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

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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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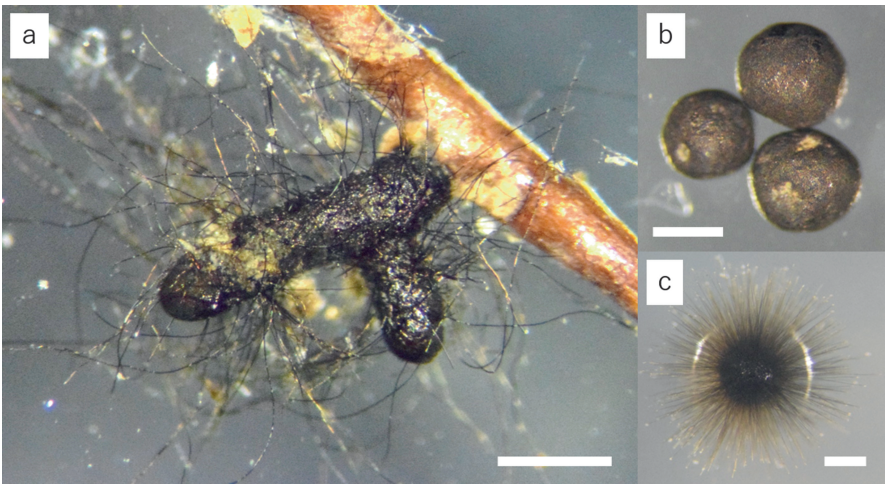

Fig. 14.1 *Cenococcum geophilum*. (**a**) An ectomycorrhizal root of *Betula ermanii* that has been colonized by *C. geophilum*. (**b**) Fresh sclerotia of *C. geophilum* after they were extracted from forest soils. (**c**) Hyphae extending from a *C. geophilum* sclerotium as it begins to grow in axenic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

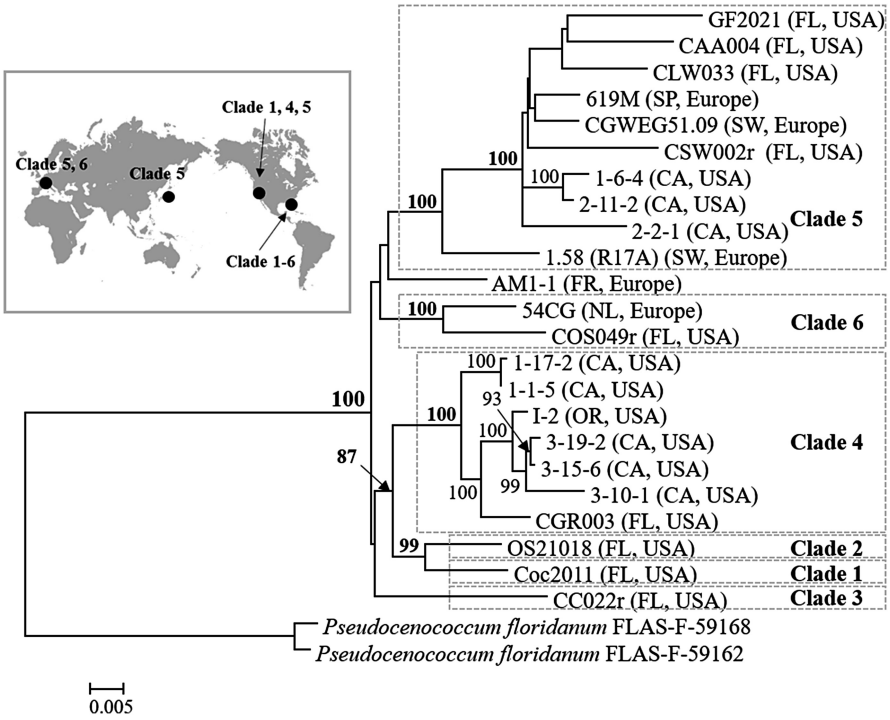

Fig. 14.2 The optimum phylogenetic tree of *C. geophilum* based on maximum likelihood analysis of seven concatenated loci (ITS, SSU, LSU, TEF, RPB1, RPB2 and GAPDH). The placement of each lineage is highlighted and clades 1–6 are named according to Obase et al. (2016a). Inset in the *upper left corner* 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

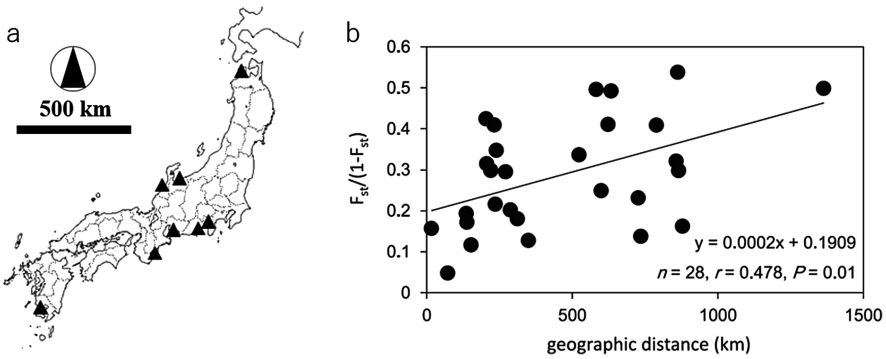

Fig. 14.3 (**a**) Locations of *Cenococcum geophilum* populations in several coastal pine forests in Japan. (**b**) Relationship between genetic differentiation among the populations of *C. geophilum* (*y*-axis) and geographic distance (*x*-axis). The figure was modified from Matsuda et al. (2015)

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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References

Agerer R (2001) Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Agerer R, Gronbach E (1988) *Cenococcum geophilum*. In: Agerer R (ed) Colour atlas of Ectomycorrhizae, Plate 11. Einhorn–Verlag, Schwäbisch Gmünd

Agerer R, Rambold G (2004–2016) DEEMY – An information system for characterization and determination of Ectomycorrhizae. Ludwig-Maximilians, München. www.deemy.de

Alexander IJ, Högberg P (1986) Ectomycorrhizas of tropical angiospermous trees. New Phytol 102:541–549

Allen MF, Egerton-Warburton LM, Allen EB, Kårén O (1999) Mycorrhizae in *Adenostoma fasciculatum* Hook. & Arn.: a combination of unusual ecto- and endo-forms. Mycorrhiza 8:225–228

Antibus RK, Croxdale JG, Miller OK, Linkins AE (1981) Ectomycorrhizal fungi of *Salix rotundifolia* III. Resynthesized mycorrhizal complexes and their surface phosphatase activities. Can J Bot 59:2458–2465

Bahram M, Põlme S, Kõljalg U, Tedersoo L (2011) A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. FEMS Microbiol Ecol 75:313–320

Bai S, Bai Y, Fang L, Liu Y (2003) Mycorrhiza of *Cenococcum geophilum* (Fr.) formed on *Ostryopsis daidiana* and mycorrhizal affection on the growth of *Ostryopsis davidiana*. Sci Silvae Sin 40:194–196

Bakshi BK, Thapar HS, Singh B (1968) Mycorrhiza in blue pine, spruce and deodar. Proc Natl Inst Sci India 84:27–34

Belfiori B, Riccioni C, Paolocci F, Rubini A (2013) Mating type locus of Chinese black truffles reveals heterothallism and the presence of cryptic species within the *T. indicum* species complex. PLoS One 8:e82353

Boehm EWA, Mugambi GK, Miller AN, Huhndorf SM, Marincowitz S, Spatafora JW, Schoch CL (2009) A molecular phylogenetic reappraisal of the Hysteriaceae, Mytilinidiaceae and Gloniaceae (Pleosporomycetidae, Dothideomycetes) with keys to world species. Stud Mycol 64:49–83

Bourne EC, Mina D, Gonçalves SC, Loureiro J, Freitas H, Muller LA (2014) Large and variable genome size unrelated to serpentine adaptation but supportive of cryptic sexuality in *Cenococcum geophilum*. Mycorrhiza 24:13–20

Branco S, Gladieux P, Ellison CC, Kuo A, LaButii K, Lipzen A, Grigoriev IV, Liao HL, Vilgalys R, Peay KG, Taylor JW, Bruns TD (2015) Genetic isolation between two recently diverged populations of a symbiotic fungus. Mol Ecol 24:2747–2758

Brearley FQ, Press MC, Scholes JD (2003) Nutrients obtained from leaf litter can improve the growth of dipterocarp seedlings. New Phytol 160:101–110

Brearley FQ, Scholes JD, Press MC, Palfner G (2007) How does light and phosphorus fertilisation affect the growth and ectomycorrhizal community of two contrasting dipterocarp species? Plant Ecol 192:237–249

Casacuberta E, González J (2013) The impact of transposable elements in environmental adaptation. Mol Ecol 22:1503–1517

Castellano MA, Trappe JM (1985) Mycorrhizal associations of five species of Monotropoideae in Oregon. Mycologia 77:499–502

Chen LH, Wei YAN, Yan XU (2007) Identification and preliminary analysis of the genetic diversity of *Cenococcum geophilum* Fr. Agric Sci China 6:956–963

Corrales A, Arnold AE, Ferrer A, Turner BL, Dalling JW (2016) Variation in ectomycorrhizal fungal communities associated with *Oreomunnea mexicana* (Juglandaceae) in a Neotropical montane forest. Mycorrhiza 26:1–17

Dahlberg A, Jonsson L, Nylund JE (1997) Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old growth Norway spruce forest in south Sweden. Can J Bot 75:1323–1335

Dickie IA, Guza RC, Krazewski SE, Reich PB (2004) Shared ectomycorrhizal fungi between a herbaceous perennial (*Helianthemum bicknellii*) and oak (*Quercus*) seedlings. New Phytol 164:375–382

Diédhiou AG, Selosse MA, Galiana A, Diabaté M, Dreyfus B, Bâ AM, De Faria SM, Béna G (2010) Multi-host mycorrhizal fungi are predominant in a Guinean tropical rainforest and shared between canopy trees and seedlings. Environ Microbiol 12:2219–2232

Dokmai P, Phosri C, Khangrang R, Suwannasai N (2015) Above- and below-ground ectomycorrhizal diversity in a pine-oak forest in northeastern Thailand. Chiang Mai J Sci 42:79–87

Douhan GW, Darren PM, Rizzo DM (2007a) Using the putative asexual fungus *Cenococcum geophilum* as a model to test how species concepts influence recombination analyses using sequence data from multiple loci. Curr Genet 52:191–201

Douhan GW, Huryn KL, Douhan LI (2007b) Significant diversity and potential problems associated with inferring population structure within the *Cenococcum geophilum* species complex. Mycologia 99:812–819

Douhan GW, Rizzo DM (2005) Phylogenetic divergence in a local population of the ectomycorrhizal fungus *Cenococcum geophilum*. New Phytol 166:263–271

Douhan GW, Vincenot L, Gryta H, Selosse MA (2011) Population genetics of ectomycorrhizal fungi: from current knowledge to emerging directions. Fungal Biol 115:569–597

Ferdinandsen C, Winge Ö (1925) *Cenococcum* Fr. A monographic study. Kongelige Vet landbohøjsk 1925:332–382

Fernandez CW, Koide RT (2013) The function of melanin in the ectomycorrhizal fungus *Cenococcum geophilum* under water stress. Fungal Ecol 6:479–486

Fernandez CW, McCormack ML, Hill JM, Pritchard SG, Koide RT (2013) On the persistence of *Cenococcum geophilum* ectomycorrhizas and its implications for forest carbon and nutrient cycles. Soil Biol Biochem 65:141–143

Fernández-Toirán LM, Águeda B (2007) Fruitbodies of *Cenococcum geophilum*. Mycotaxon 100:109–114

Fries E (1825) Systema orbis vegetabilis I. Typographia Academica, Lundae

Fujiyoshi M, Yoshitake S, Watanabe K, Murota K, Tsuchiya Y, Uchida M, Nakatsubo T (2011) Successional changes in ectomycorrhizal fungi associated with the polar willow *Salix polaris* in a deglaciated area in the high Arctic, Svalbard. Polar Biol 34:667–673

Ge ZW, Smith ME, Zhang QY, Yang ZL (2012) Two species of the Asian endemic genus *Keteleeria* form ectomycorrhizas with diverse fungal symbionts in southwestern China. Mycorrhiza 22:403–408

Geng Y, Li Z, Xia LY, Wang Q, Hu XM, Zhang XG (2014) Characterization and phylogenetic analysis of the mating-type loci in the asexual ascomycete genus *Ulocladium*. Mycologia 106:649–665

Glassman SI, Peay KG, Talbot JM, Smith DP, Chung JA, Taylor JW, Vilgalys R, Bruns TD (2015) A continental view of pine-associated ectomycorrhizal fungal spore banks: a quiescent functional guild with a strong biogeographic pattern. New Phytol 205:1619–1631

Gómez BL, Nosanchuk JD (2003) Melanin and fungi. Curr Opin Infect Dis 16:91–96

Gonçalves SC, Portugal A, Gonçalves MT, Vieira R, Martins-Loução MA, Freitas H (2007) Genetic diversity and differential in vitro responses to Ni in *Cenococcum geophilum* isolates from serpentine soils in Portugal. Mycorrhiza 17:677–686

Grand LF (1971) Tuberculate and *Cenoccocum* mycorrhizae of *Photinia* (Rosaceae). Mycologia 63:1210–1212

Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F, Smirnova T, Nordberg H, Dubchak I, Shabalov I (2014) MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res. doi:10.1093/nar/gkt1183

Haselwandter K, Read DJ (1980) Fungal associations of roots of dominant and sub-dominant plants in high-alpine vegetation systems with special reference to mycorrhiza. Oecologia 45:57–62

Hashimoto Y, Higuchi R (2003) Ectomycorrhizal and arbuscular mycorrhizal colonization of two species of floodplain willows. Mycoscience 44:339–343

Hasselquist N, Germino MJ, McGonigle T, Smith WK (2005) Variability of *Cenococcum* colonization and its ecophysiological significance for young conifers at alpine-treeline. New Phytol 165:867–873

Horton TR, Bruns TD (1998) Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*). New Phytol 139:331–339

Hrynkiewicz K, Baum C, Leinweber P (2009) Mycorrhizal community structure, microbial biomass P and phosphatase activities under *Salix polaris* as influenced by nutrient availability. Eur J Soil Biol 45:168–175

Huang J, Nara K, Zong K, Wang J, Xue S, Peng K, Shen Z, Lian C (2014) Ectomycorrhizal fungal communities associated with Masson pine (*Pinus massoniana*) and white oak (*Quercus fabri*) in a manganese mining region in Hunan province, China. Fungal Ecol 9:1–10

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. ITE Research Publication, London

Izzo A, Canright M, Bruns TD (2006) The effects of heat treatments on ectomycorrhizal resistant propagules and their ability to colonize bioassay seedlings. Mycol Res 110:196–202

Jany JL, Garbaye J, Martin F (2002) *Cenococcum geophilum* populations show a high degree of genetic diversity in beech forests. New Phytol 154:651–659

Kantvilas G, Coppins BJ (1997) *Melaspilea circumserpens* Nyl. rediscovered and referred to *Glonium*; with discussion of the provenance of some of Robert Brown's lichen specimens. Lichenologist 29:525–532

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiß M, Larsson KH (2013) Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22:5271–5277

Kück U, Pöggeler S (2009) Cryptic sex in fungi. Fungal Biol Rev 23:86–90

Lian C, Narimatsu M, Nara K, Hogetsu T (2006) *Tricholoma matsutake* in a natural *Pinus densiflora* forest: correspondence between above- and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities. New Phytol 171:825–836

Linhell D (1942) *Cenococcum graniforme* als Mykorrizabildner von Waldbäumen. Symb Bot Ups 5:1–18

LoBuglio KF (1999) Cenococcum. In: Cairney JWG, John WG, Chambers SM (eds) Ectomycorrhizal fungi key genera in profile. Springer, Berlin, pp 287–309

LoBuglio KF, Berbee ML, Taylor JW (1996) Phylogenetic origins of the asexual mycorrhizal symbiont *Cenococcum geophilum* Fr. and other mycorrhizal fungi among the ascomycetes. Mol Phylogenet Evol 6:287–294

LoBuglio KF, Rogers SO, Wang CJK (1991) Variation in ribosomal DNA among isolates of the mycorrhizal fungus *Cenococcum geophilum*. Can J Bot 69:2331–2343

LoBuglio KF, Taylor JW (2002) Recombination and genetic differentiation in the mycorrhizal fungus *Cenococcum geophilum* Fr. Mycologia 94:772–780

Malloch D, Thorn RG (1985) The occurrence of ectomycorrhizae in some species of Cistaceae in north America. Can J Bot 63:872–875

Maser C, Maser Z (1987) Notes on mycophagy in four species of mice in the genus *Peromyscus*. Great Basin Nat 47:308–313

Massicotte HB, Melville LH, Peterson RL, Luoma DL (1998) Anatomical aspects of field ectomycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bellardii* (Cyperaceae). Mycorrhiza 7:287–292

Massicotte HB, Trappe JM, Peterson RL, Melville LH (1992) Studies on *Cenococcum geophilum*. II. Sclerotium morphology, germination, and formation in pure culture and growth pouches. Can J Bot 70:125–132

Matsuda Y, Hayakawa N, Ito S (2009a) Local and microscale distributions of *Cenococcum geophilum* in soils of coastal pine forests. Fungal Ecol 2:31–35

Matsuda Y, Noguchi Y, Ito S (2009b) Ectomycorrhizal fungal community of naturally regenerated *Pinus thunbergii* seedlings in a coastal pine forest. J For Res 14:335–341

Matsuda Y, Takeuchi K, Obase K, Ito S (2015) Spatial distribution and genetic structure of *Cenococcum geophilum* in coastal pine forests in Japan. FEMS Microbiol Ecol 91. doi:10.1093/femsec/fiv108

Miller SL, Torres P, McClean TM (1994) Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. Mycologia 86:89–95

Miyamoto Y, Nara K (2016) Soil propagule banks of ectomycorrhizal fungi share many common species along an elevation gradient. Mycorrhiza 26:189–197

Molina R, Trappe JM (1982) Patterns of ectomycorrhizal host specificity and potential among Pacific northwest conifers and fungi. For Sci 28:423–458

Morris MH, Pérez-Pérez MA, Smith ME, Bledsoe CS (2008) Multiple species of ectomycorrhizal fungi are frequently detected on individual oak root tips in a tropical cloud forest. Mycorrhiza 18:375–383

Moser AM, Frank JL, D'Allura JA, Southworth D (2009) Ectomycorrhizal communities of *Quercus garryana* are similar on serpentine and nonserpentine soils. Plant Soil 315:185–194

Mühlmann O, Bacher M, Peintner U (2008) *Polygonum viviparum* Mycobionts on an alpine primary successional glacier forefront. Mycorrhiza 18:87–95

Murat C, Rubini A, Riccioni C, De la Varga H, Akroume E, Belfiori B, Guaragno M, Le Tacon F, Robin C, Halkett F, Martin F, Paolocci F (2013) Fine-scale spatial genetic structure of the black truffle (*Tuber melanosporum*) investigated with neutral microsatellites and functional mating type genes. New Phytol 199:176–187

O'Gorman CM, Fuller HT, Dyer PS (2009) Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature 457:471–474

Obase K, Cha JY, Lee JK, Lee SY, Lee JH, Chun KW (2009) Ectomycorrhizal fungal communities associated with *Pinus thunbergii* in the eastern coastal pine forests of Korea. Mycorrhiza 20:39–49

Obase K, Douhan GW, Matsuda Y, Smith ME (2016a) Revisiting phylogenetic diversity and cryptic species of *Cenococcum geophilum* sensu lato. Mycorrhiza 26:529–540

Obase K, Douhan GW, Matsuda Y, Smith ME (2016b) *Cladophialophora floridana* and *Cladophialophora tortuosa*, new species isolated from sclerotia of *Cenococcum geophilum* in forest soils of Florida, USA. Mycoscience 57:26–34

Obase K, Lee JK, Lee SY, Chun KW (2011) Diversity and community structure of ectomycorrhizal fungi in *Pinus thunbergii* coastal forests in the eastern region of Korea. Mycoscience 52:383–391

Panaccione DG, Sheets NL, Miller SP, Cumming JR (2001) Diversity of *Cenococcum geophilum* isolates from serpentine and non-serpentine soils. Mycologia 93:645–652

Peter M, Kohler A, Ohm RA, Kuo A, Krützmann J, Morin E, Arend M, Barry KW, Binder M, Choi C, Clum A, Copeland A, Grisel N, Haridas S, Kipfer T, LaButti K, Lindquist E, Lipzen A, Maire R, Meier B, Mihaltcheva S, Molinier V, Murat C, Pöggeler S, Quandt CA, Sperisen C, Tritt A, Tisserant E, Crous PW, Henrissat B, Nehls U, Egli S, Spatafora JW, Grigoriev IV, Martin FM (2016) Ectomycorrhizal ecology is imprinted in the genome of the dominant symbiotic fungus *Cenococcum geophilum*. Nat Commun 7:12662

Phosri C, Põlme S, Taylor AFS, Kõljalg U, Suwannasai N, Tedersoo L (2012) Diversity and community composition of ectomycorrhizal fungi in a dry deciduous dipterocarp forest in Thailand. Biodivers Conserv 21:2287–2298

Pöggeler S (2002) Genomic evidence for mating abilities in the asexual pathogen *Aspergillus fumigatus*. Curr Genet 42:153–160

Portugal A, Martinho P, Vieira R, Freitas H (2001) Molecular characterization of *Cenococcum geophilum* isolates from an ultramafic soil in Portugal. S Afr J Sci 97:617–619

Rubini A, Belfiori B, Riccioni C, Tisserant E, Arcioni S, Martin F, Paolocci F (2011) Isolation and characterization of MAT genes in the symbiotic ascomycete *Tuber melanosporum*. New Phytol 189:710–722

Séne S, Avril R, Chaintreuil C, Geoffroy A, Ndiaye C, Diédhiou AG, Sadio O, Courtecuisse R, Sylla SN, Selosse MA, Bâ A (2015) Ectomycorrhizal fungal communities of *Coccoloba uvifera* (L.) L. mature trees and seedlings in the neotropical coastal forests of Guadeloupe (Lesser Antilles). Mycorrhiza 25:547–559

Shinohara ML, LoBuglio KF, Rogers SO (1999) Comparison of ribosomal DNA ITS regions among geographic isolates of *Cenococcum geophilum*. Curr Genet 35:527–535

Smith ME, Douhan GW, Rizzo DM (2007) Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytol 174:847–863

Smith ME, Henkel TW, Aime MC, Fremier AK, Vilgalys R (2011) Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest. New Phytol 192:699–712

Spatafora JW, Owensby CA, Douhan GW, Boehm EWA, Schoch CL (2012) Phylogenetic placement of the ectomycorrhizal genus *Cenococcum* in Gloniaceae (Dothideomycetes). Mycologia 104:758–765

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R et al (2014) Global diversity and geography of soil fungi. Science 346:1256688

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Trappe JM (1962) *Cenococcum graniforme*-its distribution, ecology, mycorrhiza formation, and inherent variation. Ph.D. Thesis, University of Washington, Seattle, WA

Trappe JM (1964) Mycorrhizal host and distribution of *Cenococcum graniforme*. Lloydia 27:100–106

Trappe JM (1971) Mycorrhiza-forming ascomycetes. In: Hacskaylo E (ed) Mycorrhizae: proceedings of the first north american conference on Mycorrhizae-April 1969. U.S. Department of Agriculture Forest Service, Washington, pp 19–37

Vaario LM, Xing ST, Xie ZQ, Lun ZM, Sun X, Li YH (2006) In situ and in vitro colonization of *Cathaya argyrophylla* (Pinaceae) by ectomycorrhizal fungi. Mycorrhiza 16:137–142

Väre H, Vestberg M, Ohtonen R (1997) Shifts in mycorrhiza and microbial activity along an oroarctic altitudinal gradient in northern Fennoscandia. Arct Alp Res 29:93–104

Wu B, Nara K, Hogetsu T (2005) Genetic structure of *Cenococcum geophilum* populations in primary successional volcanic deserts on Mount Fuji as revealed by microsatellite markers. New Phytol 165:285–293

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29:2869–2876