrophytes (32 sp.)	Temperate	NM (25), AM (7)	Most hydrophytes NM	Kai and Zhiwei (2006)

(continued)

Table 21.4 (continued)

Plant (family)	Habitat	Status	Evidence	References
Parasites				
Cuscuta (2 sp.)	Temperate	GFC	Ephemeral root-like organ colonised by hyphae	Behdarvandi et al. (2015)
Cytinus (2 sp.)	Mediterranean	GFC?	Ephemeral colonisation by hyphae	De Vega et al. (2010)

here. The most important plant families in this category are the Cyperaceae, Papaveraceae and Chenopodiaceae, which seem to include a majority of NM species with a few exceptions. Many investigators have looked at roots of the Cyperaceae, which is one of the largest NM-AM plant families, but interpreting their data is difficult. For example, most of the roots examined did not contain arbuscules, but these sedge species were designated as AM due to the presence of hyphae and vesicles formed by glomalean fungi (Powell 1975; Miller et al. 1999; Muthukumar et al. 2004; Brundrett 2009). Thus, the designation of these species was based on a definition of AM that does not require arbuscules to be formed, which is contrary to the normal practice by mycorrhizal researchers. It seems that most of the sedge roots, which have been examined, have GFC but are not AM, but there may also be a few species with functional AM (see Table 21.4).

The endophytic growth of AM fungi (GFC) is common in non-host plants, but is not consistently interpreted by mycorrhizal scientists. Toju et al. (2014) found that EcM and AM fungi were present in most of the 36 tropical plants they studied, but in many cases these were obviously growing as endophytes in non-hosts. Endophytes including AM, EcM, ericoid and orchid mycorrhizal fungi seem to be common in NM plants (Brundrett 2006; Quilliam and Jones 2010; Lekberg et al. 2015). Issues also arise with the diagnosis of the roles of fungi in EcM and EcM-AM plants. (Chap. 19). These issues can be tested by using consistent definitions of mycorrhizal and NM roots when gathering new data. Other ecological categories of plants, where roots are typically NM but often contain endophytic AM fungi, include carnivores and parasites (Table 21.4).

Some published claims about mycorrhizal associations do not make sense, for example the recognition of AM in parasitic plants that lack roots at maturity (de Vega et al. 2010; Kamble and Agre 2014; Behdarvandi et al. 2015). The NM status of most parasitic plants has recently been strengthened by a genomic study by Delaux et al. (2014) which showed that *Cuscuta* and *Orobanche* had lost the symbiosis-specific genes that are normally present in mycorrhizal plants. This implies that attempts by mycorrhizal fungi to form associations with them would fail due to the inability of host cells to recognise beneficial fungi and/or form a functional symbiotic interface. There is also physiological evidence that GFC does not function like AM in roots. For example, Zhang et al. (2012) found hyphae were present in 15% of the roots of *Ceratocarpus arenarius* (Chenopodiaceae), but there were no arbuscules or increase in phosphorus content in colonised plants. They observed growth responses due to the presence of fungi, but the mechanism for this

is unclear. It is common for soil fungi to cause growth responses in glasshouse experiments using pasteurised soils, and these responses have been documented for endophytes such as Serendipitaceae as well as putative EcM fungi that failed to colonise roots (Kariman et al. 2014; Ray et al. 2015). Growth promotion by endophytic fungi seems to be fairly common under experimental conditions and perhaps can also occur in agricultural soils, but is much less likely to occur in natural habitats where a high functional diversity or microorganisms is already present. Interpreting growth responses due to fungi that do not form mycorrhizas is challenging as there are no fully effective controls in any mycorrhizal experiment (Brundrett et al. 1996; Chap. 17).

21.4 Mycorrhizal Growth Responses

It makes sense to link mycorrhizal formation to root structures and growth responses, but meta-analysis studies correlating variations in mycorrhizal colonisation may fail to detect meaningful correlations between values for mycorrhizal colonisation and other variables. In particular, it is risky to link colonisation data to soil or environmental conditions because variations between studies in methodology and sampling are likely to be major contributing factor to differences in colonisation levels. For example, some studies measure colonisation relative to total root length while others exclude woody roots, which are not susceptible to mycorrhizal formation, from total root length. In addition, mycorrhizas are very hard to detect in older roots of some species and some species of AM fungi stain very weakly so are easily overlooked. Switching to less toxic (but lower contrast) stains for microscopy may also be a factor in unreliable diagnosis of AM. Despite these limitations, some meta-analyses have detected trends between mycorrhizal colonisation intensity. For example, Treseder (2013) summarised data from many mycorrhizal experiments and found that AM colonisation was linked to plant growth and phosphorus content, but the unexplained variation was substantial. Another meta-analysis by Soudzilovskaia et al. (2015) linked mycorrhizal colonisation intensity to habitat factors, but is also likely to be strongly influenced by inconsistent methodology.

Mycorrhizal associations are balanced mutualisms where both the plant and fungus partner benefit in indirect ways (Brundrett 2004). Examples of studies where plants were grown in realistic soil conditions generally show substantial growth responses to mycorrhizas (Zangaro et al. 2000; Brundrett and Abbott 2002; Johnson et al. 2015; Koziol and Bever 2015). However, measurements of responses to inoculation at a single phosphorus level can be misleading, since nutrient response curve studies are required to quantify mycorrhizal responses (Abbott and Robson 1984). Soil fertility is important for North American prairie plants, which respond to AM in soils where soil P is a limiting factor for plant growth, but not when N supply is limiting (Johnson et al. 2015).

Within the plants which normally have AM, there are variations in mycorrhizal colonisation intensity in a continuum from sparse to intense colonisation of roots. Plants which have sparse colonisation are often referred to as facultatively mycorrhizal and usually have relatively long roots hairs (e.g. Bayliss 1975; Brundrett 1991). However, designating facultatively mycorrhizal plants is often difficult due to limited sampling and lack of standardisation of methods, as explained in Sect. 21.2 above. The Canadian deciduous forest plant species included in Fig. 21.1 all had perennial fine roots, while annual plants and geophytes which replace all their roots each year showed strong seasonal variations in mycorrhizal root length. The majority of plants in natural ecosystems have perennial roots, so annual crop plants are not very good models for studying mycorrhizas in natural ecosystems.

It has long been known that NM plants generally have longer root hairs than mycorrhizal plants, and these major differences in root form are linked to different strategies for nutrient uptake from soils (Bayliss 1975; Lambers and Teste 2013; Fig. 21.2). However, for plants with varying levels of AM colonisation, the link

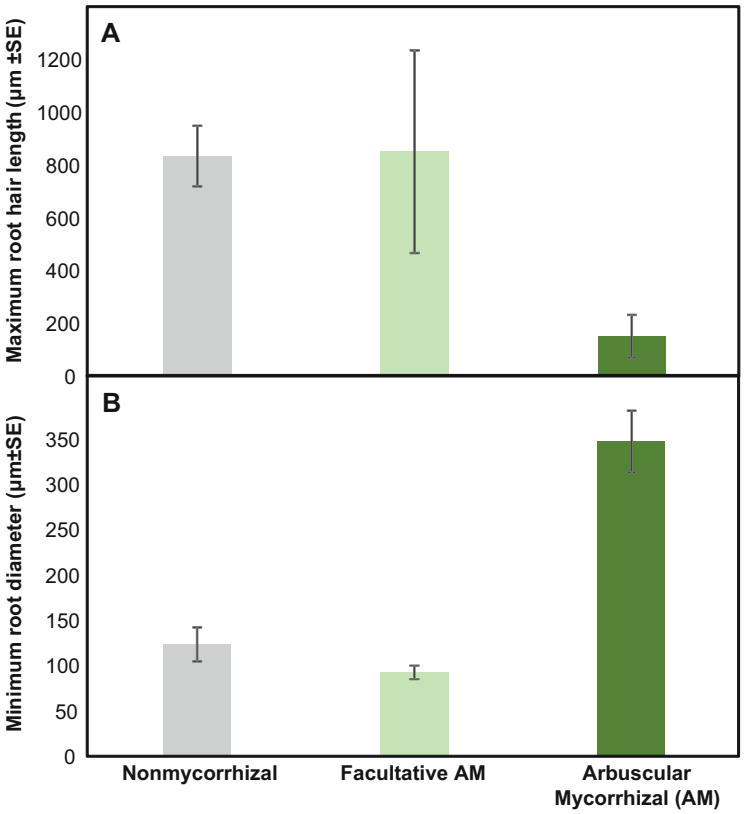

Fig. 21.2 Comparisons of root diameter, maximum root hair length and average AM colonisation levels for Canadian mycorrhizal plants (data from Brundrett and Kendrick 1988)

between mycorrhizal growth responses and root form has been questioned due to a lack of consistent data (Maherali 2014). The lack of correlation between mycorrhizal colonisation and root form in this meta-analysis probably resulted, because these properties are not measured consistently across studies, as explained above. A detailed comparison by Schweiger et al. (1995) found a strong negative correlation between mycorrhizal growth responses and the length of root hairs in pasture species and showed that root hairs were the most important root property for modelling mycorrhizal benefits.

21.5 Global and Regional Summaries of Mycorrhizal Plant Dominance

Figure 21.3 provides a global summary of the total diversity of flowering plants which are mycorrhizal. The mycorrhizal diversity of vascular plants is very similar (Fig. 21.4). About 92% of flowering plants can form mycorrhizas including 7% of species in plant families with inconsistent associations that vary with habitat or soil conditions (NM-AM). The oldest mycorrhizal association is still the most important, with over 210,000 species of AM hosts. The second largest category is orchid mycorrhizas (Orchidacae) with about 28,000 plant species, while there are >6000 plants with EcM and about 4000 species in the Ericaceae with ericoid mycorrhizas (some Ericaceae members have a type of EcM and few have AM roots). There are also >40,000 NM or NM-AM plants. The NM-AM category of plants also includes

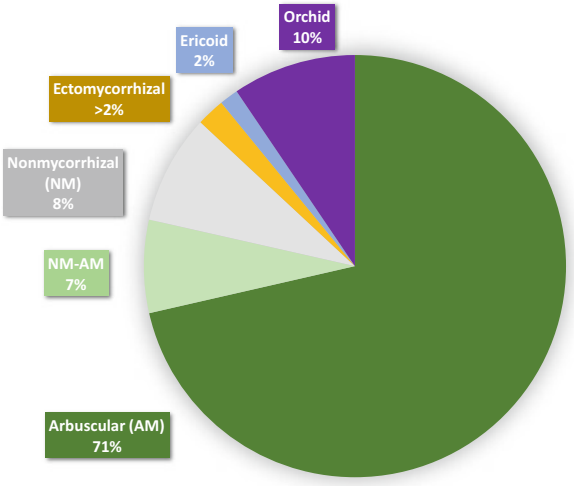

Fig. 21.3 The relative diversity of different categories of mycorrhizal plants on a global scale. All taxa of flowering plants were assigned to categories using data in the scientific literature (updated from Brundrett 2009). See text for data sources and methodology

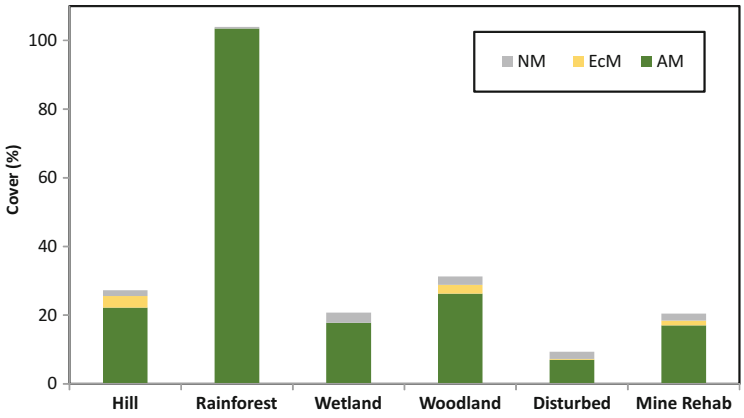

Fig. 21.4 Relative dominance of mycorrhizal and nonmycorrhizal understory plants in different major habits across a region in tropical Australia (data from Brundrett et al. 1995). Data are the relative cover of all species present in quadrats at each site (1 m^2 quadrants located at 10 m intervals along a 100 m transect) averaged by habitat type (25 transects in total)

families such as the Cyperaceae where the mycorrhizal status of many species cannot yet be resolved due to contradictory published information. Thus, it is likely that some of the families listed as NM-AM here will eventually be recognised as NM only. As shown in Table 21.3, the majority of NM-AM families are specialists that grow in habitats where mycorrhizal fungi are inhibited, so are unlikely to be consistently AM.

Other than the Orchidaceae and Ericaceae, members of most of the remaining plant families are known or expected to have AM, EcM or NM roots. Less than 1% of plants belong to families which have not been sampled for mycorrhizas and the majority of plant families have consistent mycorrhizas, so the mycorrhizal status of additional species in these families can be accurately inferred from phylogeny (Brundrett 2009). There are some orders of plants that consistently have AM roots. However, there are also a few plant families in NM-AM clades, where roots need to be sampled to resolve conflicting information (Table 21.2). Several other complex plant families, such as the Fabaceae and Myrtaceae in Australia, include AM and EcM-AM species. There are also cases where relictual associations persist in roots, such as EcM (AM) in *Eucalyptus* spp. that have AM as seedlings, but only rarely do so as adults (Chap. 17). There are also a few plants with both EcM and AM roots as adults, but these plants are normally classified as EcM. These include members of the Salicaceae in the northern hemisphere and some genera in the Fabaceae, Myrtaceae and Casuarinaceae in Australia (Chap. 19).

Lists of mycorrhizal and NM families or genera for all vascular plants can be compiled from the information in this book, which includes comprehensive lists of EcM and NM or NM-AM plants. This approach was used to provide regional summaries of numbers of mycorrhizal plants in Fig. 21.5. It is now possible to repeat these calculations for any region or habitat type with a comprehensive list of

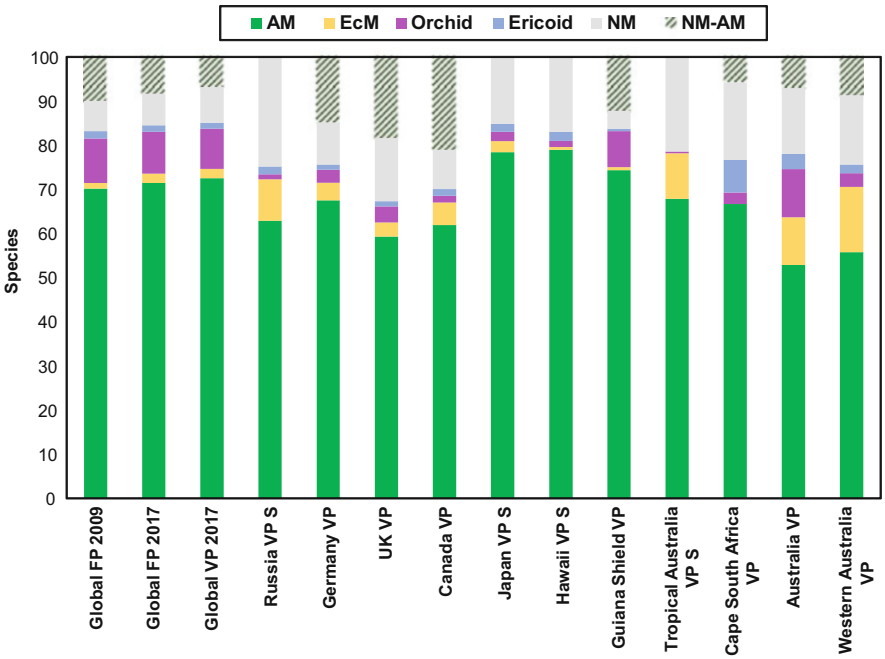

Fig. 21.5 Comparison of the relative diversity of mycorrhizal plants at regional scales for flowering plants (FP) or all vascular plants (VP). These stacked bar graphs were produced by assigning mycorrhizal status to all the species in a region based on phylogeny (see Fig. 21.3), expect for Russia, Japan, Hawaii and tropical Australia which are from large studies where roots were sampled, but do not include all species (S). These samples were from ~3000 species from Russia (Akhmetzhanova et al. 2012), 1037 spp. Japan (Maeda 1954), 147 spp. from Hawaii (Koske et al. 1992) and 247 species form tropical Australia (Brundrett et al. 1995). NM and NM-AM plants were not distinguished in some surveys

plant species. This approach can also be used in combination with more time-consuming approaches (looking at roots) to check for consistency within clades of plants or confirm the status of plants in NM-AM or EcM-AM clades (Table 21.3).

The total number of species of mycorrhizal and NM plants in Fig. 21.3 are very similar to the estimates of Brundrett (2009), but were updated using newer databases listing species of flowering plants (the two lists differ by about 5000 species). Table 21.2 also incorporates recent taxonomic changes to plant families (Angiosperm Phylogeny Group 2016), which have resulted in the consolidation of families that are closely related but in some cases ecologically different. The main changes to numbers of mycorrhizal plants since Brundrett (2009) are for recognised species in the Orchidaceae (1% larger) and NM plus NM-AM plants (also 1% larger). These values are provided for comparison in the first two columns in Fig. 21.5. The taxonomic diversity of vascular plants now seems to be relatively stable, but is still not fully resolved (Christenhusz and Byng 2016), so the estimates in Fig. 21.3 may still be subject to minor adjustments in numbers

of plant species in some families in the future. The same caveats apply to estimates of numbers of mycorrhizal plants at a regional scale based on phylogeny, as provided in Fig. 21.5. However, lists of mycorrhizal plants derived from phylogeny and lists resulting from studies of roots tend to converge when large data sets of plants growing in similar habitats are compared. For example, the examination of root samples of 2970 Russian plants (Akhmetzhanova et al. 2012) produced very similar results to estimated mycorrhizal totals for German plants based on phylogeny (Fig. 21.5). Both of these regions have similar habitats and plant diversity.

Mycorrhizal plant diversity alone does not represent the importance of associations since the status of under- and overstory plants often differs and mycorrhizal plant lists are commonly dominated by herbs and shrubs. Regional summaries of mycorrhizal species diversity become even more valuable when used in combination with relative dominance data or vegetation maps showing the importance in ecosystems where roots were sampled (Swaty et al. 2016; Fig. 17.4). However, these studies are uncommon (Brundrett 1991). As explained above, the mycorrhizal status of plant species in a region or county can be assigned using phylogeny, and this approach can be extended to datasets of plant dominance. Examples of studies which have determined the total diversity or relative dominance of mycorrhizal plants in a regional flora are provided below.

Hempel et al. (2013) and Menzel et al. (2016) assigned mycorrhizal status to 1752 plant species that occur in Germany. However, their use of the Wang and Qiu (2006) dataset resulted in about 600 misallocated species relative to family allocations in Brundrett (2009). Revised totals for mycorrhizal plants in their list are provided in Fig. 21.5. They also designated species with inconsistent mycorrhizas as facultatively mycorrhizal, but some of this variability is likely to have resulted from variations in methodology in mycorrhizal studies. Despite these potential issues, Hempel et al. (2013) found there were strong relationships between the consistency of mycorrhizal colonisation and soil and climatic factors.

Figure 21.5 includes fewer examples of mycorrhizal plant diversity in tropical habitats, but the overall dominance of AM host plants in most of these habitats has already been well documented (Brundrett 1991). One such study by Bechem et al. (2014) examined roots of 252 species of Cameroun forest trees and found most of the dominant plants in this ecosystem had AM (94%), with only 6 species with EcM (probably an overestimate - see Chap. 19) and 4 species with NM roots. At the opposite end of the global temperature gradient, the proportion of NM plants in Arctic soils increases with proximity to the pole, including both plants from NM families and species that form AM in warmer soils (Brundrett 2009; Newsham et al. 2009). Comparisons in Fig. 21.5 reinforces the idea that AM plants are generally most numerous in tropical habitats while NM plants become more important in colder climates.

Brundrett (1991) provides an overall summary of the mycorrhizal status of all the major ecosystems globally. Despite numerous mycorrhizal studies since then the overall picture has not changed much. In summary, the majority of ecosystems globally are dominated by AM host plants, which are also common in most of the

remaining habitats. Ecosystems dominated by EcM tree species are also very important, especially in northern boreal forests and Australia (Read 1991; Chap. 20). Trees or shrubs with EcM are also dominant or co-dominant in many other temperate forests, as well as some tropical and subtropical areas. Orchids are present in most ecosystems but are not dominant. Plants with ericoid mycorrhizas are also widespread, but are only dominant in a few habitat types and have centres of diversity in mountains (Schwery et al. 2015; Chap. 9). Plants with NM roots tend to be specialist that occur in harsh sites or have other nutrient uptake mechanisms (Table 21.3), but are also prevalent in arctic and alpine habitats (Brundrett 2009). Early mycorrhizal research was primarily based in the Northern Hemisphere where soils and plants are atypical on a global scale (more likely to be dominated by EcM trees, highly fertile or disturbed with many weedy plants). But this trend is gradually shifting to include a much better representation of tropical plants in mycorrhizal studies. The impacts of the Anthropocene have resulted in increasing losses of EcM or AM tree coverage with an increasing importance of NM weeds (Betekhtina and Veselkin 2011; Swaty et al. 2016).

My 2009 review predicts that new studies looking at mycorrhizal roots will often be of limited value since the status of most families is well resolved. In many cases, designating mycorrhizal status based on phylogeny will provide more accurate results than sampling roots due to issues with sample quality and the inconsistent interpretation of fungal structures. There is no evidence that the error rate for diagnosis of mycorrhizal roots has reduced since I identified this as an issue in 2009. In fact, advances in molecular techniques make it easier than ever to detect mycorrhizal fungi in NM roots. We need to acknowledge that endophytic activity by mycorrhizal fungi is common, and careful visual observations and adequate root samples are required to diagnose mycorrhizas. Many root samples are inadequate for accurately determining mycorrhizal status (due to their age, mixtures of different species, limited sampling, poor clearing and staining, etc.). These issues with methodology and diagnosis of associations in roots need to be addressed by the mycorrhizal community.

21.6 Mycorrhizal Evolutionary Trends

The evolution of mycorrhizal associations is briefly updated here, to complement information available elsewhere (Brundrett 2002, 2009). The two most common evolutionary trends for species are to switch from AM to NM roots or from AM to EcM roots with about 45,000 species of flowering plants in the former category and over 6000 in the latter. In most cases, these trends are consistent across families, but in a few cases, there are diverse root types within one family, such as the separate clades of EcM, AM or NM plants in the Australian Fabaceae (see Chap. 17). As shown in Table 21.5, there are intermediate stages in both of these evolutionary trends where plants have multifunctional roots with both EcM and AM symbioses, or can acquire nutrients directly and/or by the AM symbioses, as

Table 21.5 The two most common evolutionary trends in mycorrhizal roots (see text)

	Stage 1	Stage 2	Stage 3
A. AM to NM Evolutionary Continuum			
Stage	Obligate AM	Facultative AM or NM-AM	NM
Hyphae in root	AM fungi efficiently colonise the root cortex using longitudinal or coiling hyphae to extend colonies in roots. Hyphal growth primarily occurs in young roots	Colonisation of the root cortex is relatively inefficient in thin highly branched roots Root colonisation may be regulated by soil conditions that suppress fungal activity	Absent or diffuse and most common in older roots. If present, AM fungi typically occur in combination with other endophytic fungi
Arbuscules (Interface area)	Numerous in young roots, forming in one or more layers of cortex cells	Less numerous, inconsistently present or absent from roots	Absent or rarely present in some older roots
Vesicles (storage)	Many, few or none (fungus dependent)	Sparse or absent and highly variable	Rare or absent (roots may be short-term fungal refuges, but carbon stored is imported from elsewhere)
Root Form	Usually fairly thick (due to cortex) with short root hairs	Usually thinner and highly branched with fewer rows of cortex cells and longer root hairs than AM hosts. Roots are primarily optimised for direct nutrient uptake from soil	
Root evolution	Plants have root systems adapted for efficient mycorrhiza formation and symbiosis regulation genes responsible for recognition and formation of a host–fungus interface	The plant–fungal interface becomes less efficient due to root adaptations for direct nutrient uptake. Some symbiosis genes may be lost?	Symbioses regulation genes lost (interface nonfunctional if present) Roots further optimised for direct nutrient uptake and cluster roots may develop
B. AM to EcM Evolutionary Continuum			
Stage	AM	EcM-AM	EcM or EcM (AM)
Hyphae on root	Patchy colonisation by EcM fungi occurs on long laterals	Some lateral roots have a thin or thick mantle of hyphae	Many short lateral roots have a thick mantle of hyphae
Hartig net (interface)	Absent (hyphae may grow between epidermal cells but they do not form an interface)	Present but relatively inefficient due to root length and thickness. Arbuscules are also present, especially in longer roots	Substantially increased Hartig net area due to elongation of root cells in the epidermis
Root Form	No specialised lateral roots (roots optimised for AM or NM roles) Root form does not	Ultimate lateral roots have reduced growth rates and increased branching to allow a larger fungal interface to	Ultimate lateral roots have highly reduced growth rates and more lateral roots to increase interface area

(continued)

Table 21.5 (continued)

	Stage 1	Stage 2	Stage 3
	change in the presence of EcM fungi	form Root form is altered in the presence of EcM fungi	Root form is highly responsive to EcM fungi
Root evolution	Plants have root systems adapted for efficient AM formation and symbiosis regulation genes responsible for recognition and formation of host–fungus interface (in some cases NM or ericoid roots develop EcM)	Root system form and symbioses genes change to allow EcM formation, but roots retain adaptations for AM (or NM) root functionality	Root systems are optimised for EcM only, so AM specific genes may be lost or have altered roles. In some cases AM fungi are not fully excluded, but are usually rare or primarily found in young plants as EcM (AM) associations

determined by soil conditions. Other, less common, trends, which have occurred in one or more plant lineages, include (1) switching from NM to EcM roots, (2) from Ericoid to EcM or even AM, or (3) switching from balanced to mycoheterotrophic associations in plants with AM, EcM or orchid mycorrhizas (Brundrett 2002).

One of the strongest root evolution trends for plants that are exposed to hostile soil or environmental conditions is to develop NM-AM or NM roots. NM and NM-AM plants are more likely to be epiphytes, grow in wet, salty or cold soils or become parasitic on other plants. The alternative hypothesis (plants in these habitats lose mycorrhizas more often) has less support because families of NM plants with different ecological preferences tend to cluster together in phylogenetic trees. Evolutionary trends linked to soil conditions also include the increased importance of both EcM and NM plants in extremely infertile soils in Australia (Chap. 17).

Table 21.5 shows mycorrhizal evolution as a three-stage process starting from AM roots and progressing forward to NM or EcM roots, but there may be some cases where reversions back to AM occur. The presence of both AM and NM families in the Poales provide one example of complex evolution, as it seems likely that ancestral plants in this group had NM roots, but the Poaceae has AM roots in most species. Reports of some mycorrhizal species in the otherwise NM Cyperaceae may also represent recent switching from NM to AM or EM roots, provided that these are functional associations. It has yet to be confirmed that there are lineages of plants that have re-acquired mycorrhizal associations that descended from ancestors with fully NM roots. It is possible that plant lineages with newly acquired mycorrhizal associations do not function in the same way as ancient mycorrhizal lineages and that their associations are regulated by a different suite of symbiotic genes. The complex lineages of EM-AM plants in the Australian Fabaceae and Myrtaceae provide an excellent opportunity to investigate the functional and genetic processes in symbiotic associations of different ages (Chap. 17).

Not all plants follow the trends in Table 21.5 to their conclusion (fully EcM or fully NM roots), as there are also many plants that remain in an intermediate state

such as EcM-AM or NM-AM. These plants have retained several root functions with overlapping roles that may provide them with greater ecological flexibility, but this may come at a greater cost. In other cases, different root functions are utilised by plants at different times or in different habitats, which can be the case for hydrophytes or halophytes that have seasonal mycorrhizal associations, or for plants with NM roots as epiphytes and AM roots when growing in soil. Examples of plant families with very complex roots include the Australian Fabaceae and Casuarinaceae where some species have several types of mycorrhizas as well as a nitrogen fixing symbiosis (Chaps. 17 and 19). The ability of some plants to support multifunctional roots and remain competitive provides strong evidence that soil fertility is the most important factor limiting plant productivity in their habitats.

As explained in Chap. 17, there have been three waves of mycorrhizal evolution that started with AM in early land plants, followed by a second major phase of root functional diversification in the Cretaceous when EcM, orchid, ericoid and NM plants would have originated. The third phase of root diversification is currently underway in some habitats in response to changing soil conditions. Lineages of plants that have acquired new root traits are most common in hostile habitats. Some examples include the EcM roots of sedges in the genus *Kobresia* that grow in arctic habitats or cluster roots in some members of the Fabaceae that grow in extremely infertile soils. However, these examples are not typical of the majority of vascular plants, which have remained associated with AM fungi throughout their evolutionary history.

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