Above and Below Ground Fungal Diversity in a Hemlock-Dominated Forest Plot in Southern Ontario and the Phylogenetic Placement of a New Ascomycota Subphylum

by

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General Abstract

The objective of this thesis was to assess the diversity and community structure of fungi in a forest plot in Ontario using a variety of field sampling and analytical methods. Three broad questions were addressed: 1) How do different measures affect the resulting view of fungal diversity? 2) Do fruiting bodies and soil rDNA sampling detect the same phylogenetic and ecological groups of Agaricomycotina? 3) Will additional rDNA sampling resolve the phylogenetic position of unclassified fungal sequences recovered from environmental sampling? Generally, richness, abundance, and phylogenetic diversity (PD) correspond and identify the same dominant fungal groups in the study site, although in different proportions. Clades with longer branch lengths tend to comprise a larger proportion of diversity when assessed using PD. Three phylogenetic-based comparisons were found to be variable in their ability to

detect significant differences. Generally, the Unifrac significance measure (Lozupone et al., 2006) is the most conservative, followed by the P-test (Martin, 2002), and Libshuff library comparison (Singleton et al., 2001) with our dataset. Fruiting body collections and rDNA sampling recover largely different assemblages of fungi at the species level; however, both methods identify the same taxonomic groups at the genus-order level as well as ectomycorrhizal fungi as the dominant functional type of Agaricomycotina. This work also shows that the Soil Clone Group I (SCGI) clade is widespread in soils of diverse origins and represents a novel subphylum of Ascomycota.

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Supplementary Material

Acronyms

4c26R – Reverse primer in the LSU rDNA region used for nested PCR and sequencing in this study (Porter et al., 2007).

ACCTRAN – Accelerated transformation optimization (Swofford and Maddison, 1987) assigns character changes close to the root of the phylogenetic tree and may result in a bias toward mutations along internal rather than external branches.

ACE – Abundance-based coverage estimator used to estimate species richness based on the frequency of the ten rarest classes sampled (Chao and Lee, 1992).

AFTOL – All Fungal Tree of Life project aims to significantly enhance understanding of the evolutionary history of the Kingdom Fungi by developing large datasets of molecular and non-molecular characters. Can be accessed at: http://aftol.org/ [November 2007]

Bg – B mineral soil horizon that is gleyed (g) and characterized by grey colours and/or mottling indicating reduction in waterlogged soils with anaerobic conditions.

BLAST – Basic local alignment search tool used to find regions of similarity between sequences, in this study, we used “blastn” to search the GenBank nucleotide databases using a nucleotide query sequence (Altschul et al., 1990; Altschul et al., 1997).

BPP – Bayesian posterior probability, a measure of confidence in evolutionary relationships, the probability that a clade is true given the data, evolutionary model, and prior assumptions about model parameters (Huelsenbeck et al., 2001).

Chao1 – Abundance based richness estimator used to estimate species richness based on the number of sampled singletons and doubletons (Chao, 1987).

Chao2 – Incidence based richness estimator used to estimate species richness based on the number of sampled singletons and doubletons (Chao, 1987).

CI – Consistency index measures the overall amount of homoplasy in a dataset by calculating the minimum number of all character-state changes possible in a dataset divided by the total number of character-state changes required to most parsimoniously fit the tree being described (Sanderson and Donoghue, 1989). If there is no homoplasy, CI=1.0, and CI decreases as homoplasy increases (Kluge and Farris, 1969).

Ck – C mineral soil horizon, characterized by presence of carbonate (k), indicated by bubbling when dilute HCl is added.

Ckg – C mineral soil horizon, characterized by presence of carbonate (k), that is gleyed (g) and characterized by grey colours and/or mottling indicating reduction in waterlogged soils with anaerobic conditions.

CROX – Costa Rican oxisol (soil) samples.

DELTRAN – Delayed transformation optimization (Swofford and Maddison, 1987) postpones character changes to the tips of the phylogenetic tree.

DNA – Deoxyribonucleic acid, encodes genetic information.

dNTPs – Deoxynucleotide triphosphates are the building blocks of DNA used by DNA polymerase to form a new DNA template.

EHF – Eastern Hemlock Forest soil samples.

EXO1 – Exonuclease 1 degrades single-stranded DNA in a 3' to 5' direction.

F81+G – The Felsenstein (Felsenstein, 1981) model of nucleotide substitution that allows for unequal nucleotide base frequencies and a Gamma-shaped distribution of nucleotide substitution rates among sites (Yang, 1996).

GSP – Genealogical concordance concept involves combining multiple gene genealogies to remove the subjectivity for determining species limits (Mayden, 1997).

GTR – General time reversible model of nucleotide substitution that allows for unequal base frequencies and for all six pairs of substitutions to have different rates (Rodriguez et al., 1990; Yang et al., 1994).

HI – Homoplasy index measures the amount of homoplasy in a dataset (excluding autapomorphies) where HI = 1 – CI' (Givnish and Sytsma, 1997).

ICE – Incidence-based coverage estimator used to estimate species richness based on the frequency of the ten rarest classes sampled (Chao and Lee, 1992).

ITS – Internal transcribed spacer region of ribosomal RNA, comprised of two parts, ITS1 and ITS2, interrupted by the 5.8S rRNA gene.

ITS1 – Forward primer at the 3' end of the SSU rDNA region used for PCR and sequencing in this study (White et al., 1990).

ITS9 – Forward primer used for PCR in this study (Egger, 1995).

-lnL – Likelihood estimate for branch lengths on a phylogenetic tree.

LB – Luria Bertani agar is a nutritionally rich medium used for the growth of bacteria.

LR0R – Forward primer in the LSU rDNA region used for PCR and sequencing of in this study (Rehner and Samuels, 1994).

LR5 – Reverse primer in the LSU rDNA region used for PCR and sequencing of in this study (Hopple and Vilgalys, 1999).

LSU – Large subunit region of nuclear-encoded ribosomal RNA.

M13R – Plasmid vector reverse primer used for PCR amplification of sequence insert after cloning.

$MgCl_2$ – Magnesium chloride.

ML – Maximum likelihood estimation is a statistical method that is used here to infer a phylogeny. It suggests that a phylogenetic tree with a higher probability of reaching the observed data (DNA sequences) given a model of nucleotide substitution is preferred to a phylogenetic tree with a lower probability.

MPF – Montane Pine-Fir forest soil samples.

NCBI – National Centre for Biotechnology Information, can be accessed at: http://www.ncbi.nlm.nih.gov/ [November 2007]

nLSU1221R – Taxon-specific reverse primer in the LSU rDNA region used for PCR in this study (Schadt et al., 2003).

NS1 – Forward primer at the 5' end of the SSU rDNA region used for PCR and sequencing in this study (White et al., 1990).

NWR-LTER – Niwot Ridge Long Term Ecological Research site.

OTU – Operational taxonomic unit used as a proxy for “species”, in this study, defined as 99% sequence similarity in ca. 900 bp of the 5’ LSU rDNA region.

PAUP* – Phylogenetic Analysis Using Parsimony *and other methods; a software package (Swofford, 2002).

PAUPrat – Parsimony ratchet, as implemented in PAUP, conducts parsimony searches using a modified strategy of multiple short searches instead of one long search (Sikes and Lewis, 2001).

PBS – Parsimony bootstrap, a nonparametric method that involves randomly resampling (with replacement) characters from the original data matrix to generate pseudoreplicate matrices that are subjected to the same phylogenetic searches as the original data set. Support for a clade is calculated as the proportion of times it is obtained in pseudoreplicate searches (Alfaro et al., 2003).

PCA – Principal components analysis used to reduce multidimensional data sets to lower dimensions for analysis.

PCR – Polymerase chain reaction used to amplify DNA sequences (Mullis, 1987).

PD – Phylogenetic diversity measure developed to measure the underlying feature diversity for a subset of taxa by measuring branch lengths in a phylogram (Faith, 1992).

P-test – Phylogenetic test that uses the number of optimized character state transitions to determine whether a given character state (environment-type) significantly covaries with phylogeny (Martin, 2002).

rDNA – Ribosomal DNA, tandemly repeated units of genes that code for ribosomal RNA.

RI – Retention index is the complement of the distortion coefficient (from Farris, 1973) and reaches the maximum value of 1.0 when there is no homoplasy (Farris, 1989).

rRNA – Ribosomal RNA, a type of RNA found in ribosomes of cells.

RNA – Ribonucleic acid, similar to DNA but containing ribose instead of deoxyribose.

RTU – Recognizable taxonomic units (Rees, 1983).

SAP – Shrimp alkaline phosphatase catalyzes the release of phosphate groups from nucleotides and proteins.

SCGI – Soil Clone Group I (Ascomycota, Fungi) a well-supported subphylum-level clade present in soils of diverse origin, first reported as unknown fungal lineage ‘Group I’ by (Schadt et al., 2003).

SF102 – Spruce-Fir treeline forest soil samples collected January 2002.

SF602 – Spruce-Fir treeline forest soil samples collected June 2002.

SF702 – Spruce-Fir treeline forest soil samples collected July 2002.

SR1.5 – Primer that sits in the SSU rDNA region used for sequencing in this study (James et al., 2000).

SR6 – Primer that sits in the SSU rDNA region used for sequencing in this study (James et al., 2000).

SSU – Small subunit region of nuclear-encoded ribosomal RNA.

T7 – Plasmid vector primer used for PCR amplification of inserted sequence after cloning.

Taq – *Thermophilus aquaticus* is a bacterium that tolerates high temperatures, source of Taq DNA polymerase.

TBR – Tree bisection-reconnection is a heuristic algorithm also known as “branch-breaking” used to search treespace and involves breaking a phylogenetic tree into two parts and then reconnecting the two subtrees at all possible branches. If a better tree is found, it is kept and the procedure is repeated.

TDM – Alpine tundra dry meadow soil samples.

TFC – Tundra forest control soil samples.

TFG – Tundra forest soil samples from plots where trees had been girdled.

TSF – Treeline spruce-fir forest soil samples.

TW13 – Reverse primer used for sequencing the LSU rDNA region in this study (Taylor and Bruns, 1999).

Type I error – Statistical error, also known as “false positive”, the error of incorrectly rejecting the null hypothesis, ie., the error of accepting the alternative hypothesis when the results can be attributed to chance.

Type II error – Statistical error, also known as “false negative”, the error of failing to reject a null hypothesis when the alternative hypothesis is true.

WHF – Western hemlock forest soil samples.

1 Chapter One- General Introduction

1.1 Importance of fungi in terrestrial ecosystems

The biological community of any terrestrial ecosystem consists of an assemblage of many organisms from all the kingdoms of life. Organisms within a community have different and complex structures, activities, and relationships with each other (Cooke and Rayner, 1984). This study aims to determine the taxonomic, phylogenetic, and ecological assemblage of fungi in a hemlock-dominated forest site. There are two major functional groups of fungi in an ectotrophic forest ecosystem: saprophytes and mycorrhizas.

Saprophytic fungi play major roles in nutrient cycling in soil and plant litter. Particularly, white-rot fungi are able to decompose lignin and play a significant role in carbon cycling (de Boer et al., 2005). Some saprophytes have been studied by measuring decay rates (Frankland et al., 1990), nutrient release (Robinson et al., 1993), and spatial relocation of nutrients (Boddy, 1999; Gray et al., 1995; Lindahl et al., 1999). Saprophytes have been traditionally sampled using direct collection of fruiting bodies or culture-based methods on natural or artificial substrates. For instance, the distribution of microbial decomposers including fungi, bacteria and actinomycetes across different soil horizons in a temperate deciduous woodland was described by Frankland (1982) using microscopic observation. Basidiomycota fungi were found to be most abundant on dead plant roots, woody debris, and throughout the surface of organic and humus layers. Other fungi were found to be most abundant on dead roots in the deeper A and B soil horizons. Bacteria and actinomycetes formed a much smaller proportion of living biomass in these substrates. Also, the distribution of fungi across differently sized particles (50 - 250 µm) from a

coniferous forest soil was studied by Baath (1988) using culture-based methods. Similar species were isolated from all soil particle partitions; however, the abundance of species differed among particle-size factions. Slower-growing fungi were found to be most abundant on small particles and faster-growing fungi were found to be most abundant on larger particles. Generally, saprophytic fungi may be largely cosmopolitan (Finlay et al., 1997) but they appear to be structured on a local-scale by soil horizon and substrate affecting the composition of dominant and rare species among ecosystems (Frankland, 1998).

Mycorrhizal fungi have been recognized as providing 'keystone' ecosystem functions because of their direct access to plant carbon that drives below-ground microbial communities (Leake et al., 2005). Mycorrhizas assist plants in obtaining water and nutrients, protect plant roots from pathogens, and form below-ground networks that link above-ground plant communities (Smith and Read, 1997, Bruns et al., 2002; Horton and Bruns, 2001). Common below-ground mycorrhizal networks also link plant communities and may influence above-ground forest community development (Simard et al., 1997; Read, 1997; van der Heijden et al., 1998; Horton and Bruns, 1998; Booth, 2007).

A major group of mutualistic fungi discussed in chapter two are endomycorrhizal or arbuscular mycorrhizal fungi. There are ca. 150 described species of endomycorrhizal fungi from the Glomeromycota that associate with more than 300,000 plant species from more than 80% of extant vascular plant groups such as the Brassicaceae, Commelinaceae, Cyperaceae, Juncaceae, and Proteaceae (Kendrick, 2000; Schuβler et al., 2001). In an

endomycorrhiza, the fungus grows in or between plant cortical root cells without penetrating the plasmalemma, sometimes forming arbuscules, vesicles, extramatrical hyphae that extends outward from the root into the soil, and asexual spores (Kendrick, 2000).

A second major group of mutualistic fungi discussed in chapter three are ectomycorrhizal fungi. There are ca. 5,500 described species of ectomycorrhizal fungi (Molina et al., 1992) from at least 73 Basidiomycota genera and 16 Ascomycota genera (Kendrick, 2000) that associate with ca. 2,000 plant species from groups such as the Betulaceae, Caesalpinioideae, Dipterocarpaceae, Ericaceae, Fagaceae, Myrtaceae, Papilionoideae, Pinaceae, and Rosaceae (Smith and Read, 1997; Kendrick, 2000). In an ectomycorrhiza, the fungus forms a mantle of hyphae covering the outer parts of young roots, and it penetrates the intercellular spaces of the host cortical cells forming the Hartig net (Smith and Read, 1997).

1.2 Magnitude of fungal diversity

In a landmark publication, Hawksworth (1991) calculated that less than 5% of the total number of fungal species has been formally described and a total of 1.5 million species may exist worldwide. This estimate was based on a 1:6 ratio of plants to fungi in a well-studied region of the British Isles and a conservative estimate of plants worldwide (250,000). This estimate sparked a debate as to the true number of fungi that exist worldwide. Estimates from other authors range from 0.5 million (May, 2000) to 9.9 million (Cannon, 1997) and the basis for these and other estimates have been reviewed by

Frohlich and Hyde (1999). Although there are about 80,000 recognized fungal species (Kirk et al., 2001), 1.5 million species are often used as a conservative working figure for the total richness of fungi worldwide (Hawksworth, 2001).

Colwell and Coddington (1994) acknowledged that fungi are a hyperdiverse group; however, there are still a number of problems with generating accurate estimates for global fungal diversity. One difficulty is the sheer diversity of fungi that are detected in extensive local surveys, for example, over 500 species were recorded in the litter of five tree species in Panama (Cornejo et al., 1994). Other challenges are how to apply species concepts to fungi and a lack of knowledge concerning the prevalence of host specificity (Cannon, 1997), which varies among fungi (Bruns et al., 2002). One strategy to conduct a rapid assessment of highly diverse sites, is to train non-specialists to use recognizable taxonomic units (RTUs) (Rees, 1983) instead of species recognition which would require specialist knowledge (Cannon, 1997). Cannon (1997) also suggested the need for ecosystem and microhabitat analyses within larger areas to explore fungal niche diversity.

Current strategies for a more comprehensive direct global assessment of fungal diversity include detailed systematic studies within both taxonomic and nutritional/ecological groups including specificity in plant association, and spatial and temporal sampling of soil, litter, and the phyllosphere using nucleic acid analysis. A shortcoming of Hawksworth's (1991) estimate is that it is an indirect assessment of fungal diversity because it relies on another group of organisms (plant:fungus ratio in localized studies). A number of studies indicate a lack of correlation among species estimates from

major groups of organisms (Hammond, 1994; Prendergast et al., 1993; Yen, 1987; Oliver and Beattie, 1993; Colwell and Coddington, 1994). Instead, it is suggested that fungal species numbers may be better correlated with ecosystem architecture rather than plant species numbers (Cannon, 1997). For instance, May (1991) suggested that fungal diversity in the tropics might be more closely correlated with the physical variety of tree habitats and plant species distribution than to the number of plant species present.

Hawksworth (2001) acknowledges many of the issues raised about the methods he used to estimate total fungal diversity in his original paper (Hawksworth, 1991), particularly his indirect calculation approach. However, he cites additional studies that have inventoried specific habitat types or taxonomic groups since his original publication and he suggested that further long-term (> 20 yrs) site inventories are needed, particularly in understudied areas in the tropics (Hawksworth, 2001). He concluded that his initial 1.5 million species estimate could probably be revised upwards with the introduction of molecular studies and the detection of cryptic species, but maintains his original 1.5 million estimate as a conservative working number for the present.

1.3 Recognizing fungal "species"

Species concepts have been discussed by many authors (such as Avise and Wollenberg, 1997; Mayden, 1997; Taylor et al., 2000; Mallet, 2001; Hey, 2001; Wheeler and Meier, 2000); however, a universal definition of a species is not likely to be useful for every group of organisms nor for every study. Below, I will briefly present the most

prevalent species concepts that are currently in use in fungal systematics and have been used to various degrees in this thesis.

1.3.1 Morphological species

A morphological species is recognized by its phenetic distinctiveness and is based on classifying organisms based on overall similarity of observable traits that do not necessarily reflect evolutionary relatedness. There are about 80,000 described species of fungi (Kirk et al., 2001) that are recognized by a variety of macro- and micromorphological characters, in addition to other phenotypic traits, for instance growth at different temperatures for psychrophilic, thermophilic, or pathogenic fungi; and the production of secondary metabolites or pigments (Kuehn and Gunderson, 1963; Maheshwari et al., 2000; Kendrick, 2000; Rippon, 1988).

In this study, fruiting body identifications rely on this concept and incorporated the use of macroscopic and microscopic characteristics. A problem however, is that phenotypic plasticity can confuse the recognition of species, resulting in species complexes when the biological or phylogenetic species concepts are applied, for example, the morphological species *Armillaria mellea sensu lato* (Anderson and Stasovski, 1992).

1.3.2 Phylogenetic species

A phylogenetic species is the smallest monophyletic cluster of individuals distinct from other clusters within which there is a pattern of ancestry and descent (Cracraft, 1983). The application of this definition typically includes the need for a synapomorphy, i.e., a shared derived character present among all members of a species but not present in others (Rosen, 1978). The problem with this definition is that while it is easy to diagnose clusters, determining species limits remains subjective. Mayden (1997) introduced the idea of combining multiple gene genealogies to remove the subjectivity for determining species limits and called this the Genealogical Concordance Concept (GSP). Taylor et al. (2000) have advocated the application of this principle for fungi.

In this thesis I used mainly DNA sequence-based methods to sample fungi. Therefore, questions related to the delimitation of individual species were not as important as the amount of genetic and evolutionary distinctiveness represented by each terminal taxon in phylogenetic trees and the clustering of related taxa with shared ecological characteristics within the framework of the most recent molecular systematic studies. To reach my research objectives I opted to use molecular operational taxonomic units (OTUs) as a proxy for 'species'.

1.3.3 Operational taxonomic units

When working with DNA sequences from environmental sequence data, it is convenient to define molecular operational taxonomic units (OTUs) as a proxy for species. Experimental evidence indicates that OTUs defined by 99% sequence similarity for the LSU rDNA region is a conservative but good proxy for "species." 1% sequence variation within each OTU appears to be sufficient to account for within-species variation in the LSU rDNA region as well as for methodological errors during PCR, cloning, and sequencing (Lynch and Thorn, 2006; Schadt et al., 2003; Porter et al., 2007). The percentage of sequence-similarity cut-off used to define an OTU can be adjusted according to the DNA region used. For instance, 90-99% sequence similarity cutoffs have been used for the more variable internal transcribed spacer (ITS) rDNA region (O'Brien et al., 2005; Arnold et al., 2007).

1.4 Fungal sampling

Different groups of fungi are characterized by different life histories and strategies, resulting in differential nutrient allocation to vegetative mycelium or sexual fruiting bodies (Gardes and Bruns, 1996). Many fungal biotic surveys have used macroscopic fruiting bodies as indicators of fungal diversity, abundance, and importance (Watling, 1995; Vogt et al., 1992; Ferris and Humphrey, 1999). Fungi not seen above-ground, however, may in fact produce fruiting bodies below-ground, or simply grow in a vegetative state below-ground or within a substrate without the need to produce fruiting bodies each year. In saprophytic fungi, vegetative mycelium may completely cover the substrate being

consumed. In ectomycorrhizal fungi, extramatrical hyphae may extend outwards from ectomycorrhizal root tips into the surrounding soil. In these situations, soil or plant root tip sampling can reveal the presence of these fungi (Horton and Bruns, 2001). Other traditional surveys used culture-based methods from environmental samples (such as Arnold et al., 2000; Thorn et al., 1996) but different fungi have different recovery rates using this method (Warcup, 1965). Thus, it is not surprising that the resulting picture of a fungal community in a given ecosystem is largely affected by the sampling method used.

1.4.1 Traditional methods

Fruiting body surveys mostly recover fungi that form reproductive structures that can be detected by naked eye, thus primarily detect members of the Basidiomycota subphylum Agaricomycotina (macromycetes, or “mushrooms”). Any fruiting body that is large enough to be seen with the naked eye and held in the hand is referred to as a “macromycete.” Ascomycota fungi generally produce microscopic fruiting bodies, called ascomata that may be contained within a larger stroma, and are frequently overlooked during fruiting body surveys. Other fungi, such as the Chytridiomycota and Zygomycota produce minute microscopic fruiting bodies that are never seen in direct field surveys as well as members of the Glomeromycota that are not known to reproduce sexually.

Frequent and long-term studies are needed to assess the diversity of macromycetes (Straatsma et al., 2001; Watling, 1995). Since fruiting body development depends on light (Miller, 1967; Walker and Miller, 2002), temperature, precipitation, soil pH, and nutrient

availability, not all species fruit annually or in the same abundance and fruiting body production by basidiomycetes are known to change from season to season (Watling, 1995; Straatsma et al., 2001). By comparison, the plant community in our field site is relatively stable and predictable.

Traditionally, culture-based methods have also been used to study soil saprophytes (Malloch, 1981; Thorn et al., 1996). Numerous methods have been developed to isolate and maintain different fractions of the soil fungal community (Malloch, 1981; Warcup, 1965). Known biases of culture-based methods include the preferential isolation of fast-growing fungi such as mitosporic Ascomycota and Zygomycota moulds. Additionally, it is well known that many fungi are not cultivable using standard isolation methods, and discrepancies between studies that use culture-based methods and molecular-based methods have been found (soil fungi Bridge and Spooner, 2001; ericoid mycorrhizal roots Allen et al., 2003; beetle gut yeasts Suh et al., 2005).

1.4.2 Molecular methods

The use of culture-free methods by microbiologists resulted in the ability to delimit phylogenetic species based on SSU rDNA and discover novel lineages (Woese et al., 1977; Pace, 1997). The Bruns lab at the University of California at Berkely pioneered the use of molecular systematic methods for the study and identification of environmental fungi, particularly ectomycorrhizal fungi (Bruns et al., 1991; Gardes and Bruns, 1996; Horton and Bruns, 2001; Bruns and Shefferson, 2004). Fungal molecular systematic studies have

used DNA-based methods, such as DNA hybridization studies, restriction enzyme analysis, and particularly culture-free DNA sequence analysis to begin to unravel natural relationships. Molecular methods based on PCR have become some of the most useful approaches for identifying mycorrhizal root tips, mycelia, and fungi from environmental samples. The key was recognizing and exploiting the different levels of nucleotide conservation and variation in the ribosomal DNA gene region for phylogenetic studies (Bruns et al., 1991).

Ribosomal DNA codes for the ribosomal RNAs involved in the production of ribosomes. Each repeat consists of a transcribed region that codes for the three ribosomal RNA subunits with two internal non-coding sequences and it is separated from the next repeat unit by an intergenic spacer region (White et al., 1990). The internal transcribed spacer (ITS) is framed by the small subunit (SSU) (18S) ribosomal RNA gene and the large subunit (28S) ribosomal RNA gene and is split into two regions ITS1 and ITS2 by the intervening 5.8S ribosomal RNA gene (Gardes and Bruns, 1996; Horton and Bruns, 2001). Including the 5.8S gene, the ITS region in Basidiomycota fungi is approximately 650-900 base pairs in length (Horton and Bruns, 2001) and is suitable for genus-species level analyses (Gardes and Bruns, 1996; Horton, 2002). The SSU region is suitable for kingdom-subphylum level analyses, and the LSU region is of intermediate resolution for order-genus level analyses.

1.5 Research objectives and hypotheses

The aim of this thesis is to assess the diversity and community structure of fungi in a hemlock-dominated forest site using a variety of field sampling and analytical methods. This work consists of three parts in Chapters 2-4.

Chapter Two- LSU rDNA soil sampling: application and comparison of several phylogenetic diversity measures.

The objective of this study was to globally assess fungal community structure in two soil horizons in the study site and calculate differences in diversity using a variety of measures, including traditional diversity tests and tree-based (or phylogenetic) measures. I used explicit statistical methods to test the hypothesis that fungal diversity in two soil horizons differs. This hypothesis is based on previous studies that have observed trends in fungal distribution by depth, such as mycorrhizal fungi concentrated in surface layers of soil (Read, 1991; Neville et al., 2002).

Chapter Three- Fruiting body and soil rDNA sampling detects complementary assemblage of Agaricomycotina (Basidiomycota, Fungi) in a hemlock-dominated forest plot in southern Ontario.

The objectives of this study were threefold: 1) determine whether fruiting bodies and soil rDNA sampling detect the same phylogenetic and ecological groups of

Agaricomycotina (which are mostly "macromycetes") in our study site; 2) determine whether richness, abundance and phylogenetic diversity measures detect the same proportions of phylogenetic and ecological groups; and, 3) determine whether quick DNA-based surveys can replace traditional fruiting body collections to describe communities of Agaricomycotina taxa. I tested the hypotheses that 1) different sampling methods will recover different taxonomic groups of Agaricomycotina and that 2) traditional sampling methods only recover a subset recovered by DNA-based surveys. My assumptions were based on the results of previous studies that show macromycete collections to be biased toward the detection of taxa from the Agaricales (Watling, 1995) whereas taxonomically diverse fungi are detected using rDNA methods (O'Brien et al., 2005).

Chapter Four- Widespread occurrence and phylogenetic placement of a soil clone group adds a prominent new branch to the fungal tree of life.

The objective of this study was to determine the phylogenetic position of an unknown fungal group that was detected in our study site using LSU rDNA sequence sampling. Preliminary analyses based on 300 bp of rDNA indicated that it corresponds to a novel soil clone cluster first detected in alpine tundra soil from Colorado (Schadt et al. 2003). I tested the hypothesis that additional rDNA data would support the phylogenetic placement of this group at the order or subphylum taxonomic level among the basal Ascomycota lineages. This assumption was based on the observation that the recovered sequences had no high similarity match to sequences in the Genbank database despite the presence of all known major fungal lineages in the database (Lutzoni et al., 2004).

1.6 Study site

The study site is a 50 x 100 m forest plot in the Koffler Scientific Reserve, Ontario, Canada (43° 37' N, 79° 39' W; 258 - 261 m elevation). The reserve is located within the Oak Ridges Moraine, part of the Eastern Great Lakes lowland forests ecoregion (Ricketts et al., 1999). The soil is classified as a gleysol. Below the litter, fractionated and humus layers, the Bg mineral soil horizon is dark colored and had a blocky texture, the Ckg 1 layer is grey-orange and has a plated texture with silty sand, and the Ck 2 layer is mostly grey and contains gravel. A principal component analysis of vegetation coverage abundance indicates that there is very little variation among 200 5 m^2 subplots (Figure 1-1). Homogeneity of the understory vegetation is suggested by: 1) clustering of stands at the centre of the PCA plot; 2) principal components 1 and 2 account for a very small amount of variation, 6.2% and 5.3%, respectively; and 3) the proportional variance between principal components 1 and 2 is small suggesting that a scree plot of all principal components would have a gentle slope further indicating a clustering of stands in multidimentional space. The dominant tree species is eastern hemlock (*Tsuga canadensis*), but white cedar (*Thuja occidentalis*) and yellow birch (*Betula lutea*) are also common. The understory vegetation is dominated by lady fern (*Athyrium filix-femina*), dwarf scouring-rush (*Equisetum scirpoides*), and Canada mayflower (*Maianthemum canadense*) (Table 1-1).

1.7 Tables

Table 1-1: Vegetation coverage abundance of 41 vascular plant species in a 5000 m^2 plot.

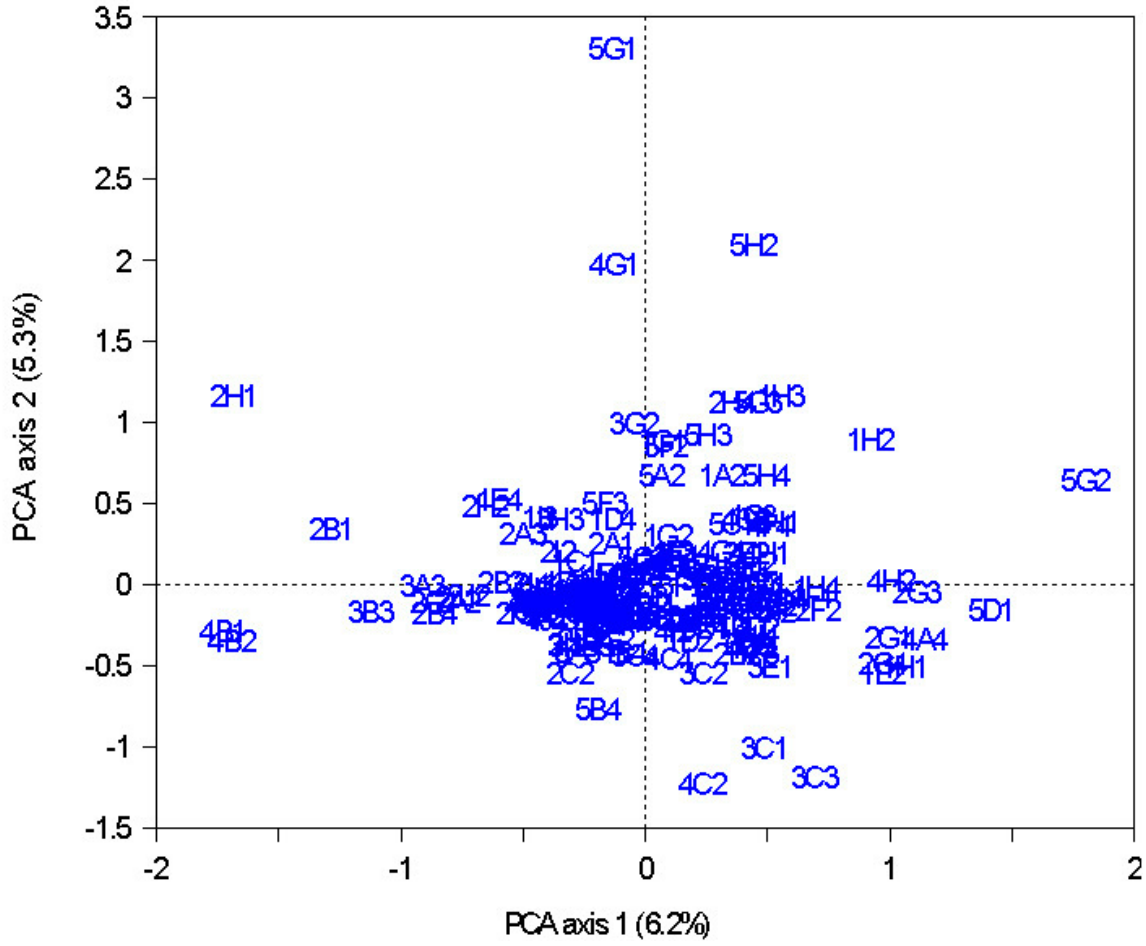
Species	Presence[a]	Mean % coverage[b]	Mean % coverage where present[c]	Maximum % coverage[d]	Lead Dominant[e]
Acer saccharum	5	0.12	4.6	10	0
Actaea rubra	7	0.16	4.34	10	1
Aralia nudicaulis	20	0.57	5.52	23	6
Arisaema atrorubens	53	1.96	7.1	23	10
Athyrium filix-femina	105	7.99	14.6	63	36
Betula lutea	63	0.79	2.4	10	5
Carex peduculata	83	8.77	20.29	88	30
Carex pennsylvanica	28	0.6	4.09	10	1
Carex platyphylla	16	0.75	9.01	41	1
Clintonia borealis	16	0.76	9.06	23	0
Cystopteris bulbifera	58	5.04	16.67	63	16
Dentaria diphylla	4	0.01	0.33	1	0
Dryopteris carthusiana	77	2.91	7.25	10	6
Dryopteris marginalis	60	2.16	6.91	23	2
Equisetum arvense	4	0.06	3.03	10	0
Equisetum scirpoides	101	14.32	27.23	88	41
Gymnocarpium dryopteris	53	2.81	10.19	41	6
Huperzia lucidula	13	0.56	8.26	63	0
Maianthemum canadense	98	3.3	6.46	23	10
Maianthemum racemosa	43	0.54	2.39	41	0
Monotropa uniflora	13	0.45	6.71	41	2
Onoclea sensibilis	40	2.06	9.9	63	1
Phegopteris connectilis	33	3.41	19.82	88	3
Polygala paucifolia	13	0.3	4.39	10	0
Polygonatum pubescens	9	0.06	1.3	10	0
Populus balsamifera	28	0.43	2.94	23	0
Prenanthes alba	3	0.01	0.4	1	0
Rhus radicans	2	0.01	0.55	1	0
Solidago flexicaulis	6	0.46	14.85	23	0
Streptopus roseus	1	0.01	1	1	0
Thalictrum dioicum	1	0.01	1	1	0
Thelypteris noveboracensis	16	0.49	5.89	10	0
Thuja occidentalis	19	0.27	2.75	10	0
Trillium erectum	53	1.98	7.16	41	1
Tsuga canadensis	77	5.62	14.02	88	13
"UNK00004"	3	0.11	7	10	0
"UNK00015"	5	0.01	0.28	1	0
"UNK00018"	1	0	0.1	0.1	0
"UNK00022"	1	0	0.1	0.1	0
"UNK000F4"	3	0.01	0.7	1	0
"UNK00110"	12	0.18	2.95	10	0

aThe total number of 5 x 5m stands from which each species was found
bThe total percent coverage abundance of each species across the entire plot of 200 stands
cThe percent coverage abundance of each species within the stands from which each species was found
dThe maximum percent coverage abundance encountered for each species within any single stand
eThe number of times any particular species had the greatest percent coverage in a stand

1.8 Figures

Figure 1-1: Principal component analysis for the coverage abundance of understory vegetation in the study site.

The coverage abundance of understory vegetation is shown to be relatively homogenous among stands.


PCA axis 2 (5.3%)
PCA axis 1 (6.2%)
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-1
0
1
2
5G1
5H2
4G1
2H1
3G2
1H2
5A2
1A2
5H4
5G2
2B1
5F3
1D4
2A3
2A1
1G2
4H2
2G3
5D1
3B3
2B4
4B1
4B2
2G4
1A4
2C2
3C2
3E1
5B4
3C1
4C2
3C3

2 Chapter Two- LSU rDNA soil sampling: application and comparison of several phylogenetic diversity measures

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2.1 Abstract

Soil was sampled from an ectotrophic forest site using two different methods: point and pooled soil sampling followed by total soil DNA extraction. Phylogenetic analyses were used to assess the diversity of fungi detected from soil. Dominant groups included: the Leotiomycetes and Sordariomycetes in the Ascomycota; the Agaricales and Pucciniomycotina in the Basidiomycota; the Glomeromycota and two unknown groups among the basal fungal lineages. The distribution of major fungal lineages and mycorrhizal fungi in two soil horizons was assessed using a measure that employs the concept of phylogenetic diversity. Richness, abundance, and phylogenetic diversity were found to largely correspond and identify the Ascomycota and basal fungi as the most diverse groups detected in our study, although in slightly different proportions. Thus, for rDNA based environmental surveys, phylogenetic diversity may be a suitable substitute for traditional diversity measures that rely on the delimitation of species or OTUs. Three phylogenetic-based comparisons were found to be variable in their ability to detect significant differences. Generally, the Unifrac significance measure is the most conservative, followed by the P-test, and Libshuff library comparison. However, we were unable to assess the impact of Type II statistical error and further simulation studies are needed to evaluate this concern. We also discuss the challenges of dealing with putatively chimeric sequences in large and phylogenetically diverse datasets.

2.2 Introduction

The “great plate count anomaly” describes the discrepancy observed between the number of bacterial colony forming units that grow in culture and the number of bacteria counted from soil using microscopic methods (Faegri et al., 1977; Staley and Konopka, 1985). Since then, it has been hypothesized that less than 1% of all soil bacteria, and only about 17% of known fungi can be isolated in culture using standard methods (Torsvik and Ovreas, 2002; Hawksworth, 1991). It is well known that many fungi are not cultivable using standard isolation methods, and discrepancies between studies that use culture-based methods and molecular-based methods have been found (ericoid mycorrhizal roots Allen et al., 2003; beetle gut yeasts Suh et al., 2005). Culture-free methods have provided a window on the fungal diversity present in environmental samples that were otherwise unknown based on culture-based methods alone (agricultural soil fungi Lynch and Thorn, 2006; leaf endophytes Arnold et al., 2007). In some cases, new deeply diverging lineages of taxa have been discovered (Vandenkoornhuyse et al., 2002; Schadt et al., 2003; Jumpponen and Johnson, 2005; Porter et al., 2007; Schmidt et al., in press).

The widespread application of DNA-based environmental sampling necessitates the refinement of techniques to analyze these data. Many studies are already starting to explore fungal communities in forest (sclerophyll, brigalow, eucalypt, mixed hardwood, coniferous), grassland, prairie, alpine tundra, montane, treeline, agricultural, and unvegetated soils using culture-free methods (Chen and Cairney, 2002; Anderson et al., 2003a; Anderson et al., 2003b; Jumpponen, 2003; Schadt et al., 2003; Jumpponen and

Johnson, 2005; O'Brien et al., 2005; Lynch and Thorn, 2006; Jumpponen, 2007; Midgley, 2007). In these studies, traditional methods of expressing diversity such as richness, abundance, collector's curves, rank-abundance curves, frequency histograms, traditional ecological indices such as the Shannon diversity index, Shannon-Weiner diversity index, Simpsons index of diversity, Simpsons evenness, Sorensen's index, Morisita-Horn index, and diversity estimators such as Jackknife, ICE, ACE, Chao1, Chao2 are used. It is becoming more common to use phylogenetic analyses and phylogenetic diversity measures (Faith, 1992). For instance, Arnold et al. (2007) used neighbor joining and Porter et al. (in review) used maximum likelihood models to estimate branch lengths to calculate phylogenetic diversity.

Traditional measures of diversity are problematic with rDNA data because of uncertain species definitions and the reliance on artificial operational taxonomic units (OTUs) based on sequence similarity. The advantage of phylogenetic diversity (PD) is that it takes into account the evolutionary breadth of samples without having to reduce the data into artificial groups for comparison. The concept of phylogenetic diversity was first developed for the field of conservation biology to measure the underlying feature diversity of a subset of taxa (Faith, 1992; Faith, 1994; Sechrest et al., 2002). Phylogenetic-based measures appear well-suited for the analysis of DNA-data.

DNA-based datasets have the advantage of being generated relatively rapidly, compared with traditional culture-based or collection-based datasets. Some advantages for incorporating DNA-data include the ability to: 1) perform comparative phylogenetic

analyses; 2) develop primers and probes for further targeted studies; and, 3) use phylogenetic-based methods for assessing diversity and detecting significant differences among samples. The objectives for this study are to: 1) identify the major groups of fungi present in the field site; 2) assess the diversity of the major taxonomic and ecological groups of fungi in two soil horizons using richness, abundance, and phylogenetic diversity; and 3) assess whether there are significant differences in the distribution of soil fungi in two soil horizons using phylogenetic-based tests.

2.3 Methods

2.3.1 Site and soil sampling

The study site is a 5000 m^2 forest plot in the Koffler Scientific Reserve, Ontario, Canada (43° 37' N, 79° 39' W) and is dominated by eastern hemlock (*Tsuga canadensis*) and white cedar (*Thuja occidentalis*). The Reserve is located within the Oak Ridges Moraine, part of the Eastern Great Lakes lowland forests ecoregion (Ricketts et al., 1999). Additional site and soil characteristics are described elsewhere (Porter et al., in review).

Soil was sampled using two different methods. In July 2003, pooled soil sampling was conducted by collecting one soil core (2 cm diameter x 20 cm depth) every 10 m across a 100 m transect of the plot. A total of 311 g wet weight of the B-horizon was separated from 335 g wet weight of the C-horizon. The soil within each horizon was thoroughly mixed together by sieving while large roots and rocks were removed. Fresh soil

was stored at 4°C for 24 hours prior to DNA extraction. The remaining soil was then stored at -20°C. In May 2004, point soil sampling was conducted by collecting a single soil core (2 cm diameter x 42 cm depth) from the same field site. The core was immediately partitioned into 2 cm sections and stored separately at -20°C.

2.3.2 DNA extraction, PCR, cloning and sequencing

Soil DNA was extracted using the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories Inc, CA) following the manufacturer's directions. Replicate extractions of 0.25 g (wet weight) of soil were used for pooled soil samples and 0.5 - 1.0 g (wet weight) of soil was used for point soil samples.

As per the manufacturer's protocol, 10 µl of template DNA from the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories Inc., CA) was used for each mixed-template PCR. The primers used for both PCR amplification and cycle sequencing are considered to be largely fungal specific and included the forward LR0R (Rehner and Samuels, 1994) and the reverse LR5 (Hopple and Vilgalys, 1999) primers that targets approximately 900 bp at the 5'-end of the LSU region of rDNA. The PCR amplification procedure followed that described by Vilgalys and Hester (1990) except that the extension step was extended to 2 min, the number of cycles reduced to 25, and the final extension time increased to 7 min. The quality and amount of the PCR products were visualized on ethidium bromide-stained agarose gels.

For most libraries, a single PCR reaction was used to generate the clone libraries. For two libraries, however, five PCR reactions were pooled prior to cloning. Amplicons were cleaned with the use of the QiaQuick PCR Purification Kit (Qiagen Inc., ON) following the manufacturer's protocol and cloned using the QIAGEN PCR cloning kit (Qiagen Inc., ON) with QIAGEN EZ competent cells (Qiagen Inc., ON) following the manufacturer's protocol. Transformed cells were plated on LB agar plates with ampicillin and blue/white selection to select for bacteria with a plasmid containing an inserted sequence.

Colony PCR was conducted using whole cells from white colonies added directly to the PCR cocktail described above with an addition 10 µl of ddH_20 per reaction. Amplicons were cleaned as described above with a final elution of dd H_2O to yield approximately 40 ng DNA / 4 µl for cycle sequencing with the Big Dye Terminator Kit v3.1 (Applied Biosystems, CA). Cycle-sequencing products were purified using gel-filtration chromatography with a Sephadex slurry in Centri-Sep spin columns (Princeton Separations, NJ) and run on an ABI PRISM 3100 genetic analyzer (Applied Biosystems, CA). Forward and reverse chromatograms were assembled using Sequencher 4.1 (Gene Codes Corporation, MI).

2.3.3 Checking for chimeric sequences

The presence of chimeric sequences in clone libraries was tested using two methods. First, the chimera check program available through the Ribosomal Database Project II was used and their database was supplemented with sequences from our own

clone libraries (Cole et al., 2003). Second, the first half and second half of each sequence were used to create separate phylogenetic trees and taxa were visually checked for stability in phylogenetic placement.

2.3.4 Classifying rDNA sequences from soil

The primers we used were largely fungal-specific, however, we used sequence similarity and phylogenetic analyses to identify and exclude non-fungal sequences. In order to facilitate the classification of unknown soil sequences, the BioPerl toolkit (available at http://bio.perl.org/) was used to write a script to submit a batch of sequences to the NCBI nucleotide BLAST tool to retrieve the top hit sequence, description and percentage similarity for each query sequence. In order to facilitate global phylogenetic analyses of all soil sequences, the script created a fasta-formatted file with all non-redundant top BLAST hits (script available from T.M. Porter). After careful examination of preliminary phylogenetic trees (data not shown) sequences were sorted into three datasets: Ascomycota, Basidiomycota, and basal fungi (Glomeromycota + Chytridiomycota + Zygomycota).

2.3.5 Operational taxonomic units

The dataset is composed of all soil sequences sorted into operational taxonomic units (OTUs) based on 99% sequence similarity in lieu of species. This level of sequence

similarity cut-off has been used in other studies using LSU sequence data (Schadt et al. 2003; Lynch and Thorn, 2006). OTU-assignment involved conducting multiple pairwise sequence comparisons using Sequencher 4.1 (Gene Codes Corporation, MI). A single sequence representing each OTU from soil was included in the final phylogenetic analyses. Richness was assessed by counting the total number of OTUs. Abundance was assessed by counting the total number of randomly sequenced clones that comprised each OTU.

2.3.6 Assessing sampling effort

We assessed whether we saturated the sampling of soil fungi in our site by plotting a collector's curve using EstimateS 7.00 (Colwell 2003). OTUs were used as a proxy for species and sampling effort was assessed using the total number of randomly sampled sequences from clone libraries. We sampled the data without replacement and performed 1000 randomizations. Second-order Jackknife and Chao2 estimators were used for comparison to assess the proportion of total estimated diversity that we detected in our site. These estimators were specifically selected for their ability to provide the least biased estimates for small numbers of samples (Colwell and Coddington, 1994).

2.3.7 Phylogenetic based methods

Phylogenetic analyses were conducted for several subsets of the data: 1) Ascomycota taxa, 2) Basidiomycota taxa, 3) basal fungal taxa (Glomeromycota +

Chytridiomycota + Zygomycota), 4) all fungi from the B-horizon (4 - 6 cm) and all fungi from the C-horizon (40 - 42 cm). All Ascomycota taxa were aligned in a single matrix with reference sequences from each major lineage selected from recent studies (Lutzoni et al., 2004; Porter et al., 2007; Sugiyama et al., 2006). Only one sequence, selected on the basis of sequence quality and length, representing each OTU was included in the analysis and deposited in GenBank with accession numbers xxxxxxx-xxxxxxx. Trees were rooted with three taxa from the Basidiomycota. All lineages were labeled according to the convention used by Lutzoni et al. (2004) and Porter et al. (2007). All Basidiomycota taxa were aligned in a single matrix with reference sequences from each major lineage selected from Binder and Hibbett (2002). Only one sequence representing each OTU was included in the analysis. Trees were rooted with three taxa from the Ascomycota. All lineages were labeled according to the phylogenetic classification used by Hibbett et al. (2007). All basal fungal taxa were aligned in a single matrix with reference sequences from each major lineage selected from James et al. (2006). Only one sequence representing each OTU was included in the analysis. Trees were rooted with two metazoan taxa. All lineages were labeled according to the convention used by James et al. (2006).

A maximum parsimony heuristic search was conducted using the parsimony ratchet (Nixon 1999) as implemented in PAUP using the PAUPrat module (Sikes and Lewis, 2001). Analyses were run on a 28-processor Linux Beowulf cluster. Parsimony bootstrap values were calculated in PAUP* by running 1000 parsimony bootstrap replicates with fast stepwise addition.

2.3.7.1 Phylogenetic diversity measure

In addition to richness and abundance as diversity indicators, we also used a phylogenetic measure that incorporates information in branch lengths on a phylogram containing taxa from B-horizon (4-6 cm) and C-horizon (40-42 cm) soil only. To avoid the problems associated with comparing artificial groups, Faith (1992, 1994) proposed the use of branch lengths in phylogenetic trees for comparative diversity studies (phylogenetic diversity, PD). Faith (1994) calculated PD by adding the branch lengths for a subset of taxa and dividing this by the branch lengths for all taxa, expressing PD as a percentage of total diversity. We have adapted this formula for calculating PD by using maximum-likelihood (ML) to determine the tree score for all the taxa in the tree and for subsets of taxa in the tree. Maximum-likelihood was used to avoid the arbitrary choice of ACCTRAN or DELTRAN optimization when parsimony is used as an optimality criterion. Modeltest 3.06 (Posada and Crandall, 1998) was used to determine the ML model of DNA evolution that best fit the data and suggested the use of a model with the following parameters to estimate branch lengths and tree scores: base frequencies (A=0.2929, C= 0.2002, G=0.2838, T=0.2231), with a rate matrix (1.000 2.5667 1.000 1.000 6.0830), proportion invariable sites set to 0.1082, with a gamma distribution shape parameter set to 0.6532. This model was applied in PAUP to calculate the ML score for one of the most parsimonious trees from the parsimony ratchet analysis. The relative amount of phylogenetic diversity (ML score) captured by various data partitions (soil horizon, ecology) was calculated by pruning the tree without re-optimizing branch lengths.

2.3.7.2 Testing for significance using phylogenetic based measures

To test whether there is a significant difference between the phylogenetic and ecological distributions of taxa recovered from B-horizon (4-6 cm) and C-horizon (40-42 cm) soil, we used two tests implemented in UniFrac (Lozupone et al., 2006). Using the P-test, soil horizon and ecology was mapped onto the most parsimonious trees recovered from a parsimony analysis conducted in PAUP (Martin, 2002). The number of transitions between sample-type in the most parsimonious trees are counted and compared with a null distribution generated by counting the number of transitions in 100 randomly generated trees. The P-test indicates whether the taxa sampled from fruiting bodies and soil are significantly clustered on the tree. This was compared with the UniFrac significance test, where the amount of branch length unique to a single sample-type is expressed as a fraction of total branch length in a parsimony tree that we generated in PAUP (Lozupone and Knight, 2005). This is compared with a null distribution generated by randomly assigning sample-type to taxa on the tree, measuring the fraction of unique branch length, and permuting this 100 times. The UniFrac significance measure indicates the probability that taxa sampled from fruiting bodies or soil have more unique branch length than expected by chance. For both tests, parsimony analyses in PAUP included sequences from all samples reflecting the abundance of fruiting bodies and soil rDNA sequences actually sampled. Subsets of our dataset were assessed by pruning taxa from one most parsimonious tree. Libshuff library comparison (Singleton et al., 2001) was also conducted as implemented using webLibshuff (Henriksen, 2004). This tool is used to determine whether two clone libraries are significantly different from each other using a distance

matrix, a calculation for coverage based on the proportion of unique samples in a library, in conjunction with the Cramer von-Mises statistic to calculate the difference in coverage between two libraries (Singleton et al., 2001). This value is then compared with a null distribution composed of 1000 replicates of the above procedure for randomly generated libraries.

2.4 Results

A list of all 1093 soil rDNA sequences and their corresponding top BLAST matches are available as supplementary material.

Figure 2-1 shows one of 649 most parsimonious trees with length 2280 recovered from a parsimony ratchet search for the Ascomycota clones (CI = 0.3654, HI = 0.6346, RI = 0.7396). The analysis included 148 Ascomycota sequences, each representing an OTU, 85 reference taxa that were pruned from the final tree, and 3 Basidiomycota outgroup taxa. 1200 characters were aligned, 628 characters from ambiguously aligned regions and large gapped regions were excluded from the analysis. 572 characters were included, 106 were constant, 128 were variable but parsimony uninformative, and 338 were parsimony informative.

Figure 2-2 shows one of 278 most parsimonious trees with length 1249 recovered from a parsimony ratchet search for the Basidiomycota clones (CI = 0.3475, HI = 0.6525, RI = 0.6747). The analysis included 97 Basidiomycota sequences, each representing an

OTU, 30 reference taxa that were pruned from the final tree, and 3 Ascomycota outgroup taxa. 1196 characters were aligned with 618 characters excluded from the analysis. 578 characters were included, 287 were constant, 79 were variable but parsimony uninformative, and 212 were parsimony informative.

Figure 2-3 shows one of 596 most parsimonious trees with length 1485 recovered from a parsimony ratchet search for the basal fungi clones (CI = 0.4013, HI = 0.5987, RI = 0.5850). The analysis included 65 basal fungal sequences, each representing an OTU, 13 reference taxa that were pruned from the final tree, and 2 metazoan outgroup taxa. 1235 characters were aligned with 725 characters excluded from the analysis. 510 characters were included, 137 were constant, 117 were variable but parsimony uninformative, and 256 were parsimony informative. There are at least two major clades that we were unable to classify.

Figure 2-4 shows one of 668 most parsimonious trees with length 2771. 1249 characters were aligned and 703 characters were excluded. 546 characters were included. 336 fungal sequences from the B (4-6 cm) and C (40-42 cm) soil horizons were analyzed, two of which had high sequence similarity to metazoan taxa and were used to root the tree. The major fungal lineages analyzed in Table 2-1 and Table 2-2 are indicated on the tree. Figure 2-5 shows the collector's curve and two diversity richness estimators, Chao 2 and second-order Jackknife, for the most densely sampled soil horizons. In Figure 2-5, samples labeled 2C are from a single soil core at a depth of 4-6 cm and a total of 106 OTUs (219 sequences) were sampled. Samples labeled 20C are from a single soil core at

a depth of 40-42 cm and a total of 100 OTUs (180 sequences) were sampled. Each curve shows positive slopes for the observed number of OTUs and for the estimators indicating that we have not saturated fungal sampling in these two soil layers.

Table 2-1 shows the results of using three measures to assess the diversity of four fungal lineages. All three measures find a greater diversity of Ascomycota and basal fungi. All three measures also find slightly more Basidiomycota from the C-soil horizon and more basal fungi from the B-soil horizon. The phylogenetic diversity measure reveals that the basal fungi contribute the greatest proportion of diversity, in contrast with the richness and abundance measures that determine that the Ascomycota contribute the most to overall diversity in the two sampled layers. Table 2-2 shows a comparison among three phylogenetic-based tests of significance among the two samples. Unifrac significance does not find any significant differences between the diversity of each fungal lineage in each soil horizon, whereas, the Libshuff library comparison finds that each major fungal lineage is significantly distributed between soil horizons.

Table 2-3 shows the results of using richness, abundance, and phylogenetic diversity to assess whether there is a significant difference among the distribution of mycorrhizal taxa between two soil horizons. Although values are shown for non-mycorrhizal taxa, this category includes both true saprophytes on a variety of substrates in addition to a few parasites in order to simplify the comparison and will not be considered in detail here. Richness finds a similar number of OTUs are distributed between both soil layers, abundance finds that more mycorrhizal OTUs were sampled from the C soil horizon,

however, phylogenetic diversity finds a greater proportion of mycorrhizal diversity is present in the B soil horizon. Table 2-4 compares whether the Unifrac, P-test, and Libshuff tests find significant differences among the distribution of mycorrhizal taxa in two soil layers. None of these tests found a significant difference with the proportion of mycorrhizal OTUs present in two soil horizons.

2.5 Discussion

2.5.1 Phylogenetic distribution of soil rDNA OTUs

The nLSU region used in this study was sufficient to sort all the fungal sequences from soil into major lineages (Figure 2-1 to Figure 2-3). In order to maximize the number of characters used in each analysis, the dataset was split into three separate alignments. Although good statistical support is shown for the monophyly of most major lineages within each phylogenetic tree, the arrangement among these clades showed little support. This is a result of using only a single gene region in these analyses.

In the Ascomycota, the Leotiomycetes 1, Sordariomycetes, and SCGI have the greatest number of OTUs detected from soil. The Leotiomycetes 1 clade sensu Lutzoni et al. (2004) include all apothecial ascomycetes, cup-shaped ascomata, and Erysiphales that produce gymnothecial ascomata. Previous fruiting body surveys in this site also recorded abundant fructifications of *Peziza* spp. during the spring. The vast majority of "pyrenomycetes" are members of the Sordariomycetes. Traditional fruiting body surveys

would have overlooked these minute fructifications. The SCGI clade (also known as Group I *sensu* Schadt et al., 2003) was first reported from alpine tundra soil from Colorado, USA using sequenced-based methods, and has recently been shown to have a widespread distribution in vegetated soils from diverse origins (Schadt et al., 2003; Porter et al., 2007). This group is an anomaly among the fungi since it is the only group known exclusively from environmental rDNA sequences.

In the Basidiomycota, the Agaricales and Pucciniomycotina have the greatest number of OTUs detected from soil. Since the Agaricales is the largest order in the Agaricomycotina with over 8,400 described species (Hawksworth et al., 1995), this is not surprising, and the most likely sources of rDNA detected from soil are vegetatively growing hyphae and spores. One-third of described Basidiomycota belong to subphylum Pucciniomycotina (formerly known as the Urediniomycetes) (Bauer et al., 2006; Swann and Taylor, 1995). About 7000 of these, or ca. 90%, belong to a single order, the Pucciniales (rust fungi formerly known as the Uredinales) (Kirk et al., 2001). The remaining taxa represent a variety of putative saprotrophs and parasites of plants, animals, and fungi (Aime et al., 2006). In this study, the Pucciniomycotina is composed of at least two large clades related to the Platygloeales in the Pucciniomycetes and to the Erythrobasidiales in the Cystobasidiomycetes sensu Hibbett et al. (2007). The most likely sources of rDNA detected from soil are overwintering spores or yeasts.

Among the basal fungi, the largest number of OTUs were detected from the arbuscular-mycorrhiza forming Glomeromycota. This is expected because white cedar

(*Thuja occidentalis*) was a dominant species in the study site and is known to form arbuscular mycorrhizal associations (Kendrick, 2000; Blaszkowski, 1994). The peduncled sedge (*Carex pedunculata*) was also a relatively dominant understory species in the field site and is known to form arbuscular mycorrhizal associations (Muthukumar et al., 2004). Additionally, two phylogenetically divergent clades, labeled Unknown I and II, were detected in this study. These may represent novel lineages and warrant further investigation using additional reference taxa and molecular markers to refine their phylogenetic placement and for comparison with other environmental sampling studies similar to what has been done for the Soil Clone Group I (SCGI) clade in the Ascomycota (Porter et al., 2007).

2.5.2 Detection of rDNA from obligate biotrophs

We detected fungi that are likely to be actively growing in soil, but also fungi from dormant propagules. For instance, many taxa in the Leotiomycetes 1 clade (Lutzoni et al., 2004), Pucciniomycotina and Ustilaginomycetes are plant pathogens, detected from our soil samples. Plant pathogens from these groups are known to produce symptoms on aerial plant tissue such as leaves and stems, however, we detected the presence of these groups directly from sifted soil. This may be explained by the detection of over-wintering spores and sclerotia capable of surviving periods of inactivity. For instance, sclerotia of *Sclerotinium cepivorum* have been known to survive burial in soil up to 20 years (Coley-Smith et al., 1990).

Additionally, we detected large groups of obligately mycorrhizal fungi such as endomycorrhizal fungi from the Glomeromycota, and numerous ectomycorrhizal lineages within the Basidiomycota such as the Agaricales, Boletales, Russulales, Thelephorales, and Cantharellales. Despite removing large roots and other organic debris from our soil samples we were still able to detect these taxa, either from dormant spores or from extramatrical fungal mycelia from colonized root tips. Interestingly, root tips colonized by ectomycorrhizal fungi have been described as most abundant in organic and upper layers of soil (Neville, 2002; Buee et al., 2007; Izzo et al., 2005). In our field site, there was only a very thin A horizon filled with large roots that were cleared away prior to soil collection. Despite removing this potentially abundant source of ectomycorrhizal fungi, we were still able to detect the presence of several major taxonomic lineages of mycorrhizal fungi.

2.5.3 Comparison of three measures of diversity

A comparison of two traditional measures of diversity and phylogenetic diversity shows that each measure identifies the same dominant groups. This suggests that for rDNA based environmental surveys, phylogenetic diversity may be a suitable substitute for richness and abundance measures that rely on the delimitation of species or artificial OTUs. The two most dominant groups of soil fungi are the Ascomycota and basal fungal lineages (Table 2-1). The results of culture-based methods do generally recover Ascomycota taxa preferentially when using soil dilution plate methods with standard media (Malloch, 1981). Three phylogenetic-based comparisons among the fungal lineages detected from two soil horizons tests are variable in their ability to detect significant

differences. Generally, the Unifrac significance measure is most conservative, followed by the P-test, and Libshuff library comparison. The Unifrac test did not detect any significant differences among the comparisons we were interested in (Table 2-2 and Table 2-4).

2.5.4 Statistical error

Although we have stated that the three phylogenetic-based tests are variable in their ability to detect significant differences, the underlying assumption is that there actually is a significant difference between the taxonomic and ecological distribution of fungi between two soil horizons that can be detected using our methods (Table 2-2 and Table 2-4). Type I (false-positive) and type II (false-negative) statistical error needs to be taken into consideration. When hypothesis testing, we set the type I error rate, level of significance, to 0.05. Increasing the specificity of the test from 0.95 to 0.99 reduces the amount of type I error, but increases the amount of type II error. It may be possible for simulation studies to assess the power of these tests.

2.5.5 Identifying putatively chimeric sequences

Progress from the All Fungal Tree of Life (AFTOL) project has contributed numerous LSU rDNA sequences essential for comparison with environmental sequences (such as Lutzoni et al., 2004; James et al., 2006). For the phylogenetic analysis of all Ascomycota and Basidiomycota taxa, inclusion of a variety of basal sequences and outgroup taxa was

essential to identify putatively chimeric sequences. A number of basal Basidiomycota and Ascomycota sequences were excluded from the analyses because they were difficult to classify and could not be differentiated from putatively chimeric sequences. It is possible that some of these may have been classified if we had included members of the basal Basidiomycota classes Wallemiomycetes and Entorrhizomycetes for comparison (Hibbett et al., 2007; Matheny et al., 2006; Zalar et al., 2005). For the phylogenetic analysis of basal fungi, additional reference taxa are needed.

Chimeric sequences may be formed when a prematurely terminated amplicon re-anneals to another DNA strand in the PCR mixture, and is copied to completion and amplified during subsequent PCR cycles (Hugenholtz and Huber, 2003). Despite using two methods to detect chimeric sequences, they were often difficult to identify. The output from the Chimera Check program was often ambiguous and the output from suspected novel taxa or chimeric sequences did not seem to differ significantly from sequences obtained directly from reference taxa whose sequences were obtained from pure cultures or fruiting bodies.

For instance, in the Ascomycota dataset, suspected chimeric sequences would consistently cluster at the base of the phylogeny with the Basidiomycota outgroup taxa; in the Basidiomycota dataset, the same suspected chimeric sequences clustered at the base of the phylogeny with the Ascomycota outgroup taxa. Basal placement of these sequences is consistent when maximum parsimony and neighbor joining methods are used, and may be the result of long branch attraction. This situation is similar to that observed by

Jumpponen (2007) who concluded that there were chimeric portions of DNA at the ends of amplicons causing these divergent sequences to cluster at the base of his phylogenetic tree.

Because of the difficulty with identifying chimeric sequences, particularly those formed from sequences of closely related taxa, and when chimeras are formed from more than two fragments, I propose several hypotheses to explain the origin of these sequences: 1) these are basal Ascomycota, 2) these are basal Basidiomycota, 3) these are paralogs, or 4) these are chimeric sequences. The best way to distinguish among these hypotheses would be to analyze more data, that is, include additional reference taxa to the phylogenetic analysis as these become available, and to include additional characters in the analysis, such as from the neighboring ITS and SSU rDNA regions. For simplicity, these taxa were excluded from Figure 2-1 and Figure 2-2. This problem is certainly not unique to Fungi and microbiologists have long recognized the problems caused by putatively chimeric sequences (Hugenholtz and Huber, 2003).

2.5.6 Known distribution of fungi by depth

Mycorrhizal fungi are known to be associated with specific types of soil environments (Read, 1991). Neville (2002) showed that arbuscular mycorrhizae and ectomycorrhizae associated with *Populus tremuloides* are differentially distributed in surface soil layers by direct microscopic examination of root tips at different depths. Root tip examinations of ectomycorrhizal fungi associated with *Populus tremuloides* show that

they are most abundant in shallow organic soils from 0 to 10 cm in depth (Neville, 2002). We did not sample the A horizon in our site because it was very thin and saturated with large roots which were cut away prior to soil collection. We did, however, sample taxa from the B and C soil horizons and we grouped ectomycorrhizal and arbuscular mycorrhizal taxa into a single category of mycorrhizal taxa. When we looked at soil sampled from 4 – 6 cm versus 40 – 42 cm depth, we were unable to detect a difference in the number of OTUs of mycorrhizal fungi using molecular methods. The abundance of mycorrhizal taxa appeared to be greater in deeper soil; however, the phylogenetic diversity of mycorrhizal taxa was slightly greater in surface soil. These differences may be accounted for by the nature of the gleysol soil we studied, a soil type often saturated with water, which may skew patterns of ectomycorrhizal taxon distribution seen in other soil types.

Future studies looking at the distribution of fungi by depth using molecular methods, should correlate soil depth with soil horizon and root tip abundance. Alternatively, total soil rRNA as opposed to rDNA could be extracted to help distinguish between living/viable fungi and dead/non-viable fungi present in soil. A greater amount of sampling may improve the chances of detecting significant differences among samples. Ultra-high throughput methods such as pyrosequencing may make this feasible.

2.6 Tables

Table 2-1: Comparison among three measures of diversity for the major fungal taxonomic groups detected from two soil horizons.

	4-6 cm	40-42 cm	Combined
Richness (OTUs)			
Basidiomycota	14	20	31
Ascomycota	35	35	58
SCGI	7	7	12
Basal	36	27	51
Abundance (sequences)			
Basidiomycota	21	58	79
Ascomycota	66	65	131
SCGI	14	15	29
Basal	68	56	124
Phylogenetic Diversity (-lnL)			
Basidiomycota	1761.35	2454.29	2896.90
Ascomycota	3093.43	2985.74	4135.78
SCGI	962.14	981.85	1114.53
Basal	5210.93	5020.27	7735.38

Table 2-2: Results from three phylogenetic-based tests of significance comparing two soil horizon samples.

Asterisk indicates values that are significant at 0.05.

	4-6 cm versus 40-42 cm
Unifrac significance	
Basidiomycota	0.25
Ascomycota	0.39
SCGI	0.11
Basal	0.54
P-test	
Basidiomycota	0.07
Ascomycota	0.01*
SCGI	0.44
Basal	0.04*
Libshuff library comparison	
Basidiomycota	0.026*
Ascomycota	0.001*
SCGI	0.001*
Basal	0.002*

Table 2-3: Comparison among three measures of diversity for the major fungal ecological groups detected from two soil horizons.

	4-6 cm	**40-42 cm**	**Combined**
Richness (OTUs)			
Mycorrhizal	11	12	20
Non-mycorrhizal	58	57	94
Uncertain ecology	17	15	30
Abundance (sequences)			
Mycorrhizal	14	45	59
Non-mycorrhizal	118	109	227
Uncertain ecology	24	26	50
Phylogenetic Diversity (-lnL)			
Mycorrhizal	2275.76	1829.94	2808.21
Non-mycorrhizal	6503.09	6672.21	9878.75
Uncertain ecology	2038.05	2733.85	3590.64

Table 2-4: Comparison among three phylogenetic-based tests of significance between two soil horizon samples.

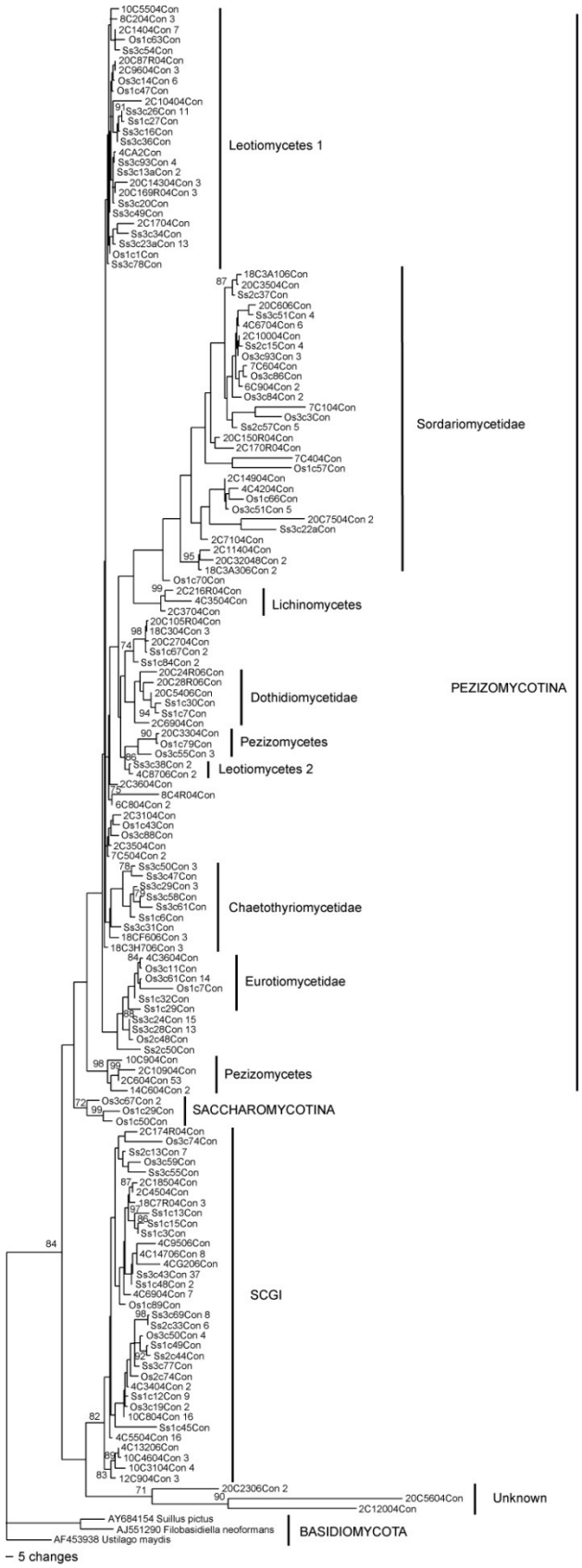
Asterisk indicates values that are significant at 0.05.

	4-6 cm versus 40-42 cm
Unifrac significance	
Mycorrhizal	0.62
Non-mycorrhizal	0.33
Uncertain ecology	0.01*
P-test	
Mycorrhizal	0.36
Non-mycorrhizal	0.01*
Uncertain ecology	0.01*
Libshuff library comparison	
Mycorrhizal	0.10
Non-mycorrhizal	0.001*
Uncertain ecology	0.003*

2.7 Figures

Figure 2-1: Phylogenetic distribution of all Ascomycota OTUs.

One of 649 most parsimonious trees with length 2280 (CI = 0.3654, HI = 0.6346, RI = 0.7396). Clades are labeled according to the convention used in Lutzoni et al. (2004) and Porter et al. (2007). OTUs are labeled according to the clone library they were sequenced from, followed by the number of sequences in each 99% contig (OTU). Bootstrap support is shown as a percentage at the nodes.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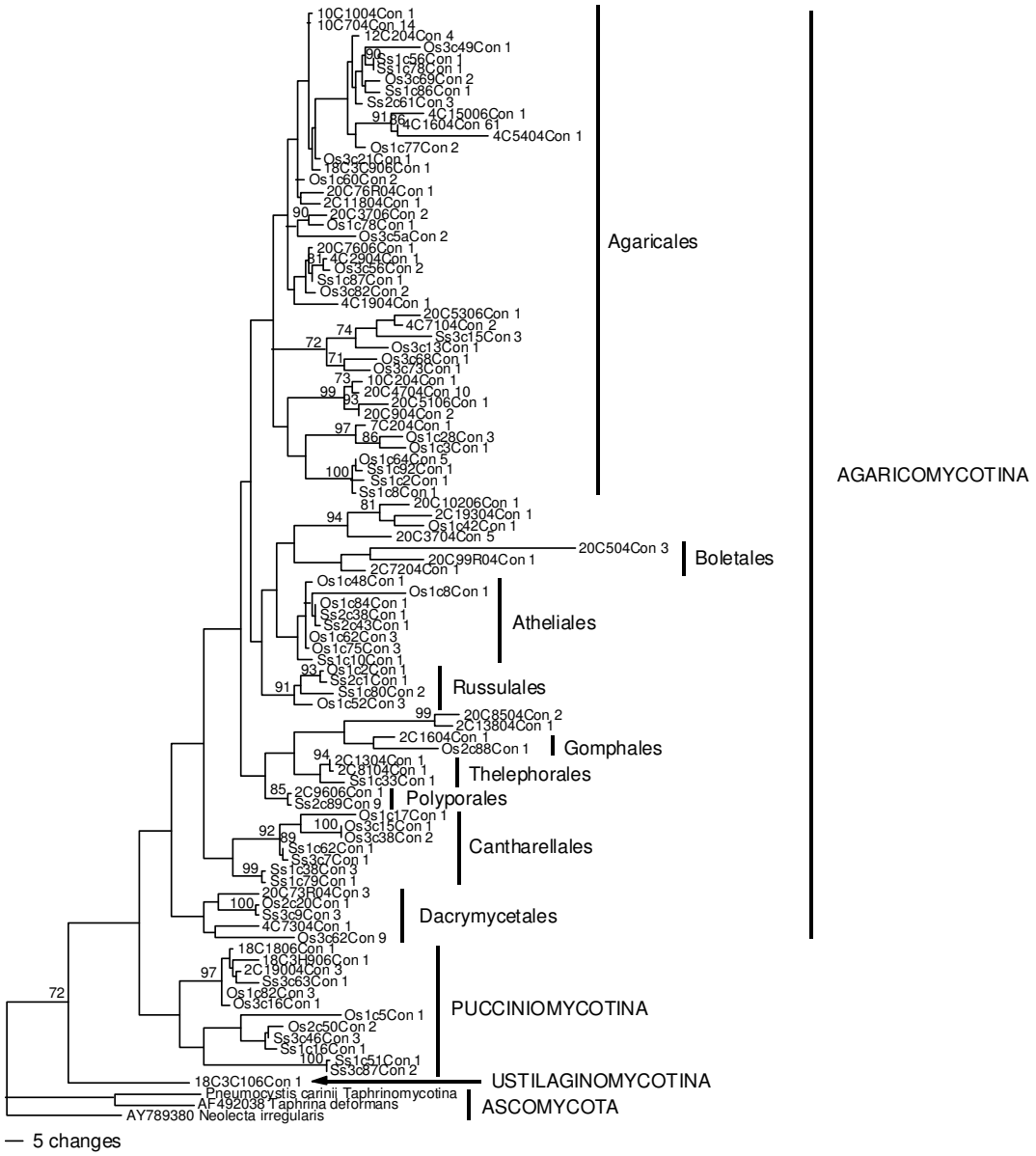
8C204Con 3
2C1404Con 7
Os1c63Con
Ss3c54Con
20C87R04Con
2C9604Con 3
Os3c14Con 6
Os1c47Con
2C10404Con
Ss3c26Con 11
Ss1c27Con
Ss3c16Con
Ss3c36Con
4CA2Con
Ss3c93Con 4
Ss3c13aCon 2
20C14304Con 3
20C169R04Con 3
Ss3c20Con
Ss3c49Con
2C1704Con
Ss3c34Con
Ss3c23aCon 13
Os1c1Con
Ss3c78Con
Leotiomycetes 1
18C3A106Con
20C3504Con
Ss2c37Con
20C806Con
Ss3c51Con 4
4C6704Con 6
2C10004Con
Ss2c150Con 4
Os3c93Con 3
7C604Con
Os3c86Con
6C3904Con 2
Os3c84Con 2
7C104Con
Os3c3Con
Ss2c57Con 5
20C150R04Con
2C170R04Con
7C404Con
Os1c57Con
2C14904Con
4C4204Con
Os1c66Con
Os3c51Con 5
20C7504Con 2
Ss3c22aCon
2C7104Con
2C11404Con
20C32048Con 2
18C3A306Con 2
Os1c70Con
2C216R04Con
4C3504Con
2C3704Con
Sordariomycetidae
Lichinomycetes
20C105R04Con
18C304Con 3
20C2704Con
Ss1c67Con 2
Ss1c84Con 2
20C24R06Con
20C28R06Con
20C5406Con
Ss1c30Con
Ss1c7Con
2C6904Con
Dothidiomycetidae
20C3304Con
Os1c79Con
Os3c55Con 3
Pezizomycetes
Ss3c38Con 2
4C8706Con 2
Leotiomycetes 2
2C3604Con
8C4R04Con
6C804Con 2
2C3104Con
Os1c43Con
Os3c88Con
2C3504Con
7C504Con 2
PEZIZOMYCOTINA
Ss3c50Con 3
Ss3c47Con
Ss3c29Con 3
Ss3c58Con
Ss3c61Con
Ss1c6Con
Ss3c31Con
18CF606Con 3
18C3H706Con 3
Chaetothyriomycetidae
4C3604Con
Os3c11Con
Os3c61Con 14
Os1c7Con
Ss1c32Con
Ss1c29Con
Ss3c24Con 15
Ss3c28Con 13
Os2c48Con
Ss2c50Con
Eurotiomycetidae
10C904Con
2C10904Con
2C604Con 53
14C604Con 2
Pezizomycetes
Os3c57Con 2
Os1c29Con
Os1c50Con
SACCHAROMYCOTINA
2C174R04Con
Os3c74Con
Ss2c13Con 7
Os3c59Con
Ss3c55Con
2C18504Con
2C4504Con
18C7R04Con 3
Ss1c13Con
Ss1c15Con
Ss1c3Con
4C9506Con
4C14706Con 8
4CG206Con
Ss3c43Con 37
Ss1c48Con 2
4C6904Con 7
Os1c89Con
Ss3c69Con 8
Ss2c33Con 6
Os3c50Con 4
Ss1c46Con
Ss2c44Con
Ss3c77Con
Os2c74Con
4C3404Con 2
Ss1c12Con 9
Os3c19Con 2
10C804Con 16
Ss1c45Con
4C5504Con 16
4C132060on
10C4604Con 3
10C3104Con 4
12C904Con 3
SCGI
20C2306Con 2
20C5604Con
2C12004Con
Unknown
AY684154 Suillus pictus
AJ551290 Filobasidiella neoformans
AF453938 Ustilago maydis
BASIDIOMYCOTA
– 5 changes

Figure 2-2: Phylogenetic distribution of all Basidiomycota OTUs.

One of 278 most parsimonious trees with length 1249 (CI = 0.3475, HI = 0.6525, RI = 0.6747). Clades are named according to the classification of Hibbett et al., (2007). OTUs are labeled according to the clone library they are sequenced from, followed by the number of sequences in each 99% contig (OTU). Bootstrap support is shown as a percentage at the nodes.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Os3c49Con 1
Ss1c56Con 1
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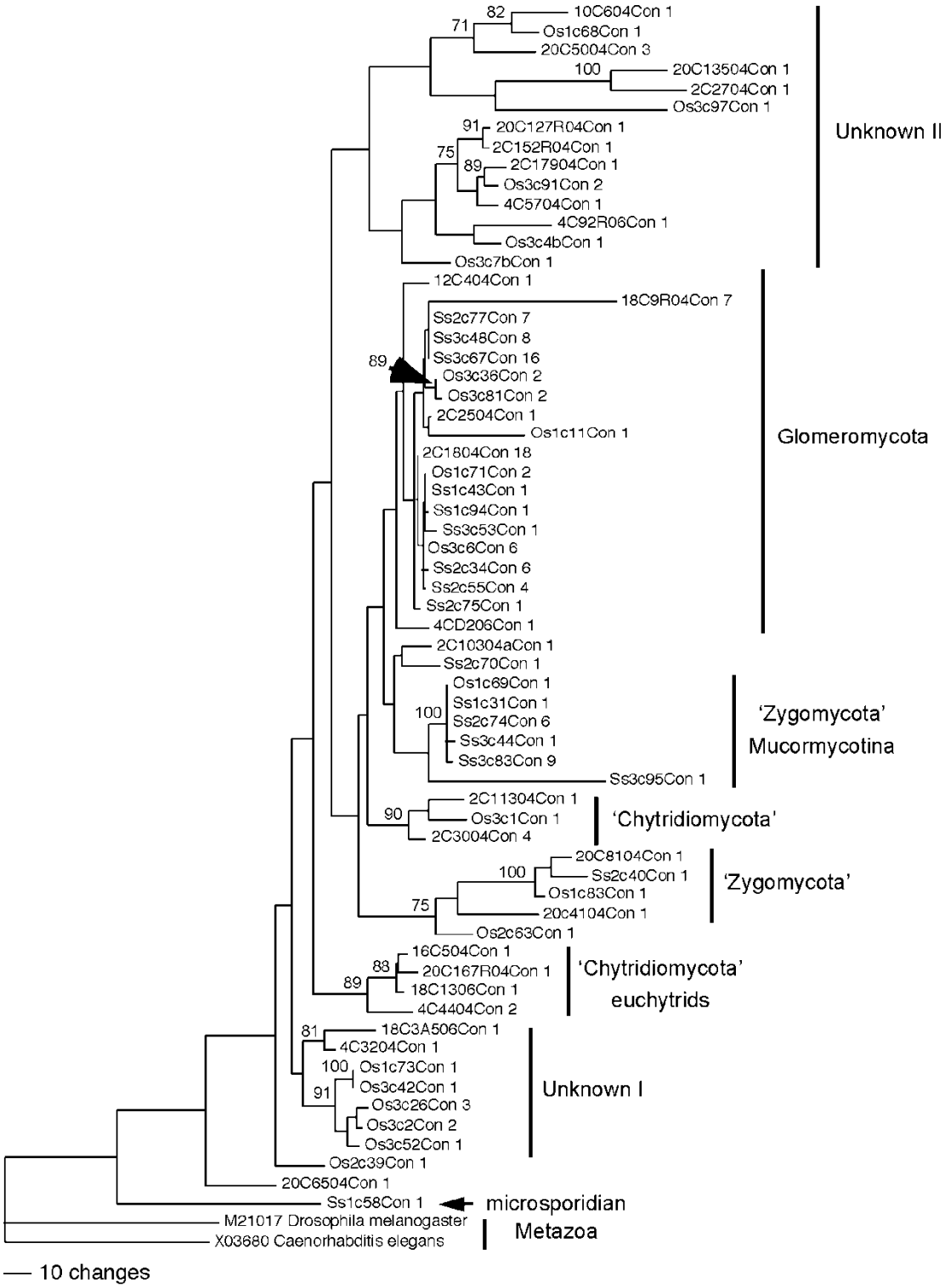
Os3c69Con 2
Ss1c86Con 1
Ss2c61Con 3
4C15006Con 1
4C1604Con 61
4C5404Con 1
Os1c77Con 2
Os3c21Con 1
18C3C906Con 1
Os1c60Con 2
20C76R04Con 1
2C11804Con 1
20C3706Con 2
Os1c78Con 1
Os3c5aCon 2
20C7606Con 1
4C2904Con 1
Os3c56Con 2
Ss1c87Con 1
Os3c82Con 2
4C1904Con 1
20C5306Con 1
4C7104Con 2
Ss3c15Con 3
Os3c13Con 1
Os3c68Con 1
Os3c73Con 1
10C204Con 1
20C4704Con 10
20C5106Con 1
20C904Con 2
7C204Con 1
Os1c28Con 3
Os1c3Con 1
Os1c64Con 5
Ss1c92Con 1
Ss1c2Con 1
Ss1c8Con 1
20C10206Con 1
2C19304Con 1
Os1c42Con 1
20C3704Con 5
20C504Con 3
20C99R04Con 1
2C7204Con 1
Os1c48Con 1
Os1c8Con 1
Os1c84Con 1
Ss2c38Con 1
Ss2c43Con 1
Os1c62Con 3
Os1c75Con 3
Ss1c10Con 1
Os1c2Con 1
Ss2c1Con 1
Ss1c80Con 2
Os1c52Con 3
20C8504Con 2
2C13804Con 1
2C1604Con 1
Os2c88Con 1
2C1304Con 1
2C8104Con 1
Ss1c33Con 1
2C9606Con 1
Ss2c89Con 9
Os1c17Con 1
Os3c15Con 1
Os3c38Con 2
Ss1c62Con 1
Ss3c7Con 1
Ss1c38Con 3
Ss1c79Con 1
20C73R04Con 3
Os2c20Con 1
Ss3c9Con 3
4C7304Con 1
Os3c62Con 9
18C1806Con 1
18C3H06Con 1
2C19004Con 3
Ss3c63Con 1
Os1c82Con 3
Os3c16Con 1
Os1c5Con 1
Os2c50Con 2
Ss3c46Con 3
Ss1c16Con 1
Ss1c51Con 1
Ss3c87Con 2
18C3C106Con 1
Pneumocystis carinii Taphrinomycotina
AF492038 Taphrina deformans
AY789380 Neolecta irregularis
— 5 changes
Agaricales
Boletales
Atheliales
Russulales
Gomphales
Thelephorales
Polyporales
Cantharellales
Dacrymycetales
PUCCINIOMYCOTINA
USTILAGINOMYCOTINA
ASCOMYCOTA
AGARICOMYCOTINA

Figure 2-3: Phylogenetic distribution of all basal fungal OTUs.

One of 596 most parsimonious trees with length 1485 (CI = 0.4013, HI = 0.5987, RI = 0.5850). Clades are named according to the convention used by James et al. (2006). OTUs are labeled according to the clone library they are sequenced from, followed by the number of sequences in each 99% contig (OTU). Bootstrap support is shown as a percentage at the nodes.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Os1c68Con 1
20C5004Con 3
20C13504Con 1
2C2704Con 1
Os3c97Con 1
20C127R04Con 1
2C152R04Con 1
2C17904Con 1
Os3c91Con 2
4C5704Con 1
4C92R06Con 1
Os3c4bCon 1
Os3c7bCon 1
Unknown II
12C404Con 1
18C9R04Con 7
Ss2c77Con 7
Ss3c48Con 8
Ss3c67Con 16
Os3c36Con 2
Os3c81Con 2
2C2504Con 1
Os1c11Con 1
2C1804Con 18
Os1c71Con 2
Ss1c43Con 1
Ss1c94Con 1
Ss3c53Con 1
Os3c6Con 6
Ss2c34Con 6
Ss2c55Con 4
Ss2c75Con 1
4CD206Con 1
Glomeromycota
2C10304aCon 1
Ss2c70Con 1
Os1c69Con 1
Ss1c31Con 1
Ss2c74Con 6
Ss3c44Con 1
Ss3c83Con 9
Ss3c95Con 1
'Zygomycota'
Mucormycotina
2C11304Con 1
Os3c1Con 1
2C3004Con 4
'Chytridiomycota'
20C8104Con 1
Ss2c40Con 1
Os1c83Con 1
20c4104Con 1
Os2c63Con 1
'Zygomycota'
16C504Con 1
20C167R04Con 1
18C1306Con 1
4C4404Con 2
'Chytridiomycota'
euchytrids
18C3A506Con 1
4C3204Con 1
Os1c73Con 1
Os3c42Con 1
Os3c26Con 3
Os3c2Con 2
Os3c52Con 1
Unknown I
Os2c39Con 1
20C6504Con 1
Ss1c58Con 1
microsporidian
M21017 Drosophila melanogaster
X03680 Caenorhabditis elegans
Metazoa
10 changes

Figure 2-4: Phylogenetic distribution of all sequences obtained from the B (4-6 cm) and C (40-42 cm) soil horizons.

One of 668 most parsimonious trees with length 2771. Only the lineages assessed for phylogenetic diversity and significance differences are labeled.

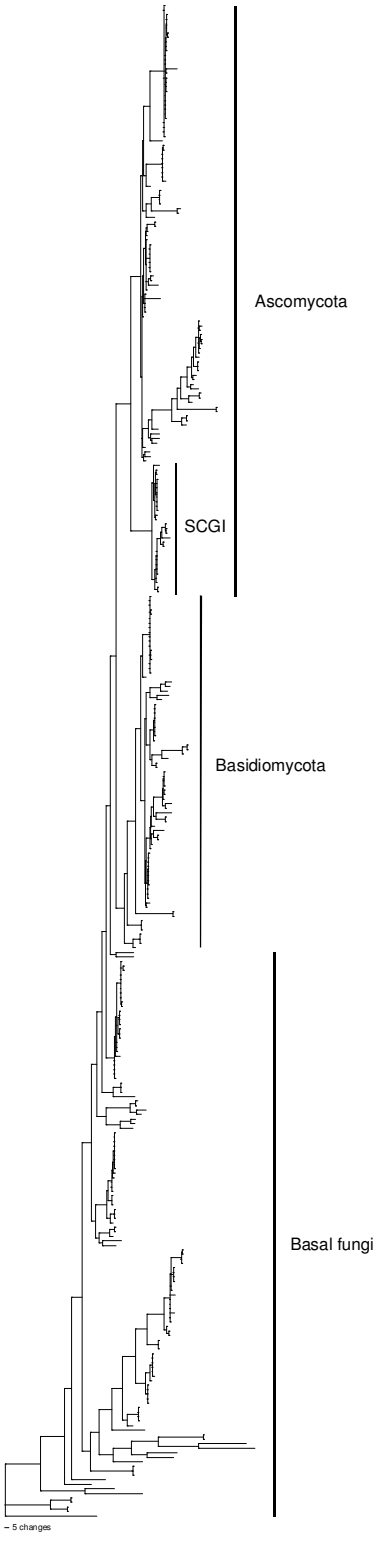
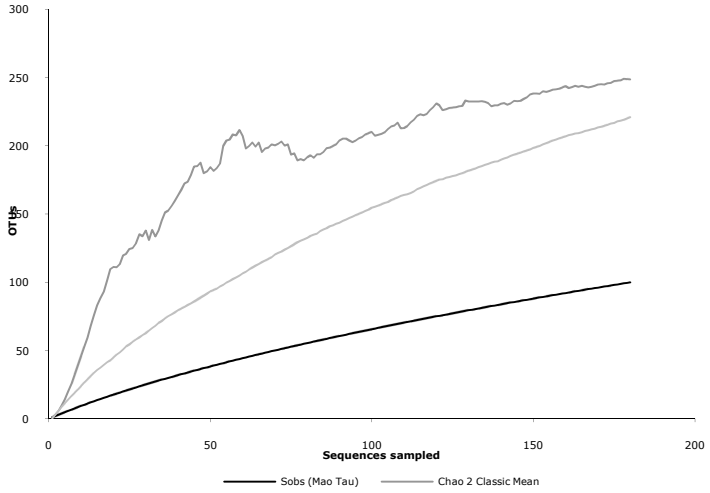

Ascomycota
SCGI
Basidiomycota
Basal fungi
5 changes

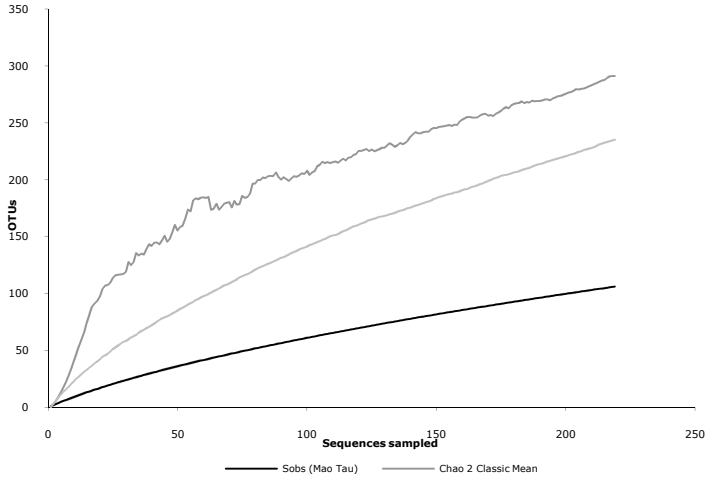
Figure 2-5: Collector's curves and two richness estimators for soil sampled from the B and C horizons.

Collector's curves and two richness estimators, Chao2 and second order Jackknife, are plotted for A) 2C soil core samples spanning a depth of 4-6cm; and B) 20C soil core samples spanning a depth of 40-42 cm.

A)


300
250
200
150
100
50
0
OTUs
0
50
100
150
200
Sequences sampled
Sobs (Mao Tau)
Chao 2 Classic Mean

B)


350
300
250
200
150
100
50
0
OTUs
0
50
100
150
200
250
Sequences sampled
Sobs (Mao Tau)
Chao 2 Classic Mean

3 Chapter Three- Fruiting body versus soil rDNA sampling: rapid rDNA survey sufficient to detect major taxonomic and ecological groups of the Agaricomycotina (Basidiomycota, Fungi) present in an ectotrophic forest

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3.1 Abstract

This is the first study to assess the diversity and community structure of the Agaricomycotina in an ectotrophic forest using above-ground fruiting body surveys as well as soil rDNA sampling. We recovered 132 molecular OTUs, or "species", from fruiting bodies and 65 from soil, with little overlap. Fruiting body sampling primarily recovered fungi from the Agaricales, Russulales, Boletales and Cantharellales. Many of these species are ectomycorrhizal and form large fruiting bodies. Soil rDNA sampling recovered fungi from these groups in addition to taxa overlooked during the fruiting body survey from the Atheliales, Trechisporales and Sebacinales. Species from these groups form inconspicuous, resupinate, and corticioid fruiting bodies. Soil sampling also detected fungi from the Hysterangiales that form their fruiting bodies underground. Generally, fruiting body and soil rDNA samples recover largely different assemblages of fungi at the species level; however, both methods identify the same dominant genera and both show ectomycorrhizal fungi are the dominant guild. Richness, abundance, and phylogenetic diversity (PD) identify the Agaricales as the dominant fungal group above and below-ground; however, we find that lineages with long branches may account for a greater proportion of total diversity using the PD measure compared with richness and abundance. Unless an exhaustive inventory is required, DNA-based sampling may provide a quick first assessment of the dominant taxonomic and ecological groups of fungi in forest soil.

3.2 Introduction

In the kingdom Fungi, taxa from the phylum Basidiomycota, subphylum Agaricomycotina, form the bulk of the macromycetes and represent about 20% of all described fungal species (ca. 14,000 out of ca. 72,000; Hawksworth, 2001). The Agaricomycotina includes many morphologically diverse taxa with equally diverse functional roles. For instance, mycorrhizas link above-ground plant communities, help plants aquire additional nutrients and water, as well as protect plant roots from pathogens (Smith and Read, 1997). Mycorrhizal taxa that uptake heavy metals from soils are of particular interest for land reclamation (Gadd, 1993). Ectomycorrhizas are the predominant type formed in the Agaricomycotina with plants from families such as the Pinaceae, Betulaceae, and Fagaceae (Kendrick, 2000; Smith and Read, 1997). Saprophytic species, on the other hand, are abile to break down recalcitrant compounds such as cellulose and lignin, consequently they play a major role in carbon-cycling (Kendrick, 2000).

Traditionally, the study of fungal diversity and community structure in forest ecosystems has relied on two different sampling techniques: 1) a direct method involving the collection of macromycetes, and 2) an indirect method involving the isolation of spores and mycelia in culture from soil samples. Since many members of the Agaricomycotina form abundant above-ground macroscopic fruiting bodies, the first technique has often been used to survey fungal diversity and ecology (Watling, 1995; Straatsma et al., 2001). The second technique is largely biased towards the detection of fungi that grow quickly in culture using standard methods, and consequently recovers many Ascomycota and

Zygomycota species that grow as moulds (Warcup, 1965; Gams, 1992). Culture-based techniques are also problematic because of the large number of species that are not culturable using standard isolation techniques (Torsvik and Ovreas, 2002).

DNA fingerprinting techniques such as restriction fragment length polymorphisms (RFLPs) or single-strand conformation polymorphism analysis (SSCP) have proven useful for environmental studies of microbes, but these methods provide limited taxonomic resolution when compared to direct sequence comparison (such as Schwieger and Tebb, 1998; Horton and Bruns, 2001). Most key systematic studies in fungi have employed nucleotide sequence data from ribosomal RNA genes (rDNA) (such as Bruns et al., 1991; Lutzoni et al., 2004; James et al., 2006; Hibbett et al., 2007). Subsequently, molecular identification systems such as DNA barcoding in fungi are largely based on sequence data from these genes (such as Gardes et al., 1991; Kowalchuk, 1997; El Karkouri et al., 2007; Frøslev et al., 2007; Summerbell et al., 2007). These and other studies have indicated that sequence data from the internal transcribed spacer (ITS) region of nuclear-encoded ribosomal (rDNA) genes are fast evolving and good molecular markers at the species level, but are of limited value for broad-phylogenetic analyses and cannot conclusively identify unknown sequences that lack close relatives in a database for comparison. In contrast, sequences from the small subunit rDNA region (SSU, or 16-18S rDNA) are more conserved and can only provide subphylum-family level identification. Sequence variation at the 5'-end of the large subunit rDNA region (LSU, or 25-28S rDNA) is intermediate between ITS and SSU sequence variation, and thus provides an appropriate level of resolution for systematics at the family-order-genus, and sometimes, to the species level.

LSU data have been widely used in systematic studies of the Agaricomycotina and large nucleotide sequence databases exist for these fungi to facilitate the identification of unknown environmental sequences in combination with phylogenetic analyses (Moncalvo et al., 2002).

The purpose of this study was to investigate and compare how two different sampling methods affect our assessment of Agaricomycotina diversity and community structure within a forest ecosystem. We directly compared the diversity of fruiting body morphospecies collected using traditional field methods and their corresponding LSU OTUs with that of LSU sequences sampled randomly from soil clone libraries. Specifically, we were interested in the following questions: 1) Do fruiting bodies and soil rDNA sampling detect the same phylogenetic taxa and ecological groups? 2) Do richness, abundance, and phylogenetic diversity measures detect the same proportions of phylogenetic taxa and ecological groups? 3) Can quick, DNA-based surveys replace time-consuming and laborious collection methods traditionally used for describing communities of Agaricomycotina taxa in a forest ecosystem?

3.3 Materials and Methods

3.3.1 Study site

Our study site is a 50 x 100m forest plot in the Koffler Scientific Reserve, Ontario, Canada (43° 37' N, 79° 39' W; 846-857 ft elevation). The Reserve is located within the Oak

Ridges Moraine, part of the Eastern Great Lakes lowland forests ecoregion (Ricketts et al., 1999). The soil is classified as a gleysol and is characterized by a thick Bg mineral soil horizon and Ckg 1 and Ck 2 layers intermixed with rocks and gravel. The dominant tree species is eastern hemlock (*Tsuga canadensis*) but white cedar (*Thuja occidentalis*) and yellow birch (*Betula lutea*) are also common. The understory vegetation is dominated by lady fern (*Athyrium filix-femina*), dwarf scouring-rush (*Equisetum scirpoides*), and Canada mayflower (*Maianthemum canadense*).

3.3.2 Fruiting body sampling

From July 2002 to December 2003, collection trips were made 2-3 times per week during the peak fruiting period (August – December), and monthly thereafter (December – July). We focused on collecting macromycetes including mainly above-ground fruiting bodies and crusts. Fruiting bodies were collected from living and dead wood, forest litter, and directly from the forest floor. Fresh tissue samples were transferred to 1.5 ml tubes containing 500 µl of either 2xCTAB buffer or 2.5% SDS extraction buffer (Zolan and Pukkila, 1986; Lee and Taylor, 1990) and stored at room temperature until DNA extraction. Fruiting bodies were photographed and identified at least to the genus level using morphological characters, then dried and stored in boxes. Collections were then sorted into morphotypes and identifications to the species level were made using macro- and microscopic characters and guided by phylogenetic analyses of LSU and/or ITS sequences.

3.3.3 Soil sampling

Soil was sampled using two different methods to test whether a point or pooled soil sampling method would detect the same fungal taxa. Pooled soil sampling was conducted in July 2003 by taking one soil core (2 cm diameter x 20 cm depth) every 10 m across a 100 m transect of the plot. A total of 311 g wet weight of the B horizon was separated from 335 g wet weight of the C horizon. The soil within each horizon was thoroughly mixed together by sieving while large roots and rocks were removed. Fresh soil was stored at 4°C for 24 hours prior to DNA extraction. The remaining soil was then stored at -20°C. Point sampling was conducted in May 2004 by collecting a single soil core (2 cm diameter x 42 cm depth). The core was immediately partitioned into 2 cm sections and stored separately at -20°C.

3.3.4 DNA extractions

Fruiting bodies were processed using standard miniprep protocols (Zolan and Pukkila, 1986; Lee and Taylor, 1990) that were modified for combined use with the FastPrep Instrument (Qbiogene, CA) to improve the physical break down of cell tissue. When using the FastPrep Instrument, we combined 0.1 and 1 mm zirconia beads with 500 µl of DNA lysis buffer. Tissues were homogenized with 3 replicates of 45 s of shaking at speed setting 6.0 (tubes were kept 2 min on ice between each shaking), followed by a 2 min centrifugation at 4°C at 10,000 rpm. After the addition of 500 µl of 24:1 chloroform-

isoamyl alcohol, the procedure was repeated. DNAs were then precipitated with 600 µl 4°C isopropanol, washed twice with -20°C 80% ethanol followed by 95% ethanol, re-suspended in 50 µl of distilled-deionized (dd) H_20, and stored at –20°C. Soil DNA was extracted using the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories Inc, CA) following the manufacturer's directions. Replicate extractions of 0.25 g (wet weight) of soil were used for pooled soil samples and 0.5-1.0 g (wet weight) of soil was used for point soil samples.

3.3.5 PCR amplification, cloning and sequencing

At least one LSU sequence was generated for each fruiting body morphospecies. The primers used for both PCR amplification and cycle sequencing are considered to be largely fungal specific and included the forward LR0R (Rehner and Samuels, 1994) and the reverse LR5 (Hopple and Vilgalys, 1999) primers that targets approximately 900 bp at the 5'-end of the LSU region of rDNA. The PCR amplification procedure follows that described by Vilgalys and Hester (1990). The quality and amount of the PCR products were visualized on ethidium bromide-stained agarose gels. Amplicons were cleaned with the use of the QiaQuick PCR Purification Kit (Qiagen Inc., ON) following the manufacturer's protocol with a final elution in dd H_2O to yield approximately 40 ng DNA/4 µl for cycle sequencing with the Big Dye Terminator Kit v3.1 (Applied Bisystems, CA). Cycle-sequenced products were purified using gel-filtration chromatography with a Sephadex slurry in Centri-Sep spin columns (Princeton Separations, NJ). Samples were run on an ABI PRISM 3100 genetic analyzer (Applied Biosystems, CA). Forward and reverse chromatograms were assembled using Sequencher 4.1 (Gene Codes Corporation, MI).

As per the manufacturer's protocol, 10 µl of template DNA from the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories Inc., CA) was used for each mixed-template PCR. The thermal cycler program for PCR amplification was the same as for the fruiting bodies except that the extension step was extended to 2 min, the number of cycles reduced to 25, and the final extension time increased to 7 min. For most libraries, a single PCR reaction was used to generate the clone libraries. For two libraries, however, five PCR reactions were pooled prior to cloning. These products were then concentrated and cleaned as described above then cloned using the QIAGEN PCR cloning kit (Qiagen Inc., ON) with QIAGEN EZ competent cells (Qiagen Inc., ON) following the manufacturer's protocol. Transformed cells were plated on LB agar plates with ampicillin and blue/white selection. Culture PCR was conducted using whole cells from white colonies and directly added to the PCR cocktail (Vilgalys and Hester, 1990) supplemented with 10 µl of dd H_20 per reaction. Sample cleaning and sequencing are as described above for fruiting bodies.

3.3.6 DNA Sequence-similarity based methods:

3.3.6.1 Identifying basidiomycete DNA sequences from soil

The primers we used were largely fungal-specific, however, sequence similarity and phylogenetic analyses were used to detect non-fungal sequences and specifically identify taxa from the Agaricomycotina. To facilitate the classification of unknown soil sequences, the BioPerl toolkit (available at http://bio.perl.org/) was used to write a script to submit a

batch of sequences to the NCBI nucleotide BLAST tool to retrieve the top hit sequence, description and percentage similarity for each query sequence. To facilitate phylogenetic sorting of all soil sequences, the script created a fasta-formatted file with all non-redundant top hit sequences (script available from T.M. Porter). After careful examination of preliminary phylogenetic trees (data not shown), only sequences with clear affinities to the Basidiomycota were kept for further analyses.

3.3.6.2 Screening for chimeric sequences

The presence of PCR- and cloning-generated chimeric sequences in our data set was checked using two different methods: 1) the Chimera Check program available from the Ribosomal Database Project at Michigan State University (http://rdp8.cme.msu.edu/html/index.html) after uploading additional sequences from our own libraries to supplement their LSU database, and 2) by systematically performing BLAST searches in GenBank with the first-half, followed by the second-half of each sequence, and comparing the two taxonomy reports to look for consistency in taxonomic placement.

3.3.6.3 Operational taxonomic units (OTUs)

Our molecular dataset was composed of fruiting body and soil LSU rDNA sequences that were sorted into operational taxonomic units (OTUs) based on 99% sequence similarity in lieu of species. This level of sequence similarity cut-off has been used in other

studies using environmental LSU sequence data (Lynch and Thorn, 2006; Schadt et al., 2003) as a conservative proxy for “species” estimates since empirical observations suggest that the range of intraspecific LSU sequence divergence in the Agaricomycotina is 0-1%. This cut-off value is applied here for both convenience and to account for PCR-generated, cloning, and sequencing errors. OTU-assignment was conducted from multiple pairwise sequence comparisons using Sequencher 4.1 (Gene Codes Corporation, MI). A single sequence representing each OTU recovered from fruiting body and soil sampling was included in the final alignment. OTUs were labeled with the taxonomic name of morphologically identified fruiting bodies, or in the case of soil sequences from the genus of the top BLAST hit in the GenBank database.

3.3.6.4 Assessing sampling effort

We assessed the sampling effort of Agaricomycotina taxa in our study site by plotting collector’s curves using EstimateS 7.00 (Colwell, 2003) with 1,000 randomizations sampling without replacement, and two diversity estimators ICE (Chazdon et al., 1998) and Chao2 (Chao, 1987), using OTUs as a proxy for species. Sampling effort was assesed by counting the total number of observed fruiting body collections or the total number of sequences randomly sampled from all clone libraries.

3.3.7 Phylogenetic methods:

The dataset was composed of a single representative OTU sequence from fruiting body collections and soil samples, and was automatically aligned using ClustalX 1.83 (Thompson et al., 1997) followed by manual editing in Se-Al 2.0 (Rambaut, 1996). Hypervariable, indel-rich regions with problematic alignment were excluded from the analyses. We analyzed the data using two methods: 1) two-step parsimony heuristic search method and 2) parsimony heuristic search using the parsimony ratchet (Nixon 1999) as implemented in PAUP using the PAUPrat module (Sikes and Lewis, 2001). Phylogenetic trees were rooted using our soil-recovered sequences that had high similarity to the Pucciniomycotina and Ustilaginomycotina, that are basal members of the Basidiomycota.

The two-step phylogenetic analysis was conducted in a manner similar to that described by Binder and Hibbett (2002) in PAUP (Swofford, 2002) using parsimony as the optimality criterion. First, tree searches were conducted with 100 random addition sequence replicates, saving no more than 10 trees per replicate, with TBR branch-swapping. A second search was conducted with the same settings, except that the most parsimonious trees from the first search were used as starting trees and ‘maxtrees’ was set to 5000.

The parsimony ratchet approach utilized the default settings in the PAUPrat module except that the basic program was modified to describe branch lengths for each tree produced. Branch robustness was assessed using 1,000 full heuristic bootstrap replicates

with the following settings: 100 random addition sequence replicates with TBR branch swapping, keeping 10 trees per replicate. We included taxa representing the major basidiomycete evolutionary lineages and classified all of our samples according to the phylogenetic classification of the fungi recently proposed by Hibbett et al. (2007).

3.3.7.1 Test for significance

To test whether there is a significant difference between the phylogenetic distributions of taxa recovered from fruiting bodies versus soil, we used the P-test (Martin 2002) implemented in UniFrac (Lozupone et al., 2006). In the P-test, a sample-type (fruiting body or soil) is mapped onto the most parsimonious tree and the number of transitions is counted and compared with a null distribution of transitions in 100 randomly generated trees. The P-test indicates whether the taxa sampled from fruiting bodies and soil are significantly clustered on the tree.

3.3.7.2 Measuring phylogenetic diversity

In addition to richness and abundance as diversity indicators, we also used a phylogenetic diversity measure (PD) as first proposed by Faith (1994). That measure, in contrast to richness OTU enumeration that assumes all taxa have equal weight, uses the information content in branch lengths on a phylogram that represents the timing and amount of evolutionary change among taxa. Since taxa classified at similar taxonomic ranks do not necessarily represent equivalent amounts of genetic variation, comparisons based on richness and abundance measures alone may not accurately reflect the

underlying genetic diversity present among taxa. To avoid the problems associated with comparing artificial groups, Faith (1994) was the first to propose the use of PD for comparative diversity studies.

Faith (1994) proposed to calculate PD by adding the branch lengths for a subset of taxa and dividing this by the branch lengths for all taxa, expressing PD as a percentage of total diversity. We have adapted this formula for calculating PD by using maximum-likelihood (ML) to determine the tree score for all the taxa in the tree and for subsets of taxa in the tree. Maximum-likelihood was used to avoid the arbitrary choice of ACCTRAN or DELTRAN optimization when parsimony is used as an optimality criterion. Modeltest 3.06 (Posada and Crandall, 1998) was used to determine the ML model of DNA evolution that best fits the data and suggested the use of a F81+G model with the following parameters to estimate branch lengths and tree scores: base frequencies (A=0.4043, C= 0.1639, G=0.1654, T=0.2664), all rates equal, proportion of invariable sites set to 0, with a gamma distribution shape parameter set to 1.6296. This model was applied in PAUP to calculate the ML score from the first most parsimonious tree found in the parsimony ratchet analysis. The relative amount of phylogenetic diversity (ML score) captured by various data partitions (sample-type, ecology) was calculated by pruning the tree without re-optimizing branch lengths.

3.4 Results

3.4.1 Fruiting body sampling

Two years of extensive fruiting body surveys in the study site yielded 451 collections corresponding to 132 OTUs (Table 3-1). In the majority of cases, multiple collections of the same morphospecies clustered in the same OTU. Occasionally, however, LSU sequences from multiple collections of the same morphospecies would segregate into more than one OTU. Conversely, there were also instances of different morphospecies whose LSU sequences clustered together in the same OTU. As a result, our 132 OTUs correspond to 134 morphotaxa in 55 genera (Supplementary Material). Voucher specimens are deposited at the Royal Ontario Museum Fungarium (TRTC). LSU rDNA sequences will be deposited to Genbank.

3.4.2 Soil rDNA sampling

The LSU rDNA libraries generated from pooled and point soil core sampling were combined. Although ca. 1,200 sequences were sampled in total, only sequences corresponding to Agaricomycotina taxa are analyzed here. We retrieved 167 Agaricomycotina sequences corresponding to 65 OTUs from 14 libraries (Table 3-2). LSU sequences will be deposited to Genbank. The top blast hit in the Genbank database for each sequence is listed as supplementary material. We recovered fewer duplicate

sequences using soil from ten pooled soil cores (1 OTU:1.9 clones) compared with using soil from a single soil core (1 OTU:3.2 clones). No conclusive evidence for chimeric sequences was detected.

A comparison of the distribution of the sequences from soil from our first library to our final analysis including all 14 libraries shows a high level of similarity in detecting the same dominant taxonomic orders (Figure 3-4). After sampling 72 sequences from our first soil clone library, 28 sequences were found to belong to the Agaricomycotina (16 OTUs). In this library, we found members of the orders Agaricales (38%), Cantharellales (19%), and Atheliales (19%) to be most abundant. After sampling ca. 1,200 sequences from soil, we found the same orders to be most abundant (55%, 10%, 6% respectively).

3.4.3 Assessing sampling effort

Twice as many OTUs (132) were recovered from our labour intensive two-year fruiting body survey compared with our quick sampling of soil rDNA (65 OTUs). Figure 3-1 shows the sampling curves for the Basidiomycota in our plot from both fruiting bodies and soil. Both accumulation curves have a positive slope indicating that more taxa should be found with increased sampling. The species diversity estimator ICE levels off and predicts 180 fruiting body OTUs suggesting this study has sampled 73% of all the fruiting bodies in our plot. The species diversity estimator ICE and Chao2 for soil sequences shows a stronger positive slope indicating that additional OTUs will be found with further sampling.

3.4.4 Minimal overlap between samples

When frequency histograms of all OTUs were compared, few taxa were found to be abundant and sampled more than 10 times. The majority of OTUs were found in a long 'tail' of taxa that were rare and were only sampled once or twice (Figure 3-3). There was minimal overlap (13 OTUs) between taxa sampled from fruiting bodies and soil. There was also minimal overlap (4 OTUs) between taxa sampled from ten pooled soil cores and a single soil core. We found no taxon overlap between fruiting body sampling, pooled soil sampling and point soil sampling combined.

3.4.5 Phylogenetic analyses

The data matrix has 192 sequences aligned in 1,007 positions. Of these, 238 ambiguously aligned sites were excluded from the analyses. Of the 769 included characters, 249 were constant, 126 were variable but parsimony uninformative, and 394 were variable and parsimony informative. The two-step parsimony analysis yielded a single most parsimonious tree with a tree length score of 4417 (CI = 0.2185, HI = 0.7815, RI = 0.6136). The parsimony ratchet search yielded 118 most parsimonious trees with a tree length score of 4412 (CI=0.2187, HI=0.7813, RI=0.6142). Figure 3-2 shows one of 118 most parsimonious trees from the parsimony ratchet analysis. Thickened branches indicate parsimony bootstrap support greater than 70%.

All taxa were labeled according to the classification proposed by Hibbett et al. (2007), except for a single '*Stephanospora'* sequence from soil because of the uncertain placement of this taxon in previous systematic studies. Taxa that are successfully recovered as monophyletic in our analysis are labeled with a black bar. Groups that are resolved as paraphyletic in our analysis are labeled with a grey bar.

3.4.6 Measuring diversity

The richness, abundance, and phylogenetic diversity of the Agaricomycotina sampled in our study plot are shown in Table 3-3. This study identified a total of 184 OTUs representing 17 out of 21 described orders of the Agaricomycotina. When fruiting body and soil samples are considered together, members of the Agaricales clearly represent the majority of samples, and to a lesser extent, members of the Russulales and Boletales. Members of the Hymenochaetales, Gomphales, Geastrales, Dacrymycetales and Tremellales were detected from fruiting bodies but not from soil samples. Members of the Atheliales, Phallales, Hysterangiales, Trechisporales, Sebacinales and Filobasidiales were detected from soil but not from the fruiting body survey. Members of the Gloeophyllales, Corticiales, Auriculariales or Cystofilobasidiales were not detected in this study.

We tested for significant differences among the proportion of taxa detected from fruiting bodies and soil using the P-test (Martin, 2002) and the UniFrac significance test (Lozupone and Knight, 2005). Since p-values calculated by the P-test appeared to be more conservative with this data set these values are shown in Table 3-3. A greater

proportion of taxa were found from fruiting body sampling (p-value ≤ 0.01). This difference is attributed mainly to taxa from the Agaricales (p-value ≤ 0.01), Polyporales (p-value ≤ 0.01) and Cantharellales (p-value = 0.02) that are preferentially detected from fruiting bodies.

3.4.7 Ecological assessment

Most OTUs were classified as mycorrhizal or saprophytic, largely corresponding to their generic taxonomy (Moncalvo et al., 2002). Since only a few species of the Agaricomycotina are parasitic or pathogenic, for simplification we considered them here as 'saprophytes' in the broad sense. A few OTUs were also classified as possessing "uncertain" ecology. The proportion of each ecological type across fruiting body and soil samples was assessed using a combination of three diversity measures: richness, abundance and phylogenetic diversity (Table 3-4). When fruiting body and soil samples are considered together, 55-58% of our sampled taxa are putatively mycorrhizal, 32-38% are saprophytic, and 8-20% have uncertain ecological functions. There was little overlap between fruiting body and soil samples that are putatively mycorrhizal (9 OTUs, 5%) or saprophytic (4 OTUs, 2%). A greater proportion of mycorrhizal and saprophytic taxa were detected from fruiting bodies in this study (p-value ≤ 0.01).

3.5 Discussion

3.5.1 LSU-rDNA phylogeny

As expected with the LSU rDNA region, there was enough variation to identify most Agaricomycotina soil sequences to the family-genus levels, and often to the species level. The phylogenetic grouping of taxa in Figure 3-2 is largely in agreement with the recent multi-gene phylogenies reported in Hibbett et al. (2007), Binder et al. (2005) and Larsson et al. (2004); however, there is little resolution among these clades and some misplacements in our analysis. For instance, the Agaricales are not resolved as monophyletic, but many subclades within the euagarics are monophyletic as expected (Moncalvo et al., 2002). Also, the Polyporales appear paraphyletic in our analysis, but the putative monophyly of this group was never strongly supported (Hibbett and Thorn, 2001; Binder and Hibbett, 2002; Binder et al., 2005). We attribute these inconsistencies to the exclusion of variable regions in the sequence alignment across such a broad range of Agaricomycotina and the use of only a single gene region in our analyses.

3.5.2 Using phylogeny to predict ecology

Many recent molecular phylogenies for the Agaricomycotina have shown a strong correlation between monophyletic clades and major ecological traits. The “MOR” project for Agaricomycotina classification (available at http://mor.clarku.edu/) describes the

predominant ecological habits in the major clades (Hibbett et al., 2005). Identification of environmental samples can also be facilitated by referring to the "UNITE" database for ectomycorrhizal taxa based on the ITS rDNA region (http://unite.ut.ee/) (Koljalg et al., 2005). Moncalvo et al. (2002) points out that, within the Agaricales, monophyly of subclades are often supported with shared ecological traits. We also found here that many clades are characterized by taxa with shared ecological characteristics, such as those described by Singer (1986).

3.5.3 Does fruiting body and soil rDNA sampling detect the same taxonomic and ecological groups?

We detected fruiting bodies in this study that have been previously shown to be ectomycorrhizal symbionts with hemlock. In this study, approximately 55% of the Agaricomycotina sampled are putatively mycorrhizal (Table 3-4). We recovered several taxa previously determined to be associated with the roots of western hemlock (*Tsuga heterophylla*) in pure culture synthesis experiments and field observations, such as *Amanita muscaria*, *A. vaginata*, *Boletus edulis*, *Cantharellus cibarius*, *Craterellus tubaeformis*, *Laccaria laccata*, *Lactarius deliciosus*, *L. scrobiculatus* and *Paxillus involutus* (Kropp and Trappe, 1982). Although these taxa are documented ectomycorrhizal partners with western hemlock in North America, these fungal species also have a worldwide distribution and are found associated with other ectotrophic tree species in temperate and boreal forests.

3.5.3.1 Mycorrizal taxa

The importance of mycorrhizal fungi to individual plants and plant communities is well established (Smith and Read, 1997; van der Heijden, 2002). Although the majority of mycorrhizal taxa recovered in this study are ectomycorrhizal fungi, we also detected members of the Sebacinales known to form a diverse array of symbiotic associations such as orchid mycorrhizas, ectomycorrhizas, ericoid mycorrhizas, and jungermannioid mycorrhizas (Taylor et al., 2003; Weiss et al., 2004). Interestingly, we did not survey any orchids in our study site but they are present in other parts of the reserve.

In this study, mycorrhizal fruiting bodies and rDNA from soil showed little overlap at the OTU or 'species' level. Nine of thirteen OTUs detected from both fruiting bodies and soil rDNA were ectomycorrhizal taxa: *Laccaria laccata*, *Inocybe* spp., *Amanita muscaria*, *Lactarius deliciosus*, *Hydnum* sp., *Clavulina cristata*, and *Craterellus tubaeformis*. From a total of 101 ectomycorrhizal taxa, 28 were detected only from soil and 64 were detected only from fruiting bodies. In a pioneering fungal molecular ecology study, Gardes and Bruns (1996) also described a distinct lack of overlap between ectomycorrhizal taxa that were dominant on plant root tips compared with taxa that were dominant as above-ground fruiting bodies in a pine forest in California. Notably, we recorded 35 occurrences of the ectomycorrhizal mushroom *Tricholoma imbricatum* above-ground but this taxon was not detected at all from any of our soil samples. Conversely, we found an OTU from soil that belongs to the ectomycorrhizal genus '*Inocybe'* to be highly abundant from soil (32 sequences randomly sampled from 6 different soil clone libraries) but that was absent from

fruiting body collections. Taxa from the Atheliales, characterized by inconspicuous resupinate fruiting bodies, with sequences highly similar to the ectomycorrhizal genus '*Piloderma'* were detected exclusively from soil. This lack of overlap may be due, in part, to the separation in time and space of soil core and fruiting body sampling, as well as to an insufficient amount of soil clone sampling and field search for cryptic sporocarps. In fact, it is only recently that extensive fruiting body and root tip sampling has found significant overlap between the above-ground and below-ground ectomycorrhizal community in a California oak stand (Smith et al., 2007).

3.5.3.2 Saprophytic taxa

While ectomycorrhizal fungi have been the subject of many studies in forest ecosystems because of their important role in plant health, the diversity of saprophytic and parasitic fungi has been given little attention (Thorn et al., 1996; Lynch and Thorn, 2006). This is the first study we are aware of to also directly compare the above- and below-ground diversity of saprophytic fungi using fruiting body collections and soil rDNA sampling.

Similar to observations with mycorrhizal fungi, we found little overlap in the occurrence of saprophytes detected from both fruiting bodies and soil sequences. Only four of thirteen OTUs recovered by both sampling methods were saprophytes: *Hygrocybe conica*, *Entoloma sinuatum* (two unique OTUs), and *Marasmius scorodonius*. From a total of 58 saprophytic taxa, 11 OTUs were recovered only from soil and 53 OTUs were recovered only from fruiting bodies (Table 3-4). For instance, we found *Entoloma*

nidorosum (18 observed fruiting body collections) extremely common above ground but not detected at all below ground. *E. nidorosum* was collected from leaf litter and buried wood, often in areas covered with an abundant litter layer. Conversely, we also found a taxon highly similar to the known saprophytic/parasitic genus '*Ganoderma'* to be common from soil (9 randomly sampled sequences from soil) but not sampled at all from fruiting bodies. It is interesting to note, however, that *Ganoderma tsugae* was observed fruiting in our study site two years after the study period.

Above-ground versus below-ground saprophyte diversity can be difficult to assess using traditional methods, requiring the use of specialized techniques such as soil-washing and culturing using specialized media to exclude mould growth (Thorn et al., 1996; Warcup, 1965; Malloch, 1981). We were interested in how successful molecular methods would be for assessing the diversity of soil saprophytes compared with the results typically found using standard soil fungi cultivation methods. Generally, we sampled a greater richness and abundance of saprophytes from fruiting bodies than from soil in our study. Similar findings for ectomycorrhizal fungi sampled from fruiting bodies and soil also showed a different composition of above-ground and below-ground ectomycorrhizal fungi and a greater richness of fruiting bodies detected compared with below-ground sampling (Dahlberg et al., 1997). These observations are likely due to the differences in spatial scales actually sampled during fruiting body surveys compared with below-ground sampling. In future studies, additional rDNA sampling from woody debris and forest litter layer may facilitate the detection of Agaricomycotina taxa whose fruiting bodies were

collected on these substrates. Do richness, abundance, and phylogenetic diversity measures detect the same proportions of taxonomic and ecological groups?

Generally, all three diversity measures recover similar dominant and common groups but in different proportions. All three measures identify the Agaricales as the dominant Agaricomycotina group (49-62%) among both fruiting body and soil samples. The Agaricales is taxonomically the best collected order of the Agaricomycotina with over 8,400 described species (Hawksworth et al., 1995). Members of the Agaricomycotina represent a mixture of saprophytic and mycorrhizal types. All three measures also identify taxa from the Russulales and Boletales as common (7.1 - 13%), not surprising considering these fungi are known to be largely ectomycorrhizal with trees that are present in our field site such as hemlock, birch and willow.

The phylogenetic diversity measure attributed a greater proportion of diversity to members of the Cantharellales and Sebacinales compared with richness or abundance measures (Table 3-3). This is likely due to long branch lengths representing a greater amount of genetic divergence among these taxa. Whereas taxa from the Cantharellales form large fruiting bodies, taxa from the orchid-mycorrhizal Sebacinales do not and were detected only from soil in this study. Since the abundance of clones in a library does not necessarily represent the true diversity of a taxon in soil due to PCR and cloning bias, phylogenetic diversity represents an alternative method to assess diversity among environmental samples.

3.5.4 Can quick, DNA-based surveys replace the laborious collection methods traditionally used for describing Agaricomycotina communities?

We found that fruiting body and soil sampling recover different OTUs at the species taxonomic level; however, both methods do recover the same dominant taxonomic orders and ecological groups. For instance, we retrieved 96 random clones from our first soil clone library corresponding to 28 Agaricomycotina sequences (16 OTUs) (Table 3-2). 12 of these OTUs (75%) correspond to known ectomycorrhizal fruiting bodies from the genera *Inocybe*, *Craterellus*, *Lactarius*, *Amanita*, *Clavulina* and *Hydnum* or are closely related to known ectomycorrhizal genera such as '*Russula'*, '*Cortinarius'* and '*Piloderma'* detected from soil. Based on this we predicted that the majority of Agaricomycotina detected from soil would also be ectomycorrhizal taxa. After sampling a total of ca. 1200 clones randomly sequenced from soil corresponding to 167 Agaricomycotina sequences (65 OTUs), 37 of these OTUs (57%) were assigned to genera with predominantly ectomycorrhizal ecology (Table 3-4). Our results clearly show that fruiting body and soil sampling recovers dramatically different subsets of fungi at the OTU-species level (Figure 3-3); however, both methods do detect the same dominant taxonomic fungal orders (Table 3-3) and ecological groups (Table 3-4). Traditional fungal surveys in forest ecosystems are laborious, time consuming, and biased toward detecting macromycetes (Watling, 1995). If an exhaustive fungal inventory is needed; however, then sequencing-based methods should be used in addition to traditional fruiting body surveys. If only the dominant, and presumably most ecologically significant, fungal groups need to be identified then a soil rDNA survey may provide a sufficient first assessment of the fungal assemblage in a forest site.

3.5.5 Current and future fungal community studies

Studies using PCR-based methods to survey fungal communities are just beginning to unravel the complexity of taxonomic and ecological guilds present in different types of soil. For instance, studies have started to document the fungal communities in habitats from a variety of forest soils (sclerophyll, brigalow, eucalypt, pine, mixed hardwood), grasslands, agricultural and unvegetated soils (Chen and Cairney, 2002; Anderson et al., 2003a, b; Schadt et al., 2003; Jumpponen, 2003; Jumpponen and Johnson, 2005; O'Brien et al., 2005; Lynch and Thorn, 2006; Jumpponen, 2007; Midgley et al., 2007). As a consequence of this type of research, several novel lineages at the family-subphylum levels have been discovered (Schadt et al., 2003; Porter et al., 2007; Schmidt et al., in press). An important advantage of culture-free methods is that they can be used to develop an ecological profile of a community relatively quickly without having to use specialized techniques to detect biotrophs versus saprotrophs.

Future studies should also consider using fruiting body surveys and culture-independent methods to sample fungi that are functionally and ecologically active in wood. For instance, it has been shown that while ectomycorrhizal euagarics and ascomycetes appeared more frequently in mineral soil, members of the Thelephorales, Atheliales and Sebacinales are commonly found on woody debris (Tedersoo et al., 2003). It is possible to test the hypothesis that rotten wood may be a reservoir of mycorrhizal inoculum for seedlings (Kropp, 1982). Hemlock seedlings may become established on 'nurse logs' and over time the log substrate decomposes leaving a hollow at the base of the mature tree.

This situation is hypothesized to be a form of escape from hemlock seed predators and competition in forest soil and leaf litter (Long et al., 1998; O'Hanlon-Manners and Kotanen, 2004).

DNA-based environmental surveys are likely to become increasingly informative with the continued growth of well-annotated reference sequences in public databases that are essential for making accurate and meaningful comparisons. Future high throughput sequencing techniques such as pyrosequencing may eventually reduce the cost and increase the rate of processing environmental samples. In the coming years, continued PCR-based surveys of different fractions of the environment such as soil, leaf litter, wood, and water may help to clarify the specific ecological niches and reservoirs that fungal mutualists, saprotrophs, parasites and pathogens occupy.

3.5.6 Acknowledgements

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3.6 Tables

Table 3-1: Summary of fruiting body sampling.

Collection Year	# Fruting bodies sampled	# Morphospecies	# Sequences[c]	# OTUs[d]
2002	262[a]	98	148	84
2003	189[b]	83	83	65
Total	451	134	231	132

[a]collections from the field

[b]collections from the field and observations from 5x5m subplots

[c]from a single fruiting body, at least one per morphospecies

[d]based on 99% pair-wise sequence similarity comparisons as implemented in Sequencher

Table 3-2: Summary of soil sampling.

Sampling Strategy	**Library**	**# Agaricomycotina sequences[b] per library**	**# OTUs**
Ten pooled soil cores	Os1	28	16
	Ss1	19	13
	Os2	3	3
	Ss2	6	4
	Os3[a]	17	10
	Ss3[a]	5	3
Subtotal		78	41
One soil core	2c	19	12
	4c	14	5
	6c	1	1
	7c	1	1
	10c	19	7
	12c	1	1
	14c	1	1
	20c	33	12
Subtotal		89	28
Grand Total		168	65

[a]Products from 5 mixed-template PCRs were pooled prior to ligation

[b]Determined from preliminary phylogenetic sorting of library sequences (data not shown)

Table 3-3: Comparison of diversity measurements for fruiting body and soil sampling.

Richness is expressed as the number of OTUs sequenced from fruiting body collections and soil rDNA extracts, abundance is expressed as the total number of fruiting body collections observed and the total number of rDNA sequences randomly sampled from soil clone libraires and phylogenetic diversity is expressed as the negative natural log likelihood score (-lnL). P-values are provided for all relevant fruiting body versus soil diversity comparisons calculated using the P-test (Martin, 2002).

Order	**Richness (# OTUs)**				**Abundance (# samples)**			**Phylogenetic diversity (PD)[a] (-lnL)**			**P-test significance[b] (P-value)**
	Subtotal	**Fruiting bodies**	**Fruiting bodies and Soil**	**Soil**	**Subtotal**	**Fruiting bodies**	**Soil**	**Subtotal**	**Fruiting bodies**	**Soil**	**Fruiting bodies versus soil**
Boletales	13	11	0	2	49	47	2	2,470.19	2,290.78	1,275.10	1.00
Atheliales	4	0	0	4	10	0	10	1,396.41	0.00	1,396.41	n/a
Agaricales	114	79	9	26	367	272	95	9,784.60	7,673.97	4,601.83	<= 0.01
Russulales	16	12	1	3	81	74	7	2,201.74	2,041.94	1,385.30	0.24
Thelephorales	6	3	0	3	7	4	3	1,624.86	1,263.77	1,322.71	0.07
Polyporales	7	6	0	1	25	16	9	1,869.48	1,820.02	-	<= 0.01
Hymenochaetales	1	1	0	0	1	1	0	-	-	0.00	n/a
Phallales	1	0	0	1	1	0	1	-	0.00	-	n/a
Hysterangiales	1	0	0	1	1	0	1	-	0.00	-	n/a
Gomphales	1	1	0	0	1	1	0	-	-	0.00	n/a
Geastrales	1	1	0	0	1	1	0	-	-	0.00	n/a
Trechisporales	2	0	0	2	3	0	3	1,208.16	0.00	1,208.16	n/a
Cantharellales	9	3	3	3	44	26	18	3,612.61	2,790.83	2,706.71	0.02
Sebacinales	3	0	0	3	5	0	5	1,498.98	0.00	1,498.98	n/a
Dacrymycetales	1	1	0	0	4	4	0	-	-	0.00	n/a
Tremellales	1	1	0	0	5	5	0	-	-	0.00	n/a
Filobasidiales	2	0	0	2	12	0	12	1,347.07	0.00	1,347.07	n/a
Stephanospora	1	0	0	1	1	0	1	-	0.00	-	n/a
Subtotal	184	119	13	52	618	451	167	20,109.82	14,217.57	10,233.99	<= 0.01

[a]"-" indicates that PD value could not be calculated when there are less than 3 taxa to be compared

[b]Indicates whether samples are signifnicantly clustered on the tree

Table 3-4: Mycorrhizal status of fruiting body and soil samples analyzed with respect to the proportion of total richness, frequency, and phylogenetic diversity (PD) recovered from the field site.

Mycorrhizal status	**Richness (#OTUs)**				**Abundance (# samples)**			**Phylogenetic diversity (PD) (-lnL)**			**P-test significance[d] (p-value)**
	Subtotal	**Fruiting bodies**	**Fruiting body and soil**	**Soil**	**Subtotal**	**Fruiting bodies**	**Soil**	**Subtotal**	**Fruiting bodies**	**Soil**	**Fruiting bodies versus soil**
Mycorrhizal[a]	101	64	9	28	366	290	76	11,469.12	8,688.52	6,402.51	<= 0.01
Saprobic[b]	68	53	4	11	193	159	34	7,737.32	6,561.13	3,056.76	<= 0.01
Uncertain ecology[c]	15	2	0	13	59	2	57	3,996.35	1,382.71	3,564.89	1.00
Subtotal	184	119	13	52	618	451	167	20,109.82	14,217.57	10,233.99	<= 0.01

[a]Largely ectomycorrhizal taxa, except for members of the Sebacinales which form mycorrhizae with orchids

[b]Mostly saprobic but also includes a few parasitic taxa such as Armillaria and Ganoderma

[c]Taxa whose ecology are not well studied

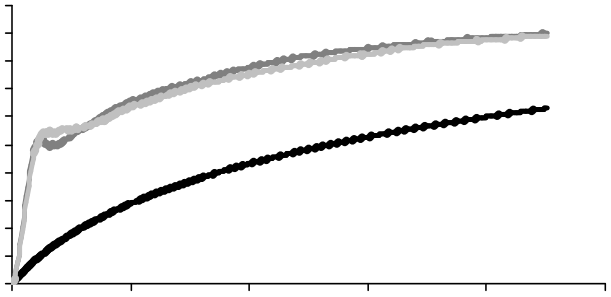
[d]Indicates whether samples are significantly clustered on the tree

3.7 Figures

Figure 3-1: Collectors curves for fruiting body and soil samples.

Each dataset was resampled 1000 times without replacement and the number of observed samples (Mao Tao Sobs) were plotted. The means for two richness estimators, ICE and Chao2, are plotted for comparison. A) Collectors curve for all Basidiomycota fruiting body collections observed. B) Collectors curve for all Basidiomycota OTUs sampled from soil clone libraries.

A)



B)

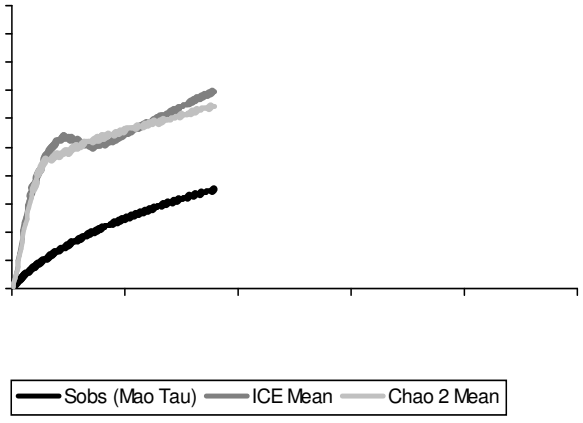


Figure 3-2: One of 118 most parsimonious parsimony ratchet trees showing all OTUs sampled from fruiting body and soil samples with a score of 4412 (CI=0.2187, HI=0.7813, RI=0.6142).

Support from 1000 parsimony bootstrap replicates is shown by thickened branches. Black bars indicate monophyletic groups, grey bars indicate paraphyletic groups. Clade naming convention is from Hibbett et al. (2007). Sequences from fruiting bodies are labeled in black with the prefix “TM02” or “TM03” followed by the species identification, soil sequences are labeled in red with the library name followed by the ‘Genus’ of the top BLAST hit. Numbers in square brackets indicate abundance: fruiting body abundance (f), soil abundance (s). Bolded labels indicate taxa detected from fruiting bodies and soil.

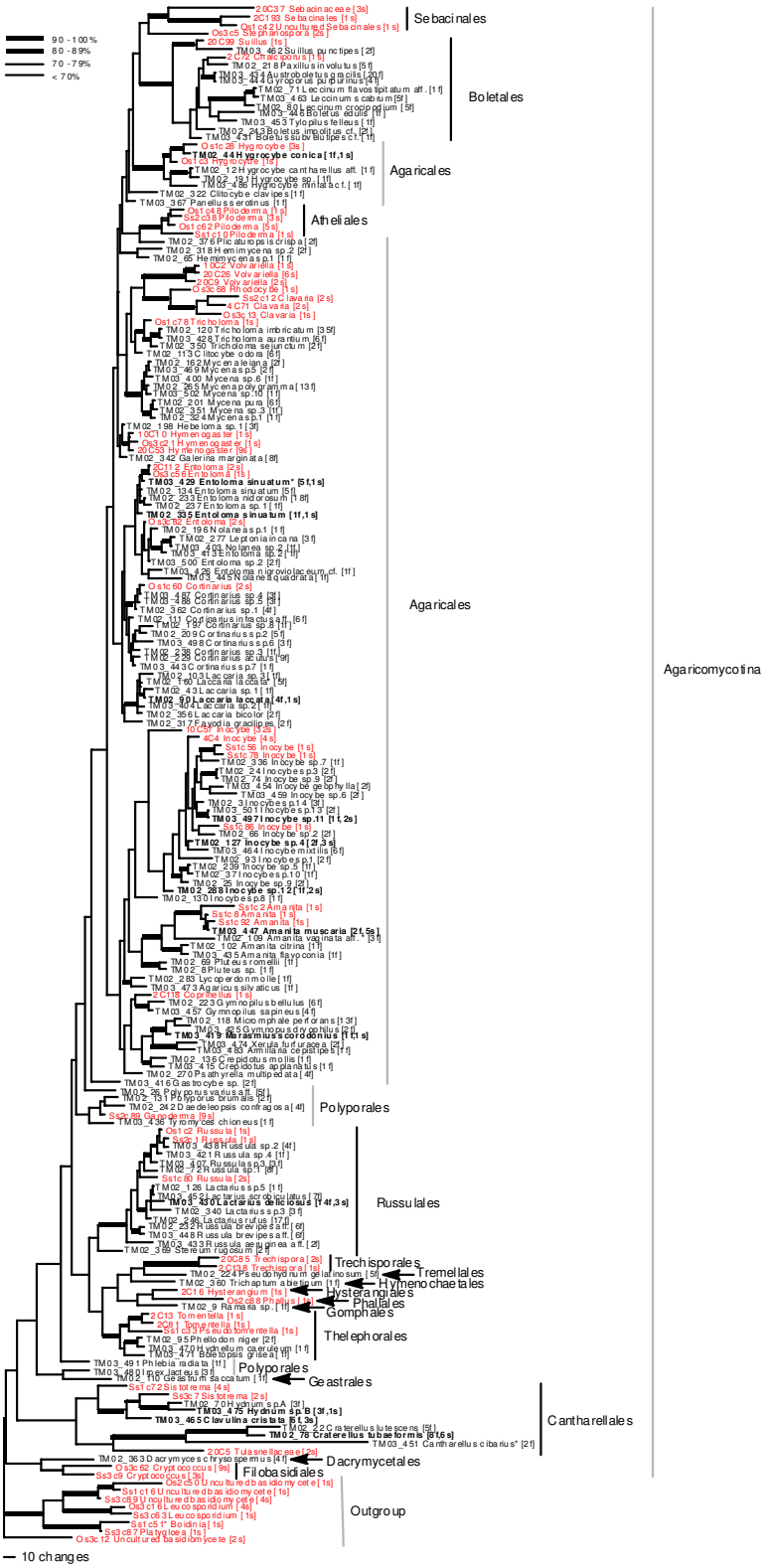

90 - 100%
80 - 89%
70 - 79%
< 70%
Sebacinales
Boletales
Agaricales
Athéliales
Agaricales
Agaricomycotina
Polyporales
Russulales
Trechisporales
Tremellales
Hymenochaetales
Hysterangiales
Phallales
Gomphales
Thelephorales
Polyporales
Geastrales
Cantharellales
Dacrymycetales
Filobasidiales
Outgroup
10 changes

Figure 3-3: Histogram of Agaricomycotina OTUs sampled from fruiting bodies and soil.

Grey bars indicate fruiting body abundance and black bars indicate soil clone abundance.

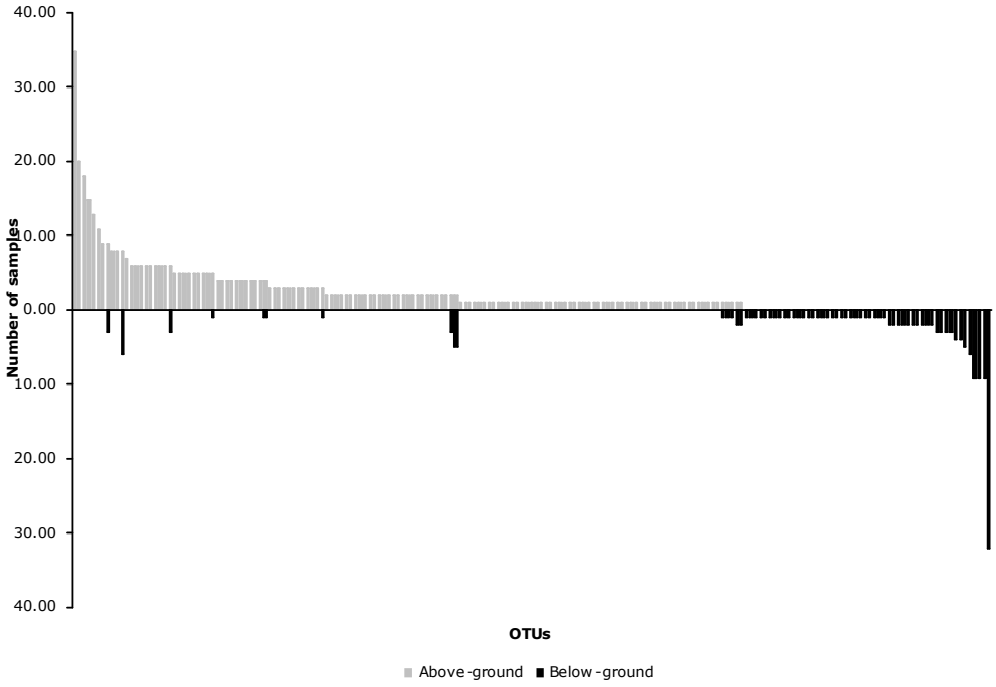

40.00
30.00
20.00
10.00
0.00
10.00
20.00
30.00
40.00
Number of samples
OTUs
Above-ground
Below-ground

Figure 3-4: Proportion of samples that belong to each Agaricomycotina taxonomic order for each accumulated soil clone library.

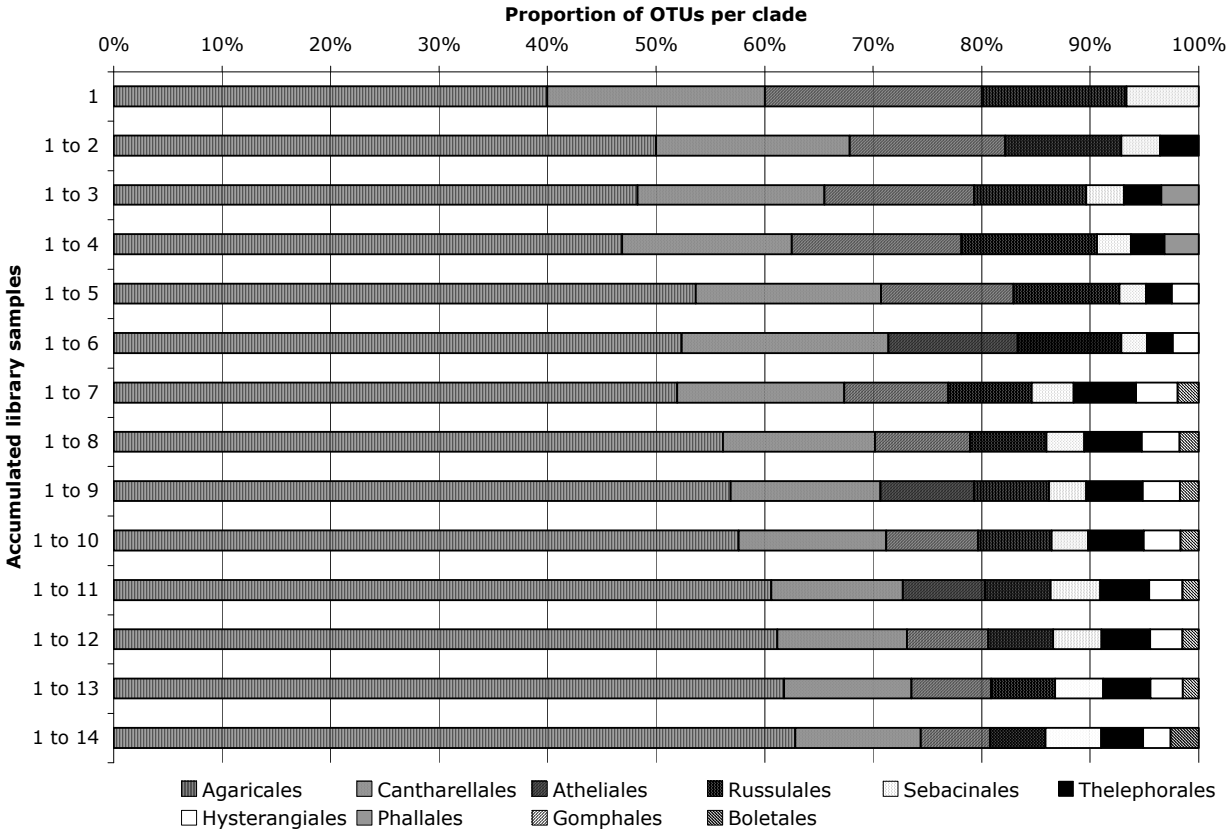

Proportion of OTUs per clade
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Accumulated library samples
1
1 to 2
1 to 3
1 to 4
1 to 5
1 to 6
1 to 7
1 to 8
1 to 9
1 to 10
1 to 11
1 to 12
1 to 13
1 to 14
Agaricales
Cantharellales
Atheliales
Russulales
Sebacinales
Thelephorales
Hysterangiales
Phallales
Gomphales
Boletales

Figure 4

4 Chapter Four- Widespread occurrence and phylogenetic placement of a soil clone group adds a prominent new branch to the fungal tree of life

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4.1 Abstract

Fungi are one of the most diverse groups of Eukarya and play essential roles in terrestrial ecosystems as decomposers, pathogens and mutualists. This study unifies disparate reports of unclassified fungal sequences from soils of diverse origins and anchors many of them in a well-supported clade of the Ascomycota equivalent to a subphylum. We refer to this clade as Soil Clone Group I (SCGI). We expand the breadth of environments surveyed and develop a taxon–specific primer to amplify 2.4 kbp rDNA fragments directly from soil. Our results also expand the known range of this group from North America to Europe and Australia. The ancient origin of SCGI implies that it may represent an important transitional form among the basal Ascomycota groups. SCGI is unusual because it currently represents the only major fungal lineage known only from sequence data. This is an important contribution towards building a more complete fungal phylogeny and highlights the need for further work to determine the function and biology of SCGI taxa.

4.2 Introduction

Diversity estimates suggest that less than 5% of the 1.5 million fungal species postulated to exist have been formally described (Hawksworth, 1991; Hawksworth, 2001). Although higher-level novel lineages have been frequently detected in the Bacteria and Archaea using molecular methods (Borneman and Triplett, 1997; Pace, 1997; Handelsman, 2004; Hugenholtz and Pace, 1996; Woese et al., 1986; Dawson and Pace, 2002), this is a relatively rare occurrence in the Fungi (Vandenkoornhuyse et al., 2002; Schadt et al., 2003) where species-level novelty is more commonly detected in systematic studies. This is due to the widespread application of culture-free methods to study natural microbial ecosystems by microbiologists.

It has been predicted that new fungal species or groups are more likely to be discovered from poorly-studied habitats, particularly from the tropics (Hawsworth, 2001). It was thus surprising when unclassified groups of fungi were discovered from temperate soils, since mycologists have intensively studied them using direct fungal isolation of hyphae and indirect isolation of dormant propagules in culture for many years (Schadt et al., 2003; Malloch, 1981; Warcup, 1965). Environmental DNA sampling strategies have revolutionized our understanding of the diversity of Bacteria and Archaea in water, sludge, sediments and soil, but only occasionally have the diversity of microscopic eukaryotes been examined using culture and morphology independent methods (Dawson and Pace, 2002; Venter et al., 2004; Luo et al., 2005; Moon-van der Staay et al., 2001; Lopez-Garcia et al., 2001). The development and refinement of molecular tools have also prompted a

resurgence of fungal community studies (O'Brien et al., 2005; Horton and Bruns, 2001). Identification of environmental sequences is made possible by the expansion of multi-locus datasets and development of bioinformatic tools to facilitate high-throughput sequence analysis for phylogenetic identification (Pennisi, 2005; Bruns, 2006). Preliminary studies using this approach have revealed many "unclassified" sequences that cannot be directly compared with known fungi or each other, either because the regions under study are too small (0.3 – 0.8 kb) and divergent, or target non-overlapping rDNA regions such as SSU 18S rDNA, internal transcribed spacer (ITS) regions, or LSU 28S rDNA (Schadt et al., 2003; Vandenkoornhuyse et al., 2002; Jumpponen and Johnson, 2005).

This study is the first to attempt to reconcile the relationships among the many "unclassified" fungal sequences that have been recovered from independent studies. To address this issue, we chose to further characterize the LSU Soil Group I clade (Ascomycota, Fungi) originally recorded from alpine tundra soil in Colorado, which was highly divergent from known fungal taxa (Schadt et al., 2003). To accomplish this we vastly broadened the diversity of soil environments surveyed to include four new locations in the Americas, and we developed a taxon-specific primer and a nested-PCR technique to generate a 2.4 kb rDNA fragment from Group I members that encompassed portions of the SSU, ITS, and LSU rDNA regions. From these long sequences we retrieved many similar fragments from GenBank and determined the phylogenetic placement of Group I in the fungal tree of life. This allows us to present a first assessment of the taxonomic diversity, evolutionary relationships, geographic distribution and ecology of these newly discovered fungi that we refer to as Soil Clone Group I (SCGI).

4.3 Materials and Methods

4.3.1 Site Characteristics and Soil Sampling

10-15 cm of upper soils were collected from four locations: Treeline Forest. This is a forested site dominated by *Picea engelmannii* and *Pseudotsuga menziesii* at 3,325 m (10,900 ft) elevation at the forest-tundra ecotone in Colorado. This site is part of the Niwot Ridge Long Term Ecological Research Site (NWR-LTER) which is 35 km west of Boulder, Colorado. Soils originated from equal composites of samples taken randomly from within five, 5m radius plots taken on three dates; January 25th, 2001 from under winter snow pack (SF102), during spring snowmelt on June 11th, 2002 (SF602) and in summer after the soils had dried out on July 10th (SF702) and were used for separate DNA extraction and rDNA clone library generation. Montane Forest. This is a lodgepole pine stand in an aggrading *Pinus contorta*, *Abies lasiocarpa*, *Picea engelmannii* forest at an elevation of 3,050 m (10,000 ft) ~2 km east of the forest-tundra ecotone at the NWR-LTER. The site, soil characteristics and ongoing studies have been described (Monson et al., 2002; Scott-Denton et al., 2003). Samples from 5 control plots (TFC) and the 5 plots where the trees had been girdled (TFG) were taken randomly on July 31st, 2002, for DNA extraction and rDNA clone library generation. Costa Rican Oxisol. This site is located in tropical southwest Costa Rica at a 8°43'N, 83°37'W, ~5 km inland near the town of Drake (Agujitas). The characteristics of the site have been described (Townsend et al., 2002; Cleveland et al., 2002). Ten samples were taken August 31st, 2001, from a forested, P limited, highly-weathered oxisol (CROX) plot. Temperate Coniferous Forest. This is a

mixed forest heavily dominated by Eastern Hemlock (*Tsuga canadensis*) in southern Ontario, Canada (44°37'N, 79°39'W). The study site is a 50 m x 100 m plot in the Koffler Scientific Reserve situated within the Oak Ridges Moraine. Soil was collected in July 2003 by taking ten soil cores across a 100 transect. For each soil core the B and C horizons were separated, corresponding soil horizons were pooled and mixed by sieving, removing rocks and large roots. In May 2004, a single soil core was sampled from within the plot and soil was separated into 2 cm deep portions. Separate DNA extractions and clone libraries were created for each pooled soil horizon sample and 2cm deep portion.

4.3.2 DNA extraction, amplification, cloning and sequencing

DNA from the Colorado and Costa Rican soils was extracted and purified as described previously (Schadt et al., 2003). A portion of the LSU rDNA region was targeted for amplification using the primers ITS9 (Egger, 1995) and nLSU1221R (Schadt et al., 2003). PCR amplification reactions consisted of: 2.75 mM $MgCl_2$, 800 µM dNTPs, 25 µg BSA, 0.5 µM each primer, 1X Taq PCR buffer and 1.875U Taq Polymerase (Promega, Maddison, WI USA), and 45 ng template DNA. To avoid biases and artefacts in PCR amplification, 8 replicate reactions (25 µL) of each sample were prepared and the total PCR cycle number kept low (Polz and Cavanaugh, 1998; Suzuki and Giovannoni, 1996; Qiu et al., 2001). Thermocycling used a Mastercycler Gradient (Eppendorf, Hamburg Germany) with initial denaturation at 95°C for 1 min; 28 cycles of denaturation at 94°C for 45 sec, annealing at 60°C for 50 sec, and extension at 72°C for 1 min and 45 sec (+1 sec/cycle); and a final extension step of 72°C for 5 min. Amplification products were combined and

cloned using the TOPO-TA PCR 2.1 kit (Invitrogen, Carlsbad, CA USA). At least 100 colonies were selected from each cloned sample. Plasmids were extracted using a standard miniprep modified for 96 well plates and inserts were amplified using vector primers T7 and M13R. PCR product was digested with 1 µL each of EXO1 and SAP (USB, Cleveland, OH USA) to remove unincorporated primers and dNTPs. 2 µL of purified PCR product was used for sequencing reactions with 3.2 pmol of the primers LR0R (Rehner and Samuels, 1994) and TW13 (Taylor and Bruns, 1999) and 2 µL ABI BigDye Ready Reaction Mix (Foster City, CA USA) in a total volume of 12 µL. Reactions were purified using DyeEx-96 kits (Qiagen, Valencia, CA USA) and processed on an ABI3700 (Foster City, CA USA) at the ISU DNA Sequencing Facility (Ames, IA USA).

DNA for the Ontario samples were extracted using the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories, Inc, CA USA). 0.5 g (wet weight) of soil was used per sample. Replicate extractions were performed with various portions of hemlock forest soil and used to generate a total of twelve clone libraries. The first 900 bp of the 5'- nLSU region was amplified using LR0R and LR5 (Vilgalys and Hester, 1990) primers. PCR amplification reactions consisted of: 5 mM dNTPs, 25 µg BSA, 0.4 µM each primer, 1X PCR buffer (Qiagen Inc, ON Canada), 2.5U Taq DNA polymerase and 0.1-10 ng template DNA. Thermalcycling used a PTC-100 (MJ Research, Waltham, MA USA) or a GeneAmp 9700 (Applied Biosystems, CA USA) with 25 cycles of denaturation at 95°C for 45 sec, annealing at 50°C for 45 sec, extension at 72°C for 2 min and a final extension step of 72°C for 5 min. Amplicons were purified using the Qiaquick PCR Purification Kit and cloned using Qiagen PCR Cloning Kit with Qiagen EZ Competent Cells (Qiagen Inc, ON Canada). Colony PCR

was used to amplify 50 to 100 colonies per plate by picking cells from a single colony using a pipette tip and adding this directly into the PCR cocktail above. The thermal cycler program used was as above, but with 36 cycles, with a 72°C extension step for 1.5 min. BigDye Terminator v3.1 (Applied Biosystems, CA USA) chemistry was used for cycle sequencing. Reactions were purified using gel filtration with 600 µL of Sephadex (G-50 fine) slurry in Centri-Sep columns (Princeton Separations, Inc., NJ USA), dried in a heated vacuum centrifuge at 45-65°C and run on an ABI Prism 3100 automated sequencer.

4.3.3 SCGI taxon-specific nested PCR and sequencing

Nested PCR was used to increase specificity, by reducing background amplification due to non-specific primer binding, with bulk DNA from soil. The first reaction used the primers NS1 (White et al., 1990) and LR5 to amplify a long stretch of rDNA spanning the SSU, ITS, and 5'-LSU regions. The PCR mixtures and thermal conditions used were as described above for Ontario soils except that the extension time was increased to 2.5 minutes. We designed a taxon-specific primer in the D2 divergent domain of the LSU rDNA region (4c26R = 5'-CAGCGTCCTAGGAAGAAC-3'). This primer was used with the SSU primer NS1 to specifically amplify ca. 2.4 kb rDNA. PCR products were purified, cloned and sequenced as described above for the Ontario soils using the following sequencing primers: NS1, SR1.5 and SR6 (James et al., 2000), ITS1 (White et al., 1990) and 4c26R.

4.3.4 Checking for chimeric sequences and pseudogenes

The presence of chimeric sequences in the data set was checked using two methods: (1) the Chimera Check program (Cole et al., 2003), after uploading additional sequences from our clone libraries; (2) we systematically performed BLAST searches in GenBank with the first-half, followed by the second-half of each sequence, and compared the two taxonomy reports to look for consistency. We were unable to detect the presence of any chimeric sequences in our final dataset using these methods. Also, SCGI sequences do not appear to be pseudogenes because rates of sequence evolution of the ITS1 and ITS2 regions were much faster than in the 5.8S gene, as expected (Bailey et al., 2003).

4.3.5 Phylogenetic analyses

Sequences were assembled using Sequencher 4.0 (Gene Codes, Ann Arbor MI), and alignments were manually optimized using Se-Al 2.0 (Rambaut, 1996). The placement of SCGI was determined by assembling a dataset of representative Ascomycota taxa from Lutzoni et al. (2004) with SSU and LSU data from our 2.4 kbp SCGI rDNA sequences. We conducted a Bayesian analysis using MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003) on a 28-processor Linux Beowulf cluster using a general time-reversible model of DNA substitution with the following settings: six classes of nucleotide substitutions, gamma rate amongst sites, four Monte-Carlo Markov chains run for five million generations starting from random trees, and sampling one tree every 100 generations. The first 1,000 sampled trees

were discarded (burn-in). The resulting 50% majority-rule tree was computed and visualized in PAUP* 4.0b10 (Swofford, 2002). Parsimony bootstrap values were calculated in PAUP* by running 1000 full heuristic bootstrap replicates with the following settings: 100 random addition sequence replicates with TBR branch swapping, and keeping 1 tree per replicate.

The structure within SCGI was determined by including the greatest number of SCGI taxa with overlapping sequences in a single analysis. We aligned 144 SCGI taxa with 7 reference taxa across 380 bp of the 5'-LSU rDNA region and rooted the tree using *Pneumocystis carinii*. We conducted a parsimony ratchet analysis (Nixon, 1999) as implemented in PAUP using the PAUPrat module (Sikes and Lewis, 2001) using the default module settings. The form of the best model was determined using MrModeltest 2.2 (Nylander, 2004) and Bayesian support was assessed using the settings described above in MrBayes.

4.4 Results

The newly designed SCGI taxon-specific primer (4c26R = 5'-CAGCGTCCTAGGAAGAAC-3') was successfully used in a semi-nested PCR amplification to produce a 2.4 kbp region of rDNA. This strategy generated fragments that span nearly the entire SSU, complete 5.8S gene and ITS spacer regions, as well as the 5'-end of LSU ribosomal genes. This allowed us to unite previously disparate data, greatly broadening this survey, and to firmly root the SCGI group within the Ascomycota. Sequences

generated for this study have been submitted to GenBank with accession numbers EU179545 - EU179599 and EU179933 - EU180016.

We confirmed the relatedness of our newly generated SCGI rDNA 2.4 kbp sequences with those originally reported by Schadt et al. (2003) by conducting a maximum parsimony bootstrap analysis which indicated their monophyly with 100% statistical support (Supplementary Material). Bayesian posterior probability (BPP) is expressed on the interval 0.0 to 1.0. Parsimony bootstrap (PBS) is expressed as a percentage of trees in which a node is found. Analyses including the newly produced 2.4 kpb fragment indicate that the SCGI clade represents a distinct subphylum-level monophyletic group with 1.0 BPP (Fig. 1). The SSU+LSU combined alignment included 2,364 characters and resolved the SCGI lineage as a clade independent of the three currently recognized subphyla. The Pezizomycotina subphylum is monophyletic (1.0 BPP / 99% PBS), the Pezizomycotina-Saccharomycotina subphyla are monophyletic (0.98 BPP / 95% PBS), and the Pezizomycotina-Saccharomycotina-SCGI clades are monophyletic (0.99 BPP / 100% PBS). There were no strongly supported conflicts (i.e. greater than 70% PBS) with the grouping of taxa into clades recognized in the literature.

We then retrieved “unclassified” environmental fungal sequences from GenBank using BLAST and confirmed their relatedness to the SCGI clade by conducting a series of maximum parsimony bootstrap analyses using only the characters overlapping with our newly generated 2.4 kb rDNA fragments (Fig. 2 and Supplementary Material). The analyses that included only short stretches of rDNA consistently recovered a moderate to

well supported monophyletic SCGI clade with 62-100% PBS, but could not unambiguously classify these sequences in the fungal tree of life (Supplementary Material).

When we assessed the phylogenetic structure within the SCGI clade we found SCGI taxa to be widespread and common within soils from across North and Central America, occurring in forest and alpine tundra soil in Colorado (USA), forested soil in Ontario (Canada), as well in a tropical forest soil in Costa Rica (Fig. 3). The relative abundance of these clones within the overall fungal libraries from the surveyed localities ranged from 6.9 to 27% (Table 4-1).

Parsimony and Bayesian analyses of the LSU region homologous to bases 190 - 549 in *Saccharomyces cerevisiae* (J01355) in all SCGI taxa for which we have these sequences revealed many well-supported clades (Fig. 3). The most parsimonious tree had a tree length of 662 (CI=0.4789, HI=0.5211, RI=0.8565) and is shown in Figure 4-3 with Bayesian support greater than 0.90 at the nodes. The form of the best model determined by MrModeltest is as follows: equal base frequencies with a GTR model of DNA substitution with gamma distributed rate variation across sites. Nearly all of the tip clades within SCGI clustered by sampling location, similarly, sequences from any one sampling location are distributed across SCGI. In contrast, Bayesian analyses of the SSU and 5.8S regions of SCGI taxa resolved very few well-supported nodes (Supplementary Material).

We also compared the maximum pairwise sequence differences represented within the recognized Ascomycota subphyla and SCGI (Supplementary Material). We found that

the maximum percent pairwise sequence differences in the Pezizomycotina ranged from 10.2 - 19.7%, in the Saccharomycotina from 2.2 - 12.1%, in the Taphrinomycotina from 5.3 – 17.3%, and within SCGI from 6.3 - 20% for the SSU and LSU rDNA regions analyzed in this study.

4.5 Discussion

4.5.1 SCGI phylogenetic placement and sequence divergence

The total length of rDNA analyzed in this study (2.4 kbp) far exceeds the sampling norm for this type of fungal survey (0.3 to 1 kbp). This greater number of characters, combined with a backbone phylogeny based on the All Fungal Tree of Life (AFTOL) phylogeny, clearly highlights the significance of the SCGI clade as a novel subphylum of Ascomycota (Fig. 1). In fact, except for the placement of the novel SCGI lineage, this tree topology largely conforms to the rDNA AFTOL phylogeny for the Ascomycota that is itself also independently supported by analyses of protein-coding genes (Lutzoni et al., 2004; Liu et al., 1999). SCGI is clearly distinct from the known Ascomycota subphyla: the Pezizomycotina, Saccharomycotina, and Taphrinomycotina. This suggests that SCGI may harbour a unique biology and ecology that is as yet entirely unstudied. To date, this group is only known from its rDNA, which is unusual at a time when most fungi have already been sorted into major evolutionary lineages (Lutzoni et al., 2004; James et al., 2006). This clearly indicates the need to update the most current fungal classification (Hibbett et al., 2007).

We found that SCGI taxa are significant soil components that may comprise up to 27% of soil fungal sequences sampled in this and other independent studies (Table 4-1 and Supplementary Material). Clearly, SCGI taxa comprise a substantial portion of soil fungi that was overlooked by traditional sampling methods. The repeated recovery of SCGI from so many environmental DNA-based studies emphasizes the need to recognize SCGI as an integral component of a comprehensive fungal phylogeny.

Even with the limited SCGI sampling conducted to date, the maximum pairwise sequence differences among SCGI taxa, 6.3-20%, is comparable to the maximum pairwise sequence differences found within the other recognized Ascomycota subphyla, 2.2-19.7% (Supplementary Material). This amount of sequence divergence may indicate potentially high taxon diversity within SCGI. Figure 4-3 shows that many SCGI taxa cluster according to sampling site. If we were to use 99% sequence similarity to delimit operational taxonomic units (OTUs) as a proxy for species, as used in similar studies for the LSU rDNA region (such as Schadt et al., 2003), then 144 SCGI sequences in Figure 4-3 represents 38 OTUs. Additionally, each OTU is comprised of clone sequences from a single site. A frequency histogram of OTUs versus sequences sampled shows that two-thirds of OTUs are rare taxa sampled only once or twice (data not shown). These results suggest that we have only just begun to sample the diversity of SCGI taxa in the soils analyzed in this study, making a comprehensive geographic assessment of SCGI diversity difficult to assess at this point.

4.5.2 Current theory of Ascomycota evolution

It has been hypothesized that the most basal Ascomycota are the Taphrinomycotina (Sugiyama et al., 2006; James et al., 2006; Liu et al., 1999), recognized relatively recently from molecular evidence (Nishida and Sugiyama, 1994; Liu and Hall, 2004). Yeasts and filamentous hyphae characterize the vegetative stage whereas the sexual stage is characterized by unitunicate asci and a lack of well-formed ascomata (one exception is *Neolecta*). Lifestyles range from obligate plant pathogens, animal pathogens, to saprobic fission yeasts (Berbee, 2001). Two well-known exemplars include the plant pathogen *Taphrina deformans* and *Pneumocystis carinii*, a cause of pneumonia in immune-compromised patients.

The Saccharomycotina and Pezizomycotina are the next two subphyla to arise (Lutzoni et al., 2004; Liu et al., 2006). The Saccharomycotina are composed of about 1,000 species with basal forms that are filamentous, others that are true budding yeasts, and more derived forms that show filamentous-yeast dimorphism (Suh et al., 2006). Most are free-living, but one genus is known to contain animal pathogens (*Candida*), and one genus contains plant pathogens (*Eremothecium*) (Berbee, 2001). The sexual stage is also characterized by the lack of ascomata and the formation of unitunicate asci. Some well-known members include the baker's and brewer's yeast, *Saccharomyces cerevisiae*, as well as the opportunistic human pathogen, *Candida albicans*.

The Pezizomycotina is the largest subphylum and includes greater than 27, 000 described species (Kirk et al., 2001). A five-gene phylogeny strongly supported the monophyly of the Pezizomycotina (Spatafora et al., 2006). When the sexual stage is known, it is characterized by the formation of ascomata. Generally, the "higher" ascomycetes show more complex morphology and include most lichen-forming fungi (Gargas et al., 1995). Some well-known forms include the edible delicacy, *Morchella*, the morel mushroom and the ubiquitous *Penicillium* and *Aspergillus* moulds.

4.5.3 Transitional nature of SCGI

The Taphrinomycotina and the Saccharomycotina is intersected by SCGI in our phylogeny (Fig. 1). It is unlikely that SCGI taxa produce a macroscopic fruiting body, based on the observed lack of well-formed ascomata in the Taphrinomycotina and lack of ascomata in the Saccharomycotina. This would explain why SCGI taxa have not been detected in previous studies using traditional fruiting body collection methods or methods that rely on morphology for identification and classification. If SCGI taxa are obligately biotrophic, as are many of the pathogens in the Taphrinomycotina, this would explain why these taxa have been overlooked in studies using conventional culture-based isolation techniques. Although we have made no rigorous attempt to culture SCGI taxa directly from soil, if our hypothesis about an obligately symbiotic lifestyle is true, it will not be possible to find a living representative of SCGI without its host.

Based on the ancient origin of SCGI among the basal Ascomycota, this group may have diversified before the origin of the complex Ascomycete sexual reproductive structure, the ascoma, which is a defining characteristic among sexually reproducing members of the Pezizomycotina. Further study of the biology and function of SCGI would help to further develop a holistic evolutionary theory of Ascomycota fungi. This study is the first to convincingly place SCGI in the Ascomycota phylogeny with good statistical support. For now the morphological characteristics and metabolic and ecological properties associated with SCGI remain unknown and can only be speculated from comparison with their closest evolutionary relatives.

4.5.4 Known geographical and environmental range of SCGI

We have expanded the known range of SCGI from a single North American alpine tundra location (Schadt et al., 2003), to other sites within the Americas, Europe and Australia (Figs. 2-3). Members of SCGI were often very prominent among the percentage of overall fungal clones present, often comprising more than 10% of the total number of clones (Table 4-1 and Supplementary Material). This abundance in clone libraries suggests a numerical abundance in soils, however further experiments using in situ hybridization or other methods will be needed to confirm this (Amann et al., 1995). Using the 2.4 kbp region characterized in this study it was also possible to make comparisons across a number of studies utilizing ITS and SSU rDNA markers. These studies confirmed that SCGI members are commonly detected from soil and ectomycorrhizal root tips in North America from both western and eastern Canadian provinces, from the western, middle, and

eastern states of the United States, with a southerly range that extends into Costa Rica (Schadt et al., 2003; O'Brien et al., 2005; White et al., 1990; Izzo et al., 2005; Pringle et al., 2000). SCGI members are also found in the same habitats in Europe from Scotland in the United Kingdom, with their occurrence extending eastwards and northwards to Lithuania, Sweden and Norway (Anderson et al., 2003; Menkis et al., 2005; Rosling et al., 2003). SCGI members are also found in Australia in soils from New South Wales (Chen and Cairney, 2002). The cosmopolitan distribution of these taxa on three separate continents is consistent with a hypothesis of a relatively flexible and adaptable biology capable of surviving in subsurface environments in a variety of both temperate and tropical soils.

It is also worthy to note which studies using similar PCR-based methods to study environmental samples have not detected members of the SCGI clade. Although SCGI taxa may be widespread in studies from alpine tundra, forest, and grassland soils as well as from ectomycorrhizal plant roots, despite exhaustive GenBank searches we did not detect these sequences in studies from insect guts, above-ground plant endophytes, grass roots, Sargasso Sea water, or sulphide-rich springs (Suh et al., 2005; Higgins et al., 2007; Arnold et al., 2007; Vandenkoornhuyse et al., 2002; Venter et al., 2004; Luo et al., 2005). Similarly, SCGI taxa have not been detected from soil sampled from the forefront of the Lyman glacier nor from unvegetated alpine talus soils (Meyer and S.S., unpublished; Jumpponen, 2003). These observations collectively suggest that an active rhizosphere may be an essential requirement for SCGI taxa.

4.5.5 Other novel fungal groups

While we were able to unify many previously uncharacterized sequences with SCGI from direct phylogenetic comparison with our 2.4 kb rDNA fragments, such as the 'Unknown Soil Fungi' clade from Jumpponen and Johnson (2005; Supplementary Material), other unknown fungal groups detected from soil have yet to be properly classified. For instance, Vandenkoornhuyse et al. (2002) sampled SSU rDNA from grass roots and they recovered two unclassified groups labeled 'IV' and 'V' in the Ascomycota. Group V clearly clusters in the Pezizomycotina in their analysis. When we tested the relationship of their Group IV with our SCGI clade, they failed to cluster together (Supplementary Material). Additionally, Schadt et al. (2003) recovered two additional unknown lineages in the Ascomycota labeled 'Group II' and 'Group III' but these are clearly nested within the Pezizomycotina in their supplementary analyses and fail to nest with SCGI in our analyses (Supplementary Material). As a result, these unknown fungal lineages previously identified from grass roots and alpine tundra soil are more likely to represent species- to family-level novelty within the Pezizomycotina (Schadt et al., 2003; Jumpponen and Johnson, 2005).

4.5.6 Conclusions

Similar to the recognition of the phylum-level status of the Glomeromycota (Schußler et al., 2001) and the inclusion of the Microsporidiomycota (Keeling et al., 2003) in the Fungi, the SCGI clade should also be recognized as a significant contribution towards reconstructing a more complete fungal phylogeny and elucidating the nature of the "missing

fungi". This highlights a large gap in our knowledge of Ascomycota and emphasizes the need for further studies to characterize these fungi using fluorescent hybridization, metagenomic and targeted culture-based methods.

4.5.7 Acknowledgements

We thank J. Skillman and S. Margaritescu for laboratory assistance and B. Saville for helpful discussion. This work was supported by grants from the Discovery Program at the Natural Sciences and Engineering Research Council of Canada and from the Canadian Fund for Innovation (J.-M. Moncalvo), the NSF Microbial Observatories Program (S. Schmidt and A. Martin), and the Department of Energy Office of Science as part of the consortium for research into Carbon Sequestration in Terrestrial Ecosystems (CSiTE) (C. Schadt).

4.6 Tables

Table 4-1: Proportion of LSU SCGI clones recovered from each site.

Site	Number of SCGI Clones	Total Clones Analyzed	Percent
Tundra, Colorado, USA (Schadt et al. 2003)	13	125	10.4%
Treeline, Colorado, USA	18	207	8.7%
Montane, Colorado, USA	23	85	27%
Oxisol, Costa Rica	12	50	24%
Temperate, ON Canada	75	1093	6.9%

4.7 Figures

Figure 4-1: Bayesian consensus tree for the SSU + LSU combined rDNA analysis.

The data set includes 65 taxa and 2,364 SSU and LSU rDNA characters, and is rooted with *Pneumocystis carinii*. Class and subclass labels largely follow the convention used by Lutzoni et al. (2004). Bayesian support is shown above the branch and parsimony bootstrap support is shown below branches of interest.

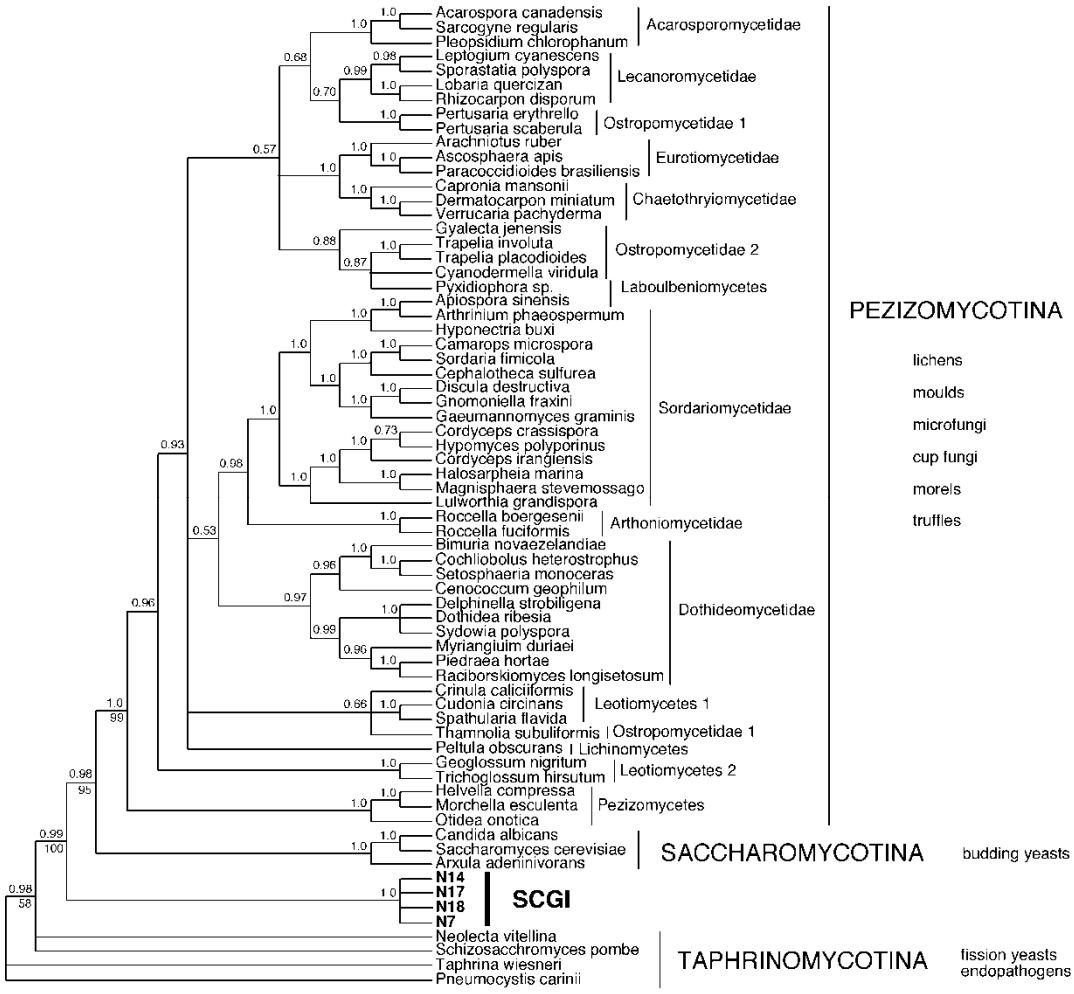
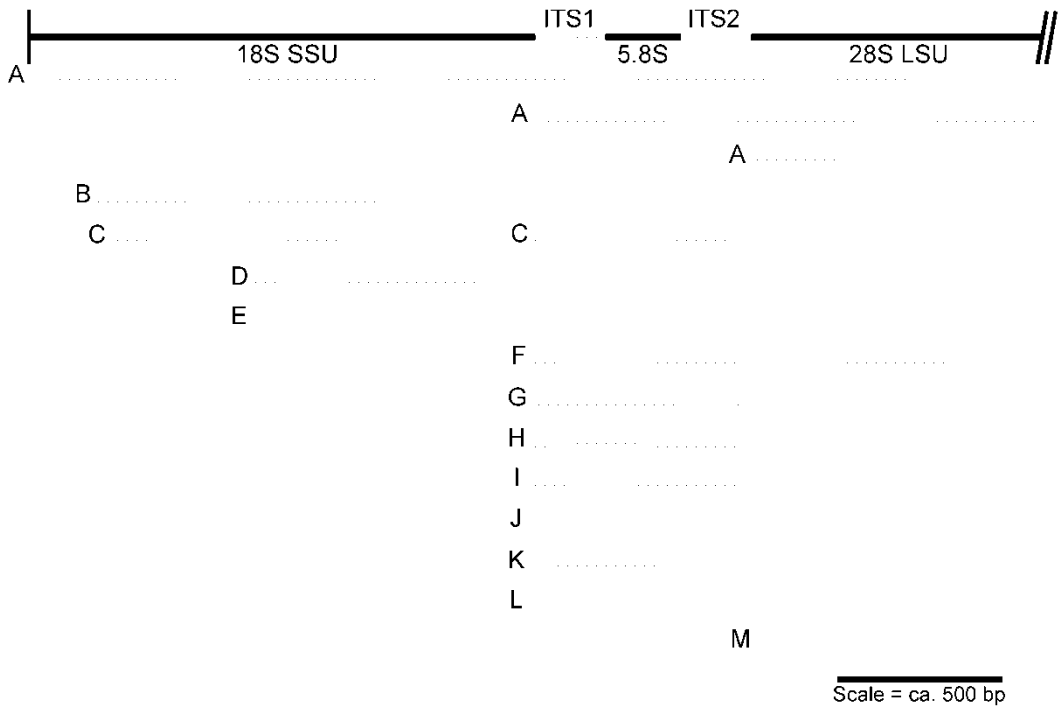

Acarospora canadensis
Sarcogyne regularis
Pleopsidium chlorophanum
Acarosporomycetidae
Leptogium cyanescens
Sporastatia polyspora
Lobaria quercizan
Rhizocarpon disporum
Lecanoromycetidae
Pertusaria erythrello
Pertusaria scaberula
Ostropomycetidae 1
Arachniotus ruber
Ascosphaera apis
Paracoccidioides brasiliensis
Eurotiomycetidae
Capronia mansonii
Dermatocarpon miniatum
Verrucaria pachyderma
Chaetothryiomycetidae
Gyalecta jenensis
Trapelia involuta
Trapelia placodioides
Ostropomycetidae 2
Cyanodermella viridula
Pyxidiophora sp.
Laboulbeniomycetes
Apiospora sinensis
Arthrinium phaeospermum
Hyponectria buxi
Camarops microspora
Sordaria fimicola
Cephalotheca sulfurea
Discula destructiva
Gnomoniella fraxini
Gaeumannomyces graminis
Cordyceps crassispora
Hypomyces polyporinus
Cordyceps irangiensis
Halosarpheia marina
Magnisphaera stevemossago
Lulworthia grandispora
Sordariomycetidae
Roccella boergesenii
Roccella fuciformis
Arthoniomycetidae
Bimuria novaezelandiae
Cochliobolus heterostrophus
Setosphaeria monoceras
Cenococcum geophilum
Delphinella strobiligena
Dothidea ribesia
Sydowia polyspora
Myriangium duriaei
Piedraea hortae
Raciborskiomyces longisetosum
Dothideomycetidae
Crinula caliciiformis
Cudonia circinans
Spathularia flavida
Leotiomycetes 1
Thamnolia subuliformis
Ostropomycetidae 1
Peltula obscurans
Lichinomycetes
Geoglossum nigritum
Trichoglossum hirsutum
Leotiomycetes 2
Helvella compressa
Morchella esculenta
Otidea onotica
Pezizomycetes
PEZIZOMYCOTINA
lichens
moulds
microfungi
cup fungi
morels
truffles
Candida albicans
Saccharomyces cerevisiae
Arxula adeninivorans
SACCHAROMYCOTINA
budding yeasts
N14
N17
N18
N7
SCGI
Neolecta vitellina
Schizosaccharomyces pombe
Taphrina wiesneri
Pneumocystis carinii
TAPHRINOMYCOTINA
fission yeasts
endopathogens

Figure 4-2: Schematic diagram of partial rDNA array showing the SSU, ITS1, 5.8S, ITS2, and 5'-LSU regions.

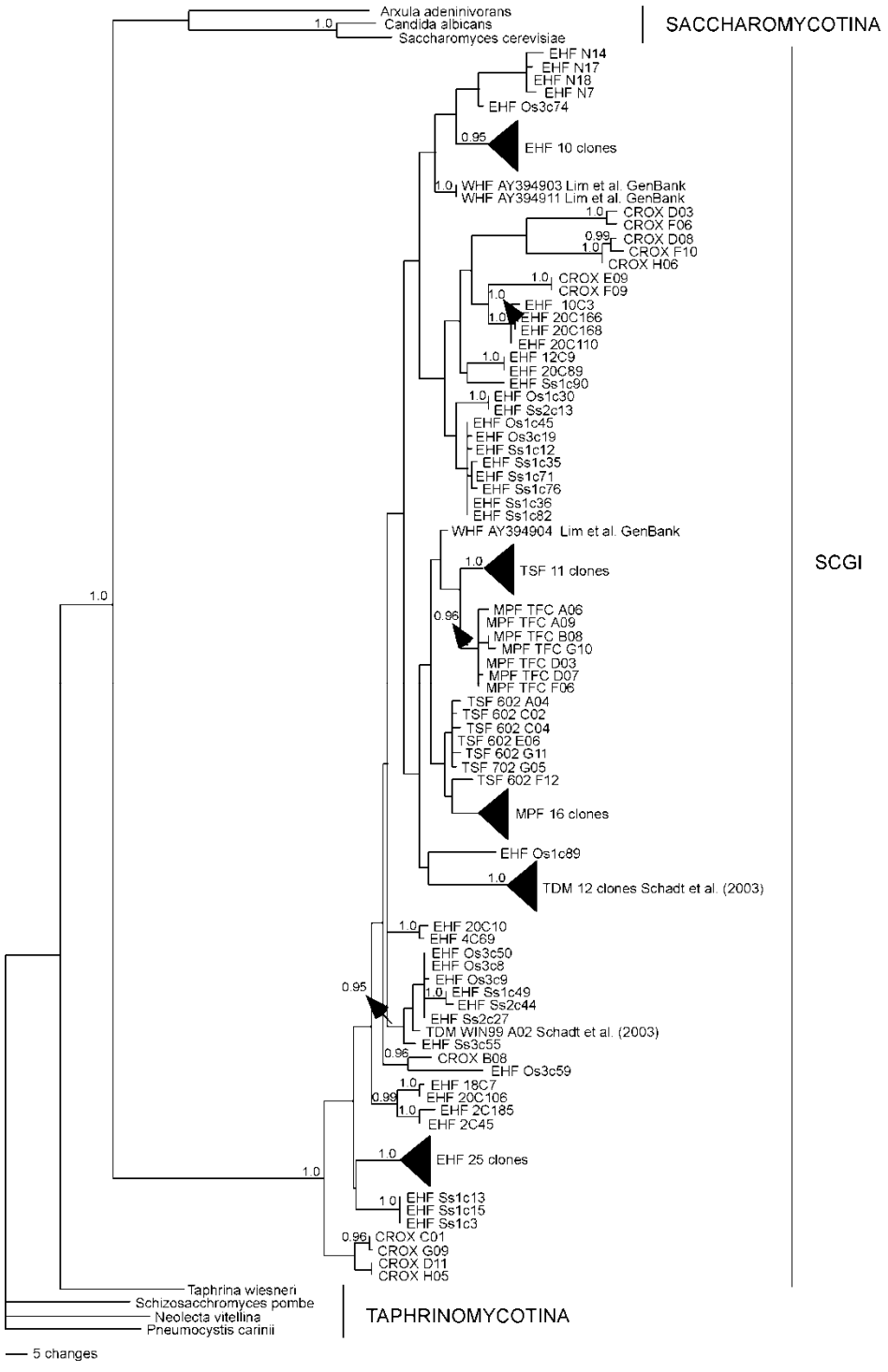
Schematic diagram of the partial rDNA regions sampled for SCGI taxa. Sequences from this study, as well as sequences from GenBank, are shown. Several sequences were from unpublished studies such as: F = Lim et al., GenBank AY394903 and AY394904; and I = Korkama et al., GenBank DQ233843 and DQ233781.



	Study	Location	Source
A	This study	Ontario, Canada	Temperate hemlock forest soil
		Colorado, USA	Montane pine-fir forest soil
			Treeline spruce-fir forest soil
		Costa Rica	Tropical forest oxisol
B	Vandenkoornhuyse et al. (2002)	France	*Arrhenatherum elatius* grass roots
C	O'Brien et al. (2005)	North Carolina, USA	Pine forest soil
			Hardwood forest soil
D	Anderson et al. (2003)	Scotland, UK	Grassland soil
E	Jumpponen and Johnson (2005)	Kansas, USA	Tallgrass prairie soil and rhizosphere
F	Lim et al. GenBank	British Columbia, Canada	Mycorrhizal hemlock root tips
G	Chen and Cairney (2002)	NSW, Australia	Sclerophyll forest soil
H	Izzo et al. (2005)	California, USA	Ectomycorrhizal root tips
I	Korkama et al. GenBank	Norway	Norway spruce root tips
J	Menkis et al. (2005)	Lithuania	Ectomycorrhizal *Pinus sylvestris* roots
K	Pringle et al. (2000)	North Carolina, USA	*Acaulospora colossica* fungal spore
L	Rosling et al. (2003)	Sweden	Ectomycorrhizal plant roots
M	Schadt et al. (2003)	Colorado, USA	Alpine tundra dry-meadow soil

Figure 4-3: Parsimony tree for the LSU region showing all SCGI taxa and several basal Ascomycota reference taxa.

The data set includes 151 taxa and 352 included characters corresponding to bases 190 - 549 in *Saccharomyces cerevisiae* (J01355). Samples from six locations are shown: Eastern hemlock forest (EHF), Western hemlock forest (WHF), Costa Rican oxisol (CROX), Treeline spruce-fir forest (TSF), Montane pine-fir forest (MPF), and Alpine tundra dry meadow (TDM). The tree was rooted with *Pneumocystis carinii*. Bayesian support greater than 0.90 is shown above the branches at the nodes.


Arxula adeninivorans
Candida albicans
Saccharomyces cerevisiae
SACCHAROMYCOTINA
EHF N14
EHF N17
EHF N18
EHF N7
EHF Os3c74
EHF 10 clones
WHF AY394903 Lim et al. GenBank
WHF AY394911 Lim et al. GenBank
CROX D03
CROX F06
CROX D08
CROX F10
CROX H06
CROX E09
CROX F09
EHF 10C3
EHF 20C166
EHF 20C168
EHF 20C110
EHF 12C9
EHF 20C89
EHF Ss1c90
EHF Os1c30
EHF Ss2c13
EHF Os1c45
EHF Os3c19
EHF Ss1c12
EHF Ss1c35
EHF Ss1c71
EHF Ss1c76
EHF Ss1c36
EHF Ss1c82
WHF AY394904 Lim et al. GenBank
TSF 11 clones
SCGI
MPF TFC A06
MPF TFC A09
MPF TFC B08
MPF TFC G10
MPF TFC D03
MPF TFC D07
MPF TFC F06
TSF 602 A04
TSF 602 C02
TSF 602 C04
TSF 602 E06
TSF 602 G11
TSF 702 G05
TSF 602 F12
MPF 16 clones
EHF Os1c89
TDM 12 clones Schadt et al. (2003)
EHF 20C10
EHF 4C69
EHF Os3c50
EHF Os3c8
EHF Os3c9
EHF Ss1c49
EHF Ss2c44
EHF Ss2c27
TDM WIN99 A02 Schadt et al. (2003)
EHF Ss3c55
CROX B08
EHF Os3c59
EHF 18C7
EHF 20C106
EHF 2C185
EHF 2C45
EHF 25 clones
EHF Ss1c13
EHF Ss1c15
EHF Ss1c3
CROX C01
CROX G09
CROX D11
CROX H05
Taphrina wiesneri
Schizosacchromyces pombe
Neolecta vitellina
Pneumocystis carinii
TAPHRINOMYCOTINA
5 changes

5 Chapter Five- General Discussion and Conclusion

The objective of this thesis was to assess the diversity and community structure of fungi in a hemlock-dominated forest plot in southern Ontario using a variety of field sampling and analytical methods. I addressed three broad questions:

1) How do different measures of biological diversity affect the resulting view of rDNA fungal diversity in my study site?

Results presented in Chapter 1 indicates that richness, abundance, and phylogenetic measures of diversity were found to largely correspond and identify the Ascomycota and basal fungi as the dominant groups detected in our study, although in slightly different proportions. These results suggest that for rDNA-based environmental surveys, phylogenetic diversity may be a suitable substitute for traditional diversity measures that rely on the use of subjective species concepts or artificial delimitation of OTUs based on sequence similarity cutoffs. Phylogenetic analyses indicate that the dominant groups in the study site are: the Leotiomycetes and Sordariomycetes in the Ascomycota; the Agaricales and Pucciniomycotina in the Basidiomycota; as well as the Glomeromycota and two unidentified lineages among the basal fungi. Three phylogenetic-based comparisons were found to be variable in their ability to detect significant differences. Generally, the Unifrac significance measure is the most conservative, followed by the P-test, and Libshuff library comparison. Thus, care should be taken when interpreting the results from such tests to detect significant differences among samples.

2) Do fruiting bodies and soil rDNA sampling detect the same phylogenetic and ecological groups of Agaricomycotina in the study site?

Results presented in Chapter 2 indicates that fruiting body collections and rDNA sampling recover largely different assemblages of fungi at the species level; however, both methods identify the same dominant fungi at the genus-order level as well as ectomycorrhizal fungi as the prevailing type. The dominant groups in the study site are the Agaricales, Russulales, Boletales, and Cantharellales. Additionally, rDNA sampling detected members of the Atheliales, Trechisporales, Sebacinales, and Hysterangiales that were overlooked in the fruiting body survey. Richness, abundance, and phylogenetic diversity tend to identify the same dominant fungal groups; however lineages that are evolutionary divergent, containing many long branches, such as the Cantharellales may account for a greater proportion of total diversity when using the PD measure. We suggest that rDNA-based sampling may be sufficient for assessing the dominant taxonomic and ecological groups of Agaricomycotina in forest soil. If a complete inventory is required, then rDNA-based methods should be combined with fruiting body surveys to detect the greatest number of rare taxa.

3) Will additional rDNA sampling resolve the phylogenetic position of unclassified fungal sequences recovered from environmental sampling?

Results presented in Chapter 3 show the existence of a Soil Clone Group equivalent to novel Ascomycota Group I first described from alpine tundra soil (Schadt et al., 2003),

that we refer to as SCGI. Members of this group were detected in our study site from LSU rDNA sampling. This work shows that SCGI is widespread in soils of diverse types and represents a novel subphylum of Ascomycotina. This is a major finding because this is group contributes to the search for the “missing fungi” and it is the only fungal lineage known only from sequence data.

In the following sections I further discuss the general issues pertaining to biological diversity assessment in fungi and recommendations for further studies.

5.1 Promises and pitfalls regarding the use of rDNA sequences in biological diversity estimates

Environmental surveys rely on the availability of well-annotated reference sequences such as those generated from the All Fungal Tree of Life project and UNITE (Lutzoni et al., 2004; James et al., 2006; Hibbett et al., 2007; Koljalg et al., 2005). There are many studies that have generated environmental sequences from sclerophyll, brigalow, eucalypt, pine, mixed hardwood, grassland, agricultural and unvegetated soils (Chen & Cairney 2002; Anderson et al. 2003a,b; Schadt et al. 2003; Jumpponen 2003; Jumpponen & Johnson 2005; O’Brien et al. 2005; Lynch & Thorn 2006; Jumpponen 2007; Midgley et al. 2007). Within these studies, meaningful analyses of these data rely on comparison with reference sequences. With the growing amount of environmental sequences accumulating in public databases, it is only a matter of time before data mining and improved bioinformatics tools allow for comparison across independent studies.

5.1.1 Overestimation of rDNA variation

Multiple copies of rRNA genes are distributed in tandem arrays across the genome (Rooney and Ward, 2005). Generally, when these arrays of rRNA genes are compared, greater sequence similarity is found within a species than between species. This suggests that multiple rRNA genes within a species do not evolve independently of each other and are considered similar in sequence due to concerted evolution (Liao, 1999; Li, 1997). Despite this, within-species heterogeneity in rRNA genes has been shown in the plasmodium, flat worm, the *Streptomyces* bacterium, oak, and an apicomplexan (Gunderson, 1987; Carranza et al., 1996; Rooney, 2004; Muir et al., 2001; Ueda et al., 1999). Several mechanisms for maintaining this heterogeneity include misincorporation of nucleotide bases during DNA replication, horizontal gene transfer, hybridization, and nucleolar dominance in hybrids (Ueda et al., 1999; Muir et al., 2001).

Within-species heterogeneity of rDNA has also been detected in fungi (Karen et al., 1997; O'Donnell and Cigelnik, 1997; Glen et al., 2001; Horton, 2002; Pawlowska and Taylor, 2004; Rooney and Ward, 2005). In these studies, polymorphic rDNA within a species is found mainly in the ITS and mitochondrial LSU rDNA regions. Intra-specific polymorphism in nuclear LSU rDNA has not been widely reported in fungi, except with studies of Glomeromycota that are known to contain many heterogenous nuclei (Clapp et al., 2001).

The concern for PCR-based environmental sampling is that rare sequence indels or substitutions could be artificially increased during the stochastic PCR and cloning amplification procedures (such as in Avis et al., 2006). This is particularly important in fungal ecology studies where PCR-based methods are used to characterize species composition and diversity. The calculation of species richness equally weights all taxa, even those with only small amounts of variation. This effect can be moderated by using operational taxonomic units based on some percentage of sequence similarity that can account for small amounts of natural or introduced variation. For instance, the clustering of sequences with 99% sequence identity into OTUs. In this case, 1% variation within an OTU may account both for within-species variation as well as for a small amount of PCR and cloning error such as single nucleotide polymorphisms. Across a 900 bp region, this would allow for up to 9 bp changes.

Phylogenetic based diversity measures that utilize the information contained in branch lengths would represent related sequences with minimal variation among them (natural or otherwise) with short branch lengths, and would represent more divergent sequences with a lot of variation among them with long branch lengths. Thus, the effect of small amounts of rDNA heterogeneity may not affect the resulting view of fungal community structure as greatly if phylogenetic diversity measures are used compared with richness.

Horton (2002) stated that rDNA heterogeneity recovered from the ITS region does not adequately explain the lack of overlap between above-ground and below-ground ectomycorrhizal fungi. This is in agreement with observations from this study, because

small amounts of rDNA heterogeneity within a species would be compensated for by the use of 1% variation allowed within the operational taxonomic units used. Additionally, even if the LSU rDNA sequences we analyzed from soil exhibited an exaggerated level of polymorphism due to PCR and cloning, it was still not sufficient to exceed the diversity detected from fruiting body collections alone.

5.1.2 Chimeric sequences

PCR-generated artifacts, particularly in combination with cloning, can result in a biased view of community diversity, overestimation of community diversity, and the detection of sequences that do not exist in the template mix (Wintzingerode et al., 1997; Qiu et al., 2001; Hugenholtz and Huber, 2003). Chimeric sequences may be formed when a partially copied amplicon reanneals to an unrelated DNA fragment that is then fully copied and amplified during subsequent PCR cycles (Hugenholtz and Huber, 2003). There are several programs available to check for the presence of chimeric sequences in libraries including Chimera Check (Cole et al., 2003), Pintail (Ashelford et al., 2005) and Mallard (Ashelford et al., 2006). The Chimera Check program does not identify chimeras, but it does provide output to help the user identify when different parts of the query sequence are similar to different organisms in the database. In this thesis, it was very challenging to determine unambiguously whether a query sequence was chimeric using this method. In contrast, chimeric sequences could be detected with greater certainty by analyzing different partitions of the dataset to check for phylogenetic stability.

Overall, the occurrence of chimeric sequences in our libraries was very rare. In contrast, Jumpponen (2007) found greater than 30% of cloned sequences were chimeric. In particular, it was found that the ends of cloned amplicons contained chimeric regions detected using the Chimera Check program. Chimeric sequences tended to cluster basal to existing classes of fungi and the removal of these chimeric regions resulted in changing phylogenies. It was suggested that increasing the extension time and reducing the number of cycles during mixed-template PCR could significantly alleviate chimera formation (Qiu et al., 2001). Our mixed-template thermal cycling protocol conformed with these suggestions as demonstrated by the rarity of detectable chimeric sequences in our dataset as well as a reduced number of redundant sequences sampled from clone libraries. For instance, the ratio of samples to OTUs in our study was 2:1 to 3:1 whereas in the Jumpponen (2007) study the ratio was 5:1. The implication for future studies is that chimeric sequences, even with carefully optimized mixed template PCR thermal cycling conditions, can occur and potentially lead to the false detection of novel taxa (Hugenholtz and Huber, 2003). This is especially problematic when a chimeric sequence is formed from templates of closely related organisms. As a rule, careful screening of sequences using automated tools for large batches of sequences as well as careful manual checks for individual sequences should be performed. For potentially novel taxa, validation using additional lines of evidence is required (discussed below).

5.1.3 Pyrosequencing

Perhaps the next revolution in molecular ecology is the continuing development and application of pyrosequencing. Pyrosequencing technology involves the release of inorganic pyrophosphate with every nucleotide added, resulting in luminescence that is recorded. Pyrosequencing is also referred to as massively parallel pyrosequencing since thousands of short (100-200 bp) DNA sequence reads can be generated in a single run in a few hours without the need for cloning (454 Life Sciences; Huse et al., 2007). Already, the diversity and population structure of microbes in deep sea water have been surveyed using this platform (Sogin et al., 2006; Huber et al., 2007). It is perhaps not surprising then, that with greater sampling afforded by pyrosequencing, the phylogenetic diversity of these deep sea communities was found to be 1-2 orders of magnitude greater than previous work that used shot-gun cloning to sample the Sargasso Sea (Sogin et al., 2006; Venter et al., 2004). Already, the largest assessment of bacteria in soil has surveyed more than 53,000 SSU rDNA sequences using pyrosequencing (Roesch et al., 2007). Additionally, a pyrosequencing analysis of two microbial communities from water and sediments from a mine in Minnesota, USA found important differences among the metabolic potential of taxa detected from each site (Edwards et al., 2006). These studies successfully illustrate the feasibility of pyrosequencing methods with a variety of environmental samples. Pyrosequencing technology may also be applied to resequence genomes and sequencing new genomes for systematic studies that may one day include whole genome comparisons. In this thesis, it was not possible to saturate the sampling of soil fungi, thus it was difficult to detect differences in the composition and number of taxa present in soil from

different horizons. The implication for future studies is that the opportunity to find novel taxa, survey rare taxa, and detect significant differences between samples is increased with larger sample sizes.

5.2 Differences between above and below ground diversity

One explanation for the discrepancy between taxa dominant as above ground fruiting bodies and below ground from environmental samples may be due to differential allocation to either vegetative growth or fruiting body production among species. It has been shown for ectomycorrhizal taxa that there is minimal overlap between fruiting bodies and ectomycorrhizal root tips, and that ectomycorrhizal diversity is greater below-ground (Gardes and Bruns, 1996; Dahlberg et al., 1997). The implication for future studies is that even with increased sampling, there may always be a mismatch between the above-ground and below-ground communities reflecting the life history differences of Agaricomycotina taxa (Horton, 2002).

An alternative explanation for the minimal taxon overlap between the above and below-ground Agaricomycotina detected in this thesis may be due to insufficient sampling and sampling bias. Agaric taxa that were not sampled in this study include: 1) taxa that did not produce above-ground fruiting bodies during the sampling visits; 2) mycorrhizal taxa intimately-associated with below-ground plant roots; and 3) saprobic taxa specifically associated with leaf litter or the upper organic layer. It has been shown that fungal diversity and abundance in soil is greatest in the upper organic layers (Buee et al., 2007;

Christensen, 1989; Neville et al., 2002). In the field, the organic layer was very thin and contained many large roots that were cut away prior to collecting soil cores. Additionally, large roots and rocks were removed during soil sieving of bulked samples. The removal of the leaf litter and organic layer, in addition to roots from bulked soil, may have excluded a large fraction of taxa from detection.

Although some studies have shown a distinct lack of overlap between the above-ground and below-ground ectomycorrhizal community (Gardes and Bruns, 1996; Dahlberg et al., 1997), other studies have shown that it is still possible to detect extramatrical mycelia from mycorrhizal roots in soil using PCR-based methods (Landeweert et al., 2005; Guidot et al., 2001; Guidot et al., 2002). It has been shown that mycelia from *Hebeloma cylindrosporum* can be detected immediately beneath fruiting bodies, but that growth is irregular, and mycelia are not detected in soil further than 50 cm away and mycorrhizas are not detected further than 20 cm away (Guidot et al., 2002; Guidot et al., 2001). These studies illustrate the usefulness of PCR-based methods towards the detection of fungi in soil; however, they also acknowledge the challenges involved due to the patchy growth of extramatrical hyphae, and the different amounts of extramatrical hyphae produced by different mycorrhizas (Agerer, 2001).

Many of these difficulties lie in the scale of the problem. Soil is a heterogeneous and opaque substrate that may harbour tremendous diversity compared with the same scale above-ground. For instance, a single gram of soil may contain 10 billion bacterial cells or up to 66,900 m of fungal hyphae (Faegri et al., 1977; Baath and Soderstrom, 1979).

Agaricomycotina taxa that produce above-ground reproductive fruiting bodies are sampled on a spatial scale similar to plants, whereas, vegetative growth in soil occurs on a spatial scale more similar to that used to sample bacteria. It has been shown that bacterial species composition differs among soil fractions such as light, heavy, and rhizosphere soil (Blackwood and Paul, 2003). In this thesis, Agaricomycotina fruiting bodies were sampled in a 5000 m^2 field site and rDNA was sampled from a total of 11 soil cores (2 cm diameter x 20 - 40 cm depth). The discrepancy among these sampling protocols was a likely contributor to the different composition of taxa detected by each method.

5.3 Evaluation of bioinformatic methods used to study fungal diversity

The Unifrac suite is a good example of multiple, easily accessible phylogenetic diversity tools available with a minimum amount of manual formatting. Other current tools in the literature stand-alone and require time-consuming file formatting. Tests and tools such as the F Statistic, Libshuff, Treeclimber (Schloss and Handlesman, 2006a), and SONS (Schloss and Handlesman, 2006b) are also relevant for community analyses and would make good additions to a phylogenetic suite of tools such as Unifrac to facilitate data exploration using multiple methods. Particularly with the introduction of massive datasets from pyrosequencing studies, bioinformatic tools that can automate the analysis of large amounts of sequence information with a minimum amount of manual formatting will be essential.

The collector's curve is commonly used to assess sampling effort and is normally displayed as a plot of species versus sampling effort (Colwell and Coddington, 1994). A natural extension of the growing interest in phylogenetic diversity measures used in conservation and ecology studies would be to modify a traditional collector's curve to plot some measure of phylogenetic diversity versus sampling effort. This can be done easily using a single phylogenetic tree and randomly adding/removing taxa from this tree and plotting the resulting branch length. Although this curve was not presented in the thesis chapters, the idea has been explored and presented at conferences. The challenge is automating this calculation to randomly resample the data and/or the trees 100 or 1000 times to generate confidence intervals.

5.4 Characterizing novel taxa

Fungal community surveys are continually finding novel taxa from the species to subphylum level (Vandenkoornhuyse et al., 2002; Schadt et al., 2003; Jumpponen and Johnson, 2006; Porter et al., 2007). This is similar to what microbiologists using culture-free methods have already found for prokaryotic taxa (Handlesman, 2004; Pace, 1997). Microbiologists are no longer limited by morphology to diagnose morphological species, nor sexual reproduction to diagnose biological species, nor even living organisms in culture to detect their presence and activity in environmental samples. The next step is to visualize these novel taxa using fluorescent in situ hybridization, converting the novel sequences already generated from these studies into probes, as has already been done with prokaryotes (Sekiguchi et al., 1999; Baker et al., 2007; Burggraf et al., 1994; Dedysh et al.,

2001). Also, targeted culturing of these novel taxa should be attempted using non-standard methods and media, as has been developed for previously uncultured bacteria (Ferrari et al., 2005; Stevenson et al., 2004). Additionally, the continuation of metagenomic approaches to unravel the genomes of organisms not yet cultured may reveal the presence of novel enzymes and biomolecules for biotechnology (Schmeisser et al., 2007; Voget et al., 2003; Rondon et al., 2000; Daniel, 2004).

5.5 Conclusion

If less than 5% of all fungi have been formally described (Hawksworth, 1991; 2001), the question that remains is: where are the missing fungi? Since 1991, numerous studies have proceeded to study fungal diversity in a diverse assortment of habitats. From this thesis alone, we have detected fruiting body samples that remain unidentified to the species level using ITS data despite extensive phylogenetic analyses and taxon sampling, such as *Hydnum* sp.A and *Hydnum* sp.B (Moncalvo et al., 2006), we have detected a novel fungal subphylum (Schmidt et al., In press; Porter et al., 2007), additionally we have identified two unique clades of basal fungi whose identity needs to be further explored (Porter et al., 2007). These results point to unknown areas of the fungal tree of life, despite extensive recent systematic treatment (Hibbett et al., 2007), and highlights the need for continued study at a variety of taxonomic levels using both traditional and molecular approaches.

6 Supplementary Material

6.1 Chapter 2

Table 6-1: List of 1093 LSU rDNA sequences from soil and top BLAST hits from GenBank sorted by taxonomic group.

Group	Sequence Label	GenBank Top Hit	Accession #	Bit Score	E-value	% Similarity
Alveolata	10C3304	Uncultured fungus clone	DQ900977	698	0	93.5
Alveolata	12C1004	Oxytricha longa	AF508763	256	1.00E-64	97.2
Alveolata	14C304	Uncultured ciliate	DQ086735	246	1.00E-61	95.4
Alveolata	20C109R04	Oxytricha longa	AF508763	252	2.00E-63	97.2
Alveolata	20C121R04	Oxytricha longa	AF508763	252	2.00E-63	97.2
Alveolata	20C12404	Uncultured ciliate	DQ086741	595	1.00E-166	99.3
Alveolata	20C5104	Oxytricha longa	AF508763	252	2.00E-63	97.2
Alveolata	20C68R04	Halteria grandinella	AF508759	997	0	91.0
Alveolata	2C10804	Uroleptus gallina	AF508779	504	3.00E-139	95.3
Alveolata	2C11004	Uncultured ciliate	DQ086735	272	2.00E-69	97.4
Alveolata	2C11704	Spathidium amphoriforme	AF223570	1199	0	94.4
Alveolata	2C121R04	Spathidium amphoriforme	AF223570	567	2.00E-158	96.9
Alveolata	2C122R04	Spathidium amphoriforme	AF223570	567	2.00E-158	96.9
Alveolata	2C163R04	Spathidium amphoriforme	AF223570	1104	0	92.9
Alveolata	2C17304	Hypotrichida sp.	AF508778	274	5.00E-70	97.4
Alveolata	2C204	Spathidium amphoriforme	AF223570	1132	0	93.0
Alveolata	2C7604	Spathidium amphoriforme	AF223570	1106	0	93.2
Alveolata	4C1504	Oxytricha sp.	AF508769	1319	0	93.8
Alveolata	4C4804	Uroleptus gallina	AF508779	529	6.00E-147	95.7
Alveolata	4C5004	Oxytricha longa	AF508763	278	3.00E-71	97.4
Alveolata	4C6004	Oxytricha sp.	AF508769	1394	0	94.9
Alveolata	4C6104	Spathidium amphoriforme	AF223570	406	5.00E-110	91.3
Alveolata	Os1c24	Hypotrichida sp.	AF508778	256	1.00E-64	95.0
Alveolata	Os1c55	Oxytricha longa	AF508763	266	1.00E-67	97.3
Alveolata	Os2c72	Engelmanniella mobilis	AF508757	597	3.00E-167	97.4
Alveolata	Os3c11	Atoxoplasma sp.	AY283870	159	3.00E-35	89.3
Alveolata	Os3c53	Oxytricha longa	AF508763	274	5.00E-70	97.4
Alveolata	Ss1c55	Oxytricha longa	AF508763	264	5.00E-67	97.3
Alveolata	Ss1c59	Oxytricha longa	AF508763	264	5.00E-67	97.3
Alveolata	Ss3c40	Hypotrichida sp.	AF508778	270	8.00E-69	95.2
Alveolata	Ss3c82	Hypotrichida sp.	AF508778	270	8.00E-69	95.2
Apusomonadidae	2C9306	Apusomonas proboscidea	DQ980467	813	0	95.3
Ascomycota	10C104	Peziza michelii	AF335149	1602	0	98.6
Ascomycota	10C1104	Peziza michelii	AF335149	1588	0	98.5
Ascomycota	10C1304*	Peziza michelii	AF335149	1602	0	98.6
Ascomycota	10C1704	Uncultured mycorrhizal fungus	AY394911	654	0	91.2
Ascomycota	10C2104	Uncultured mycorrhizal fungus	AY394911	656	0	92.4
Ascomycota	10C2204	Uncultured mycorrhizal fungus	AY394911	658	0	91.2
Ascomycota	10C2504	Uncultured mycorrhizal fungus	AY394911	646	0	90.9
Ascomycota	10C2904	Uncultured mycorrhizal fungus	AY394911	646	0	90.9
Ascomycota	10C3004	Uncultured mycorrhizal fungus	AY394911	640	2.00E-180	90.8
Ascomycota	10C304	Peziza michelii	AF335149	1602	0	98.6
Ascomycota	10C3104	Uncultured mycorrhizal fungus	AY394904	567	4.00E-158	89.4
Ascomycota	10C3604	Uncultured mycorrhizal fungus	AY394911	646	0	91.0
Ascomycota	10C4104	Umbilicaria nylanderiana	AY603119	611	2.00E-171	86.8
Ascomycota	10C4404	Peziza michelii	AF335149	1564	0	98.2
Ascomycota	10C4604*	Uncultured ectomycorrhizal	DQ497955	549	8.00E-153	92.4

		fungus				
Ascomycota	10C4704	Peziza michelii	AF335149	1586	0	98.6
Ascomycota	10C5204	Peziza michelii	AF335149	1578	0	98.5
Ascomycota	10C5404	Peziza michelii	AF335149	1570	0	98.3
Ascomycota	10C5504*	Cudoniella sp.	AY789370	1651	0	99.5
Ascomycota	10C5604	Uncultured mycorrhizal fungus	AY394911	646	0	90.9
Ascomycota	10C5904	Uncultured mycorrhizal fungus	AY394911	654	0	92.4
Ascomycota	10C804*	Uncultured mycorrhizal fungus	AY394911	674	0	91.4
Ascomycota	10C904*	Terfezia claveryi	AY500558	1275	0	93.6
Ascomycota	12C104	Uncultured mycorrhizal fungus	AY394904	660	0	91.0
Ascomycota	12C904*	Uncultured ectomycorrhizal fungus	DQ497955	559	9.00E-156	94.1
Ascomycota	14C104	Chromocleista malachitea	AB000621	1481	0	97.1
Ascomycota	14C504	Neofabraea alba	AY064705	1344	0	95.1
Ascomycota	14C604*	Peziza succosa	AF335166	1651	0	99.3
Ascomycota	14C704	Uncultured mycorrhizal fungus	AY394904	660	0	91.0
Ascomycota	16C704	Potebniamyces pyri	DQ470949	1334	0	94.6
Ascomycota	16C904	Uncultured mycorrhizal fungus	AY394911	652	0	91.4
Ascomycota	18C12806	Uncultured mycorrhizal fungus	AY394904	513	3.00E-142	89.4
Ascomycota	18C16006	Uncultured mycorrhizal fungus	AY394904	521	1.00E-144	89.6
Ascomycota	18C1606	Uncultured mycorrhizal fungus	AY394904	521	1.00E-144	89.6
Ascomycota	18C18006	Uncultured mycorrhizal fungus	AY394904	513	3.00E-142	89.4
Ascomycota	18C2106	Uncultured mycorrhizal fungus	AY394911	656	0	91.6
Ascomycota	18C2206	Crinula caliciiformis	AY544680	1223	0	95.3
Ascomycota	18C25b06	Cylindrocarpon cylindroides	AY283551	1558	0	99.9
Ascomycota	18C27a06	Peziza michelii	AF335149	1332	0	98.3
Ascomycota	18C27b06	Neofabraea malicorticis	AY544662	1344	0	96.9
Ascomycota	18C2A206	Ombrophila violacea	AY789365	1279	0	96.1
Ascomycota	18C2C306	Hyponectria buxi	AY083834	1207	0	95.4
Ascomycota	18C304*	Repetophragma goidanichii	DQ408574	1524	0	97.6
Ascomycota	18C3A106*	Hydropisphaera erubescens	AF193230	1292	0	96.3
Ascomycota	18C3A306*	Hyponectria buxi	AY083834	1249	0	95.6
Ascomycota	18C3D106	Peziza michelii	AF335149	1407	0	98.1
Ascomycota	18C3E106	Neofabraea malicorticis	AY544662	1332	0	96.9
Ascomycota	18C3E306	Crinula caliciiformis	AY544680	1223	0	95.3
Ascomycota	18C3F106	Crinula caliciiformis	AY544680	1189	0	94.6
Ascomycota	18C3F706	Neofabraea malicorticis	AY544662	1318	0	96.7
Ascomycota	18C3F906	Crinula caliciiformis	AY544680	1187	0	94.6
Ascomycota	18C3H506	Neofabraea malicorticis	AY544662	1324	0	96.9
Ascomycota	18C3H706*	Crinula caliciiformis	AY544680	1223	0	95.3
Ascomycota	18C7R04*	Uncultured fungus	AY179731	541	2.00E-150	93.7
Ascomycota	18CA606	Uncultured mycorrhizal fungus	AY394904	500	4.00E-138	89.2
Ascomycota	18CB1106	Hydrocina chaetocladia	AY789412	1233	0	96.4
Ascomycota	18CB506	Fungal endophyte	DQ979459	1310	0	98.0
Ascomycota	18CC1106	Hydrocina chaetocladia	AY789412	1225	0	96.3
Ascomycota	18CF606*	Leuconeurospora pulcherrima	AF096193	1193	0	94.7
Ascomycota	18CG806	Crinula caliciiformis	AY544680	1227	0	95.3
Ascomycota	20C0306	Peziza michelii	AF335149	1409	0	98.2
Ascomycota	20C0406	Peziza michelii	AF335149	1411	0	98.2
Ascomycota	20C1004*	Uncultured mycorrhizal fungus	AY394911	567	4.00E-158	88.9
Ascomycota	20C105R04	Repetophragma goidanichii	DQ408574	1402	0	97.1
Ascomycota	20C106R04	Uncultured fungus	AY179731	549	5.00E-153	94.0
Ascomycota	20C110R04	Uncultured mycorrhizal fungus	AY394904	583	4.00E-163	89.8
Ascomycota	20C11306	Uncultured ectomycorrhizal fungus	DQ497955	549	5.00E-153	93.8
Ascomycota	20C115R04	Peziza michelii	AF335149	1320	0	98.1
Ascomycota	20C120R04	Peziza michelii	AF335149	1328	0	98.2
Ascomycota	20C126R04	Chromocleista malachitea	AB000621	1465	0	97.0
Ascomycota	20C128R04	Uncultured mycorrhizal fungus	AY394904	682	0	93.5
Ascomycota	20C1306	Peziza michelii	AF335149	1419	0	98.3
Ascomycota	20C130R04	Repetophragma goidanichii	DQ408574	1433	0	97.5
Ascomycota	20C134R04	Peziza michelii	AF335149	1312	0	97.9
Ascomycota	20C14304*	Potebniamyces pyri	DQ470949	1356	0	94.9
Ascomycota	20C149R04	Uncultured mycorrhizal fungus	AY394911	684	0	91.4

Ascomycota	20C1506	Neonectria radicicola var. radicicola	AY283552	1463	0	98.6
Ascomycota	20C150R04*	Cordyceps inegoensis	AB027368	1128	0	93.6
Ascomycota	20C152R04	Neonectria radicicola var. radicicola	AY283552	1441	0	98.3
Ascomycota	20C1606	Hydrocina chaetocladia	AY789412	1324	0	97.4
Ascomycota	20C16104	Cylindrocarpon cylindroides	AY283551	1675	0	99.9
Ascomycota	20C164R04	Peziza michelii	AF335149	1320	0	98.1
Ascomycota	20C166R04	Uncultured mycorrhizal fungus	AY394904	575	1.00E-160	89.6
Ascomycota	20C168R04	Uncultured mycorrhizal fungus	AY394904	575	1.00E-160	89.6
Ascomycota	20C169R04*	Potebniamyces pyri	DQ470949	1340	0	96.0
Ascomycota	20C1706	Hydrocina chaetocladia	AY789412	1308	0	97.1
Ascomycota	20C1806	Hydrocina chaetocladia	AY789412	1302	0	96.6
Ascomycota	20C183R04	Uncultured mycorrhizal fungus	AY394904	690	0	93.8
Ascomycota	20C186R04	Repetophragma goidanichii	DQ408574	1372	0	97.5
Ascomycota	20C2306*	Cordierites frondosa	AY789354	180	5.00E-42	89.0
Ascomycota	20C2404	Peziza michelii	AF335149	1588	0	98.6
Ascomycota	20C24R06*	Davidiella tassiana	DQ678074	1637	0	99.5
Ascomycota	20C2506	Hydrocina chaetocladia	AY789412	1299	0	96.7
Ascomycota	20C2704*	Repetophragma goidanichii	DQ408574	1604	0	99.3
Ascomycota	20C28R06*	Phyllosticta sp.	DQ377929	1606	0	99.4
Ascomycota	20C304	Uncultured mycorrhizal fungus	AY394904	670	0	93.6
Ascomycota	20C3204*	Pestalotiopsis sp.	DQ195795	1669	0	99.9
Ascomycota	20C3304*	Scutellinia subhirtella	DQ220423	1501	0	97.2
Ascomycota	20C3504*	Nectria sesquicillii	AF193241	1554	0	98.6
Ascomycota	20C4406	Cylindrocarpon cylindroides	AY283551	1505	0	100.0
Ascomycota	20C4606	Trichoderma viride	AY291123	1485	0	99.4
Ascomycota	20C4706	Peziza michelii	AF335149	1409	0	98.2
Ascomycota	20C5006	Peziza michelii	AF335149	1413	0	98.2
Ascomycota	20C5406*	Sydowia polyspora	DQ678058	1693	0	100.0
Ascomycota	20C5506	Uncultured mycorrhizal fungus	AY394904	537	2.00E-149	89.9
Ascomycota	20C5604*	Collema flaccidum	AY424213	184	3.00E-43	93.0
Ascomycota	20C5704	Peziza michelii	AF335149	1588	0	98.6
Ascomycota	20C5904	Uncultured mycorrhizal fungus	AY394904	636	3.00E-179	92.5
Ascomycota	20C5906	Chromocleista malachitea	AB000621	1308	0	96.8
Ascomycota	20C606*	Aphysiostroma stercorarium	AF543792	1362	0	97.4
Ascomycota	20C6106	Bulgaria inquinans	DQ470960	1241	0	95.6
Ascomycota	20C6306	Peziza michelii	AF335149	1404	0	98.1
Ascomycota	20C6504*	Schizosaccharomyces pombe	DQ442711	272	2.00E-69	95.3
Ascomycota	20C6606	Hydrocina chaetocladia	AY789412	1294	0	96.5
Ascomycota	20C6806	Neofabraea malicorticis	AY544662	1310	0	96.6
Ascomycota	20C69R04	Hydrocina chaetocladia	AY789412	1316	0	96.4
Ascomycota	20C70R04	Peziza michelii	AF335149	1320	0	98.1
Ascomycota	20C71R04	Peziza michelii	AF335149	1320	0	98.1
Ascomycota	20C7404	Peziza michelii	AF335149	1602	0	98.6
Ascomycota	20C7406	Geoglossum nigritum	AY544650	1156	0	95.1
Ascomycota	20C7504*	Nectria ventricosa	AF228361	268	3.00E-68	94.3
Ascomycota	20C77R04	Uncultured mycorrhizal fungus	AY394904	692	0	91.3
Ascomycota	20C7906	Neofabraea malicorticis	AY544662	1292	0	96.2
Ascomycota	20C83R04	Chromocleista malachitea	AB000621	1465	0	97.0
Ascomycota	20C84R04	Lanatonectria flavolanata	DQ119565	1346	0	95.5
Ascomycota	20C87R04*	Neofabraea malicorticis	AY544662	1246	0	93.9
Ascomycota	20C88R04	Uncultured mycorrhizal fungus	AY394904	666	0	90.8
Ascomycota	20C89R04	Uncultured ectomycorrhizal fungus	DQ497955	559	6.00E-156	94.1
Ascomycota	20C906	Cordierites frondosa	AY789354	180	5.00E-42	89.0
Ascomycota	20C9504*	Inonotus hispidus	AF518623	246	1.00E-61	92.2
Ascomycota	2C10004*	Nectria radicicola	U17415	1618	0	99.5
Ascomycota	2C10104	Farrowia seminuda	AF286410	1475	0	97.4
Ascomycota	2C10204	Hydrocina chaetocladia	AY789412	1465	0	97.0
Ascomycota	2C10404*	Neobulgaria pura	DQ257365	1451	0	96.5
Ascomycota	2C10604	Sarcogyne regularis var. regularis	AY640964	646	0	87.6
Ascomycota	2C10904*	Peziza michelii	AF335149	1421	0	96.1
Ascomycota	2C11404	Apiospora setosa	AY346259	1570	0	98.6

Ascomycota	2C11604	Lanatonectria flavolanata	DQ119565	1346	0	95.6
Ascomycota	2C12004*	Neolecta irregularis	DQ470986	107	8.00E-20	98.3
Ascomycota	2C1204	Peziza michelii	AF335149	1606	0	98.6
Ascomycota	2C123R04	Cylindrocarpon cylindroides	AY283551	1578	0	99.6
Ascomycota	2C124R04	Chromocleista malachitea	AB000621	1443	0	97.2
Ascomycota	2C125R04	Chromocleista malachitea	AB000621	1443	0	97.2
Ascomycota	2C126R04	Peziza michelii	AF335149	1497	0	98.5
Ascomycota	2C12704	Uncultured mycorrhizal fungus	AY394904	646	0	92.8
Ascomycota	2C128R04	Chromocleista malachitea	AB000621	1443	0	97.2
Ascomycota	2C131R04	Peziza michelii	AF335149	1497	0	98.5
Ascomycota	2C132R04	Peziza michelii	AF335149	1489	0	98.4
Ascomycota	2C137R04	Chromocleista malachitea	AB000621	1443	0	97.2
Ascomycota	2C1404*	Hydrocina chaetocladia	AY789412	1493	0	97.0
Ascomycota	2C14704*	Trichosporon brassicae	DQ377685	268	4.00E-68	94.7
Ascomycota	2C14904*	Chaetomium homopilatum	AF286404	1695	0	99.9
Ascomycota	2C166R04	Sarcogyne regularis var. regularis	AY640964	609	7.00E-171	87.3
Ascomycota	2C167R04	Sarcogyne regularis var. regularis	AY640964	609	7.00E-171	87.3
Ascomycota	2C1704*	Neofabraea alba	AY064705	1294	0	94.3
Ascomycota	2C170R04	Magnisphaera spartinae	AY150221	1298	0	95.8
Ascomycota	2C171R04	Cudoniella clavus	DQ470944	1372	0	96.5
Ascomycota	2C174R04*	Uncultured mycorrhizal fungus	AY394904	579	6.00E-162	90.9
Ascomycota	2C175R04	Sarcogyne regularis var. regularis	AY640964	609	7.00E-171	87.3
Ascomycota	2C176R04	Sarcogyne regularis var. regularis	AY640964	593	4.00E-166	86.9
Ascomycota	2C182R04	Peziza michelii	AF335149	1497	0	98.5
Ascomycota	2C184R04	Cylindrocarpon cylindroides	AY283551	1584	0	99.6
Ascomycota	2C18504*	Uncultured mycorrhizal fungus	AY394904	513	3.00E-142	89.5
Ascomycota	2C189R04	Bulgaria inquinans	DQ470960	1459	0	96.3
Ascomycota	2C192R04	Potebniamyces pyri	DQ470949	1217	0	94.7
Ascomycota	2C195R04	Sarcogyne regularis var. regularis	AY640964	601	2.00E-168	87.1
Ascomycota	2C198R04	Pestalotiopsis yunnanensis	DQ657883	1637	0	100.0
Ascomycota	2C200R04	Neonectria radicicola var. radicicola	AY283552	1600	0	99.9
Ascomycota	2C207R04	Sarcogyne regularis var. regularis	AY640964	601	2.00E-168	87.1
Ascomycota	2C2104	Uncultured mycorrhizal fungus	AY394911	650	0	90.9
Ascomycota	2C213R04	Peziza michelii	AF335149	1497	0	98.5
Ascomycota	2C216R04*	Venturia chlorospora	DQ384101	831	0	88.4
Ascomycota	2C2704*	Scutellospora nigra	AY900498	168	3.00E-38	89.7
Ascomycota	2C2804	Chromocleista malachitea	AB000621	1526	0	97.2
Ascomycota	2C304	Peziza michelii	AF335149	1598	0	98.5
Ascomycota	2C3104*	Crinula caliciiformis	AY544680	1292	0	94.1
Ascomycota	2C3204	Peziza michelii	AF335149	1610	0	98.6
Ascomycota	2C3404	Peziza michelii	AF335149	1598	0	98.5
Ascomycota	2C3504*	Crinula caliciiformis	AY544680	1386	0	95.4
Ascomycota	2C3604*	Crinula caliciiformis	AY544680	1193	0	92.8
Ascomycota	2C3704*	Venturia chlorospora	DQ384101	872	0	88.6
Ascomycota	2C4304	Peziza michelii	AF335149	1602	0	98.6
Ascomycota	2C4504*	Uncultured mycorrhizal fungus	AY394904	553	4.00E-154	90.3
Ascomycota	2C604*	Peziza michelii	AF335149	1602	0	98.4
Ascomycota	2C6904*	Aquaticheirospora sp.	AY736378	1499	0	97.8
Ascomycota	2C7104*	Coniochaetidium savoryi	AY346276	1473	0	97.2
Ascomycota	2C7504	Chromocleista malachitea	AB000621	1443	0	96.5
Ascomycota	2C7804	Lanatonectria flavolanata	DQ119565	1328	0	95.3
Ascomycota	2C7904	Sarcogyne regularis var. regularis	AY640964	646	0	87.6
Ascomycota	2C8204	Bulgaria inquinans	DQ470960	1445	0	96.3
Ascomycota	2C8506	Uncultured mycorrhizal fungus	AY394904	664	0	93.6
Ascomycota	2C8604	Peziza michelii	AF335149	1588	0	98.6
Ascomycota	2C8606	Nectria ventricosa	AF228361	268	3.00E-68	94.3
Ascomycota	2C8706	Pseudeurotium zonatum	DQ470988	1461	0	99.3
Ascomycota	2C9004	Hydrocina chaetocladia	AY789412	1457	0	96.8
Ascomycota	2C9104	Peziza michelii	AF335149	1580	0	98.5
Ascomycota	2C9106	Hydrocina chaetocladia	AY789412	1324	0	97.3
Ascomycota	2C9206	Inonotus hispidus	AF518623	246	1.00E-61	92.2
Ascomycota	2C9304	Peziza michelii	AF335149	1588	0	98.6
Ascomycota	2C9604*	Neofabraea alba	AY064705	1358	0	95.3

Ascomycota	4C10006	Uncultured mycorrhizal fungus	AY394904	557	2.00E-155	90.4
Ascomycota	4C10106	Uncultured mycorrhizal fungus	AY394911	652	0	92.2
Ascomycota	4C10206*	Uncultured mycorrhizal fungus	AY394911	516	4.00E-143	89.9
Ascomycota	4C10306	Uncultured mycorrhizal fungus	AY394904	513	3.00E-142	89.4
Ascomycota	4C10506	Uncultured mycorrhizal fungus	AY394904	640	2.00E-180	92.6
Ascomycota	4C11006	Uncultured mycorrhizal fungus	AY394911	642	0	90.8
Ascomycota	4C1104	Peziza michelii	AF335149	1592	0	98.6
Ascomycota	4C11406	Uncultured mycorrhizal fungus	AY394904	557	2.00E-155	90.4
Ascomycota	4C12106	Trichoderma viride	AY291123	1481	0	99.4
Ascomycota	4C12206	Uncultured mycorrhizal fungus	AY394904	513	3.00E-142	89.4
Ascomycota	4C12406	Uncultured mycorrhizal fungus	AY394904	505	7.00E-140	89.2
Ascomycota	4C12606	Uncultured mycorrhizal fungus	AY394904	513	3.00E-142	89.4
Ascomycota	4C12706	Uncultured mycorrhizal fungus	AY394904	541	1.00E-150	90.0
Ascomycota	4C12806	Uncultured mycorrhizal fungus	AY394904	513	3.00E-142	89.4
Ascomycota	4C13206*	Uncultured mycorrhizal fungus	AY394904	491	1.00E-135	93.9
Ascomycota	4C13406	Uncultured mycorrhizal fungus	AY394904	557	2.00E-155	90.4
Ascomycota	4C13906	Uncultured mycorrhizal fungus	AY394904	507	2.00E-140	89.2
Ascomycota	4C14106	Uncultured mycorrhizal fungus	AY394904	652	0	90.7
Ascomycota	4C14406	Uncultured mycorrhizal fungus	AY394904	652	0	90.7
Ascomycota	4C14506	Uncultured mycorrhizal fungus	AY394904	648	0	92.8
Ascomycota	4C14706*	Uncultured mycorrhizal fungus	AY394904	517	3.00E-143	89.4
Ascomycota	4C14806	Lanatonectria flavolanata	DQ119565	1152	0	95.0
Ascomycota	4C15106	Uncultured mycorrhizal fungus	AY394904	617	3.00E-173	91.9
Ascomycota	4C2004	Sarcogyne regularis var. regularis	AY640964	630	2.00E-177	87.3
Ascomycota	4C2504	Peziza michelii	AF335149	1586	0	98.5
Ascomycota	4C2604	Uncultured mycorrhizal fungus	AY394904	498	2.00E-137	89.0
Ascomycota	4C2704	Peziza michelii	AF335149	1588	0	98.5
Ascomycota	4C3104	Umbilicaria nylanderiana	AY603119	648	0	87.3
Ascomycota	4C3304	Peziza michelii	AF335149	1558	0	98.3
Ascomycota	4C3404*	Uncultured mycorrhizal fungus	AY394911	694	0	91.5
Ascomycota	4C3504*	Fungal endophyte	DQ979460	710	0	88.9
Ascomycota	4C3604*	Chromocleista malachitea	AB000621	1364	0	95.0
Ascomycota	4C3704	Uncultured mycorrhizal fungus	AY394911	670	0	91.3
Ascomycota	4C3804	Uncultured mycorrhizal fungus	AY394904	670	0	91.0
Ascomycota	4C4004	Umbilicaria nylanderiana	AY603119	640	2.00E-180	87.2
Ascomycota	4C4204*	Chaetomium globosum	AF286403	1495	0	97.2
Ascomycota	4C4704	Sarcogyne regularis var. regularis	AY640964	642	0	87.5
Ascomycota	4C4904	Uncultured mycorrhizal fungus	AY394904	678	0	91.2
Ascomycota	4C5504*	Uncultured mycorrhizal fungus	AY394904	678	0	90.9
Ascomycota	4C5604	Peziza michelii	AF335149	1598	0	98.5
Ascomycota	4C5804	Peziza michelii	AF335149	1598	0	98.5
Ascomycota	4C5904	Uncultured mycorrhizal fungus	AY394911	670	0	92.5
Ascomycota	4C6204	Sarcogyne regularis var. regularis	AY640964	636	3.00E-179	87.4
Ascomycota	4C6404	Sarcogyne regularis var. regularis	AY640964	636	3.00E-179	87.4
Ascomycota	4C6604	Uncultured mycorrhizal fungus	AY394911	688	0	92.7
Ascomycota	4C6704*	Cylindrocarpon cylindroides	AY283551	1667	0	99.9
Ascomycota	4C6804	Sarcogyne regularis var. regularis	AY640964	628	8.00E-177	87.3
Ascomycota	4C6904*	Uncultured mycorrhizal fungus	AY394904	561	2.00E-156	90.4
Ascomycota	4C7204	Uncultured mycorrhizal fungus	AY394904	670	0	93.6
Ascomycota	4C7604	Sarcogyne regularis var. regularis	AY640964	636	3.00E-179	87.4
Ascomycota	4C7704	Peziza michelii	AF335149	1590	0	98.4
Ascomycota	4C7904	Sarcogyne regularis var. regularis	AY640964	638	8.00E-180	87.5
Ascomycota	4C8006	Peziza succosa	AF335166	1481	0	99.1
Ascomycota	4C8106	Uncultured mycorrhizal fungus	AY394911	656	0	92.3
Ascomycota	4C8206	Uncultured mycorrhizal fungus	AY394904	521	1.00E-144	89.6
Ascomycota	4C8406	Uncultured mycorrhizal fungus	AY394911	509	4.00E-141	89.9
Ascomycota	4C8606	Uncultured mycorrhizal fungus	AY394904	505	7.00E-140	89.2
Ascomycota	4C8706*	Geoglossum nigritum	AY544650	1164	0	95.1
Ascomycota	4C8806	Uncultured mycorrhizal fungus	AY394904	498	2.00E-137	89.0
Ascomycota	4C9306	Uncultured mycorrhizal fungus	AY394911	652	0	92.2
Ascomycota	4C9506*	Pezicula carpinea	DQ470967	687	0	90.2
Ascomycota	4C9706	Uncultured ectomycorrhizal fungus	DQ497955	547	2.00E-152	92.4
Ascomycota	4C9906	Uncultured mycorrhizal fungus	AY394911	640	2.00E-180	92.4

Ascomycota	4CA2*	Hydrocina chaetocladia	AY789412	1292	0	97.3
Ascomycota	4CC206	Uncultured mycorrhizal fungus	AY394904	513	3.00E-142	89.4
Ascomycota	4CD106	Peziza michelii	AF335149	1417	0	98.4
Ascomycota	4CE106	Uncultured mycorrhizal fungus	AY394904	505	7.00E-140	89.2
Ascomycota	4CE206	Uncultured mycorrhizal fungus	AY394911	664	0	92.6
Ascomycota	4CF206	Peziza michelii	AF335149	1437	0	98.1
Ascomycota	4CG106	Uncultured mycorrhizal fungus	AY394904	505	7.00E-140	89.2
Ascomycota	4CG206*	Uncultured mycorrhizal fungus	AY394911	414	2.00E-112	87.2
Ascomycota	4CH206	Uncultured mycorrhizal fungus	AY394904	557	2.00E-155	90.4
Ascomycota	6C1004	Uncultured mycorrhizal fungus	AY394911	644	0	90.9
Ascomycota	6C104	Uncultured mycorrhizal fungus	AY394904	682	0	91.2
Ascomycota	6C204	Uncultured mycorrhizal fungus	AY394904	682	0	91.2
Ascomycota	6C304	Peziza michelii	AF335149	1598	0	98.5
Ascomycota	6C404	Peziza michelii	AF335149	1598	0	98.5
Ascomycota	6C504	Peziza michelii	AF335149	1594	0	98.6
Ascomycota	6C604	Peziza michelii	AF335149	1602	0	98.6
Ascomycota	6C804*	Pseudeurotium zonatum	DQ470988	1620	0	98.9
Ascomycota	6C904*	Epichloe amarillans	U57680	1471	0	97.3
Ascomycota	7C104*	Swampomyces armeniacus	AY858951	490	5.00E-135	94.1
Ascomycota	7C404	Carpoligna pleurothecii	AF064646	1247	0	93.5
Ascomycota	7C504*	Crinula caliciiformis	AY544680	1469	0	95.9
Ascomycota	7C604*	Hypomyces completus	AF213028	1669	0	100.0
Ascomycota	7C704	Chromocleista malachitea	AB000621	1503	0	97.1
Ascomycota	8C204*	Cudoniella clavus	DQ470944	1469	0	96.7
Ascomycota	8C4R04*	Pseudeurotium zonatum	DQ470988	617	3.00E-173	95.5
Ascomycota	8C504	Cudoniella clavus	DQ470944	1469	0	96.7
Ascomycota	Os1c1*	Hydrocina chaetocladia	AY789412	1390	0	95.9
Ascomycota	Os1c12	Peziza michelii	AF335149	1580	0	98.4
Ascomycota	Os1c16	Neofabraea alba	AY064705	1310	0	94.6
Ascomycota	Os1c19	Trichoderma viride	AY291123	1552	0	99.0
Ascomycota	Os1c21	Sarcogyne regularis var. regularis	AY640964	642	0	87.5
Ascomycota	Os1c22	Neofabraea malicorticis	AY544662	1481	0	96.8
Ascomycota	Os1c27	Myxotrichum deflexum	AY541491	1471	0	96.5
Ascomycota	Os1c29*	Babjevia anomala	DQ518970	1631	0	99.0
Ascomycota	Os1c30	Uncultured mycorrhizal fungus	AY394904	662	0	92.9
Ascomycota	Os1c33	Chromocleista malachitea	AB000621	1479	0	97.1
Ascomycota	Os1c41	Sarcogyne regularis var. regularis	AY640964	642	0	87.5
Ascomycota	Os1c43	Crinula caliciiformis	AY544680	1255	0	93.9
Ascomycota	Os1c45	Uncultured mycorrhizal fungus	AY394911	628	8.00E-177	90.8
Ascomycota	Os1c47*	Cudoniella sp.	AY789377	1518	0	97.1
Ascomycota	Os1c50*	Lipomyces doorenjongii	DQ518974	2333	0	98.9
Ascomycota	Os1c56	Candida santjacobensis	DQ442701	1217	0	93.2
Ascomycota	Os1c57*	Pyriculariopsis parasitica	DQ341514	327	4.00E-86	88.0
Ascomycota	Os1c63*	Mollisia cinerea	DQ470942	1388	0	96.0
Ascomycota	Os1c66*	Apodus deciduus	AY681165	1622	0	99.6
Ascomycota	Os1c7*	Penicillium montanense	AF527058	955	0	100.0
Ascomycota	Os1c70*	Cryptadelphia groenendalensis	AY281103	1072	0	91.2
Ascomycota	Os1c79*	Scutellinia sp.	DQ220422	1516	0	97.2
Ascomycota	Os1c89*	Uncultured mycorrhizal fungus	AY394904	644	0	90.2
Ascomycota	Os2c10	Myxotrichum deflexum	AY541491	1455	0	96.3
Ascomycota	Os2c2	Epichloe amarillans	U57680	1287	0	97.3
Ascomycota	Os2c21	Bulgaria inquinans	DQ470960	1447	0	96.2
Ascomycota	Os2c24	Bulgaria inquinans	DQ470960	1457	0	96.2
Ascomycota	Os2c48*	Talaromyces emersonii	DQ010015	1261	0	95.4
Ascomycota	Os2c57	Exophiala salmonis	AF050274	1661	0	99.5
Ascomycota	Os2c74*	Uncultured mycorrhizal fungus	AY394911	587	4.00E-164	90.2
Ascomycota	Os2c9*	Chaunopycnis pustulata	AF373282	248	3.00E-62	97.2
Ascomycota	Os3c10	Myxotrichum deflexum	AY541491	1495	0	96.8
Ascomycota	Os3c11*	Chromocleista malachitea	AB000621	1378	0	95.5
Ascomycota	Os3c12*	Hyaloscypha daedaleae	AY789415	1608	0	98.6
Ascomycota	Os3c14*	Bulgaria inquinans	DQ470960	1479	0	96.2
Ascomycota	Os3c19*	Uncultured mycorrhizal fungus	AY394911	668	0	90.7
Ascomycota	Os3c20	Exophiala pisciphila	DQ823101	1731	0	99.6
Ascomycota	Os3c22	Gyromitra esculenta	AJ544208	1513	0	96.4

Ascomycota	Os3c23	Gyromitra esculenta	AJ544208	1509	0	96.3
Ascomycota	Os3c24	Chaetomium sphaerale	AF286407	1522	0	97.2
Ascomycota	Os3c29	Talaromyces emersonii	DQ010015	1380	0	96.2
Ascomycota	Os3c3*	Verticillium dahliae	AF104926	1479	0	96.6
Ascomycota	Os3c35	Myxotrichum deflexum	AY541491	1475	0	96.4
Ascomycota	Os3c39	Myxotrichum deflexum	AY541491	1493	0	96.4
Ascomycota	Os3c40	Myxotrichum deflexum	AY541491	1493	0	96.4
Ascomycota	Os3c43	Talaromyces emersonii	DQ010015	1378	0	96.1
Ascomycota	Os3c45	Myxotrichum deflexum	AY541491	1475	0	96.4
Ascomycota	Os3c50*	Uncultured mycorrhizal fungus	AY394911	686	0	91.4
Ascomycota	Os3c51*	Chaetomium sphaerale	AF286407	1520	0	97.1
Ascomycota	Os3c55*	Gyromitra esculenta	AJ544208	1493	0	96.1
Ascomycota	Os3c59*	Uncultured mycorrhizal fungus	AY394904	504	3.00E-139	90.2
Ascomycota	Os3c61*	Chromocleista malachitea	AB000621	1516	0	96.7
Ascomycota	Os3c67*	Candida santjacobensis	DQ442701	1235	0	93.5
Ascomycota	Os3c74*	Uncultured mycorrhizal fungus	AY394904	660	0	93.8
Ascomycota	Os3c77	Hydrocina chaetocladia	AY789412	1499	0	96.7
Ascomycota	Os3c79*	Melanelia panniformis	AJ421430	246	1.00E-61	94.9
Ascomycota	Os3c8	Uncultured mycorrhizal fungus	AY394911	698	0	91.7
Ascomycota	Os3c83	Hydrocina chaetocladia	AY789412	1479	0	96.4
Ascomycota	Os3c84	Beauveria brongniartii	AB027381	1659	0	98.9
Ascomycota	Os3c86*	Hypomyces chrysospermus	AB027385	1526	0	97.3
Ascomycota	Os3c88*	Crinula caliciiformis	AY544680	1382	0	95.0
Ascomycota	Os3c90	Talaromyces emersonii	DQ010015	1384	0	96.0
Ascomycota	Os3c93*	Neonectria radicicola var. radicicola	AY283552	1600	0	98.8
Ascomycota	Os3c94	Chromocleista malachitea	AB000621	1532	0	97.0
Ascomycota	Ss1c12*	Uncultured mycorrhizal fungus	AY394911	626	3.00E-176	90.6
Ascomycota	Ss1c13*	Uncultured mycorrhizal fungus	AY394904	460	4.00E-126	89.4
Ascomycota	Ss1c15*	Uncultured fungus	AY179731	444	3.00E-121	90.9
Ascomycota	Ss1c21	Talaromyces emersonii	DQ010015	1338	0	95.6
Ascomycota	Ss1c27*	Myxotrichum deflexum	AY541491	1352	0	95.4
Ascomycota	Ss1c29*	Monascus purpureus	DQ782908	1273	0	94.8
Ascomycota	Ss1c3*	Uncultured mycorrhizal fungus	AY394904	509	5.00E-141	88.8
Ascomycota	Ss1c30*	Sydowia polyspora	DQ678058	1487	0	98.0
Ascomycota	Ss1c32*	Chromocleista malachitea	AB000621	1497	0	97.3
Ascomycota	Ss1c35a	Uncultured mycorrhizal fungus	AY394911	638	8.00E-180	92.5
Ascomycota	Ss1c36	Uncultured mycorrhizal fungus	AY394911	644	0	91.2
Ascomycota	Ss1c42	Uncultured mycorrhizal fungus	AY394911	603	5.00E-169	90.2
Ascomycota	Ss1c45*	Uncultured ectomycorrhizal fungus	DQ481984	486	7.00E-134	93.6
Ascomycota	Ss1c47	Talaromyces emersonii	DQ010015	1352	0	96.2
Ascomycota	Ss1c48*	Uncultured mycorrhizal fungus	AY394904	507	2.00E-140	89.4
Ascomycota	Ss1c49*	Uncultured mycorrhizal fungus	AY394911	660	0	91.4
Ascomycota	Ss1c52	Venturia chlorospora	DQ384101	1604	0	98.8
Ascomycota	Ss1c6*	Lasallia pustulata	AY300839	1231	0	93.5
Ascomycota	Ss1c61	Umbilicaria nylanderiana	AY603119	624	1.00E-175	86.9
Ascomycota	Ss1c63	Repetophragma goidanichii	DQ408574	1457	0	97.1
Ascomycota	Ss1c64*	Uncultured ectomycorrhizal fungus	DQ497955	573	4.00E-160	94.4
Ascomycota	Ss1c67	Repetophragma goidanichii	DQ408574	1493	0	97.5
Ascomycota	Ss1c69	Lasallia pustulata	AY300839	1300	0	94.6
Ascomycota	Ss1c7*	Sydowia polyspora	DQ678058	1588	0	98.6
Ascomycota	Ss1c70	Uncultured mycorrhizal fungus	AY394911	646	0	90.9
Ascomycota	Ss1c71	Uncultured mycorrhizal fungus	AY394911	660	0	91.6
Ascomycota	Ss1c73	Farrowia seminuda	AF286410	1491	0	97.5
Ascomycota	Ss1c74	Byssochlamys nivea	AY176750	1259	0	95.9
Ascomycota	Ss1c75	Talaromyces emersonii	DQ010015	1348	0	96.2
Ascomycota	Ss1c76	Uncultured mycorrhizal fungus	AY394911	636	3.00E-179	92.2
Ascomycota	Ss1c81	Talaromyces emersonii	DQ010015	1334	0	96.1
Ascomycota	Ss1c82	Uncultured mycorrhizal fungus	AY394911	642	0	90.8
Ascomycota	Ss1c84*	Venturia chlorospora	DQ384101	1598	0	98.7
Ascomycota	Ss1c85	Beauveria brongniartii	AB027381	1671	0	99.8
Ascomycota	Ss1c88	Uncultured mycorrhizal fungus	AY394911	638	8.00E-180	90.7

Ascomycota	Ss1c90	Uncultured ectomycorrhizal fungus	DQ497955	531	2.00E-147	92.6
Ascomycota	Ss1c93	Talaromyces emersonii	DQ010015	1340	0	95.9
Ascomycota	Ss2c13*	Uncultured mycorrhizal fungus	AY394904	644	0	92.6
Ascomycota	Ss2c15*	Neonectria radicicola var. radicicola	AY283552	1657	0	99.8
Ascomycota	Ss2c17	Talaromyces emersonii	DQ010015	1346	0	96.0
Ascomycota	Ss2c21	Talaromyces emersonii	DQ010015	1342	0	95.9
Ascomycota	Ss2c27	Uncultured mycorrhizal fungus	AY394911	664	0	91.3
Ascomycota	Ss2c33*	Uncultured mycorrhizal fungus	AY394911	646	0	90.9
Ascomycota	Ss2c37*	Nectria sesquicillii	AF193241	1465	0	97.1
Ascomycota	Ss2c44*	Uncultured mycorrhizal fungus	AY394911	634	1.00E-178	90.5
Ascomycota	Ss2c49	Talaromyces emersonii	DQ010015	1350	0	96.1
Ascomycota	Ss2c5	Neonectria radicicola var. radicicola	AY283552	1671	0	99.9
Ascomycota	Ss2c50*	Byssochlamys nivea	AY176750	504	3.00E-139	90.8
Ascomycota	Ss2c51	Talaromyces emersonii	DQ010015	1346	0	95.9
Ascomycota	Ss2c57*	Lanatonectria flavolanata	DQ119565	1370	0	95.5
Ascomycota	Ss2c76	Talaromyces emersonii	DQ010015	1370	0	96.1
Ascomycota	Ss2c81	Uncultured mycorrhizal fungus	AY394911	638	8.00E-180	90.7
Ascomycota	Ss2c82	Neonectria radicicola var. radicicola	AY283552	1645	0	99.6
Ascomycota	Ss2c87	Talaromyces emersonii	DQ010015	1378	0	96.2
Ascomycota	Ss3c12	Talaromyces emersonii	DQ010015	1370	0	96.2
Ascomycota	Ss3c13a*	Hyphodiscus hymeniophilus	DQ227263	1423	0	95.6
Ascomycota	Ss3c13b	Lasallia pustulata	AY300839	1302	0	94.3
Ascomycota	Ss3c16*	Myxotrichum deflexum	AY541491	1449	0	96.2
Ascomycota	Ss3c17*	Talaromyces emersonii	DQ010015	1374	0	95.8
Ascomycota	Ss3c18a	Neofabraea malicorticis	AY544662	1497	0	96.0
Ascomycota	Ss3c18b	Talaromyces emersonii	DQ010015	1354	0	96.0
Ascomycota	Ss3c19	Talaromyces emersonii	DQ010015	1358	0	96.2
Ascomycota	Ss3c20*	Hydrocina chaetocladia	AY789412	1417	0	95.8
Ascomycota	Ss3c21	Myxotrichum deflexum	AY541491	1471	0	96.4
Ascomycota	Ss3c22a*	Neonectria radicicola var. radicicola	AY283552	1154	0	100.0
Ascomycota	Ss3c22b	Myxotrichum deflexum	AY541491	1503	0	96.5
Ascomycota	Ss3c23a*	Neofabraea malicorticis	AY544662	1465	0	96.6
Ascomycota	Ss3c23b	Hyphodiscus hymeniophilus	DQ227263	1425	0	95.8
Ascomycota	Ss3c24*	Talaromyces emersonii	DQ010015	1384	0	96.0
Ascomycota	Ss3c26*	Myxotrichum deflexum	AY541491	1487	0	96.3
Ascomycota	Ss3c28*	Talaromyces emersonii	DQ010015	1407	0	96.3
Ascomycota	Ss3c29*	Lasallia pustulata	AY300839	1350	0	94.6
Ascomycota	Ss3c3	Talaromyces emersonii	DQ010015	1380	0	96.0
Ascomycota	Ss3c31*	Aleurodiscus farlowii	AY039323	1651	0	99.2
Ascomycota	Ss3c34*	Porpidia soredizodes	AY532965	1057	0	91.0
Ascomycota	Ss3c35	Chaetomium sphaerale	AF286407	1528	0	97.2
Ascomycota	Ss3c36*	Myxotrichum deflexum	AY541491	1542	0	97.0
Ascomycota	Ss3c38*	Geoglossum glutinosum	AY789310	1715	0	99.2
Ascomycota	Ss3c39	Geoglossum glutinosum	AY789310	1744	0	99.6
Ascomycota	Ss3c41	Talaromyces emersonii	DQ010015	1376	0	96.0
Ascomycota	Ss3c43*	Umbilicaria nylanderiana	AY603119	648	0	87.3
Ascomycota	Ss3c47*	Phialophora verrucosa	AF050283	1536	0	97.4
Ascomycota	Ss3c49*	Hyaloscypha daedaleae	AY789415	1528	0	97.4
Ascomycota	Ss3c50*	Exophiala pisciphila	DQ823101	1731	0	99.6
Ascomycota	Ss3c51*	Hypocrea lutea	AB027384	1663	0	99.2
Ascomycota	Ss3c54*	Hydrocina chaetocladia	AY789412	1520	0	97.4
Ascomycota	Ss3c55*	Uncultured mycorrhizal fungus	AY394911	749	0	92.6
Ascomycota	Ss3c58*	Lasallia pustulata	AY300839	1263	0	93.3
Ascomycota	Ss3c59	Umbilicaria nylanderiana	AY603119	648	0	87.3
Ascomycota	Ss3c61*	Lasallia pustulata	AY300839	1302	0	94.5
Ascomycota	Ss3c62	Talaromyces emersonii	DQ010015	1392	0	96.2
Ascomycota	Ss3c64	Myxotrichum deflexum	AY541491	1513	0	96.6
Ascomycota	Ss3c65	Talaromyces emersonii	DQ010015	1402	0	96.2
Ascomycota	Ss3c69*	Uncultured mycorrhizal fungus	AY394911	664	0	90.7

Ascomycota	Ss3c70	Uncultured mycorrhizal fungus	AY394911	664	0	90.7
Ascomycota	Ss3c71	Umbilicaria nylanderiana	AY603119	632	5.00E-178	87.0
Ascomycota	Ss3c73	Talaromyces emersonii	DQ010015	1380	0	96.2
Ascomycota	Ss3c75	Uncultured mycorrhizal fungus	AY394911	648	0	90.4
Ascomycota	Ss3c77*	Uncultured mycorrhizal fungus	AY394911	836	0	94.4
Ascomycota	Ss3c78*	Crinula caliciiformis	AY544680	1431	0	95.5
Ascomycota	Ss3c79	Talaromyces emersonii	DQ010015	1372	0	96.1
Ascomycota	Ss3c80	Talaromyces emersonii	DQ010015	1405	0	96.2
Ascomycota	Ss3c81	Uncultured mycorrhizal fungus	AY394911	672	0	90.9
Ascomycota	Ss3c84*	Talaromyces emersonii	DQ010015	1384	0	96.0
Ascomycota	Os3c9	Uncultured mycorrhizal fungus	AY394911	662	0	91.1
Ascomycota	Ss3c90	Umbilicaria nylanderiana	AY603119	640	2.00E-180	87.2
Ascomycota	Ss3c93*	Hydrocina chaetocladia	AY789412	1546	0	97.4
Basal	10C2004	Mortierella verticillata	DQ273794	1645	0	95.8
Basal	10C604	Mortierella verticillata	DQ273794	272	3.00E-69	86.8
Basal	12C404	Mortierella polycephala	AF113464	545	1.00E-151	94.4
Basal	14C904	Mortierella verticillata	DQ273794	1676	0	95.9
Basal	16C404	Mortierella verticillata	DQ273794	1679	0	96.0
Basal	18C1306	Uncultured eukaryote	AY332056	281	2.00E-72	95.1
Basal	18C1406	Glomus sp.	AJ271925	367	2.00E-98	89.8
Basal	18C1506	Spiromyces minutus	AF031070	278	2.00E-71	94.9
Basal	18C25a06	Mortierella verticillata	DQ273794	1043	0	97.6
Basal	18C3A506	Nowakowskiella sp.	DQ273798	385	2.00E-103	91.8
Basal	18C3A706	Mortierella verticillata	DQ273794	1511	0	99.5
Basal	18C3B506	Mortierella verticillata	DQ273794	1211	0	94.8
Basal	18C3E706	Mortierella verticillata	DQ273794	1513	0	99.6
Basal	18C3H306	Mortierella verticillata	DQ273794	1211	0	94.8
Basal	18C6R04	Mortierella verticillata	DQ273794	1998	0	99.7
Basal	18C9R04	Mortierella verticillata	DQ273794	1669	0	98.2
Basal	18CA506	Glomus sp.	AJ271925	438	1.00E-119	90.8
Basal	18CG606	Mortierella verticillata	DQ273794	1509	0	99.6
Basal	20C104	Glomus sp.	AJ271925	783	0	90.3
Basal	20C107R04	Rhizophydium sp.	DQ485552	975	0	91.4
Basal	20C11106	Rhizophydium sp.	DQ485560	1316	0	97.4
Basal	20C1406	Rhizophydium sp.	DQ485552	912	0	90.9
Basal	20C145R04	Glomus sp.	AJ271925	1170	0	96.1
Basal	20C146R04	Mortierella verticillata	DQ273794	1251	0	95.0
Basal	20C15104	Glomus sp.	AJ271925	297	4.00E-77	90.2
Basal	20C167R04	Uncultured eukaryote	AY332056	281	3.00E-72	95.1
Basal	20C178R04	Glomus sp.	AJ271925	775	0	90.1
Basal	20C185R04	Mortierella verticillata	DQ273794	1515	0	99.1
Basal	20C188R04	Mortierella verticillata	DQ273794	1168	0	94.2
Basal	20C204	Glomus sp.	AJ271925	783	0	90.3
Basal	20C2206	Rhizophydium sp.	DQ485552	946	0	91.2
Basal	20C3004	Glomus sp.	AJ271925	751	0	89.4
Basal	20C3006	Umbelopsis fusiformis	AB090296	250	7.00E-63	94.6
Basal	20C3104	Glomus sp.	AJ271925	765	0	90.1
Basal	20C3106	Mortierella verticillata	DQ273794	1515	0	99.6
Basal	20C3306	Glomus sp.	AJ271925	749	0	89.8
Basal	20C3404	Glomus sp.	AJ271925	1152	0	96.1
Basal	20C3804	Rhizophydium sp.	DQ485552	967	0	91.3
Basal	20C4006	Mortierella verticillata	DQ273794	1156	0	94.2
Basal	20C4104	Kuzuhaea moniliformis	DQ273796	262	3.00E-66	93.8
Basal	20C4106	Mortierella verticillata	DQ273794	1207	0	94.7
Basal	20C4404	Monoblepharella sp.	AY546687	404	2.00E-109	91.7
Basal	20C4504	Mortierella verticillata	DQ273794	1626	0	95.6
Basal	20C4904	Glomus sp.	AJ271925	763	0	90.1
Basal	20C5004	Smittium tronadorium	DQ367505	222	2.00E-54	92.9
Basal	20C5504	Rhizophydium sp.	DQ485552	975	0	91.4
Basal	20C61R04	Rhizophydium sp.	DQ485552	975	0	91.4
Basal	20C6404	Mortierella polycephala	AF113464	264	5.00E-67	96.2
Basal	20C67R04	Glomus sp.	AJ271925	1170	0	96.1
Basal	20C78R04	Mortierella verticillata	DQ273794	1526	0	99.1
Basal	20C8104	Rhopalomyces elegans	DQ273795	325	2.00E-85	96.4

Basal	2C10304b	Mortierella verticillata	DQ273794	507	2.00E-140	95.0
Basal	2C10504	Rhizophydium sp.	DQ485552	975	0	91.4
Basal	2C1104	Glomus sp.	AJ271925	1150	0	96.1
Basal	2C133R04	Mortierella verticillata	DQ273794	1122	0	94.3
Basal	2C13904	Glomus sp.	AJ271925	305	2.00E-79	90.6
Basal	2C15304	Rhizophydium sp.	DQ485560	1594	0	99.8
Basal	2C155R04	Rhizophydium sp.	DQ485552	967	0	91.3
Basal	2C15904	Chytridium sp.	DQ273831	513	4.00E-142	95.6
Basal	2C160R04	Mortierella verticillata	DQ273794	1098	0	93.9
Basal	2C161R04	Rhizophydium sp.	DQ485552	700	0	90.4
Basal	2C164R04	Rhizophydium sp.	DQ485552	975	0	91.8
Basal	2C165R04	Rhizophydium sp.	DQ485552	983	0	91.9
Basal	2C168R04	Rhizophydium sp.	DQ485552	975	0	91.4
Basal	2C1804	Mortierella verticillata	DQ273794	1697	0	96.1
Basal	2C181R04	Mortierella verticillata	DQ273794	1146	0	94.6
Basal	2C188R04	Mortierella verticillata	DQ273794	1135	0	94.3
Basal	2C19104	Powellomyces sp.	DQ273776	1374	0	93.7
Basal	2C194R04	Mortierella verticillata	DQ273794	1146	0	94.6
Basal	2C197R04	Rhizophydium sp.	DQ485552	1477	0	99.7
Basal	2C2004	Mortierella verticillata	DQ273794	1628	0	95.5
Basal	2C204R04	Mortierella verticillata	DQ273794	1122	0	94.3
Basal	2C205R04	Mortierella verticillata	DQ273794	1138	0	94.5
Basal	2C206R04	Smittium tronadorium	DQ367505	222	2.00E-54	92.9
Basal	2C20804	Powellomyces sp.	DQ273776	295	2.00E-76	96.5
Basal	2C209R04	Smittium tronadorium	DQ367505	222	2.00E-54	92.9
Basal	2C210R04	Mortierella verticillata	DQ273794	1090	0	93.8
Basal	2C211R04	Mortierella verticillata	DQ273794	1090	0	93.8
Basal	2C217R04	Mortierella verticillata	DQ273794	1423	0	99.2
Basal	2C218R04	Rhizophydium sp.	DQ485552	1477	0	99.7
Basal	2C2204	Monoblepharella sp.	AY546687	422	1.00E-114	92.0
Basal	2C2504	Mortierella verticillata	DQ273794	880	0	95.1
Basal	2C2904	Rhizophydium sp.	DQ485552	959	0	91.1
Basal	2C3004*	Conidiosporomyces ayresii	AY819017	367	5.00E-98	91.0
Basal	2C3804	Rhizophydium sp.	DQ485552	1382	0	98.1
Basal	2C3904	Kappamyces sp.	DQ485542	1245	0	96.6
Basal	2C4004	Mortierella verticillata	DQ273794	1990	0	99.7
Basal	2C504	Chytridium sp.	DQ273831	264	6.00E-67	91.9
Basal	2C7404	Mortierella verticillata	DQ273794	1951	0	99.3
Basal	2C7704	Glomus sp.	AJ271925	1144	0	95.9
Basal	2C8004	Paraglomus occultum	DQ273827	478	2.00E-131	93.8
Basal	2C8804	Mortierella verticillata	DQ273794	1671	0	95.9
Basal	2C8806	Glomus sp.	AJ271925	306	9.00E-80	90.6
Basal	2C8906	Rhizophydium sp.	DQ485560	1425	0	99.3
Basal	2C9006	Glomus cf. claroideum	AY639334	117	7.00E-23	86.0
Basal	2C9204	Chytriomyces angularis	DQ273815	488	2.00E-134	94.0
Basal	2C9504	Mortierella verticillata	DQ273794	1675	0	96.1
Basal	2C9506	Mortierella verticillata	DQ273794	1209	0	94.8
Basal	2C9506	Mortierella verticillata	DQ273794	1209	0	94.8
Basal	2C9704	Mortierella verticillata	DQ273794	1965	0	99.5
Basal	2C9904	Rhizophlyctis harderi	DQ273775	628	8.00E-177	96.7
Basal	4C1404	Glomus sp.	AJ271925	1152	0	96.1
Basal	4C1704	Mortierella verticillata	DQ273794	1624	0	96.1
Basal	4C3204	Nowakowskiella sp.	DQ273798	677	0	94.5
Basal	4C4304	Mortierella verticillata	DQ273794	1675	0	95.9
Basal	4C4404	Glomus sp.	AJ854649	274	6.00E-70	96.3
Basal	4C8906	Basidiobolus ranarum	AF113452	262	2.00E-66	95.6
Basal	4C8906	Basidiobolus ranarum	AF113452	262	2.00E-66	95.6
Basal	4CD206	Dissophora decumbens	AF157187	448	1.00E-122	91.1
Basal	7C304	Rhizophydium sp.	DQ485552	1469	0	99.6
Basal	8C304	Mortierella verticillata	DQ273794	1631	0	95.5
Basal	Os1c1	Mortierella verticillata	DQ273794	1608	0	95.5
Basal	Os1c11	Mortierella sp.	DQ273786	759	0	92.5
Basal	Os1c26*	Uncultured eukaryote	AY332057	149	2.00E-32	83.1
Basal	Os1c39	Mortierella verticillata	DQ273794	1649	0	95.6

Basal	Os1c40	Mortierella verticillata	DQ273794	1982	0	99.6
Basal	Os1c49	Mortierella verticillata	DQ273794	1976	0	99.6
Basal	Os1c68	Triparticalcar arcticum	DQ273826	299	1.00E-77	87.2
Basal	Os1c69	Umbelopsis roseonana	AB090302	1114	0	96.8
Basal	Os1c71	Mortierella verticillata	DQ273794	1649	0	95.6
Basal	Os1c73	Rhizophydium sp.	DQ485560	1570	0	99.4
Basal	Os1c74	Mortierella verticillata	DQ273794	1966	0	99.4
Basal	Os1c81	Rhizophydium sp.	DQ485552	1493	0	100.0
Basal	Os1c83	Rhopalomyces elegans	DQ273795	327	5.00E-86	95.5
Basal	Os1c85	Mortierella verticillata	DQ273794	1633	0	95.6
Basal	Os1c91*	Trichosporon brassicae	DQ377685	252	2.00E-63	93.6
Basal	Os1c93	Umbelopsis roseonana	AB090302	1249	0	99.2
Basal	Os2c13	Mortierella verticillata	DQ273794	1998	0	99.8
Basal	Os2c39	Uncultured fungus	DQ901000	690	0	95.6
Basal	Os2c4	Mortierella verticillata	DQ273794	1241	0	94.1
Basal	Os2c63	Piptocephalis corymbifera	AY546690	618	1.00E-173	94.7
Basal	Os3c1	Mortierella verticillata	DQ273794	442	1.00E-120	93.2
Basal	Os3c10	Mortierella verticillata	DQ273794	2008	0	99.8
Basal	Os3c2	Rhizophydium sp.	DQ485552	967	0	91.3
Basal	Os3c26	Rhizophydium sp.	DQ485552	1477	0	99.7
Basal	Os3c30	Mortierella verticillata	DQ273794	2000	0	99.3
Basal	Os3c31	Mortierella verticillata	DQ273794	2024	0	99.4
Basal	Os3c32	Mortierella verticillata	DQ273794	2008	0	99.3
Basal	Os3c33	Mortierella verticillata	DQ273794	1679	0	95.6
Basal	Os3c34	Mortierella verticillata	DQ273794	1655	0	95.6
Basal	Os3c36	Mortierella verticillata	DQ273794	1441	0	93.1
Basal	Os3c37	Mortierella verticillata	DQ273794	2020	0	99.6
Basal	Os3c41	Umbelopsis roseonana	AB090302	1257	0	99.4
Basal	Os3c42	Rhizophydium sp.	DQ485560	1467	0	97.8
Basal	Os3c48	Mortierella verticillata	DQ273794	1485	0	93.2
Basal	Os3c52	Rhizophydium sp.	DQ273837	682	0	88.1
Basal	Os3c6	Mortierella verticillata	DQ273794	1673	0	95.6
Basal	Os3c60*	Herpobasidium filicinum	AY512850	266	2.00E-67	94.8
Basal	Os3c65	Mortierella verticillata	DQ273794	1663	0	95.6
Basal	Os3c7	Umbelopsis roseonana	AB090302	1257	0	99.4
Basal	Os3c7	Uncultured fungus	DQ900993	258	4.00E-65	93.3
Basal	Os3c80	Mortierella verticillata	DQ273794	1568	0	94.5
Basal	Os3c81	Mortierella verticillata	DQ273794	1580	0	94.4
Basal	Os3c95	Mortierella verticillata	DQ273794	1999	0	99.5
Basal	Ss1c31	Umbelopsis roseonana	AB090302	1164	0	98.2
Basal	Ss1c43	Uncultured eukaryote	AY332053	1152	0	97.3
Basal	Ss1c5	Umbelopsis roseonana	AB090302	1241	0	99.1
Basal	Ss1c83	Umbelopsis roseonana	AB090302	1233	0	99.1
Basal	Ss1c94	Dissophora decumbens	AF157187	1213	0	96.3
Basal	Ss2c20	Umbelopsis roseonana	AB090302	1241	0	99.2
Basal	Ss2c26	Umbelopsis roseonana	AB090302	1251	0	99.2
Basal	Ss2c3	Umbelopsis roseonana	AB090302	1241	0	99.2
Basal	Ss2c32	Rhizophydium sp.	DQ485552	975	0	91.4
Basal	Ss2c34	Mortierella verticillata	DQ273794	1612	0	95.3
Basal	Ss2c35	Mortierella verticillata	DQ273794	1968	0	99.5
Basal	Ss2c39	Mortierella verticillata	DQ273794	1968	0	99.5
Basal	Ss2c40	Rhopalomyces elegans	DQ273795	327	5.00E-86	96.4
Basal	Ss2c55	Mortierella verticillata	DQ273794	1604	0	95.3
Basal	Ss2c56	Umbelopsis roseonana	AB090302	1249	0	99.2
Basal	Ss2c70	Mortierella verticillata	DQ273794	523	4.00E-145	95.9
Basal	Ss2c74	Umbelopsis roseonana	AB090302	1241	0	99.2
Basal	Ss2c75	Uncultured zygomycete	EF027378	1065	0	98.5
Basal	Ss2c77	Mortierella verticillata	DQ273794	1915	0	99.1
Basal	Ss3c10	Mortierella verticillata	DQ273794	1997	0	99.1
Basal	Ss3c11	Mortierella verticillata	DQ273794	2030	0	99.5
Basal	Ss3c14	Mortierella verticillata	DQ273794	2000	0	99.7
Basal	Ss3c19	Mortierella verticillata	DQ273794	2032	0	99.6
Basal	Ss3c30	Mortierella verticillata	DQ273794	408	2.00E-110	90.9
Basal	Ss3c4	Umbelopsis roseonana	AB090302	1263	0	99.4

Basal	Ss3c44	Uncultured Mucorales	DQ273563	1279	0	99.3
Basal	Ss3c48	Mortierella verticillata	DQ273794	2020	0	99.4
Basal	Ss3c53	Mortierella verticillata	DQ273794	1292	0	90.9
Basal	Ss3c6	Mortierella verticillata	DQ273794	2034	0	99.6
Basal	Ss3c60	Umbelopsis roseonana	AB090302	1257	0	99.4
Basal	Ss3c66	Mortierella verticillata	DQ273794	2020	0	99.7
Basal	Ss3c67	Mortierella verticillata	DQ273794	2022	0	99.6
Basal	Ss3c68	Mortierella verticillata	DQ273794	2004	0	99.5
Basal	Ss3c72	Mortierella verticillata	DQ273794	2038	0	99.6
Basal	Ss3c83	Umbelopsis roseonana	AB090302	1255	0	99.2
Basal	Ss3c85	Mortierella verticillata	DQ273794	2030	0	99.5
Basal	Ss3c86	Umbelopsis roseonana	AB090302	1257	0	99.4
Basal	Ss3c92	Mortierella verticillata	DQ273794	2020	0	99.7
Basal	Ss3c94	Umbelopsis roseonana	AB090302	1263	0	99.4
Basal	Ss3c95	Mucor hiemalis f. hiemalis	AY706246	966	0	93.0
Basal	Ss3c96	Mortierella verticillata	DQ273794	2012	0	99.3
Basidiomycota	10C1004*	Hymenogaster griseus	DQ133941	1723	0	99.2
Basidiomycota	10C1204	Volvariella hypopithys	AF261532	1091	0	90.9
Basidiomycota	10C1604	Inocybe sp.	AY380402	1725	0	99.6
Basidiomycota	10C1804	Inocybe sp.	AY380402	1731	0	99.6
Basidiomycota	10C1904	Tremellodendron sp.	AY745701	1505	0	96.5
Basidiomycota	10C204*	Volvariella hypopithys	AF261532	1068	0	90.6
Basidiomycota	10C2304	Sebacinaceae sp.	AJ534931	1536	0	97.8
Basidiomycota	10C2404	Inocybe sp.	AY380402	1707	0	99.5
Basidiomycota	10C2604	Volvariella gloiocephala	AY745710	1082	0	91.1
Basidiomycota	10C2704	Tremellodendron sp.	AY745701	1522	0	96.9
Basidiomycota	10C2804	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	10C3204	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	10C3404	Tremellodendron sp.	AY745701	1532	0	97.0
Basidiomycota	10C3804	Volvariella hypopithys	AF261532	1094	0	91.0
Basidiomycota	10C3904	Inocybe sp.	AY380402	1731	0	99.6
Basidiomycota	10C4004	Inocybe sp.	AY380402	1715	0	99.5
Basidiomycota	10C404	Inocybe lanatodisca	AY380382	1750	0	99.7
Basidiomycota	10C4304	Inocybe lanatodisca	AY380382	1671	0	99.7
Basidiomycota	10C4504	Inocybe sp.	AY380402	1731	0	99.6
Basidiomycota	10C4804	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	10C4904	Hymenogaster olivaceus	AF336256	1735	0	99.9
Basidiomycota	10C504	Inocybe sp.	AY380402	1752	0	99.6
Basidiomycota	10C5104	Hymenogaster olivaceus	AF336256	1735	0	99.9
Basidiomycota	10C5304	Inocybe lanatodisca	AY380382	1739	0	99.7
Basidiomycota	10C5704	Inocybe sp.	AY380402	1731	0	99.7
Basidiomycota	10C5804	Volvariella hypopithys	AF261532	1086	0	91.0
Basidiomycota	10C6004	Inocybe sp.	AY380402	1735	0	99.7
Basidiomycota	10C704*	Hymenogaster olivaceus	AF336256	1742	0	100.0
Basidiomycota	12C204*	Inocybe candidipes	AY239019	1491	0	96.9
Basidiomycota	12C604	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	12C704	Inocybe sp.	AY380402	1723	0	99.4
Basidiomycota	14C404	Inocybe candidipes	AY239019	1491	0	96.9
Basidiomycota	16C504*	Pycnobasidium sp.	AY323905	272	3.00E-69	94.8
Basidiomycota	18C1806*	Kriegeria eriophori	AY745728	1400	0	98.0
Basidiomycota	18C26a06	Inocybe sp.	AY380402	1479	0	99.4
Basidiomycota	18C3A906	Inocybe sp.	AY380402	1493	0	99.5
Basidiomycota	18C3B706	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	18C3C106*	Ustilago tritici	DQ094784	1441	0	98.6
Basidiomycota	18C3C306	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	18C3C706	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	18C3C906*	Agaricales sp.	AF261623	1296	0	96.9
Basidiomycota	18C3D306	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	18C3E506	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	18C3G706	Inonotus hispidus	AF518623	236	1.00E-58	91.6
Basidiomycota	18C3H906*	Leucosporidium scottii	AY646098	1185	0	94.6
Basidiomycota	18CC1006	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	18CC606	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	18CD1006	Inonotus hispidus	AF518623	244	4.00E-61	92.2

Basidiomycota	20C10006	Inocybe sp.	AY380402	1497	0	99.6
Basidiomycota	20C101R04	Inonotus hispidus	AF518623	236	1.00E-58	91.6
Basidiomycota	20C10206*	Sebacina vermifera	DQ983815	900	0	90.4
Basidiomycota	20C106	Inocybe sp.	AY380402	1489	0	99.5
Basidiomycota	20C10606	Inocybe sp.	AY380402	1489	0	99.5
Basidiomycota	20C10906	Uncultured basidiomycete	DQ341889	1001	0	96.8
Basidiomycota	20C11006	Hymenogaster olivaceus	AF336256	1518	0	99.9
Basidiomycota	20C11406	Hymenogaster olivaceus	AF336256	1560	0	100.0
Basidiomycota	20C114R04	Fomes fomentarius	DQ208419	1695	0	99.3
Basidiomycota	20C117R04	Fomes fomentarius	DQ208419	1695	0	99.3
Basidiomycota	20C127R04*	Uncultured fungus clone	AY179609	351	3.00E-93	87.8
Basidiomycota	20C1304	Inocybe lanatodisca	AY380382	1742	0	99.7
Basidiomycota	20C131R04	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	20C132R04	Inocybe sp.	AY380402	1530	0	99.5
Basidiomycota	20C13504*	Urediniomycete sp.	DQ363323	196	1.00E-46	92.3
Basidiomycota	20C136R04	Inocybe sp.	AY380402	1538	0	99.6
Basidiomycota	20C1404	Inocybe lanatodisca	AY380382	1741	0	99.7
Basidiomycota	20C148R04	Inocybe sp.	AY380402	1538	0	99.6
Basidiomycota	20C1504	Volvariella hypopithys	AF261532	1096	0	91.0
Basidiomycota	20C1604	Inocybe sp.	AY380402	1742	0	99.7
Basidiomycota	20C160R04	Fomes fomentarius	DQ208419	1695	0	99.3
Basidiomycota	20C163R04	Volvariella hypopithys	AF261532	1086	0	91.0
Basidiomycota	20C165R04	Hymenogaster olivaceus	AF336256	1538	0	99.7
Basidiomycota	20C170R04	Hymenogaster olivaceus	AF336256	1546	0	99.9
Basidiomycota	20C172R04	Cryptococcus gastricus	DQ645512	1671	0	99.7
Basidiomycota	20C173R04	Cryptococcus gastricus	DQ645512	1671	0	99.7
Basidiomycota	20C176R04	Hymenogaster olivaceus	AF336256	1546	0	99.9
Basidiomycota	20C1804	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	20C182R04	Uncultured mycorrhiza	AY634130	1138	0	92.1
Basidiomycota	20C2004	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	20C2006	Uncultured fungus	AY179609	159	2.00E-35	90.9
Basidiomycota	20C2104	Stephanospora caroticolor	AF518652	1491	0	96.6
Basidiomycota	20C2106	Inocybe sp.	AY380402	1483	0	99.6
Basidiomycota	20C2604	Volvariella hypopithys	AF261532	1096	0	91.0
Basidiomycota	20C3604	Trichosporon brassicae	DQ377685	268	4.00E-68	94.7
Basidiomycota	20C3704*	Tremellodendron sp.	AY745701	1542	0	97.2
Basidiomycota	20C3706*	Tricholoma fulvum	AY207309	1528	0	100.0
Basidiomycota	20C3806	Tricholoma fulvum	AY207309	1528	0	100.0
Basidiomycota	20C3906*	Trichosporon brassicae	DQ377685	268	3.00E-68	94.7
Basidiomycota	20C4206	Uncultured basidiomycete	DQ341889	993	0	96.6
Basidiomycota	20C4604	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	20C4704*	Volvariella hypopithys	AF261532	1096	0	91.0
Basidiomycota	20C4906	Inocybe sp.	AY380402	1503	0	99.6
Basidiomycota	20C504*	Uncultured mycorrhiza	AY634130	1179	0	92.5
Basidiomycota	20C5106*	Uncultured basidiomycete	DQ341889	1007	0	96.7
Basidiomycota	20C5204	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	20C5206	Inonotus hispidus	AF518623	236	1.00E-58	91.6
Basidiomycota	20C5304	Hymenogaster olivaceus	AF336256	1742	0	100.0
Basidiomycota	20C5306*	Clavaria redoleoalii	DQ284906	1084	0	93.4
Basidiomycota	20C5606*	Uncultured fungus	AY179609	159	2.00E-35	90.9
Basidiomycota	20C6004	Inocybe candidipes	AY239019	1467	0	96.5
Basidiomycota	20C604	Inocybe sp.	AY380402	1760	0	99.7
Basidiomycota	20C6206	Hymenogaster olivaceus	AF336256	1509	0	100.0
Basidiomycota	20C7006	Cryptococcus terricolus	AJ510144	1221	0	99.7
Basidiomycota	20C704	Inocybe lanatodisca	AY380382	1778	0	100.0
Basidiomycota	20C7106	Inocybe lanatodisca	AY380382	1511	0	99.7
Basidiomycota	20C7204	Leucosporidium antarcticum	DQ785787	1475	0	96.0
Basidiomycota	20C7206*	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	20C73R04*	Cryptococcus gastricus	DQ645512	1671	0	99.7
Basidiomycota	20C74C4104	Inocybe lanatodisca	AY380382	1778	0	100.0
Basidiomycota	20C7606*	Entoloma alpicola	AF261302	1362	0	97.9
Basidiomycota	20C76R04*	Laccaria ochropurpurea	AF261494	1645	0	98.7
Basidiomycota	20C79R04	Trechispora alnicola	AY635768	1509	0	98.8
Basidiomycota	20C8006	Hymenogaster olivaceus	AF336256	1518	0	99.9

Basidiomycota	20C804	Inocybe sp.	AY380402	1760	0	99.7
Basidiomycota	20C8206	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	20C82R04	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	20C8306	Hymenogaster olivaceus	AF336256	1524	0	100.0
Basidiomycota	20C8406	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	20C8504*	Trechispora alnicola	AY635768	1651	0	98.9
Basidiomycota	20C904*	Volvariella gloiocephala	AY745710	1098	0	91.1
Basidiomycota	20C91R04	Hymenogaster olivaceus	AF336256	1649	0	99.9
Basidiomycota	20C92R04	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	20C96R04	Volvariella hypopithys	AF261532	1082	0	90.9
Basidiomycota	20C98R04	Hymenogaster olivaceus	AF336256	1546	0	99.9
Basidiomycota	20C99R04*	Suillus luteus	AY586715	1520	0	99.9
Basidiomycota	2C10304a	Conidiosporomyces ayresii	AY819017	359	1.00E-95	90.9
Basidiomycota	2C11204	Entoloma alpicola	AF261302	1463	0	96.0
Basidiomycota	2C11304*	Itersonilia perplexans	DQ667161	398	2.00E-107	92.6
Basidiomycota	2C11504	Conidiosporomyces ayresii	AY819017	363	8.00E-97	90.9
Basidiomycota	2C11804*	Coprinellus micaceus	AY207182	1729	0	99.7
Basidiomycota	2C1302C134 R04	Inocybe lanatodisca	AY380382	1669	0	99.8
Basidiomycota	2C1304*	Tomentella botryoides	AY586717	1711	0	99.1
Basidiomycota	2C136R04	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	2C13804*	Trechispora farinacea	AF347089	1564	0	99.0
Basidiomycota	2C141R04	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	2C142R04	Inocybe sp.	AY380402	1647	0	99.5
Basidiomycota	2C14304*	Ramaria corrugata	AY586707	291	2.00E-75	98.7
Basidiomycota	2C144R04	Fomes fomentarius	DQ208419	1516	0	99.4
Basidiomycota	2C146R04*	Inonotus hispidus	AF518623	230	6.00E-57	91.7
Basidiomycota	2C148R04	Fomes fomentarius	DQ208419	1509	0	99.2
Basidiomycota	2C15004	Trichosporon brassicae	DQ377685	268	4.00E-68	94.7
Basidiomycota	2C1504	Uncultured fungus	AY179609	583	4.00E-163	95.3
Basidiomycota	2C15104	Leucosporidium antarcticum	DQ785787	1479	0	96.1
Basidiomycota	2C152R04*	Uncultured fungus	AY179609	306	7.00E-80	90.8
Basidiomycota	2C1604*	Hysterangium stoloniferum	AF336259	1475	0	96.2
Basidiomycota	2C180R04	Inonotus hispidus	AF518623	236	1.00E-58	91.6
Basidiomycota	2C19004*	Leucosporidium antarcticum	DQ785787	1475	0	96.0
Basidiomycota	2C19304*	Sebacina vermifera	AY505549	1275	0	93.4
Basidiomycota	2C199R04	Inonotus hispidus	AF518623	244	4.00E-61	92.2
Basidiomycota	2C201R04	Fomes fomentarius	DQ208419	1509	0	99.2
Basidiomycota	2C202R04	Inonotus hispidus	AF518623	244	2.00E-61	92.2
Basidiomycota	2C21404	Fomes fomentarius	DQ208419	1516	0	99.4
Basidiomycota	2C215R04	Entoloma undatum	AY207199	1629	0	99.5
Basidiomycota	2C6504	Fomes fomentarius	DQ208419	1697	0	99.0
Basidiomycota	2C7204*	Chalciporus piperatus	DQ534648	1671	0	99.2
Basidiomycota	2C8104*	Tomentella botryoides	AY586717	1629	0	98.1
Basidiomycota	2C8404	Trichosporon brassicae	DQ377685	268	4.00E-68	94.7
Basidiomycota	2C9404	Hygrocybe conica	AY684167	1530	0	96.6
Basidiomycota	2C9606*	Ganoderma tsugae	AY684163	1522	0	99.7
Basidiomycota	2C9804	Hygrocybe conica	AY684167	1461	0	96.2
Basidiomycota	4C10406	Inocybe sp.	AY380402	1491	0	99.5
Basidiomycota	4C10606	Inocybe sp.	AY380402	1489	0	99.5
Basidiomycota	4C10706	Inocybe sp.	AY380402	1491	0	99.6
Basidiomycota	4C10806	Inocybe sp.	AY380402	1489	0	99.6
Basidiomycota	4C11106	Inocybe sp.	AY380402	1489	0	99.5
Basidiomycota	4C11206	Inocybe lanatodisca	AY380382	1520	0	99.7
Basidiomycota	4C11806	Inocybe sp.	AY380402	1481	0	99.4
Basidiomycota	4C12506	Inocybe sp.	AY380402	1457	0	99.3
Basidiomycota	4C13506	Inocybe sp.	AY380402	1497	0	99.5
Basidiomycota	4C15006	Inocybe sp.	AY380402	708	0	97.9
Basidiomycota	4C1604*	Inocybe sp.	AY380402	1762	0	99.7
Basidiomycota	4C1904*	Marasmius scorodonius	AF261332	1756	0	100.0
Basidiomycota	4C2204	Inocybe sp.	AY380402	1695	0	99.5
Basidiomycota	4C2404	Inocybe sp.	AY380402	1695	0	99.5
Basidiomycota	4C2904*	Entoloma alpicola	AF261302	1570	0	97.5
Basidiomycota	4C3004	Trichosporon brassicae	DQ377685	268	4.00E-68	94.7

Basidiomycota	4C404	Inocybe candidipes	AY239019	1491	0	96.9
Basidiomycota	4c4104	Inocybe lanatodisca	AY380382	1682	0	99.9
Basidiomycota	4C4504	Inocybe sp.	AY380402	1727	0	99.4
Basidiomycota	4C5204	Inocybe lanatodisca	AY380382	1754	0	99.7
Basidiomycota	4C5404*	Inocybe lanatodisca	AY380382	1362	0	94.2
Basidiomycota	4C6304	Inonotus hispidus	AF518623	236	1.00E-58	91.6
Basidiomycota	4C6504	Uncultured basidiomycete	DQ341751	858	0	94.9
Basidiomycota	4C7004	Inocybe sp.	AY380402	1733	0	99.6
Basidiomycota	4C7104*	Uncultured basidiomycete	DQ341855	868	0	95.4
Basidiomycota	4C7304*	Cryptococcus magnus	AY953948	1227	0	96.3
Basidiomycota	4C7404	Inocybe sp.	AY380402	1729	0	99.3
Basidiomycota	4C7804	Inocybe sp.	AY380402	1741	0	99.7
Basidiomycota	4C8306	Inocybe sp.	AY380402	1453	0	99.5
Basidiomycota	4C8506	Inocybe sp.	AY380402	1505	0	99.6
Basidiomycota	4C9006	Uncultured mycorrhiza	AY634130	999	0	91.5
Basidiomycota	4C9106	Inocybe lanatodisca	AY380382	1518	0	99.9
Basidiomycota	4C92R06*	Uncultured fungus	AY179609	283	5.00E-73	89.7
Basidiomycota	4C9406	Inocybe lanatodisca	AY380382	1505	0	99.6
Basidiomycota	4C9606	Inocybe sp.	AY380402	1417	0	99.3
Basidiomycota	4CB106	Inocybe sp.	AY380402	1481	0	99.6
Basidiomycota	4CH106	Inocybe lanatodisca	AY380382	1481	0	99.6
Basidiomycota	6C704	Inocybe sp.	AY380402	1733	0	99.6
Basidiomycota	7C204*	Hygrocybe conica	AY684167	1655	0	98.0
Basidiomycota	Os1c14*	Uncultured basidiomycete	DQ341961	599	7.00E-168	95.5
Basidiomycota	Os1c17*	Hydnum rufescens	AJ406427	1610	0	99.0
Basidiomycota	Os1c2*	Uncultured Russula sp.	AB154741	1661	0	98.6
Basidiomycota	Os1c23	Cortinarius alboviolaceus	AY033136	1653	0	98.5
Basidiomycota	Os1c28*	Hygrocybe conica	AY684167	1507	0	96.1
Basidiomycota	Os1c3*	Hygrocybe conica	DQ071739	1703	0	99.0
Basidiomycota	Os1c31	Piloderma byssinum	AY586699	1505	0	97.6
Basidiomycota	Os1c32	Lactarius subpurpureus	AF218553	1697	0	98.8
Basidiomycota	Os1c35	Clavulina cristata	AM259213	1703	0	99.7
Basidiomycota	Os1c36	Inocybe pusio	AY388643	1532	0	96.9
Basidiomycota	Os1c37	Piloderma byssinum	AY586699	1505	0	97.6
Basidiomycota	Os1c42*	Sebacina vermifera	AY505549	1425	0	95.4
Basidiomycota	Os1c44	Leucosporidium scottii	AY646098	1467	0	96.3
Basidiomycota	Os1c46	Lactarius subpurpureus	AF218553	1691	0	98.8
Basidiomycota	Os1c48*	Fibulorhizoctonia sp.	AY635779	1421	0	93.8
Basidiomycota	Os1c5*	Uncultured basidiomycete	DQ273493	797	0	98.8
Basidiomycota	Os1c52*	Lactarius subpurpureus	AF218553	1667	0	98.4
Basidiomycota	Os1c54	Piloderma byssinum	AY586699	1497	0	97.5
Basidiomycota	Os1c58	Amanita muscaria	AF097367	1699	0	98.9
Basidiomycota	Os1c60*	Cortinarius alboviolaceus	AY033136	1675	0	98.8
Basidiomycota	Os1c61	Piloderma byssinum	AY586699	1475	0	97.4
Basidiomycota	Os1c62*	Piloderma byssinum	AY586699	1475	0	97.3
Basidiomycota	Os1c64*	Amanita muscaria	AF097367	1707	0	98.9
Basidiomycota	Os1c65	Cantharellus tubaeformis	AF287851	1744	0	99.2
Basidiomycota	Os1c72	Leucosporidium scottii	AY646098	1455	0	96.1
Basidiomycota	Os1c75*	Piloderma byssinum	AY586699	1459	0	97.2
Basidiomycota	Os1c76	Cantharellus tubaeformis	AF287851	1750	0	99.2
Basidiomycota	Os1c77*	Inocybe pusio	AY388643	1546	0	96.9
Basidiomycota	Os1c78*	Tricholoma apium	AY586721	1582	0	97.8
Basidiomycota	Os1c8*	Uncultured Atheliaceae	DQ273486	745	0	98.5
Basidiomycota	Os1c80	Cantharellus tubaeformis	AF287851	1744	0	99.2
Basidiomycota	Os1c82*	Leucosporidium scottii	AY646098	1469	0	96.2
Basidiomycota	Os1c84*	Fibulorhizoctonia sp.	AY635779	1602	0	93.3
Basidiomycota	Os1c90	Cryptococcus terricolus	AJ510144	1221	0	99.7
Basidiomycota	Os1c92	Cantharellus tubaeformis	AF287851	1661	0	99.3
Basidiomycota	Os2c20*	Christiansenia pallida	AJ406403	1074	0	93.1
Basidiomycota	Os2c50*	Uncultured basidiomycete	DQ273493	977	0	97.7
Basidiomycota	Os2c77	Cryptococcus neoformans	L14067	1195	0	92.1
Basidiomycota	Os2c88*	Mutinus elegans	AY574643	926	0	95.0
Basidiomycota	Os3c12*	Uncultured basidiomycete	DQ341961	632	5.00E-178	95.7
Basidiomycota	Os3c13*	Clavaria argillacea	AY463395	1122	0	91.4

Basidiomycota	Os3c15*	Clavulina cristata	AM259213	1735	0	99.8
Basidiomycota	Os3c16*	Leucosporidium scottii	AY646098	1491	0	96.1
Basidiomycota	Os3c21*	Hymenogaster decorus	AF336255	1697	0	99.0
Basidiomycota	Os3c25*	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	Os3c28	Trichosporon brassicae	DQ377685	268	4.00E-68	94.7
Basidiomycota	Os3c3	Uncultured fungus	AY179609	505	9.00E-140	95.0
Basidiomycota	Os3c38*	Clavulina cristata	AM259213	1733	0	99.8
Basidiomycota	Os3c4a*	Salal associated fungal clone	AY112932	605	1.00E-169	94.1
Basidiomycota	Os3c4b*	Trichosporon brassicae	DQ377685	270	8.00E-69	94.3
Basidiomycota	Os3c49*	Inocybe cf. serrata	AY380392	1164	0	92.4
Basidiomycota	Os3c54*	Cantharellus tubaeformis	AF287851	1788	0	99.2
Basidiomycota	Os3c56	Entoloma alpicola	AF261302	1576	0	97.3
Basidiomycota	Os3c5a*	Stephanospora caroticolor	AF518652	1503	0	96.6
Basidiomycota	Os3c5b*	Trichosporon brassicae	DQ377685	268	3.00E-68	94.7
Basidiomycota	Os3c62*	Cryptococcus neoformans	L14067	1215	0	92.0
Basidiomycota	Os3c63	Cantharellus tubaeformis	AF287851	1778	0	99.0
Basidiomycota	Os3c68*	Rhodocybe paurii	AY286004	1080	0	90.6
Basidiomycota	Os3c69*	Inocybe leiocephala	AY380383	1639	0	97.6
Basidiomycota	Os3c70	Inocybe leiocephala	AY380383	1651	0	97.7
Basidiomycota	Os3c73*	Ramariopsis kunzei	DQ284902	1029	0	95.3
Basidiomycota	Os3c75*	Trichosporon brassicae	DQ377685	268	4.00E-68	94.7
Basidiomycota	Os3c76	Trichosporon brassicae	DQ377685	268	3.00E-68	94.7
Basidiomycota	Os3c82*	Entoloma undatum	AY207199	1742	0	99.6
Basidiomycota	Os3c85	Cryptococcus podzolicus	AF075481	1229	0	99.8
Basidiomycota	Os3c87	Cryptococcus neoformans var. neoformans	AE017342	1229	0	92.2
Basidiomycota	Os3c92	Cryptococcus neoformans var. neoformans	AE017342	1215	0	91.9
Basidiomycota	Os3c96	Cryptococcus neoformans var. neoformans	AE017342	1219	0	92.2
Basidiomycota	Os3c97*	Cantharellales sp.	DQ915469	143	1.00E-30	88.6
Basidiomycota	Ss1c10*	Tylospora asterophora	AY463480	1364	0	94.7
Basidiomycota	Ss1c16*	Uncultured basidiomycete	DQ273493	1017	0	98.4
Basidiomycota	Ss1c18	Amanita muscaria	AF097367	1645	0	98.4
Basidiomycota	Ss1c2*	Amanita muscaria	DQ060887	835	0	94.3
Basidiomycota	Ss1c25	Russula densifolia	AB154704	1683	0	99.2
Basidiomycota	Ss1c33*	Tomentella fibrosa	AM412301	1489	0	96.6
Basidiomycota	Ss1c35b*	Sistotrema biggsiae	AM259217	1100	0	95.8
Basidiomycota	Ss1c38*	Sistotrema biggsiae	AM259217	1610	0	98.5
Basidiomycota	Ss1c41	Amanita muscaria	AF097367	1655	0	98.4
Basidiomycota	Ss1c50	Platygloea disciformis	AY629314	478	2.00E-131	94.3
Basidiomycota	Ss1c51*	Platygloea disciformis	AY629314	365	2.00E-97	92.1
Basidiomycota	Ss1c56*	Inocybe lacera	AY038318	1723	0	99.4
Basidiomycota	Ss1c57	Sistotrema biggsiae	AM259217	1629	0	98.7
Basidiomycota	Ss1c58*	Epulorhiza anaticula	AY243520	1471	0	98.1
Basidiomycota	Ss1c60	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	Ss1c62*	Sistotrema alboluteum	AJ606042	1495	0	96.8
Basidiomycota	Ss1c72	Sistotrema biggsiae	AM259217	1616	0	98.7
Basidiomycota	Ss1c78*	Inocybe lacera	AY038318	1610	0	98.5
Basidiomycota	Ss1c79*	Sistotrema biggsiae	AM259217	1560	0	98.1
Basidiomycota	Ss1c8*	Amanita muscaria	AF097367	1576	0	97.7
Basidiomycota	Ss1c80*	Russula densifolia	AB154704	1667	0	99.0
Basidiomycota	Ss1c86*	Inocybe flocculosa	AY380375	1435	0	95.7
Basidiomycota	Ss1c87*	Entoloma alpicola	AF261302	1633	0	98.2
Basidiomycota	Ss1c9	Amanita muscaria	AF097367	1641	0	98.6
Basidiomycota	Ss1c92*	Amanita muscaria	AF097367	1552	0	98.2
Basidiomycota	Ss2c1*	Russula sphagnophila	AF506464	1679	0	98.4
Basidiomycota	Ss2c16	Inonotus hispidus	AF518623	244	5.00E-61	92.2
Basidiomycota	Ss2c38*	Fibulorhizoctonia sp.	AY635779	1606	0	93.3
Basidiomycota	Ss2c43*	Fibulorhizoctonia sp.	AY635779	1554	0	93.2
Basidiomycota	Ss2c60	Uncultured basidiomycete	DQ273493	1047	0	99.3
Basidiomycota	Ss2c61*	Inocybe griseolilacina	AY380378	1570	0	97.5
Basidiomycota	Ss2c62	Inocybe griseolilacina	AY380378	1578	0	97.5
Basidiomycota	Ss2c89*	Fomes fomentarius	DQ208419	1741	0	99.3

Basidiomycota	Ss2c8	Inocybe griseolilacina	AY380378	1616	0	97.9
Basidiomycota	Ss3c15*	Clavaria redoleoalii	DQ284906	1269	0	93.9
Basidiomycota	Ss3c17	Clavaria redoleoalii	DQ284906	1253	0	93.7
Basidiomycota	Ss3c21	Pycnobasidium sp.	AY323905	272	3.00E-69	94.8
Basidiomycota	Ss3c25	Cryptococcus neoformans	L14067	1179	0	91.6
Basidiomycota	Ss3c27	Cryptococcus neoformans	L14067	1191	0	91.8
Basidiomycota	Ss3c46*	Uncultured basidiomycete	DQ273493	1088	0	99.1
Basidiomycota	Ss3c63*	Leucosporidium scottii	AY646098	1628	0	97.8
Basidiomycota	Ss3c7*	Sistotrema alboluteum	AJ606042	1532	0	96.7
Basidiomycota	Ss3c74	Uncultured basidiomycete	DQ273493	1096	0	99.3
Basidiomycota	Ss3c87*	Platygloea disciformis	AY629314	490	5.00E-135	94.4
Basidiomycota	Ss3c88	Cryptococcus neoformans	L14067	1183	0	91.6
Basidiomycota	Ss3c89	Uncultured basidiomycete	DQ273493	1114	0	99.7
Basidiomycota	Ss3c9*	Cryptococcus terricolus	AJ510144	1237	0	99.8
Cercozoa	14C1004	Thaumatomonas sp.	DQ980477	355	1.00E-94	88.0
Cercozoa	20C14104	Trachelocorythion pulchellum	DQ211611	289	1.00E-74	93.3
Cercozoa	20C3406	G.oviformis	X79511	157	7.00E-35	89.2
Cercozoa	2C17904*	Uncultured fungus	AY179609	422	1.00E-114	89.7
Cercozoa	2C19604	Assulina muscorum	DQ211607	287	4.00E-74	93.8
Cercozoa	2C3304	Euglypha rotunda	DQ211608	389	2.00E-104	92.2
Cercozoa	2C7304	Assulina muscorum	DQ211607	365	2.00E-97	99.5
Cercozoa	2C9406	G.oviformis	X79511	135	3.00E-28	87.3
Cercozoa	4C10906	G.oviformis	X79511	157	7.00E-35	89.2
Cercozoa	4C11906	G.oviformis	X79511	157	7.00E-35	89.2
Cercozoa	4C28R04	Bodomorpha sp.	DQ211603	254	4.00E-64	95.0
Cercozoa	4C5704*	Uncultured fungus	AY179609	446	1.00E-121	91.2
Cercozoa	Os1c86	Heteromita globosa	DQ086722	408	2.00E-110	91.3
Cercozoa	Os3c91*	Uncultured fungus	AY179609	593	6.00E-166	95.4
Cercozoa	Ss1c22	Cercomonas sp.	DQ211606	517	3.00E-143	100.0
Cercozoa	Ss1c26	Cercophora newfieldiana	AY780062	1574	0	98.8
Cercozoa	Ss3c2	Cercomonas sp.	DQ211606	535	1.00E-148	100.0
Cercozoa	Ss3c20	Thaumatomonas sp.	DQ980477	410	4.00E-111	90.5
Cercozoa	Ss3c32	Heteromita globosa	DQ086722	420	4.00E-114	91.5
Cercozoa	Ss3c8	Cercomonas sp.	DQ211606	537	3.00E-149	100.0
Cercozoa	Ss3c91	Thaumatomonas sp.	DQ980477	381	4.00E-102	87.6
Coelomata	20C11804	PREDICTED: Tribolium castaneum	XM_967210	42.1	2.1	100.0
Euglenozoa	Os3c72	Rhynchomonas nasuta	DQ086724	454	3.00E-124	93.6
Glaucocystophy-ceae	7C804	Glaucocystis nostochinearum	AY216931	250	9.00E-63	94.6
Metazoa	10C1404*	Herpetostrongylus pythonis	AM039750	399	9.00E-108	90.6
Metazoa	10C3504	Herpetostrongylus pythonis	AM039750	398	1.00E-107	90.6
Metazoa	10C4204	Herpetostrongylus pythonis	AM039750	398	1.00E-107	90.6
Metazoa	14C204	Herpetostrongylus pythonis	AM039750	391	3.00E-105	90.3
Metazoa	16C104	Mesenchytraeus rainierensis	AY227193	1876	0	96.9
Metazoa	16C204	Nausithoe rubra	AY920776	242	2.00E-60	96.5
Metazoa	16C304	Mesenchytraeus rainierensis	AY227193	1917	0	97.2
Metazoa	16C804	Mesenchytraeus rainierensis	AY227193	1863	0	96.6
Metazoa	18C1106	Milnesium sp.	AY210826	259	9.00E-66	94.4
Metazoa	18C504	Nausithoe rubra	AY920776	242	2.00E-60	96.5
Metazoa	18C8R04	Geomonhystera disjuncta	AF210408	276	2.00E-70	93.6
Metazoa	20C100R04	Cephalobus sp.	DQ903094	1522	0	99.0
Metazoa	20C104R04	Herpetostrongylus pythonis	AM039750	331	2.00E-87	89.5
Metazoa	20C1104	Cephalobus sp.	DQ903094	1974	0	99.0
Metazoa	20C11104	Isohypsibius sp.	DQ077800	307	5.00E-80	94.9
Metazoa	20C113R04	Chiloplacus sp.	DQ145634	149	2.00E-32	86.8
Metazoa	20C1204	Cephalobus sp.	DQ903094	1974	0	99.0
Metazoa	20C12904	Cephalobus sp.	DQ903094	1982	0	99.1
Metazoa	20C133R04	Mesenchytraeus rainierensis	AY303944	1344	0	96.2
Metazoa	20C1904	Cephalobus sp.	DQ903094	1974	0	99.0
Metazoa	20C2204	Acrobeloides buetschlii	DQ903081	2004	0	99.6
Metazoa	20C2804	Nausithoe rubra	AY920776	212	2.00E-51	95.4
Metazoa	20C3506	Mesenchytraeus rainierensis	AY303944	1257	0	96.0
Metazoa	20C3904	Tylencholaimus sp.	AY593028	2068	0	99.7

Metazoa	20C4204	Nausithoe rubra	AY920776	220	7.00E-54	96.2
Metazoa	20C4304	Fergusobia sp.	AY589379	175	4.00E-40	89.5
Metazoa	20C4804	Stenostomum leucops	AY157151	1275	0	90.1
Metazoa	20C4806	Mesenchytraeus rainierensis	AY303944	1257	0	96.0
Metazoa	20C5404	Cephalobus sp.	DQ903094	2030	0	99.7
Metazoa	20C5706	Cephalobus sp.	DQ903094	1465	0	99.0
Metazoa	20C5804	Stenostomum leucops	AY157151	1277	0	90.1
Metazoa	20C60R06	Haliclystus octoradiatus	AY920783	254	6.00E-64	95.0
Metazoa	20c62R04	Heterocheilus tunicatus	AF226592	228	3.00E-56	91.8
Metazoa	20C7806	Stenostomum leucops	AJ228801	823	0	88.9
Metazoa	20C90R04	Stenostomum leucops	AY157151	938	0	89.4
Metazoa	20C94R04	Herpetostrongylus pythonis	AM039750	339	1.00E-89	89.8
Metazoa	2C11104	Herpetostrongylus pythonis	AM039750	404	2.00E-109	90.7
Metazoa	2C172R04	Herpetostrongylus pythonis	AM039750	313	6.00E-82	89.4
Metazoa	2C18704	Nausithoe rubra	AY920776	242	2.00E-60	96.5
Metazoa	2C212R04	Herpetostrongylus pythonis	AM039750	313	6.00E-82	89.4
Metazoa	2C219R04	Herpetostrongylus pythonis	AM039750	313	6.00E-82	89.4
Metazoa	2C804	Herpetostrongylus pythonis	AM039750	402	9.00E-109	90.6
Metazoa	4C11306	Stenostomum leucops	AJ228801	842	0	89.1
Metazoa	4C4604	Nausithoe rubra	AY920776	234	4.00E-58	96.4
Metazoa	Os1c34	Nausithoe rubra	AY920776	224	4.00E-55	96.2
Metazoa	Os3c64	Herpetostrongylus pythonis	AM039750	422	1.00E-114	90.9
Metazoa	Ss1c1	Uncultured nematode	DQ086675	295	2.00E-76	93.9
Metazoa	Ss2c29	Carychium exiguum	AY465075	222	2.00E-54	95.6
Metazoa	Ss2c7	Nausithoe rubra	AY920776	230	7.00E-57	96.3
Rhodophyta	Os3c47	Martensia cf. fragilis	AF259449	113	1.00E-21	84.7
Stramenopiles	18C1706	Uncultured fungus	AY179608	529	5.00E-147	94.0
Stramenopiles	18C204	Chrysolepidomonas dendrolepidota	AF409121	343	6.00E-91	96.5
Stramenopiles	18C404	Sapromyces elongatus	AF119618	324	5.00E-85	97.3
Stramenopiles	20C10806	Pythium sylvaticum	AY598645	1520	0	99.9
Stramenopiles	20C125R04	Pythium sylvaticum	AY598645	1594	0	99.9
Stramenopiles	20C1906	Pythium intermedium	AY598647	1457	0	99.1
Stramenopiles	20C2904	Pythium pleroticum	AY598642	2099	0	100.0
Stramenopiles	20C63R04	Pythium sylvaticum	AY598645	1602	0	100.0
Viridiplantae	16C1004	Unidentified	AM161120	585	1.00E-163	94.3
Viridiplantae	18C3G506	Helicosporidium sp.	AF317894	309	9.00E-81	97.7
Viridiplantae	20C175R04	Pterosperma cristatum	DQ980474	236	1.00E-58	92.6
Viridiplantae	20C80R04	Pterosperma cristatum	DQ980474	236	1.00E-58	92.6
Viridiplantae	2C186R04	Neochloris aquatica	AF277653	809	0	88.7
Viridiplantae	2C1904	Heterochlamydomonas rugosa	AY206709	1633	0	99.2
Viridiplantae	2C220R04	Chloromonas clathrata	AF395508	712	0	95.3
Viridiplantae	2C4404	Heterochlamydomonas rugosa	AY206709	1633	0	99.2
Viridiplantae	Os3c58	Unidentified	AM161120	363	1.00E-96	98.5
Viridiplantae	Os3c89	Leskeodon auratus	AY452450	167	1.00E-37	91.7

6.2 Chapter 3

Table 6-2: List of 135 fruiting body species collected indicating which collections have an associated LSU sequence.

Total incidence within the plot is also shown for both collection years.

Order	Genus	Species	Accession #	Abundance			Collection ID
				2002a	2003b	Total	
Boletales	Austroboletus	gracilis		6	14	20	5, 10, 39, 86*, 87, 107, 434**
Boletales	Boletus	edulis		0	1	1	446**
Boletales	Boletus	impolitus aff.		1	1	2	243**, 417*
Boletales	Boletus	subvelutipes cf.		0	1	1	431**
Boletales	Gyroporus	purpurinus		0	4	4	444**, 461*
Boletales	Leccinum	crocipodium		4	0	4	75, 80**, 84, 305
Boletales	Leccinum	flavostipitatum aff.		1	0	1	71**
Boletales	Leccinum	scabrum		3	2	5	156, 210, 219, 463**
Boletales	Leccinum	sp.		0	1	1	432*
Boletales	Paxillus	involutus		3	2	5	89*, 218**, 222, 460*
Boletales	Suillus	punctipes		1	1	2	101*, 462**
Boletales	Tylopilus	felleus		0	1	1	453**
Agaricales	Agaricus	silvaticus		0	1	1	473**
Agaricales	Amanita	citrina		1	0	1	102**
Agaricales	Amanita	flavoconia		0	1	1	435**
Agaricales	Amanita	muscaria		0	2	2	447**
Agaricales	Amanita	vaginata aff.		2	1	3	109**, 254, 406
Agaricales	Armillaria	cepistipes		0	1	1	483**, 484*, 485*
Agaricales	Clitocybe	clavipes		1	0	1	322**
Agaricales	Clitocybe	odora		4	2	6	113**, 329*, 333*, 346*, 476*, 505
Agaricales	Cortinarius	acutus		7	2	9	154, 217, 229**, 251*, 256, 341*, 349, 467*
Agaricales	Cortinarius	infractus aff.		4	2	6	111**, 112*, 255, 359*, 456*, 472*
Agaricales	Cortinarius	sp.1		4	0	4	106, 241*, 362**, 371*
Agaricales	Cortinarius	sp.2		5	0	5	209**, 230*, 298*, 364*, 366*
Agaricales	Cortinarius	sp.3		1	0	1	238**
Agaricales	Cortinarius	sp.4		2	1	3	257, 278,487**
Agaricales	Cortinarius	sp.5		1	2	3	307, 488**
Agaricales	Cortinarius	sp.6		0	3	3	489, 492, 498**
Agaricales	Cortinarius	sp.7		0	1	1	443**
Agaricales	Cortinarius	sp.8		1	0	1	197**
Agaricales	Crepidotus	applanatus		0	1	1	415**
Agaricales	Crepidotus	mollis		1	0	1	136**
Agaricales	Entoloma	nidorosum		18	0	18	193*, 211, 231,

							233**, 234*, 250, 272, 273, 274*, 276*, 296, 308, 344, 345, 347*, 348*, 352, 353*
Agaricales	Entoloma	nigroviolaceum		0	1	1	426**
Agaricales	Entoloma	sinuatum		6	5	11	134**, 261, 284, 316, 335**, 337*, 423, 424, 429**
Agaricales	Entoloma	sp.1		1	0	1	237**
Agaricales	Entoloma	sp.2		1	2	3	240*, 500**, 413**
Agaricales	Fayodia	gracilipes		2	0	2	311, 317**
Agaricales	Galerina	marginata		6	2	8	228*, 252, 342**, 355*, 370*, 374*
Agaricales	Gastrocybe	sp.		1	1	2	92*, 416**
Agaricales	Gymnopilus	bellulus		4	2	6	223**, 225*, 306*, 327, 440*
Agaricales	Gymnopilus	sapineus		0	4	4	457**
Agaricales	Gymnopus	dryophilus		0	2	2	425**, 449*, 450*
Agaricales	Hebeloma	sp.1		1	0	1	198**
Agaricales	Hebeloma	sp.2		1	0	1	203*
Agaricales	Hebeloma	sp.3		1	0	1	338*
Agaricales	Hemimycena	sp.1		1	0	1	65**
Agaricales	Hemimycena	sp.2		2	0	2	318**, 319*
Agaricales	Hygrocybe	cantharellus aff.		1	0	1	12**
Agaricales	Hygrocybe	conica		1	0	1	44**
Agaricales	Hygrocybe	miniata cf.		0	1	1	486**
Agaricales	Hygrocybe	sp.		1	0	1	191**
Agaricales	Inocybe	geophylla		1	1	2	266, 454**
Agaricales	Inocybe	mixtilis		4	2	6	100, 104, 105, 155, 414*, 464**
Agaricales	Inocybe	sp.1		2	0	2	93**, 119
Agaricales	Inocybe	sp.2		2	0	2	64*, 66**
Agaricales	Inocybe	sp.3		2	0	2	24**, 73*
Agaricales	Inocybe	sp.4		2	0	2	127**, 159*
Agaricales	Inocybe	sp.5		1	0	1	239**
Agaricales	Inocybe	sp.6		1	1	2	94, 459**
Agaricales	Inocybe	sp.7		1	0	1	336**
Agaricales	Inocybe	sp.8		1	0	1	130**
Agaricales	Inocybe	sp.9		3	1	4	25**, 74**, 124, 504
Agaricales	Inocybe	sp.10		1	0	1	37**
Agaricales	Inocybe	sp.11		0	1	1	497**
Agaricales	Inocybe	sp.12		1	0	1	288**
Agaricales	Inocybe	sp.13		0	2	2	499, 501**
Agaricales	Inocybe	sp.14		1	2	3	3**, 506, 507
Agaricales	Laccaria	bicolor		2	0	2	213*, 356**
Agaricales	Laccaria	laccata		3	6	9	90**, 160**, 290, 409, 410*, 420*, 422*, 478*, 479*
Agaricales	Laccaria	sp.1		1	0	1	43**
Agaricales	Laccaria	sp.2		0	1	1	404**
Agaricales	Laccaria	sp.3		1	0	1	103**
Agaricales	Leptonia	incana		1	2	3	277**, 427*
Agaricales	Lycoperdon	molle		1	0	1	283**
Agaricales	Marasmius	scorodonius		0	1	1	419**
Agaricales	Micromphale	perforans		4	9	13	21, 40, 118**, 271*, 411
Agaricales	Mycena	leiana		1	1	2	162**
Agaricales	Mycena	polygramma		10	1	11	262, 263, 264*, 265**, 291, 294, 302, 315, 326, 343

Agaricales	Mycena	pura		5	1	6	199*, 201**, 214*, 275*, 287
Agaricales	Mycena	sp.1		1	0	1	324**
Agaricales	Mycena	sp.3		1	0	1	351**
Agaricales	Mycena	sp.4		1	0	1	372*
Agaricales	Mycena	sp.5		0	2	2	469**
Agaricales	Mycena	sp.6		0	1	1	400**
Agaricales	Mycena	sp.8		0	1	1	408*
Agaricales	Mycena	sp.10		0	1	1	502**
Agaricales	Nolanea	quadrata		0	1	1	445**
Agaricales	Nolanea	sp.1		1	0	1	196**
Agaricales	Nolanea	sp.2		0	1	1	403**
Agaricales	Panellus	serotinus		1	0	1	367**
Agaricales	Plicaturopsis	crispa		2	0	2	365, 376**
Agaricales	Pluteus	romellii		1	0	1	69**
Agaricales	Pluteus	sp.		1	0	1	8**
Agaricales	Psathyrella	multipedata		4	0	4	269, 270**, 282, 339*
Agaricales	Tricholoma	aurantium		4	2	6	81*, 157, 192, 227, 428**
Agaricales	Tricholoma	imbricatum		26	9	35	114, 120**, 137, 151, 153, 158, 200, 208, 220, 235*, 236*, 244*, 259, 260*, 280, 289, 293, 310, 312, 314, 323, 325, 331*, 332*, 357*, 358
Agaricales	Tricholoma	sejunctum		2	0	2	292*, 350**
Agaricales	Xerula	furfuracea		0	2	2	474**
Russulales	Lactarius	deliciosus		2	7	9	91*, 212, 430**
Russulales	Lactarius	rufus		14	1	15	216, 246**, 247, 248*, 249, 258*, 281, 285, 295, 297, 299, 334, 354, 361
Russulales	Lactarius	scrobiculatus		0	7	7	452**
Russulales	Lactarius	sp.1		2	1	3	46*, 202*, 437*
Russulales	Lactarius	sp.2		2	0	2	88*, 215
Russulales	Lactarius	sp.3		3	0	3	313, 330, 340**
Russulales	Lactarius	sp.5		1	0	1	126**
Russulales	Lactarius	subpurpureus		2	0	2	77*, 253*
Russulales	Russula	aeruginea aff.		0	2	2	433**
Russulales	Russula	brevipes aff.		2	10	12	108, 232**, 448**
Russulales	Russula	sp.1		5	3	8	72**, 79*, 85*, 245, 300, 402, 455*, 494
Russulales	Russula	sp.2		0	4	4	438**, 439*, 458*, 490
Russulales	Russula	sp.3		2	1	3	11*, 115, 407**
Russulales	Russula	sp.4		0	1	1	421**
Russulales	Stereum	rugosum		1	1	2	369**
Thelephorales	Boletopsis	grisea		0	1	1	471**
Thelephorales	Hydnellum	caeruleum		0	1	1	470**
Thelephorales	Phellodon	niger		1	1	2	95**, 442*
Polyporales	Daedaleopsis	confragosa		1	3	4	242**, 418*
Polyporales	Irpex	lacteus		1	2	3	375, 480**
Polyporales	Phlebia	radiata		0	1	1	491**
Polyporales	Polyporus	brumalis		2	0	2	131**, 378
Polyporales	Polyporus	varius aff.		1	4	5	26**
Polyporales	Tyromyces	chioneus		0	1	1	436**

Hymenochaetales	Trichaptum	abietinum		1	0	1	360**
Gomphales	Ramaria	sp.		1	0	1	9**
Geastrales	Geastrum	saccatum		1	0	1	110**
Cantharellales	Cantharellus	cibarius		0	2	2	451**
Cantharellales	Craterellus	lutescens		4	1	5	20, 22**, 38, 42, 477*
Cantharellales	Craterellus	tubaeformis	DQ898741	6	2	8	23, 41, 78**, 152, 226*, 268*, 441*, 493
Cantharellales	Clavulina	cristata	DQ898742	2	4	6	27*, 76, 465**, 466
Cantharellales	Hydnum	sp.B	DQ898743	1	2	3	123, 475**
Cantharellales	Hydnum	sp.A	DQ898744	2	1	3	6, 70**
Dacrymycetales	Dacrymyces	chrysospermus		2	2	4	363**, 379, 468*
Tremellales	Pseudohydnum	gelatinosum		2	3	5	224**, 309
Total	55	134		262	189	451	362

*LSU sequence associated with this collection

**Used to represent a unique OTU, submitted to GenBank

[a]number of fruiting bodies collections

[b]number of fruiting body observations per 5 x 5m^2 subplot

Table 6-3: List of Basidiomycota samples representing 192 OTUs from soil clone libraries and their associated top hit BLAST results from GenBank (queried August 17, 2007).

Order	OTU Label	Accession	GenBank Top Hit	Accession	Bit Score	E-value	% Identity
Boletales	20C99 Suillus [1p]		Suillus luteus	AY586715	1513	0	99.9
Boletales	2C72 Chalciporus [1p]		Chalciporus piperatus strain MB 04-001	DQ534648	1643	0	99.2
Boletales	TM02_218 Paxillus involutus [5f]		Paxillus involutus	AY612815	1459	0	100.0
Boletales	TM02_243 Boletus impolitus cf. [2f]		Xerocomus impolitus	AF139715	1552	0	97.9
Boletales	TM02_71 Leccinum flavostipitatum aff. [1f]		Leccinum flavostipitatum	AF139696	1677	0	100.0
Boletales	TM02_80 Leccinum crocipodium [5f]		Leccinum piceinum strain Lp1	DQ534614	1669	0	99.8
Boletales	TM03_431 Boletus subvelutipes cf. [1f]		Boletus coniferarum strain 7/94	AF456827	1408	0	97.2
Boletales	TM03_434 Austroboletus gracilis [20f]		Austroboletus gracilis strain 112/96	DQ534624	1469	0	96.9
Boletales	TM03_444 Gyroporus purpurinus [4f]		Austroboletus gracilis strain 112/96	DQ534624	1469	0	96.9
Boletales	TM03_446 Boletus edulis [1f]		Boletus edulis	DQ071747	1699	0	99.8
Boletales	TM03_453 Tylopilus felleus [1f]		Tylopilus felleus	AY586723	1682	0	99.7
Boletales	TM03_462 Suillus punctipes [2f]		Suillus punctipes	AY612826	1705	0	99.3
Boletales	TM03_463 Leccinum scabrum [5f]		Leccinum flavostipitatum	AF139696	1429	0	99.9
Atheliales	Os1c48 Piloderma [1b]		Piloderma lanatum voucher JS 22149 (O)	DQ469288	1602	0	98.4
Atheliales	Os1c62 Piloderma [5b]		Piloderma fallax voucher KHL 8545 (GB)	DQ469285	1606	0	98.9
Atheliales	Ss1c10 Piloderma [1b]		Piloderma lanatum voucher JS 22149 (O)	DQ469288	1374	0	95.4
Atheliales	Ss2c38 Piloderma [3b]		Piloderma lanatum voucher JS 22149 (O)	DQ469288	1465	0	96.3
Agaricales	10C57 Inocybe [32p]		Inocybe sp. PBM 2355	AY380402	1715	0	99.7
Agaricales	10C2 Volvariella [1p]		Volvariella hypopithys strain JMleg.AIME	AF261532	1039	0	90.4
Agaricales	20C26 Volvariella [6p]		Volvariella hypopithys strain JMleg.AIME	AF261532	1070	0	90.9
Agaricales	20C9 Volvariella [2b]		Volvariella gloiocephala isolate AFTOL-ID 890	AY745710	1070	0	91.0
Agaricales	2C112 Entoloma [1p]		Entoloma alpicola strain TB6415	AF261302	918	0	97.7
Agaricales	2C118 Coprinellus [1p]		Coprinellus micaceus	AY207182	1701	0	99.7
Agaricales	4C4 Inocybe [4p]		Inocybe candidipes	AY239019	1467	0	96.8
Agaricales	Os1c28 Hygrocybe [1b,2p]		Hygrocybe conica isolate AFTOL-ID 729	AY684167	1463	0	96.0
Agaricales	Os1c3 Hygrocybe [1b]		Hygrocybe conica	DQ071739	1673	0	99.0
Agaricales	Os1c60 Cortinarius [2b]		Cortinarius alboviolaceus strain IB19950329	AY033136	1631	0	98.7
Agaricales	Os1c78 Tricholoma [1b]		Tricholoma apium voucher EL37-99	DQ389736	1538	0	97.7
Agaricales	Os3c56 Entoloma [1b]		Entoloma alpicola strain	AF261302	1511	0	97.2

			TB6415				
Agaricales	Os3c68 Rhodocybe [1b]		Rhodocybe paurii	AY286004	1011	0	90.4
Agaricales	Os3c82 Entoloma [1b,1p]		Entoloma undatum	AY207199	1693	0	99.5
Agaricales	Ss1c2 Amanita [1b]		Amanita muscaria strain GAL16654	DQ060887	827	0	94.3
Agaricales	Ss1c56 Inocybe [1b]		Inocybe lacera	AY038318	1685	0	99.4
Agaricales	Ss1c78 Inocybe [1b]		Inocybe lacera	AY038318	1574	0	98.5
Agaricales	Ss1c8 Amanita [1b]		Amanita muscaria strain JM96/63	AF097367	1538	0	97.6
Agaricales	Ss1c86 Inocybe [1b]		Inocybe flocculosa	AY380375	1384	0	95.5
Agaricales	Ss1c92 Amanita [1b]		Amanita muscaria strain JM96/63	AF097367	1538	0	98.1
Agaricales	TM02_102 Amanita citrina [1f]		Amanita citrina strain JM96/61	AF097378	1731	0	100.0
Agaricales	TM02_103 Laccaria sp.3 [1f]		Laccaria ochropurpurea strain JM96/46	AF261494	1520	0	97.1
Agaricales	TM02_109 Amanita vaginata aff.* [3f]		Amanita fulva	AF097373	1590	0	97.8
Agaricales	TM02_111 Cortinarius infractus aff. [6f]		Cortinarius infractus IB19990669	AF388757	1691	0	99.4
Agaricales	TM02_113 Clitocybe odora [6f]		Clitocybe phyllophila	AY207157	1732	0	99.9
Agaricales	TM02_118 Micromphale perforans [13f]		Micromphale perforans isolate RV83/67	AF042628	1623	0	99.0
Agaricales	TM02_120 Tricholoma imbricatum [35f]		Tricholoma fulvum	AY207309	1721	0	99.9
Agaricales	TM02_127 Inocybe sp.4 [2f,3b]		Inocybe griseolilacina	AY380378	1572	0	97.8
Agaricales	TM02_134 Entoloma sinuatum [5f]		Entoloma alpicola strain TB6415	AF261302	1624	0	98.5
Agaricales	TM02_136 Crepidotus mollis [1f]		Crepidotus mollis	DQ986293	1719	0	100.0
Agaricales	TM02_160 Laccaria laccata* [5f]		Laccaria ochropurpurea strain JM96/46	AF261494	1622	0	99.3
Agaricales	TM02_162 Mycena leiana [2f]		Mycena leaiana strain DAOM167618	AF261411	1737	0	100.0
Agaricales	TM02_196 Nolanea sp.1 [1f]		Nolanea conferenda strain TB7660	AF261321	1651	0	99.0
Agaricales	TM02_197 Cortinarius sp.8 [1f]		Cortinarius illitus IB19630414	AF388751	1522	0	97.8
Agaricales	TM02_198 Hebeloma sp.1 [3f]		Hebeloma longicaudum strain DAOM176597	AF261515	1718	0	99.5
Agaricales	TM02_201 Mycena pura [6f]		Mycena pura strain JM98/136	AF261410	1659	0	99.1
Agaricales	TM02_209 Cortinarius sp.2 [5f]		Cortinarius multiformis IB19800618	AF388767	1655	0	99.0
Agaricales	TM02_223 Gymnopilus bellulus [6f]		Gymnopilus ferruginosus voucher BRV99/5	AY219596	1572	0	97.8
Agaricales	TM02_229 Cortinarius acutus [9f]		Cortinarius laetus IB19990518	AF388776	1631	0	98.6
Agaricales	TM02_233 Entoloma nidorosum [18f]		Entoloma nidorosum strain TB6263	AF261296	1729	0	100.0
Agaricales	TM02_238 Cortinarius sp.3 [1f]		Cortinarius anomalus IB19950138	AF388769	1281	0	98.0
Agaricales	TM02_239 Inocybe sp.5 [1f]		Inocybe flocculosa	AY380375	1417	0	95.9
Agaricales	TM02_24 Inocybe sp.3 [2f]		Inocybe alabamensis	AY536280	1441	0	96.0
Agaricales	TM02_25 Inocybe sp.9 [2f]		Inocybe flocculosa	AY380375	1376	0	94.9
Agaricales	TM02_265 Mycena polygramma [13f]		Mycena plumbea isolate AFTOL-ID 1631	DQ470813	1659	0	99.4
Agaricales	TM02_270 Psathyrella multipedata [4f]		Psathyrella spadicea isolate AFTOL-ID 1628	DQ470822	1673	0	99.2

Agaricales	TM02_277 Leptonia incana [3f]		Alboleptonia stylophora strain TB8475	AF261292	1505	0	96.8
Agaricales	TM02_283 Lycoperdon molle [1f]		Lycoperdon molle voucher MJ4260	DQ112566	1717	0	100.0
Agaricales	TM02_288 Inocybe sp.12 [1f,2b]		Inocybe pusio PBM 2297	AY388643	1516	0	97.0
Agaricales	TM02_3 Inocybe sp.14 [3f]		Inocybe leptocystis	AY380384	1665	0	99.2
Agaricales	TM02_317 Fayodia gracilipes [2f]		Fayodia gracilipes	DQ071744	1727	0	100.0
Agaricales	TM02_318 Hemimycena sp.2 [2f]		Hemimycena lactea	AY207209	1679	0	99.4
Agaricales	TM02_322 Clitocybe clavipes [1f]		Ampulloclitocybe clavipes strain JEJ.VA.587	AF261447	1737	0	100.0
Agaricales	TM02_324 Mycena sp.1 [1f]		Mycena rutilanthiformis isolate JM96/26	AF042606	1629	0	98.7
Agaricales	TM02_336 Inocybe sp.7 [1f]		Inocybe lanuginosa	AY038319	1705	0	99.8
Agaricales	TM02_342 Galerina marginata [8f]		Galerina marginata	AY207202	1715	0	99.8
Agaricales	TM02_350 Tricholoma sejunctum [2f]		Tricholoma apium voucher EL37-99	DQ389736	1598	0	98.2
Agaricales	TM02_351 Mycena sp.3 [1f]		Mycena pura	AY207244	1707	0	99.8
Agaricales	TM02_356 Laccaria bicolor [2f]		Laccaria bicolor isolate JM96/19	AF042588	1702	0	99.5
Agaricales	TM02_362 Cortinarius sp.1 [4f]		Cortinarius alboviolaceus strain IB19950329	AY033136	1624	0	98.5
Agaricales	TM02_43 Laccaria sp.1 [1f]		Laccaria ochropurpurea isolate AFTOL-ID 477	AY700200	1342	0	97.9
Agaricales	TM02_44 Hygrocybe conica [1f,1p]		Hygrocybe conica isolate AFTOL-ID 729	AY684167	1628	0	98.2
Agaricales	TM02_65 Hemimycena sp.7 [1f]		Pleurotopsis longinqua isolate RV95/473	AF042604	1329	0	95.9
Agaricales	TM02_66 Inocybe sp.2 [2f]		Inocybe griseolilacina	AY380378	1503	0	96.9
Agaricales	TM02_69 Pluteus romellii [1f]		Pluteus romellii isolate AFTOL-ID 625	AY634279	1550	0	99.2
Agaricales	TM02_8 Pluteus sp. [1f]		Pluteus ephebeus strain JB97/23	AF261574	1492	0	97.6
Agaricales	TM02_90 Laccaria laccata [4f,1p]		Laccaria ochropurpurea strain JM96/46	AF261494	1659	0	99.0
Agaricales	TM02_93 Inocybe sp.1 [2f]		Inocybe leptocystis	AY380384	1249	0	95.0
Agaricales	TM03_400 Mycena sp.6 [1f]		Mycena aurantiomarginata	AY207246	1645	0	99.1
Agaricales	TM03_403 Nolanea sp.2 [1f]		Alboleptonia stylophora strain TB8475	AF261292	1483	0	97.1
Agaricales	TM03_404 Laccaria sp.2 [1f]		Laccaria ochropurpurea isolate AFTOL-ID 477	AY700200	1584	0	99.3
Agaricales	TM03_413 Entoloma sp.2 [1f]		Entoloma undatum strain TB6398	AF261314	1520	0	97.0
Agaricales	TM03_415 Crepidotus applanatus [1f]		Crepidotus applanatus var. applanatus isolate MCA170	AF205694	1719	0	99.8
Agaricales	TM03_416 Gastrocybe sp. [2f]		Conocybe subpubescens	AY207177	1629	0	98.6
Agaricales	TM03_419 Marasmius scorodonius [1f,1p]		Marasmius scorodonius strain DAOM175382	AF261332	1727	0	100.0
Agaricales	TM03_425 Gymnopus dryophilus [2f]		Gymnopus dryophilus isolate AFTOL-ID 559	AY640619	1719	0	100.0
Agaricales	TM03_426 Entoloma nigroviolaceum cf. [1f]		Leptonia subserrulata strain TB6993	AF261291	1586	0	98.2
Agaricales	TM03_428 Tricholoma aurantium [6f]		Tricholoma pessundatum	AY207305	1705	0	99.7
Agaricales	TM03_435 Amanita flavoconia [1f]		Amanita franchetii	AF097381	1649	0	99.0

Agaricales	TM03_443 Cortinarius sp.7 [1f]		Cortinarius laetus IB19990518	AF388776	1711	0	99.8
Agaricales	TM03_445 Nolanea quadrata [1f]		Entoloma quadratum strain EQ7695	AF261303	1719	0	99.9
Agaricales	TM03_447 Amanita muscaria [2f,5b]		Amanita muscaria strain JM96/63	AF097367	1661	0	99.0
Agaricales	TM03_454 Inocybe geophylla [2f]		Inocybe fuscodisca	AY380376	1722	0	99.8
Agaricales	TM03_457 Gymnopilus sapineus [4f]		Gymnopilus sapineus	AY380362	1709	0	99.8
Agaricales	TM03_459 Inocybe sp.6 [2f]		Inocybe petiginosa strain DAOM174733	AF261510	1499	0	97.3
Agaricales	TM03_464 Inocybe mixtilis [6f]		Inocybe mixtilis	AY380387	1650	0	98.6
Agaricales	TM03_469 Mycena sp.5 [2f]		Mycena aurantiomarginata	AY207246	1653	0	98.9
Agaricales	TM03_473 Agaricus sylvaticus [1f]		Agaricus sylvaticus	AY207137	1717	0	99.9
Agaricales	TM03_474 Xerula furfuracea [2f]		Xerula furfuracea isolate AFTOL-ID 538	AY691890	1721	0	100.0
Agaricales	TM03_483 Armillaria cepistipes [1f]		Armillaria cepistipes strain CMW 6909	DQ338563	1714	0	99.9
Agaricales	TM03_486 Hygrocybe miniata cf. [1f]		Hygrocybe miniata strain DAOM169729	AF261452	1427	0	95.7
Agaricales	TM02_191 Hygrocybe sp. [1f]		Hygrocybe cantharellus isolate AFTOL-ID 1714	DQ457675	1047	0	91.1
Agaricales	TM03_487 Cortinarius sp.4 [3f]		Cortinarius bulliardii IB19920363	AF388782	1655	0	99.0
Agaricales	TM03_488 Cortinarius sp.5 [3f]		Cortinarius alboviolaceus strain IB19950329	AY033136	1639	0	98.7
Agaricales	TM03_498 Cortinarius sp.6 [3f]		Cortinarius elegantior IB19980248	AF388764	1579	0	99.3
Agaricales	TM03_500 Entoloma sp.2 [2f]		Entoloma undatum	AY207199	1631	0	98.7
Agaricales	TM03_501 Inocybe sp.13 [2f]		Inocybe leiocephala	AY380383	1258	0	98.0
Agaricales	TM03_502 Mycena sp.10 [1f]		Mycena tintinnabulum	AY207258	1520	0	98.9
Agaricales	TM02_237 Entoloma sp.1 [1f]		Entoloma nidorosum strain TB6263	AF261296	1354	0	98.7
Agaricales	TM03_497 Inocybe sp.11 [1f,2b]		Inocybe leiocephala	AY380383	1596	0	98.1
Agaricales	TM02_130 Inocybe sp.8 [1f]		Inocybe cf. maculata	AY038321	1602	0	98.4
Agaricales	TM02_37 Inocybe sp.10 [1f]		Inocybe pudica	AY038323	1314	0	95.0
Agaricales	TM02_74 Inocybe sp.9 [2f]		Inocybe alabamensis	AY536280	1437	0	96.0
Agaricales	TM03_429 Entoloma sinuatum* [5f,1p]		Entoloma alpicola strain TB6415	AF261302	1532	0	97.1
Agaricales	TM02_335 Entoloma sinuatum [1f,1b]		Entoloma alpicola strain TB6415	AF261302	1604	0	98.3
Agaricales	TM02_376 Plicaturopsis crispa [2f]		Plicaturopsis crispa isolate AFTOL-ID 1924	DQ470820	1694	0	99.9
Agaricales	Ss2c12 Clavaria [2b]		Clavaria redoleoalii isolate DJM1079	DQ284906	1247	0	93.9
Agaricales	4C71 Clavaria [2p]		Clavaria redoleoalii isolate DJM1079	DQ284906	1392	0	95.9
Agaricales	Os3c13 Clavaria [1b]		Clavaria argillacea	AY463395	1076	0	91.3
Agaricales	10C10 Hymenogaster [1p]		Hymenogaster griseus	DQ133941	1677	0	99.2
Agaricales	Os3c21 Hymenogaster [1b]		Hymenogaster decorus	AF336255	1657	0	99.0
Agaricales	20C53 Hymenogaster [9p]		Hymenogaster olivaceus	AF336256	1729	0	100.0
Agaricales	TM03_367 Panellus		Sarcomyxa serotina	DQ071731	1721	0	99.8

	serotinus [1f]						
Russulales	Os1c2 Russula [1b]		Russula risigallina voucher UE03.07.2003-08	DQ422022	1699	0	99.3
Russulales	Ss1c80 Russula [2b]		Russula densifolia clone:OSA-MY-1716	AB154704	1649	0	99.0
Russulales	Ss2c1 Russula [1b]		Russula aff. sapinea UE2005.09.07-03	DQ422031	1647	0	98.6
Russulales	TM02_126 Lactarius sp.5 [1f]		Lactarius citriolens voucher UE20.09.2004-03	DQ422003	1707	0	99.5
Russulales	TM02_232 Russula brevipes aff. [6f]		Russula cf. chloroides AT2003041	DQ422016	1633	0	98.1
Russulales	TM02_246 Lactarius rufus [17f]		Lactarius subsericatus voucher UE11.10.2004-8	DQ422011	1663	0	98.6
Russulales	TM02_340 Lactarius sp.3 [3f]		Lactarius citriolens voucher UE20.09.2004-03	DQ422003	1612	0	98.3
Russulales	TM02_72 Russula sp.1 [8f]		Russula emetica clone:OSA-MY-1765	AB154755	1507	0	97.0
Russulales	TM03_407 Russula sp.3 [3f]		Russula exalbicans	AY293209	1619	0	98.2
Russulales	TM03_421 Russula sp.4 [1f]		Uncultured Russula sp. clone:OSA-MY-1751	AB154741	1647	0	98.7
Russulales	TM03_430 Lactarius deliciosus [14f, 3b]		Lactarius quieticolor voucher UE10.09.2004-1	DQ422002	1651	0	98.7
Russulales	TM03_433 Russula aeruginea aff. [2f]		Russula aeruginea voucher AT2003017	DQ421999	1637	0	98.5
Russulales	TM03_438 Russula sp.2 [4f]		Russula aff. sapinea UE2005.09.07-03	DQ422031	1687	0	99.2
Russulales	TM03_448 Russula brevipes aff. [6f]		Russula aff. delica UE24.08.2004-20	DQ422005	1702	0	99.2
Russulales	TM03_452 Lactarius scrobiculatus [7f]		Lactarius citriolens voucher UE20.09.2004-03	DQ422003	1715	0	99.5
Russulales	TM02_369 Stereum rugosum [2f]		Hypsizygus tessulatus	AY293189	1687	0	99.4
Thelephorales	2C13 Tomentella [1p]		Tomentella botryoides	AY586717	1665	0	99.1
Thelephorales	2C81 Tomentella [1p]		Tomentella botryoides	AY586717	1594	0	98.1
Thelephorales	TM02_95 Phellodon niger [2f]		Phellodon niger	AY586694	1616	0	98.8
Thelephorales	TM03_470 Hydnellum caeruleum [1f]		Bankera fuligineoalba	AY586635	1663	0	99.4
Thelephorales	TM03_471 Boletopsis grisea [1f]		Boletopsis grisea	AY586636	1680	0	99.4
Thelephorales	Ss1c33 Pseudotomentella [1b]		Tomentella fibrosa	AM412301	1463	0	96.6
Polyporales	TM02_131 Polyporus brumalis [2f]		Polyporus brumalis	AJ406525	1701	0	99.7
Polyporales	Ss2c89 Ganoderma [1b,8p]		Ganoderma sp. DIS 276d	DQ674804	1685	0	99.4
Polyporales	TM02_242 Daedaleopsis confragosa [4f]		Hexagonia apiaria strain Wu 9906-13	AY351945	1695	0	99.7
Polyporales	TM02_26 Polyporus varius aff. [5f]		Hexagonia sp. Wu 9708-306	AY351938	1524	0	97.3
Polyporales	TM03_436 Tyromyces chioneus [2f]		Tyromyces chioneus	AF393080	1524	0	99.1
Polyporales	TM03_491 Phlebia radiata [1f]		Phlebia radiata	AF287885	1619	0	99.8
Polyporales	TM03_480 Irpex lacteus [3f]		Ceriporiopsis subvermispora	AF287853	1614	0	98.8
Hymenochaet-ales	TM02_360 Trichaptum abietinum [1f]		Trichaptum abietinum strain MPBTA-1	AY672927	1693	0	99.9
Phallales	Os2c88 Phallus [1b]		Mutinus elegans voucher OSC107657	AY574643	918	0	95.0
Hysterangiales	2C16 Hysterangium [1p]		Hysterangium stoloniferum	AF336259	1449	0	96.1
Gomphales	TM02_9 Ramaria sp. [1f]		Gomphus clavatus isolate AFTOL-ID 725	AY647207	1134	0	93.6
Geastrales	TM02_110 Geastrum		Geastrum saccatum	AF287859	1582	0	97.8

	saccatum [1f]						
Trechisporales	20C85 Trechispora [2p]		Trechispora alnicola isolate AFTOL-ID 665	AY635768	1586	0	98.8
Trechisporales	2C138 Trechispora [1p]		Trechispora farinacea isolate 356	AF347089	1556	0	99.0
Cantharellales	Ss1c72 Sistotrema [4b]		Sistotrema efibulatum isolate FCUG 1175	DQ898696	1663	0	99.9
Cantharellales	Ss3c7 Sistotrema [2b]		Sistotrema alboluteum isolate TAA 180259	AJ606042	1465	0	96.7
Cantharellales	TM02_22 Craterellus lutescens [5f]		Craterellus aurora	AF105304	993	0	99.2
Cantharellales	TM02_70 Hydnum sp.A [3f]	DQ898744	Hydnum sp. TM070	DQ898744	1683	0	100.0
Cantharellales	TM02_78 Craterellus tubaeformis [8f,6b]		Craterellus tubaeformis isolate TM 0268	DQ898741	1766	0	100.0
Cantharellales	TM03_451 Cantharellus cibarius* [2f]		Cantharellus cibarius isolate AFTOL-ID 971	AY745708	1657	0	99.9
Cantharellales	TM03_465 Clavulina cristata [6f,3b]	DQ898742	Clavulina cristata isolate TM 0465	DQ898742	1683	0	100.0
Cantharellales	TM03_475 Hydnum sp.B [3f,1b]	DQ898743	Hydnum sp. TM475	DQ898743	1683	0	100.0
Cantharellales	20C5 Uncultured mycorrhiza (Tulasnellaceae) [2p]		Uncultured mycorrhiza (Tulasnellaceae) 4065	AY634130	1150	0	92.3
Sebacinales	20C37 Sebacinaceae [3p]		Sebacinaceae sp. B18	AJ534931	1532	0	97.9
Sebacinales	2C193 Uncultured Sebacinales [1p]		Uncultured Sebacinales clone Permuc_6306	EF127230	1289	0	93.8
Sebacinales	Os1c42 Uncultured Sebacinales [1b]		Uncultured Sebacinales clone Permuc_6306	EF127230	1413	0	95.7
Dacrymycetal-es	TM02_363 Dacrymyces chrysospermus [4f]		Dacrymyces chrysospermus	AF287855	1594	0	99.9
Tremellales	TM02_224 Pseudohydnum gelatinosum [5f]		Pseudohydnum gelatinosum AFTOL-ID 1875	DQ520094	1645	0	98.8
Filobasidiales	Os3c62 Cryptococcus [9b]		Cryptococcus sp. HB1104 strain HB1104	AM039667	1187	0	99.8
Filobasidiales	Ss3c9 Cryptococcus [3b]		Cryptococcus terricolus strain HA1558	AJ510144	1223	0	99.8
Unassigned	Os3c5 Stephanospora [1b,1p]		Stephanospora caroticolor strain IOC-137/97	AF518652	1473	0	96.6
Outgroup	Os2c50 Uncultured basidiomycete [1b]		Uncultured basidiomycete clone S33	DQ273493	955	0	98.0
Outgroup	Ss1c16 Uncultured basidiomycete [1b]		Uncultured basidiomycete clone S33	DQ273493	1009	0	98.4
Outgroup	Ss3c89 Uncultured basidiomycete [4b]		Uncultured basidiomycete clone S33	DQ273493	1096	0	99.8
Outgroup/ Leucosporidiale s	Os3c16 Leucosporidium [4b]		Leucosporidium scottii isolate AFTOL-ID 718	AY646098	1425	0	96.0
Outgroup/ Leucosporidial-es	Ss3c63 Leucosporidium [1b]		Leucosporidium scottii isolate AFTOL-ID 718	AY646098	1557	0	97.9
Outgroup/ Corticiales	Ss1c51* Boidinia [1b]		Boidinia granulata strain Wu9209-39	AY048880	371	3.00E-99	87.9
Outgroup/ Platygloeales	Ss3c87 Platygloea [1b]		Platygloea disciformis isolate AFTOL-ID 710	AY629314	448	2.00E-122	94.0
Outgroup	Os3c12 Uncultured basidiomycete [2b]		Uncultured basidiomycete clone 146a_13 KBS-LTER	DQ341961	599	8.00E-168	95.7

6.3 Chapter 4

6.3.1 Methods

6.3.1.1 Additional phylogenetic analyses

The 5.8S matrix was constructed by adding SCGI-like sequences obtained from our study to an alignment that contained sequences representative of each major fungal phylum (Pringle et al., 2000). Because of the highly variable nature of the ITS1 and ITS2 regions, only the highly conserved 5.8S region could be aligned with confidence and is suitable for phylum-level resolution of taxa.

6.3.1.2 DNA pairwise sequence comparisons

Pairwise sequence comparisons were conducted in PAUP with the SSU and LSU regions that included taxa chosen to be representative of the Ascomycota (Lutzoni et al., 2004). For each subset of taxa (Pezizomycotina, Saccharomycotina, SCGI, Taphrinomycotina) the maximum number and percentage of pairwise sequences differences is reported.

6.3.1.3 Clustering of SCGI sequences into OTUs

SCGI sequences were sorted into operational taxonomic units (OTUs) based on 99% sequence similarity as a proxy for species. This level of sequence similarity cut-off has been used in previous studies using LSU sequence data (such as Schadt et al., 2003). Multiple pairwise sequence comparisons were conducted using Sequencher 4.1 (Gene Codes Corporation, MI).

Table 6-4: Number of SCGI taxa sampled from previously published work.

Sample Origin	# Group I sequences sampled	Total # sequences sampled	%	Reference
Bulk soil:	19	125[a]	15.2%	Schadt et al. (2003)
	18	120[b]	15%	Chen & Cairney (2002)
	11	48[c]	22.9%	Anderson et al. (2003)
	4	863[d]	0.5%	O'Brien et al. (2005)
	20	119[e]	16.8%	Jumpponen & Johnson (2005)
Bulked plant roots:	2	90[e]	2.2%	Jumpponen & Johnson (2005)
Ectomycorrhizal plant roots:	2	185[f]	1.1%	Rosling et al. (2003)
	1	150[g]	0.7%	Izzo et al. (2005)
	1	130[h]	0.8%	Menkis et al. (2005)
Fungal spore:	1	39[i]	2.6%	Pringle et al. (2000)

[a]Number of LSU sequences from 3 clone libraries

[b]Number of ITS-RFLP types from 361 ITS-PCR reactions

[c]Number of SSU sequences from 1 clone library

[d]Number of SSU sequences from 15 clone libraries

[e]Number of SSU-RFLP types from 480 SSU-PCR reactions

[f]Number of ITS sequences from 8,275 root tips examined

[g]Number of fRFLP types from 1,300 root tips examined

[h]Number of ITS sequences from 30,166 root tips examined

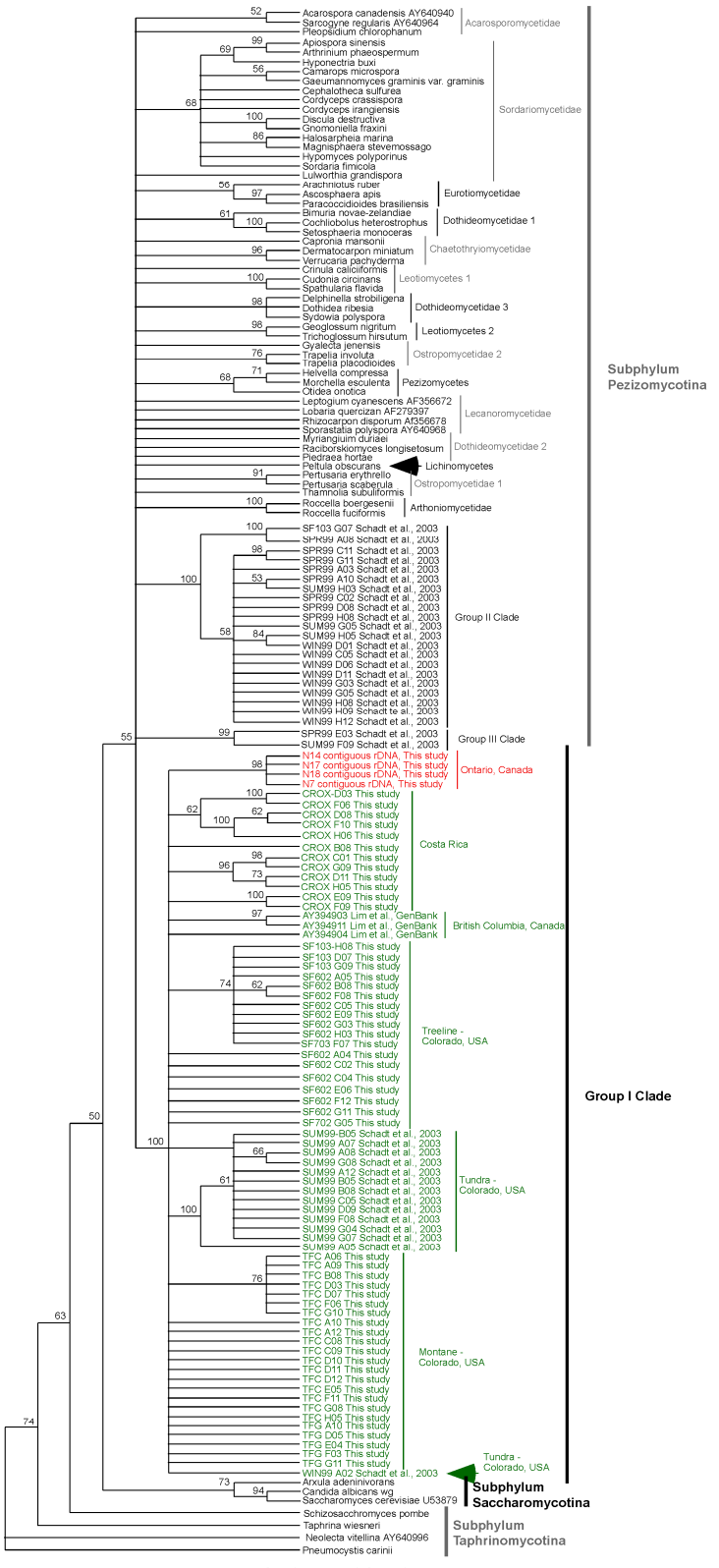
[i]Number of ITS sequences from 21 glomalean spores

Table 6-5: Maximum pairwise sequence differences among taxa in each subphylum for each partial SSU and LSU rDNA analysis conducted.

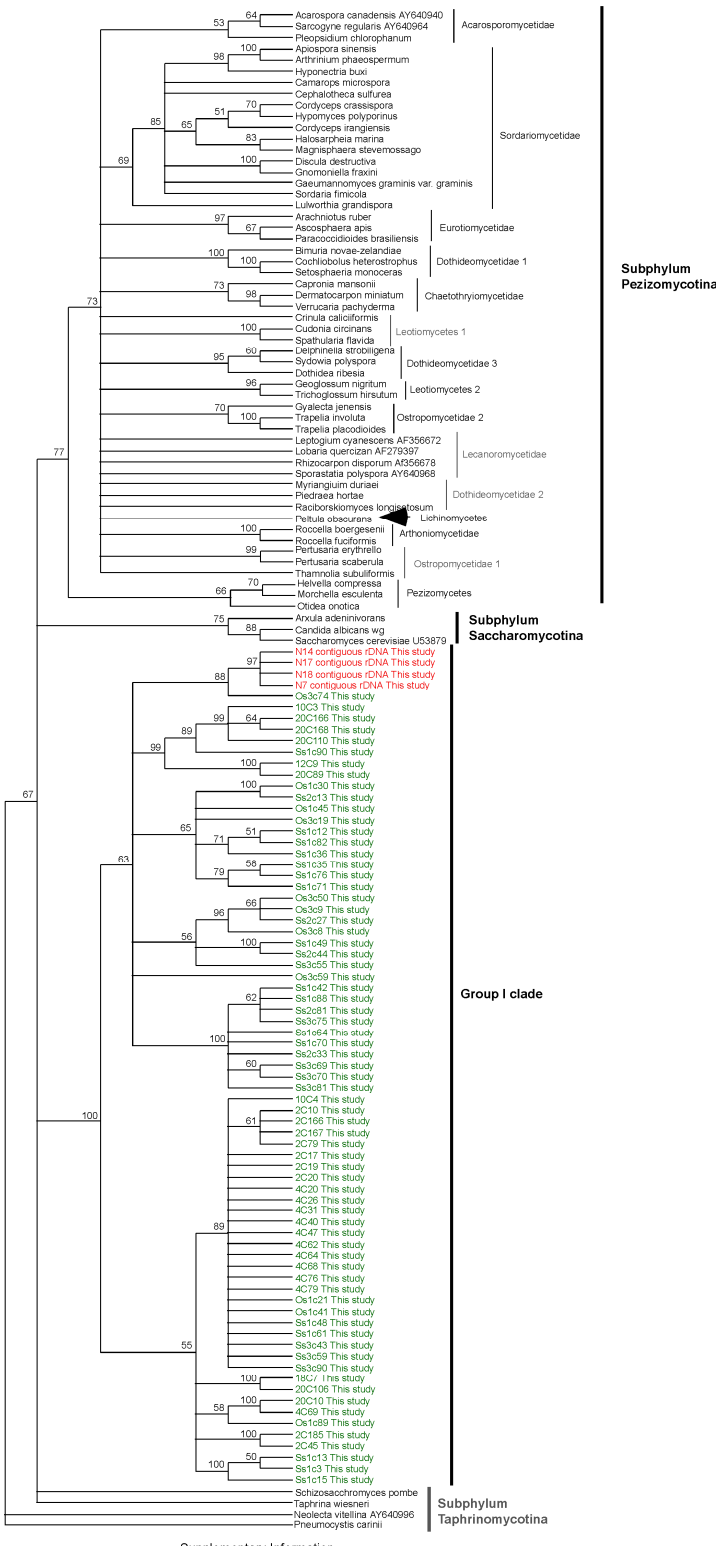
Figure	rDNA region (Number of included characters)	Subphylum	Maximum number of pairwise sequence differences	Maximum % pairwise sequence differences
5b	SSU (649 bp)	Pezizomycotina	124	19.1%
		Saccharomycotina	34	5.2%
		Taphrinomycotina	58	8.9%
		SCGI	41	6.3%
5a	SSU (713 bp)	Pezizomycotina	73	10.2%
		Saccharomycotina	16	2.2%
		Taphrinomycotina	38	5.3%
		SCGI	46	6.5%
4b	LSU (723 bp)	Pezizomycotina	135	18.7%
		Saccharomycotina	76	10.5%
		Taphrinomycotina	125	17.3%
		SCGI	63	8.7%
4a	LSU (305 bp)	Pezizomycotina	60	19.7%
		Saccharomycotina	37	12.1%
		Taphrinomycotina	45	14.8%
		SCGI	61	20.0%

Figure 6-1: LSU rDNA analyses.

A) Parsimony bootstrap consensus tree for 305 included characters from the LSU rDNA region for 158 taxa. The 2.4 kb contiguous rDNA sequences generated for this study are shown in red and SCGI taxa from Schadt et al. (2003) are shown in green. Representative Ascomycota sequences were selected from Lutzoni et al. (2004) and clades are named according to their convention. Monophyletic groups recovered in this analysis are indicated in black, otherwise they are labeled in grey. B) Parsimony bootstrap consensus tree for 723 included characters from the LSU rDNA region for 137 taxa. Our unclassified sequences are shown in green and the 2.4 kb contiguous rDNA sequences generated for this study are shown in red. Representative Ascomycota sequences were selected from Lutzoni et al. (2004) and clades are named according to their convention. Monophyletic groups recovered in this analysis are indicated in black, otherwise they are labeled in grey.



Supplementary Information
Figure 4A


Acarospora canadensis AY640940
Sarcogyne regularis AY640964
Pleopsidium chlorophanum
Acarosporomycetidae
Apiospora sinensis
Arthrinium phaeospermum
Hyponectria buxi
Camarops microspora
Cephalotheca sulfurea
Cordyceps crassispora
Hypomyces polyporinus
Cordyceps irangiensis
Halosarpheia marina
Magnisphaera stevemossago
Discula destructiva
Gnomoniella fraxini
Gaeumannomyces graminis var. graminis
Sordaria fimicola
Lulworthia grandispora
Sordariomycetidae
Arachniotus ruber
Ascosphaera apis
Paracoccidioides brasiliensis
Eurotiomycetidae
Bimuria novae-zelandiae
Cochliobolus heterostrophus
Setosphaeria monoceras
Dothideomycetidae 1
Capronia mansonii
Dermatocarpon miniatum
Verrucaria pachyderma
Chaetothryiomycetidae
Crinula caliciiformis
Cudonia circinans
Spathularia flavida
Leotiomycetes 1
Delphineilla strobilgena
Sydowia polyspora
Dothidea ribesia
Dothideomycetidae 3
Geoglossum nigritum
Trichoglossum hirsutum
Leotiomycetes 2
Gyalecta jenensis
Trapelia involuta
Trapelia placodioides
Ostropomycetidae 2
Leptogium cyanescens AF356672
Lobaria quercizan AF279397
Rhizocarpon disporum Af356678
Sporastatia polyspora AY640968
Myriangium duriaei
Dothideomycetidae 2
Piedraea hortae
Raciborskiomyces longisporosum
Peltula obscurans
Lichinomycetes
Roccella boergesenii
Roccella fuciformis
Arthoniomycetidae
Pertusaria erythrello
Pertusaria scaberula
Thamnolia subuliformis
Ostropomycetidae 1
Helvella compressa
Morchella esculenta
Otidea onotica
Pezizomycetes
Subphylum Pezizomycotina
Arxula adeninivorans
Candida albicans wg
Saccharomyces cerevisiae U53879
Subphylum Saccharomycotina
N14 contiguous rDNA This study
N17 contiguous rDNA This study
N18 contiguous rDNA This study
N7 contiguous rDNA This study
Os3c74 This study
10C3 This study
20C166 This study
20C168 This study
20C110 This study
Ss1c90 This study
12C9 This study
20C89 This study
Os1c30 This study
Ss2c13 This study
Os1c45 This study
Os3c19 This study
Ss1c12 This study
Ss1c82 This study
Ss1c36 This study
Ss1c35 This study
Ss1c76 This study
Ss1c71 This study
Os3c50 This study
Os3c9 This study
Ss2c27 This study
Os3c8 This study
Ss1c49 This study
Ss2c44 This study
Ss3c55 This study
Os3c59 This study
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Ss1c88 This study
Ss2c81 This study
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Ss1c64 This study
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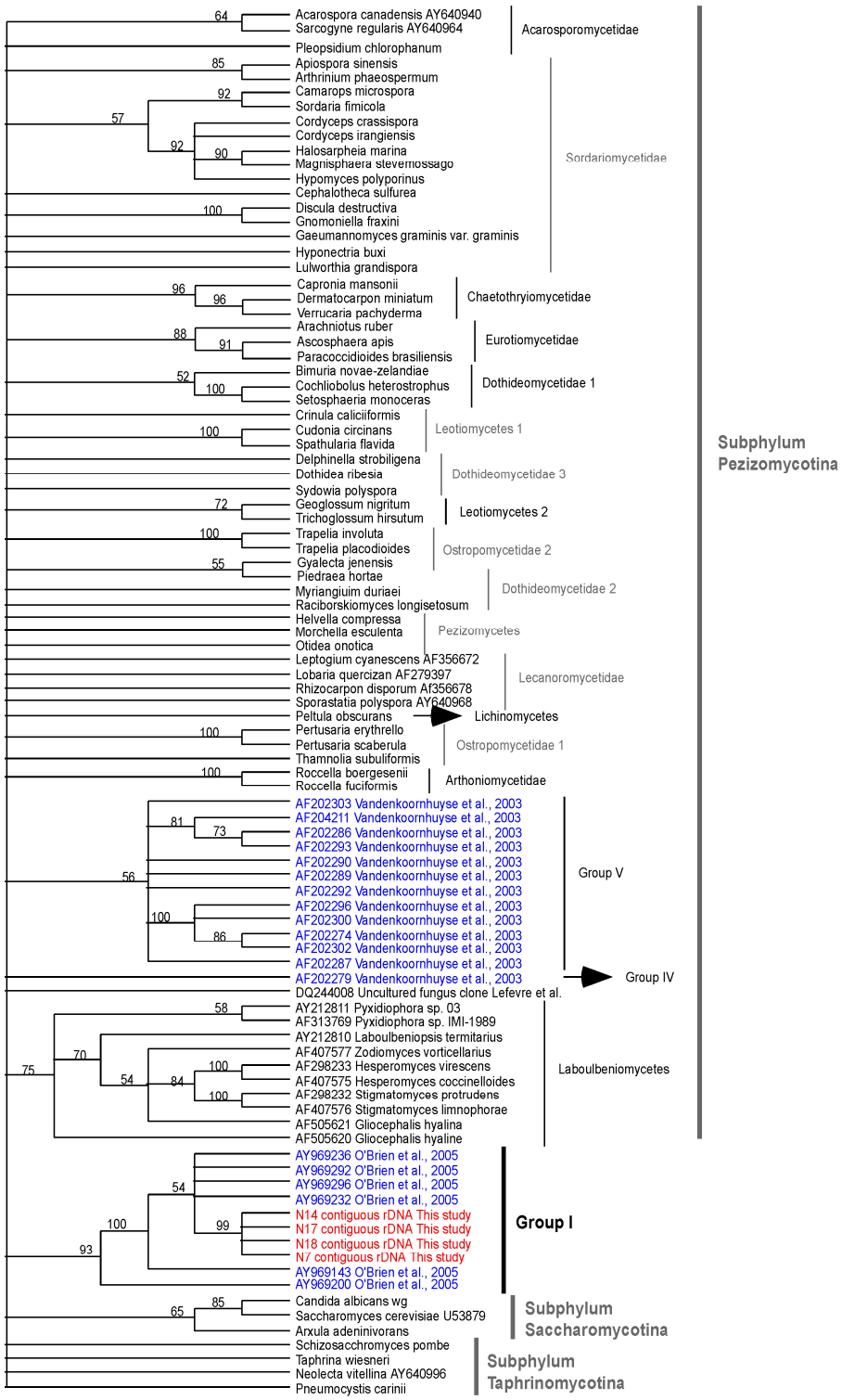
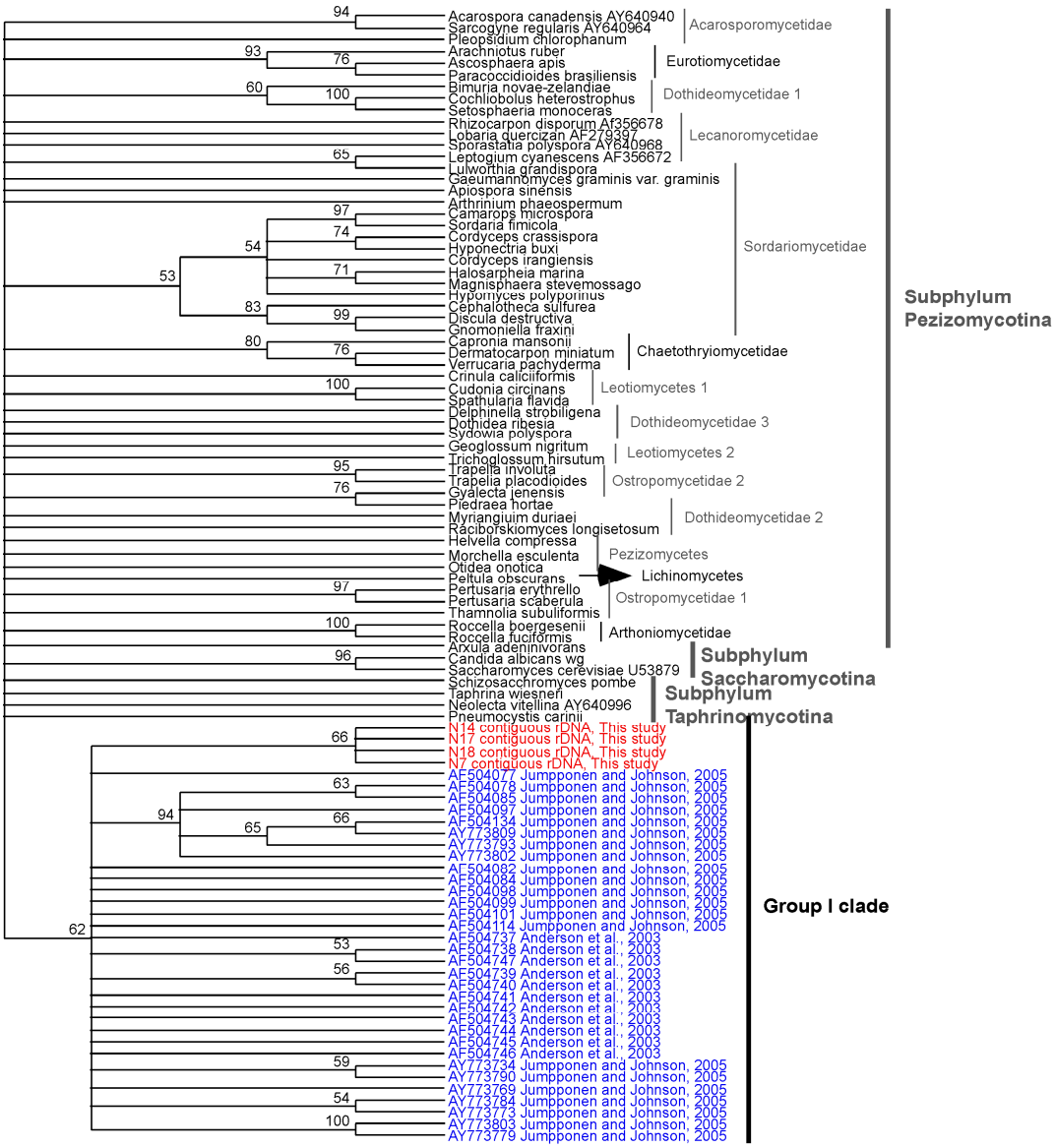
Ss2c33 This study
Ss3c69 This study
Ss3c70 This study
Ss3c81 This study
10C4 This study
2C10 This study
2C166 This study
2C167 This study
2C79 This study
2C17 This study
2C19 This study
2C20 This study
4C20 This study
4C26 This study
4C31 This study
4C40 This study
4C47 This study
4C62 This study
4C64 This study
4C68 This study
4C76 This study
4C79 This study
Os1c21 This study
Os1c41 This study
Ss1c48 This study
Ss1c61 This study
Ss3c43 This study
Ss3c59 This study
Ss3c90 This study
18C7 This study
20C106 This study
20C10 This study
4C89 This study
Os1c89 This study
2C185 This study
2C45 This study
Ss1c13 This study
Ss1c3 This study
Ss1c15 This study
Group I clade
Schizosachromyces pombe
Taphrina wiesneri
Neolecta vitellina AY640996
Pneumocystis carinii
Subphylum Taphrinomycotina

Figure 6-2: SSU rDNA analyses.

A) Parsimony bootstrap consensus tree for 818 characters from the SSU rDNA region for 94 taxa. Group IV and V sequences from Vandenkoornhuyse et al. (2002) and “unknown” environmental sequences from O’Brien et al. (2005) are shown in blue. Our 2.4 kb contiguous rDNA sequences generated for this study are shown in red. Representative sequences from the Laboulbeniomycete clade were added to the backbone alignment from Lutzoni et al. (2004) and clades are named according to their convention. Monophyletic groups recovered in this analysis are indicated in black, otherwise they are labeled in grey. B) Parsimony bootstrap consensus tree for approximately 660 bp of the SSU rDNA region for 91 taxa. “Unknown” environmental sequences from Anderson et al. (2003) and Jumpponen and Johnson (2005) are shown in blue and the 2.4 kb contiguous rDNA sequences generated for this study are shown in red. Representative Ascomycota sequences were selected from Lutzoni et al. (2004) and clades are named according to their convention. Monophyletic groups recovered in this analysis are indicated in black, otherwise they are labeled in grey. C) Bayesian consensus tree for 42 taxa (36 SCGI taxa and 6 reference taxa) and 737 included characters from the SSU rDNA region. Bayesian support is shown at the nodes.


Acarospora canadensis AY640940
Sarcogyne regularis AY640964
Acarosporomycetidae
Pleopsidium chlorophanum
Apiospora sinensis
Arthrinium phaeospermum
Camarops microspora
Sordaria fimicola
Cordyceps crassispora
Cordyceps irangiensis
Halosarpheia marina
Magnisphaera stevemossago
Hypomyces polyporinus
Sordariomycetidae
Cephalotheca sulfurea
Discula destructiva
Gnomoniella fraxini
Gaeumannomyces graminis var. graminis
Hyponectria buxi
Lulworthia grandispora
Caproniа mansonii
Dermatocarpon miniatum
Verrucaria pachyderma
Chaetothryiomycetidae
Arachniotus ruber
Ascosphaera apis
Paracoccidioides brasiliensis
Eurotiomycetidae
Bimuria novae-zelandiae
Cochliobolus heterostrophus
Setosphaeria monoceras
Dothideomycetidae 1
Crinula caliciiformis
Cudonia circinans
Spathularia flavida
Leotiomycetes 1
Delphinella strobiligena
Dothidea ribesia
Dothideomycetidae 3
Sydowia polyspora
Geoglossum nigritum
Trichoglossum hirsutum
Leotiomycetes 2
Trapelia involuta
Trapelia placodioides
Ostropomycetidae 2
Gyalecta jenensis
Piedraea hortae
Dothideomycetidae 2
Myriangiuim duriaei
Raciborskiomyces longisetosum
Helvella compressa
Morchella esculenta
Pezizomycetes
Otidea onotica
Leptogium cyanescens AF356672
Lobaria quercizan AF279397
Rhizocarpon disporum Af356678
Sporastatia polyspora AY640968
Lecanoromycetidae
Peltula obscurans
Lichinomycetes
Pertusaria erythrello
Pertusaria scaberula
Ostropomycetidae 1
Thamnolia subuliformis
Roccella boergesenii
Roccella fuciformis
Arthoniomycetidae
Subphylum
Pezizomycotina
AF202303 Vandenkoornhuyse et al., 2003
AF204211 Vandenkoornhuyse et al., 2003
AF202286 Vandenkoornhuyse et al., 2003
AF202293 Vandenkoornhuyse et al., 2003
AF202290 Vandenkoornhuyse et al., 2003
AF202289 Vandenkoornhuyse et al., 2003
AF202292 Vandenkoornhuyse et al., 2003
AF202296 Vandenkoornhuyse et al., 2003
AF202300 Vandenkoornhuyse et al., 2003
AF202274 Vandenkoornhuyse et al., 2003
AF202302 Vandenkoornhuyse et al., 2003
AF202287 Vandenkoornhuyse et al., 2003
Group V
AF202279 Vandenkoornhuyse et al., 2003
Group IV
DQ244008 Uncultured fungus clone Lefevre et al.
AY212811 Pyxidiophora sp. 03
AF313769 Pyxidiophora sp. IMI-1989
AY212810 Laboulbeniopsis termitarius
AF407577 Zodiomyces vorticellarius
AF298233 Hesperomyces virescens
AF407575 Hesperomyces coccinelloides
AF298232 Stigmatomyces protrudens
AF407576 Stigmatomyces limnophorae
AF505621 Gliocephalis hyalina
AF505620 Gliocephalis hyaline
Laboulbeniomycetes
AY969236 O'Brien et al., 2005
AY969292 O'Brien et al., 2005
AY969296 O'Brien et al., 2005
AY969232 O'Brien et al., 2005
N14 contiguous rDNA This study
N17 contiguous rDNA This study
N18 contiguous rDNA This study
N7 contiguous rDNA This study
AY969143 O'Brien et al., 2005
AY969200 O'Brien et al., 2005
Group I
Candida albicans wg
Saccharomyces cerevisiae U53879
Arxula adeninivorans
Subphylum
Saccharomycotina
Schizosacchromyces pombe
Taphrina wiesneri
Neolecta vitellina AY640996
Pneumocystis carinii
Subphylum
Taphrinomycotina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Sarcogyne regularis AY640964
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Arachniotus ruber
Ascosphaera apis
Paracoccidioides brasiliensis
Bimuria novae-zelandiae
Cochliobolus heterostrophus
Setosphaeria monoceras
Rhizocarpon disporum Af356678
Lobaria quercizan AF279397
Sporastatia polyspora AY640968
Leptogium cyanescens AF356672
Lulworthia grandispora
Gaeumannomyces graminis var. graminis
Apiospora sinensis
Arthrinium phaeospermum
Camarops microspora
Sordaria fimicola
Cordyceps crassispora
Hyponectria buxi
Cordyceps irangiensis
Halosarpheia marina
Magnisphaera stevemossago
Hypomyces polyporinus
Cephalotheca sulfurea
Discula destructiva
Gnomoniella fraxini
Capronia mansonii
Dermatocarpon miniatum
Verrucaria pachyderma
Crinula caliciiformis
Cudonia circinans
Spathularia flavida
Delphinella strobiligena
Dothidea ribesia
Sydowia polyspora
Geoglossum nigritum
Trichoglossum hirsutum
Trapelia involuta
Trapelia placodioides
Gyalecta jenensis
Piedraea hortae
Myriangium duriaei
Raciborskiomyces longisetosum
Helvella compressa
Morchella esculenta
Otidea onotica
Peltula obscurans
Pertusaria erythrello
Pertusaria scaberula
Thamnolia subuliformis
Roccella boergesenii
Roccella fuciformis
Arxula adeninivorans
Candida albicans wg
Saccharomyces cerevisiae U53879
Schizosacchromyces pombe
Taphrina wiesneri
Neolecta vitellina AY640996
Pneumocystis carinii
N14 contiguous rDNA, This study
N17 contiguous rDNA, This study
N18 contiguous rDNA, This study
N7 contiguous rDNA, This study
AF504077 Jumpponen and Johnson, 2005
AF504078 Jumpponen and Johnson, 2005
AF504085 Jumpponen and Johnson, 2005
AF504097 Jumpponen and Johnson, 2005
AF504134 Jumpponen and Johnson, 2005
AY773809 Jumpponen and Johnson, 2005
AY773793 Jumpponen and Johnson, 2005
AY773802 Jumpponen and Johnson, 2005
AF504082 Jumpponen and Johnson, 2005
AF504084 Jumpponen and Johnson, 2005
AF504098 Jumpponen and Johnson, 2005
AF504099 Jumpponen and Johnson, 2005
AF504101 Jumpponen and Johnson, 2005
AF504114 Jumpponen and Johnson, 2005
AF504737 Anderson et al., 2003
AF504738 Anderson et al., 2003
AF504747 Anderson et al., 2003
AF504739 Anderson et al., 2003
AF504740 Anderson et al., 2003
AF504741 Anderson et al., 2003
AF504742 Anderson et al., 2003
AF504743 Anderson et al., 2003
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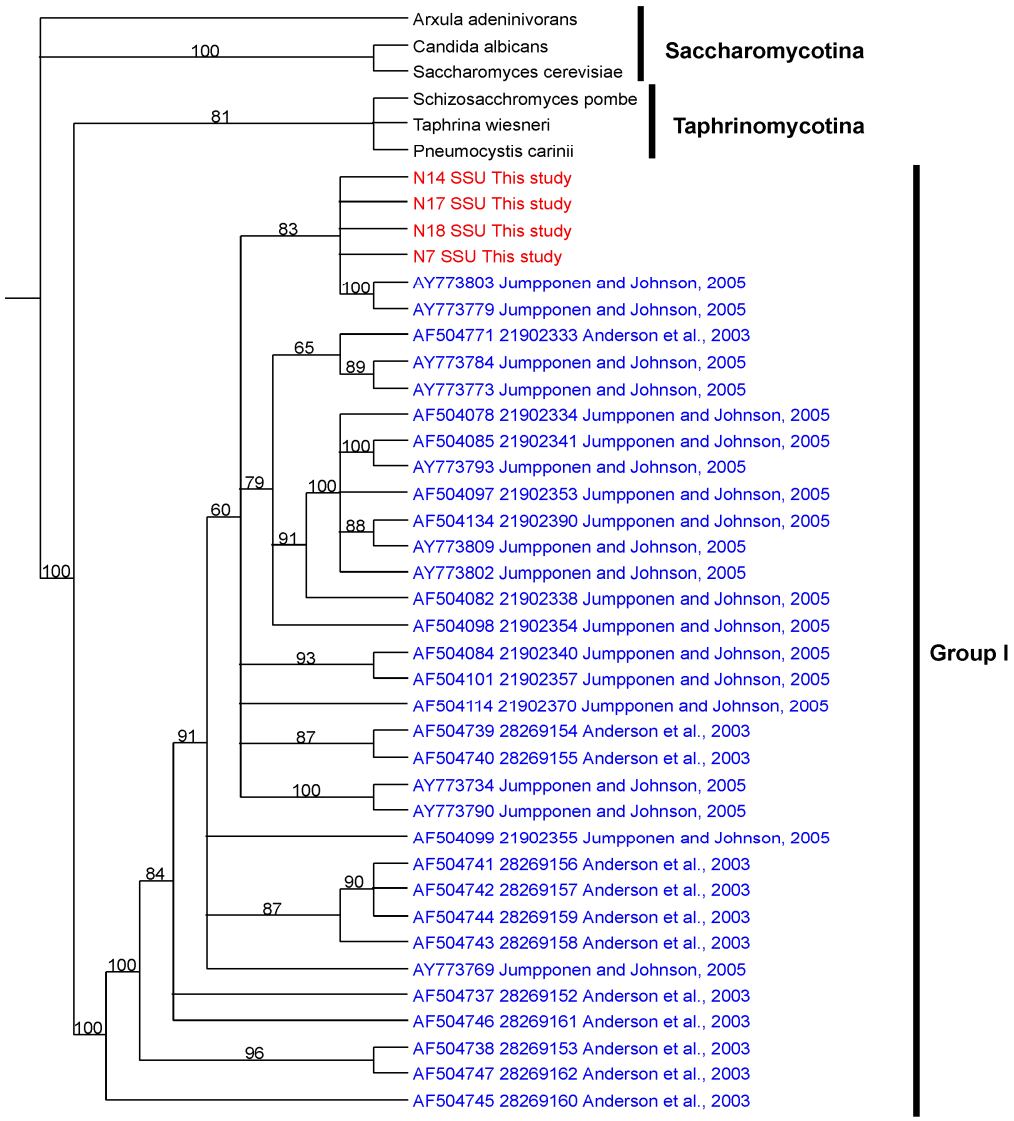
AY773734 Jumpponen and Johnson, 2005
AY773790 Jumpponen and Johnson, 2005
AY773769 Jumpponen and Johnson, 2005
AY773784 Jumpponen and Johnson, 2005
AY773773 Jumpponen and Johnson, 2005
AY773803 Jumpponen and Johnson, 2005
AY773779 Jumpponen and Johnson, 2005
Acarosporomycetidae
Eurotiomycetidae
Dothideomycetidae 1
Lecanoromycetidae
Sordariomycetidae
Chaetothryiomycetidae
Leotiomycetes 1
Dothideomycetidae 3
Leotiomycetes 2
Ostropomycetidae 2
Dothideomycetidae 2
Pezizomycetes
Lichinomycetes
Ostropomycetidae 1
Arthoniomycetidae
Subphylum Pezizomycotina
Subphylum Saccharomycotina
Subphylum Taphrinomycotina
Group I clade


Arxula adeninivorans
Candida albicans
Saccharomyces cerevisiae
Saccharomycotina
Schizosacchromyces pombe
Taphrina wiesneri
Pneumocystis carinii
Taphrinomycotina
N14 SSU This study
N17 SSU This study
N18 SSU This study
N7 SSU This study
AY773803 Jumpponen and Johnson, 2005
AY773779 Jumpponen and Johnson, 2005
AF504771 21902333 Anderson et al., 2003
AY773784 Jumpponen and Johnson, 2005
AY773773 Jumpponen and Johnson, 2005
AF504078 21902334 Jumpponen and Johnson, 2005
AF504085 21902341 Jumpponen and Johnson, 2005
AY773793 Jumpponen and Johnson, 2005
AF504097 21902353 Jumpponen and Johnson, 2005
AF504134 21902390 Jumpponen and Johnson, 2005
AY773809 Jumpponen and Johnson, 2005
AY773802 Jumpponen and Johnson, 2005
AF504082 21902338 Jumpponen and Johnson, 2005
AF504098 21902354 Jumpponen and Johnson, 2005
AF504084 21902340 Jumpponen and Johnson, 2005
AF504101 21902357 Jumpponen and Johnson, 2005
AF504114 21902370 Jumpponen and Johnson, 2005
AF504739 28269154 Anderson et al., 2003
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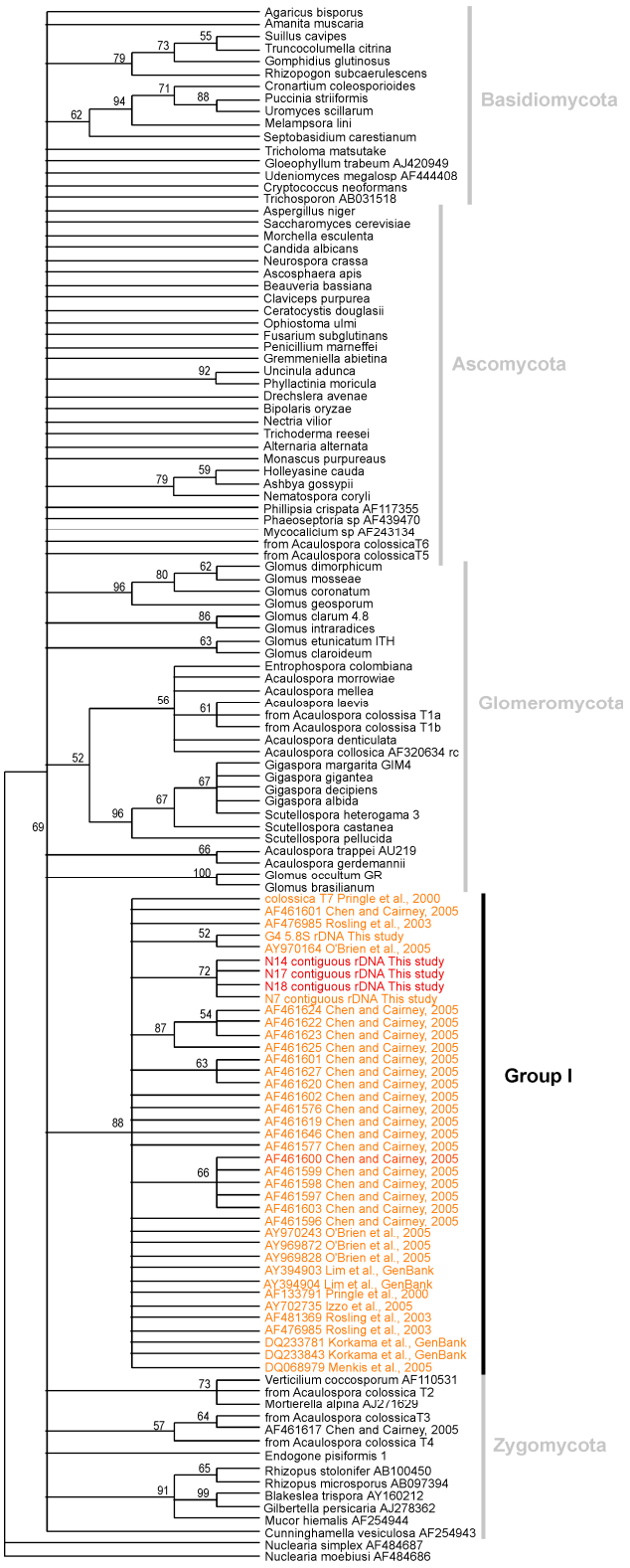
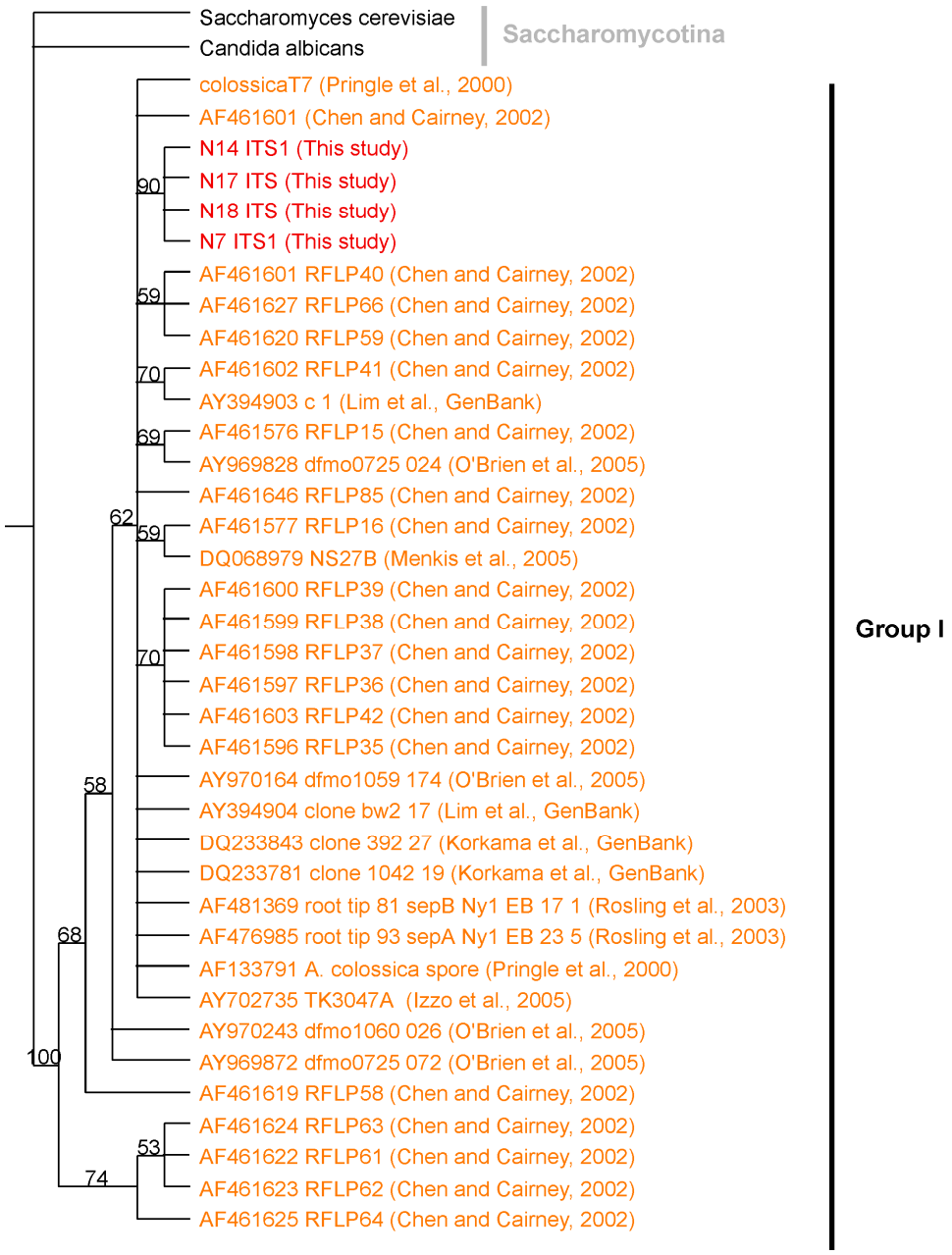
AY773734 Jumpponen and Johnson, 2005
AY773790 Jumpponen and Johnson, 2005
AF504099 21902355 Jumpponen and Johnson, 2005
AF504741 28269156 Anderson et al., 2003
AF504742 28269157 Anderson et al., 2003
AF504744 28269159 Anderson et al., 2003
AF504743 28269158 Anderson et al., 2003
AY773769 Jumpponen and Johnson, 2005
AF504737 28269152 Anderson et al., 2003
AF504746 28269161 Anderson et al., 2003
AF504738 28269153 Anderson et al., 2003
AF504747 28269162 Anderson et al., 2003
AF504745 28269160 Anderson et al., 2003
Group I

Figure 6-3: 5.8S rDNA analyses.

A) Parsimony bootstrap consensus tree for 125 taxa and 146 included characters from the 5.8S rDNA region. “Unknown” environmental sequences from several other independent studies are shown in orange and the 2.4 kb contiguous rDNA sequences generated for this study is shown in red. Representative fungal sequences are from Pringle et al. (2000). B) Bayesian consensus tree from 40 taxa (36 SCGI taxa and 4 reference taxa) and 154 included characters from the 5.8S rDNA region. Bayesian support is shown at the nodes.


Agaricus bisporus
Amanita muscaria
Suillus cavipes
Truncocolumella citrina
Gomphidius glutinosus
Rhizopogon subcaerulescens
Cronartium coleosporioides
Puccinia striiformis
Uromyces scillarum
Melampsora lini
Septobasidium carestianum
Tricholoma matsutake
Gloeophyllum trabeum AJ420949
Udeniomyces megalosp AF444408
Cryptococcus neoformans
Trichosporon AB031518
Basidiomycota
Aspergillus niger
Saccharomyces cerevisiae
Morchella esculenta
Candida albicans
Neurospora crassa
Ascosphaera apis
Beauveria bassiana
Claviceps purpurea
Ceratocystis douglasii
Ophiostoma ulmi
Fusarium subglutinans
Penicillium marneffei
Gremmeniella abietina
Uncinula adunca
Phyllactinia moricula
Drechslera avenae
Bipolaris oryzae
Nectria vilior
Trichoderma reesei
Alternaria alternata
Monascus purpureaus
Holleyasine cauda
Ashbya gossypii
Nematospora coryli
Phillipsia crispata AF117355
Phaeoseptoria sp AF439470
Mycocalicium sp AF243134
from Acaulospora colossicaT6
from Acaulospora colossicaT5
Ascomycota
Glomus dimorphicum
Glomus mosseae
Glomus coronatum
Glomus geosporum
Glomus clarum 4.8
Glomus intraradices
Glomus etunicatum ITH
Glomus claroideum
Entrophospora colombiana
Acaulospora morrowiae
Acaulospora mellea
Acaulospora laevis
from Acaulospora colossisa T1a
from Acaulospora colossisa T1b
Acaulospora denticulata
Acaulospora collosica AF320634 rc
Gigaspora margarita GIM4
Gigaspora gigantea
Gigaspora decipiens
Gigaspora albida
Scutellospora heterogama 3
Scutellospora castanea
Scutellospora pellucida
Acaulospora trappei AU219
Acaulospora gerdemannii
Glomus occultum GR
Glomus brasilianum
Glomeromycota
colossica T7 Pringle et al., 2000
AF461601 Chen and Cairney, 2005
AF476985 Rosling et al., 2003
G4 5.8S rDNA This study
AY970164 O'Brien et al., 2005
N14 contiguous rDNA This study
N17 contiguous rDNA This study
N18 contiguous rDNA This study
N7 contiguous rDNA This study
AF461624 Chen and Cairney, 2005
AF461622 Chen and Cairney, 2005
AF461623 Chen and Cairney, 2005
AF461625 Chen and Cairney, 2005
AF461601 Chen and Cairney, 2005
AF461627 Chen and Cairney, 2005
AF461620 Chen and Cairney, 2005
AF461602 Chen and Cairney, 2005
AF461576 Chen and Cairney, 2005
AF461619 Chen and Cairney, 2005
AF461646 Chen and Cairney, 2005
AF461577 Chen and Cairney, 2005
AF461600 Chen and Cairney, 2005
AF461599 Chen and Cairney, 2005
AF461598 Chen and Cairney, 2005
AF461597 Chen and Cairney, 2005
AF461603 Chen and Cairney, 2005
AF461596 Chen and Cairney, 2005
AY970243 O'Brien et al., 2005
AY969872 O'Brien et al., 2005
AY969828 O'Brien et al., 2005
AY394903 Lim et al., GenBank
AY394904 Lim et al., GenBank
AF133791 Pringle et al., 2000
AY702735 Izzo et al., 2005
AF481369 Rosling et al., 2003
AF476985 Rosling et al., 2003
DQ233781 Korkama et al., GenBank
DQ233843 Korkama et al., GenBank
DQ068979 Menkis et al., 2005
Group I
Verticilium coccosporum AF110531
from Acaulospora colossica T2
Mortierella alpina AJ271629
from Acaulospora colossicaT3
AF461617 Chen and Cairney, 2005
from Acaulospora colossica T4
Endogone pisiformis 1
Rhizopus stolonifer AB100450
Rhizopus microsporus AB097394
Blakeslea trispora AY160212
Gilbertella persicaria AJ278362
Mucor hiemalis AF254944
Cunninghamella vesiculosa AF254943
Zygomycota
Nucleariа simplex AF484687
Nucleariа moebiusi AF484686


Saccharomyces cerevisiae
Candida albicans
Saccharomycotina
colossicaT7 (Pringle et al., 2000)
AF461601 (Chen and Cairney, 2002)
N14 ITS1 (This study)
N17 ITS (This study)
N18 ITS (This study)
N7 ITS1 (This study)
AF461601 RFLP40 (Chen and Cairney, 2002)
AF461627 RFLP66 (Chen and Cairney, 2002)
AF461620 RFLP59 (Chen and Cairney, 2002)
AF461602 RFLP41 (Chen and Cairney, 2002)
AY394903 c 1 (Lim et al., GenBank)
AF461576 RFLP15 (Chen and Cairney, 2002)
AY969828 dfmo0725 024 (O'Brien et al., 2005)
AF461646 RFLP85 (Chen and Cairney, 2002)
AF461577 RFLP16 (Chen and Cairney, 2002)
DQ068979 NS27B (Menkis et al., 2005)
AF461600 RFLP39 (Chen and Cairney, 2002)
AF461599 RFLP38 (Chen and Cairney, 2002)
AF461598 RFLP37 (Chen and Cairney, 2002)
AF461597 RFLP36 (Chen and Cairney, 2002)
AF461603 RFLP42 (Chen and Cairney, 2002)
AF461596 RFLP35 (Chen and Cairney, 2002)
AY970164 dfmo1059 174 (O'Brien et al., 2005)
AY394904 clone bw2 17 (Lim et al., GenBank)
DQ233843 clone 392 27 (Korkama et al., GenBank)
DQ233781 clone 1042 19 (Korkama et al., GenBank)
AF481369 root tip 81 sepB Ny1 EB 17 1 (Rosling et al., 2003)
AF476985 root tip 93 sepA Ny1 EB 23 5 (Rosling et al., 2003)
AF133791 A. colossica spore (Pringle et al., 2000)
AY702735 TK3047A (Izzo et al., 2005)
AY970243 dfmo1060 026 (O'Brien et al., 2005)
AY969872 dfmo0725 072 (O'Brien et al., 2005)
AF461619 RFLP58 (Chen and Cairney, 2002)
AF461624 RFLP63 (Chen and Cairney, 2002)
AF461622 RFLP61 (Chen and Cairney, 2002)
AF461623 RFLP62 (Chen and Cairney, 2002)
AF461625 RFLP64 (Chen and Cairney, 2002)
90
59
70
69
62
59
70
58
68
100
53
74
Group I

7 Appendix

Fastblast.pl script to transmit a batch of fasta formatted sequences to Genbank, retrieve a record for all top hit descriptions, bit scores, e-values, % identities; as well as compile a fasta-formatted file for all non-redundant top hits.

```
#!/usr/bin/perl
# FASTBLAST.pl
#Terri Porter, May 2004, updated March 2006

#SCRIPT TO BLAST A FASTA FORMATTED TEXT FILE AGAINST GENBANK

use strict;
use warnings;
use CGI::Carp qw(fatalsToBrowser);
use Data::Dumper;
use Bio::Tools::Run::RemoteBlast;
use Bio::SearchIO;
use Bio::SeqIO;

#declare some variables
my $count = 0; #counts number of sequences to BLAST
my $i = 1; #keeps track of accession numbers in array
my $v = 1; #verbose
my $accession;

#declare arrays
my @accession;

#requires arguments to specify the sequence infile
my $infile= $ARGV[0]; #holds the filename of the input file
unless (@ARGV) { #holds arguments
	print "You need to enter a command line argument like this:
	perl <script> <inputfile>\n";
	exit;
}

#define new SeqIO object
my $seqio = Bio::SeqIO -> new (
	-file => $infile,
	-format => 'fasta')
or die "Could not create Bio::SeqIO\n";

#add these paramaters to standard blast factory object
$Bio::Tools::Run::RemoteBlast::RETRIEVALHEADER{'ALIGNMENTS'} = '5';
$Bio::Tools::Run::RemoteBlast::RETRIEVALHEADER{'HITLIST_SIZE'} = '100';
$Bio::Tools::Run::RemoteBlast::RETRIEVALHEADER{'FORMAT_TYPE'} = 'Text';

#create remote blast factory object and initialize blast parameters
my $blast_factory = Bio::Tools::Run::RemoteBlast -> new (
	'-prog' => 'blastn',
	'-data' => 'nr',
	'-expect' => '10.0',
	'-readmethod' => 'SearchIO');

#loop over all sequences input and count how many sequences are found
while (my $seq = $seqio -> next_seq) {
	$count++;

	#loop to blast each sequence, in turn, against the database
	my $job = $blast_factory -> submit_blast ($seq);
	print "Blasting sequence number $count\n";

	#loop to load rids returned for the blast job submitted
	while (my @rids = $blast_factory -> each_rid) {
	print Dumper (@rids);

		#loop over rids to check server for a result
```

```
foreach my $rid (@rids) {
	my $blast_results = $blast_factory -> retrieve_blast($rid);
#	$blast_results -> verbose (2);
	print "$blast_results\n";
	if ($blast_results == 0) { #still waiting to complete search
		print STDERR "."; #watch dots while waiting
		sleep 5; #pause between checking for results
	}
	elsif ($blast_results == (-1)) {#error returned, remove RID from stack
		print STDERR "retrieve_blast returns -1\n";
		$blast_factory -> remove_rid($rid);
	}
	#use Bio::SearchIO to return a Bio::SearchIO result object
	else {
		print "Receiving blast results...\n";
		#$blast_results -> verbose (2);
		my $result = $blast_results -> next_result();
#		$result -> verbose (2);
		print Dumper ($result);
		my $filename = $result -> query_name()."\.out";
		$blast_factory -> save_output ($filename);
		$blast_factory -> remove_rid($rid);

		#parse each blast report as it is saved
		use Bio::SearchIO;
		my $blast_report = new Bio::SearchIO (
			'-format' => 'blast',
			'-file' => $filename);
		$result = $blast_report -> next_result;

		#open file to record top hit matches for each query submitted
		open (RESULTOUT, ">>resultstable.txt") ||
		die "Could not open resultstable.txt\n\n";

		#open file to record top hits in fasta format
		open (TEMPOUT, ">>temp.out") ||
		die "Could not open temp.out\n\n";

		#using Bio::Search::Hit
		my $hit = $result -> next_hit();
		my $temp_acc = $hit -> accession();
		my $k = 0;

		#if array empty, print results to resultstable.txt and temp.out
		if (!@accession) {
			$accession[0] = $temp_acc;
			print RESULTOUT $result -> query_name(), "\t";
			print TEMPOUT ">",$hit -> accession(), "|",
					$hit -> description(), "|",
					$hit -> bits(), "|";
			print RESULTOUT  $hit -> description(), "\t",
					$hit -> accession(), "\t",
					$hit -> bits(), "\t";

			#using Bio::Search::HSP
			my $hsp = $hit -> next_hsp();
			print TEMPOUT	$hsp -> evalue(), "|",
					$hsp -> percent_identity, "\n",
					$hsp -> seq_str('sbjct'), "\n";
			print RESULTOUT  $hsp -> evalue(), "\t",
					$hsp -> percent_identity, "\n";
			close $filename;
		}

		#otherwise, check if current accession # is already in array
		else {
			foreach $accession (@accession) {
				if ($accession eq $temp_acc) {
					$k++;
				}
			}
```

```
                                        #if duplicate accession #, add to resultstable.txt only
                                        if ($k != 0) {
                                                print RESULTOUT $result -> query_name(), "\t";
                                                print RESULTOUT   $hit -> description(), "\t",
                                                                        $hit -> accession(), "\t",
                                                                        $hit -> bits(), "\t";
                                                my $hsp = $hit -> next_hsp();
                                                print RESULTOUT   $hsp -> evalue(), "\t",
                                                                        $hsp -> percent_identity, "\n";
                                                close $filename;
                                                next;
                                        }

                                        #if new accession #, print to resultstable.txt and temp.out
                                        else {
                                                $accession[$i] = $temp_acc;
                                                $i++;
                                        print RESULTOUT $result -> query_name(), "\t";
                                        print TEMPOUT ">",$hit -> accession(), "|",
                                                                $hit -> description(), "|",
                                                                $hit -> bits(), "|";
                                        print RESULTOUT   $hit -> description(), "\t",
                                                                $hit -> accession(), "\t",
                                                                $hit -> bits(), "\t";
                                        my $hsp = $hit -> next_hsp();
                                        print TEMPOUT       $hsp -> evalue(), "|",
                                                                $hsp -> percent_identity, "\n",
                                                                $hsp -> seq_str('sbjct'), "\n";
                                        print RESULTOUT   $hsp -> evalue(), "\t",
                                                                $hsp -> percent_identity, "\n";
                                        close $filename;
                                        }
                                }
                        }
                }
        }
}

#SCRIPT TO REMOVE DASHES FROM TEMPFILE IN FASTA FORMAT
#remove "-" from file

open (TEMPFILE, "temp.out") || die "Could not open temp.out\n";
open (HITFILE, ">outfile.fasta") || die "Could not open outfile.fasta\n";

my @sequence = <TEMPFILE>;

foreach my $sequence (@sequence) {
        $sequence =~ s/\-//g;
        print HITFILE $sequence;
}

close TEMPFILE;
close HITFILE;

#INSTRUCTIONS TO THE USER
print
"\n\nRemoteBlast is complete for ", $count, " sequences in your original file.\n
A new file in fasta format outfile.fasta has been created for the BLAST results.\n
A new tab-delimited file, resultstable.txt, has been created as a summary for this search.\n
Each individual blast report has also been saved as an .out file.\n";
```

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