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Impacts of agricultural disturbance on communities of selected soil fungi (Agaricomycetes)

Jessie R. Wong
The University of Western Ontario

Supervisor
Dr. R. G. Thorn
The University of Western Ontario

Graduate Program in Biology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

IMPACTS OF AGRICULTURAL DISTURBANCE ON COMMUNITIES OF SELECTED SOIL FUNGI (AGARICOMYCETES)

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By

Jessie R. Wong

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The University of Western Ontario
London, Ontario, Canada

THE UNIVERSITY OF WESTERN ONTARIO
The School of Graduate and Postdoctoral Studies

CERTIFICATE OF EXAMINATION

Supervisor

_____
Dr. R. G. Thorn

Supervisory Committee

_____
Dr. Hugh Henry

_____
Dr. Sheila Macfie

Examiners

_____
Dr. Hugh Henry

_____
Dr. Marc-André Lachance

_____
Dr. Richard Gardiner

The thesis by

Jessie Rachel Wong

entitled:

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Abstract and Keywords

The objective of this study was to use phylogeny-based and community-based analyses to compare the community composition of Agaricomycetes among four different agricultural treatments at the Kellogg Biological Station Long Term Ecological Research (KBS LTER) site. A phylogenetic tree that included 591 ribosomal DNA sequences previously obtained from KBS LTER documented the composition of Agaricomycete communities in each treatment. Sequences from KBS LTER were placed into 472 OTUs (putatively species-level operational taxonomic units defined by 99% or greater sequence similarity) and these were dominated by the Agaricales (with 330 OTUs), Cantharellales (39 OTUs), Hymenochaetales (29 OTUs), and Polyporales (23 OTUs). Multivariate statistical analyses incorporating phylogenetic information showed never tilled successional grasslands to be the most phylogenetically distinct treatment. The trend that phylotype and clade diversity decreased with increasing disturbance by tillage was consistent with results from previous individual studies emphasizing the importance of protecting remnant untilled grassland habitats.

Keywords

Agaricomycetes, agriculture, KBS, multivariate statistics, phylogenetics, soil, sustainability, tillage, UniFrac

Dedication

I dedicate this work to my supervisor, mentor and friend, Dr. Greg Thorn, without whom I would have never been able to complete my MSc. degree in a timely fashion and in such a nurturing academic environment.

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List of Abbreviations

BLAST	Basic Local Alignment Search Tool
CT	Conventional Till
DNA	Deoxyribonucleic acid
ECM	Ectomycorrhizal
GenBank	Nucleotide database of the National Center for Biotechnology Information
HTS	Historically Tilled Successional
ITS	Internal Transcribed Spacer
KBS	Kellogg Biological Station
LTER	Long Term Ecological Research
LSU	Large Subunit
MCSE	Main Cropping System Experiment
MEGA	Molecular Evolutionary Genetics Analysis
MUSCLE	Multiple Sequence Comparison by Log-Expectation
NGS	Next Generation Sequencing
NJ	Neighbour-joining
NT	No Till
NTS	Never Tilled Successional
OTU	Operational Taxonomic Unit
P-test	Parsimony test (in UniFrac)
PAUP	Phylogenetic Analysis Using Parsimony
PCoA	Principal Coordinates Analysis

PCR	Polymerase Chain Reaction
P1	Principal component 1
P2	Principal component 2
P3	Principal component 3
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SINA	SILVA INcremental Aligner
UniFrac	Unique Fraction

CHAPTER 1: INTRODUCTION

"The role of the infinitely small in nature is infinitely great."
- Louis Pasteur

Ecosystems are complex and are made up of a number of interacting biotic and abiotic components; an ecosystem is the entire biological community, from viruses to vertebrates, and the community's non-living (abiotic) environment. Each component in an ecosystem is not separate from the others but they are intertwined. In order to maintain ecosystem health, there must be an understanding of how components function individually and more importantly, how they interact with one another. Each component has an influence on the ecosystem. However, the ways in which it affects the ecosystem are intricate, as different ecosystems are made up of different sets of components acting at various degrees and multiple spatial and temporal scales. The definition of ecosystem health remains complex and we must take into account our limited knowledge and ability to provide definitive sets of measures and criteria on which to assess ecosystem health (Schaeffer et al. 1988). So, although there are many definitions of ecosystem health, a contextual and more appropriate definition, for the purpose of this study, describes ecosystem health as how stable and sustainable an ecosystem is, with regards to its ability to maintain organization and autonomy over time and be resilient to stress (Costanza 1992). More generally this means how well an ecosystem is able to respond to disturbance and how quickly it can recover from such disturbance.

Ecosystem health requires an evaluation of the components that dictate a healthy ecosystem. Terrestrial abiotic components are water, the atmosphere, sunlight, and soil. Terrestrial biotic components include plants, animals, and microorganisms and each component is sensitive to environmental and anthropogenic changes.

1.1 Soil – its role in terrestrial ecosystems

The solid matter of soil consists of mineral components derived from parent rock material and chemically complex organic components derived from living organisms (Carlile et al. 2001). A healthy soil also includes water, air, and living soil organisms (Doran et al. 1994). Soil provides an ecosystem with essential functions such as physical stability and support of aboveground biomass, filtering and buffering, water relations, and provides a habitat in which nutrient cycling may take place. Soil is of paramount importance as it supports our biosphere by supporting the production of food and fiber, and maintenance of overall ecosystem quality (Doran and Parkin 1994, Karlen et al. 1997, Doran and Zeiss 2000). It is important to consider the quality of a soil as an indicator of soil health, and its relationship to and as a basis for primary productivity. "Soil health is defined as capacity of soil to function as a vital living system, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health" (Doran and Zeiss 2000). The health of the soil largely dictates the health, resiliency, and biodiversity of a terrestrial ecosystem. Soil quality is not only important to maintain aboveground primary productivity but also overall environmental quality, as primary producers support higher trophic levels. Soil quality is controlled by chemical, physical,

and biological components. For the purpose of this study, only the physical and biological components will be considered (Kennedy and Papendick 1995, Doran 2002).

1.2 Soil organisms

The most influential soil organisms include bacteria, nematodes, and fungi. Their roles in soil structure and function have been correlated to beneficial parameters that define soil quality, including water storage, soil humus formation, decomposition and nutrient cycling, detoxification of toxicants, suppression of noxious and pathogenic organisms, and maintenance of ecosystem resiliency and soil tilth, which is defined as soil that has the proper structure and sufficient amounts of nutrients (Lynch and Bragg 1985, Tisdall 1991, Doran and Zeiss 2000, Kibblewhite et al. 2008). Soil structure is controlled in part by soil organisms, including mycorrhizal fungi whose hyphae physically entangle soil and also bind fine mineral and organic particles into aggregates with exudates such as glomalin (Miller and Jastrow 1990, Rillig 2004). Soil organisms, as determinants of soil health and ecosystem functioning, have been considered more seriously in recent years as the quality of our agricultural soils has been severely degraded through unsustainable agricultural practices such as chemical inputs in the form of pesticides, herbicides and fertilizers, as well as mechanical processes that have contributed to the loss of soil organic matter. Mechanized processes that turn or dig up soil, such as ploughing and tillage, can also lead to loss of top soil through erosion (Oldeman 1994, Kibblewhite et al. 2008). Soil organisms can be sensitive to variations in land management, are well correlated to beneficial soil functions, and can be useful for elucidating ecosystem processes (Doran and Zeiss 2000). However, soil organisms can be

difficult to study; many are difficult to observe, isolate, and identify and it is challenging or impractical to study their activities under realistic conditions (Fenchel 1992, Wardle and Giller 1996, Brussaard et al. 1997, Freckman et al. 1997). The opaque, chemically complex, and highly heterogeneous nature of soil creates difficulty when studying soil organisms (Parkin 1993, Ranjard et al. 2003). For this reason, they are often not included in ecological surveys, nor are they included as indicators of soil health. Traditionally, soil organisms have been studied at the process level, in terms of soil respiration rate and enzyme activities or very gross community measures such as total numbers of organisms (Parkinson and Coleman 1991). Detailed responses at the community level have not been studied as thoroughly (Kennedy and Smith 1995). However, the numbers of studies on soil organisms' responses, at the community level, have increased through the years (Wardle et al. 2004). Process level measurements are critical in our understanding of how soil organisms respond to environmental variations, but they may be too insensitive to detect changes on a finer scale due to the complexity of relationships within a community (Kennedy and Smith 1995). Process level measurements further lack information that could provide explanations for the patterns or changes observed.

A comprehensive understanding of soil health and ecosystem health necessitates an understanding of soil organisms and especially their responses to land management practices, as their presence has large implications for the quality and quantity of agricultural production. The composition, abundance, and activity levels of soil organisms have been shown to be markedly different in agricultural systems when compared to the natural ecosystems from which they were derived (Lavelle et al. 1994, Matson et al. 1997). In order to accurately assess land management practices and their

resulting effects on soil organisms, a variety of soil organisms must be sampled, if possible, and observed. These include soil bacteria, nematodes, and fungi, as these organisms function in nutrient cycling, decomposition, soil structure maintenance, and contribute to soil organic matter (Kibblewhite et al. 2008).

1.3 Agaricomycetes

Agaricomycetes are a class of fungi in the phylum Basidiomycota; approximately 21,000 of 30,000 accepted species of Basidiomycota are Agaricomycetes, and many of these live in soil (Hibbett et al. 2007; Kirk et al. 2008). The Agaricomycetes is a diverse class in both nutritional mode and fruiting body form (fruiting body being the macroscopic spore-bearing structure of a fungus) (Carlile et al. 2001). Among the Agaricomycetes are species that are saprotrophs (organisms that feed by decomposing organic matter) and form mutualist symbioses with the roots of various plants [ectomycorrhizae (ECM)]. Filamentous fungi, including ECM start with the basic unit of a hypha (plural hyphae), which usually consists of a chain of elongated cells, to build a highly branched three-dimensioal network called a mycelium (Bartnicki-Garcia et al. 1969, Gooday 1971, Steele and Trinci 1975). Some Agaricomycetes are important pathogens of timber, vegetable crops, and even humans (Hibbett 2006). Many species of Agaricomycetes act as primary decomposers of wood and other plant litter (Hibbett 2006, Lynch and Thorn 2006). Primary decomposers are characterized as organisms that possess the enzymes needed to degrade complex polymers, including lignin and cellulose, found in plant litter (Blanchette 1991).

Lignin degradation is the rate-limiting step in nutrient turnover in soils (Ohkuma et al. 2001). Lignin is an amorphous, heterogeneous, and highly refractive polymer of various proportions of ester-linked aromatic alcohols (*p*-coumaryl, sinapyl, and coniferyl) that is present in all plant tissues to some degree, but more abundantly in woody plant tissues (Hatakka 1994). Lignin solidifies plant cell walls, provides strength and rigidity, and protects wood from microbial attack (Hatakka 1994). It is estimated that lignin is the second most abundant aromatic compound on Earth, second to cellulose (Ohkuma et al. 2001). Cellulose is an unbranched homopolysaccharide of β(1→4) linked D-glucose that is fibrous and water-insoluble (O'Sullivan 1997). It is found in the cell walls of plants, in roots, stems, and leaves (O'Sullivan 1997). A large proportion of Agaricomycetes are the primary agents of lignocellulose degradation because these organisms posses the necessary enzymes, such as laccase, lignin peroxidases, and manganese peroxidises, needed to degrade lignin (Hatakka 1994).

There are two major modes of wood decay performed by Agaricomycetes. Brown-rot occurs when the lignin component of woody materials is not appreciably degraded and only cellulose and hemicelluloses are degraded (Morgenstern et al. 2008). In white rot, the lignin component is efficiently degraded either simultaneously or in advance of the degradation of other woody components (Morgenstern et al. 2008, Ohkuma et al. 2001). Extracellular class II peroxidases secreted by Agaricomycetes include manganese peroxidases, lignin peroxidises, and versatile peroxidases, which, along with laccases, aid in lignin depolymerization (Morgenstern et al. 2008, Ohkuma et al. 2001). Lignocellulose-degrading Agaricomycetes make plant litter more available to other fungi, or more palatable, and (together with the fungal biomass) nutritious to

detrivorous soil animals. As decayers of organic matter, Agaricomycetes help to recycle nutrients, and make them available to plants and other organisms.

Another important role that Agaricomycetes fulfill is that of a symbiont and more specifically, forming mycorrhizal associations with plants. These groups of Agaricomycetes are known as ectomycorrhizal (ECM) fungi. ECM fungi form associations with living plant root tips; a collection of fungal hyphae surrounds the root tip and penetrates the intercellular space between cortical root cells, forming what is known as a 'Hartig net' (Smith and Read 2008, Brundrett 2002). ECM fungi form associations with roots of angiosperms such as *Eucalyptus*, *Betula*, *Populus*, *Fagus*, and *Shorea* and gymnosperms such as *Pinaecea* (Brundrett 2004). ECM fungi help to transport water and nutrients; they mobilize nitrogen (N), phosphorus (P), calcium (Ca), and magnesium (Mg) from solid mineral substrates through organic acid excretion (Landeweert et al. 2001) and from organic substrates by enzymatic digestion (Bending and Read 1995, Tibbett and Sanders 2002). Fungal hyphae provide a low-cost method, relative to much more massive plant roots, that increases the volume of soil explored for soil nutrients. The diversity of roles that Agaricomycetes play are mirrored by the range in size of their fruiting bodies, from 0.1 mm in diameter to the largest fruiting body of all fungi found to date, *Fomitiporia ellipsoidea*, a polypore found in China with an estimated volume of 40.5 m^3 and weight of 400-500 kg (Dai and Cui 2011). The mycelium of another Agaricomycete, *Armillaria bulbosa*, was estimated in Michigan to occupy 15 hectares and to weigh approximately 9700 kg (Smith et al. 1992). Mycelia (individuals) of Agaricomycetes range from centimeters to tens or hundreds of meters in diameter, very large when compared to moulds and yeasts, which range from a few µm to

tens of μm in diameter (Walker 1998). Given the various important roles of Agaricomycetes, it is necessary to study the factors that determine their presence and community composition in soil ecosystems.

1.4 Mechanisms by which agricultural practices affect fungal community structure

Agricultural expansion has been called the single most destructive form of land-use change and one of the most significant anthropogenic alterations to the global environment (Matson et al. 1997). From 1700-1980, the total area of cultivated land increased 466% (Matson et al. 1997). From 1980 onwards, agricultural expansion has slowed, as less natural habitat is available for conversion to agricultural lands, but yields have increased dramatically. The increase was exponential during the "Green Revolution" in the 1960s and onwards, due to intensification of agriculturally managed lands, accomplished through high-yielding and pest-resistant crop varieties, chemical fertilizers and pesticides, large-scale irrigation, and mechanization of agriculture (Matson et al. 1997). Agriculture has local and global environmental consequences; these include loss of biodiversity, emissions of pollutants contributing to global climate change, habitat fragmentation and degradation, pollution of ground water, eutrophication of surrounding freshwater and/or marine habitats, increased erosion, soil acidification, and loss of soil fertility.

Agricultural soils have been better studied by mycologists than soils of undisturbed ecosystems (Carlile et al. 2001). Changes in soil fungal community structure due to agriculture can be attributed to the initial conversion of natural habitats to managed agricultural lands and subsequent land management practices, influencing soil

quality (Matson et al. 1997). Agricultural lands differ from undisturbed soils in containing artificially higher levels of N, P, and K (Carlile et al. 2001). Agricultural soils are subject to monoculture cropping systems and disturbance by cultivation of land and

chemical properties of soil that greatly alter the matrix supporting soil organisms (Kennedy and Smith 1995). A disruption and loss of diversity of beneficial soil fungi may profoundly alter biological regulation of decomposition and nutrient availability in soil.

Even in the absence of tillage, chemical changes due to inputs of fertilizers and lime (to increase soil pH) can alter species composition and plant-fungi symbioses. Both nitrogenous and phosphatic fertilizers have been reported to reduce mycorrhizal colonization of plant roots (Hayman 1980, Harinikumar and Bagyaraj 1989). Higher amounts of fertilizer may inhibit mycorrhizae due to a decreased dependence by plants when the soil is nutrient saturated (Coleman et al. 1983). Plant preference for fertilizer is due to the decreased energy expenditure to acquire N and P; comparatively, mycorrhizal associations with plant roots are more energy expensive (Coleman et al. 1983). Although plant species differ in the degree to which they support ectomycorrhizal relationships (Brundrett 2009), fertilizer application may inhibit ectomycorrhizae formation by Agaricomycetes.

Some biological functions provided by soil organisms have been substituted by land management practices that speed up or intensify the some of the same services, such as the use of fertilizers and tillage (Matson et al. 1997). These practices marginalize the free ecosystem services provided by fungi that can act to improve soil fertility and plant health. Management practices, however, can be designed to maximize the presence and function of soil biota (Matson et al. 1997).

1.5 Sustainable agriculture and its link to Agaricomycetes

Sustainable agriculture cannot be easily defined and many interpretations of the concept exist - the term itself can encompass environmental, social, economic, and public health factors and emphasis cannot be placed on any one single factor. Nonetheless, the basis of sustainable agriculture must depend on the resources that support the production of crops for food and fiber. A broad definition of sustainable agriculture states that it "…is one that, over the long term, enhances environmental quality and the resource base on which agriculture depends; provides for basic human food and fiber needs; is economically viable; and enhances the quality of life for farmers and society as a whole" (Weil 1990). For the purposes of this report, sustainable agriculture will be considered in the biological context with emphasis on environmental quality and the resources that affect environmental quality.

The first steps to sustainable agriculture must be the identification of the end goals (Doran 2002). The strategies or course by which we will attain these goals must be developed and indicators or benchmarks to mark progress will help determine if strategies are indeed working (Doran 2002). One concern surrounding sustainable agriculture is that policy-makers, conservationists, land-managers, and producers cannot agree on any one set of goals. Conflicting views hamper the process of defining specific goals and there is a need from the mycologist's and ecologist's point of view to include the enhancement or preservation of all relevant organisms that provide necessary ecosystem services, when developing strategies for sustainable agriculture.

Sustainable agriculture is ever more necessary now as our population has surpassed 7 billion. In order to preserve the resources that feed the global population,

certain practices will need to be adopted in order to maintain biological productivity for future generations. As mentioned previously, soil, the keystone of our agricultural systems, must be preserved. Also, in alignment with sustainable agricultural goals, soil must remain productive in ways that do not degrade it. Sustainable agricultural practices that can maintain soil quality include crop rotation, planting of cover crops, and reduction of tillage (Tilman et al. 2002).

Sustainable agriculture goals, with emphasis on preserving soil fungi, can include reducing the degree to which soil is conventionally tilled, known as conservation tillage. This practice reduces the severity of the physical effects at the soil surface and the top layer of soil as well as increases organic inputs through crop residue retention (Matson et al. 1997). Low-till or no till is often cited as one of the best promoters of a more complex and dynamic fungal community structure for this reason (Matson et al. 1997). Fungal hyphae remain intact and as a result, colonization of plant roots and litter is not retarded by physical alterations. As well, low-till or no till can be adopted to promote other abiotic factors that can support a complex fungal community structure. For example, reduced tillage can help to conserve water, restore soil fertility, and reduce soil erosion caused by conventional intensive management practices, which are fundamentally due to the removal of crop residue at the soil surface (Johansson et al. 1994, Alguacil et al. 2008). Reduction of tillage can also increase early-season P uptake in crops, especially in low-input farming systems (McGonigle and Miller 1996a). This is because associations between mycorrhizal fungi and crops may form earlier in the season, which contributes to improved crop growth and development (McGonigle and Miller 1996a).

Historically, fungal surveys or identification of fungal species in soil have been restricted to culture-based sampling methods and identification through morphology. These methods were largely restrictive because similar morphologies may lead to mis-identification of fungal species and culture-based methods fail to recover major groups of fungi (including saprotrophic and ectomycorrhizal Agaricomycetes) and often sample the same mould or yeast individual repeatedly (Straatsma et al. 2001). Furthermore, most groups of Agaricomycetes are difficult to study because they are hard to detect due to their transient fruiting bodies and are difficult to identify when not fruiting due to lack of distinguishing morphological characters (Hawksworth 2001, Hawksworth and Lagreca 2007, Matheny et al. 2006, Moncalvo et al. 2002). The inability to culture the majority of Agaricomycetes from soil contributes to the difficulty of assessing their presence and diversity in soils (Hawksworth 2001). However, DNA-based methods of sampling have greatly resolved these issues simply because they are reliant on soil-extracted DNA rather than visual identification. The phylogenies that result from DNA-based methods are very different from traditional morphological classifications and the use of DNA-based methods is resolving current controversies in fungal taxonomy (Hibbett 2006, Moncalvo et al. 2000).

Previous studies by Lynch (2004) and Bahnmann (2009) used DNA-based methods to assess the community composition of Agaricomycetes in Michigan agricultural soils. Bahnmann (2009) repeated DNA-based methods used by Lynch (2004) and concluded that DNA-based surveys of Agaricomycetes are repeatable across years.

1.6 Phylogenetics and multivariate statistics

One way to study changes at the community level is through the use of phylogenetics. Phylogenetics is the study of evolutionary relationships of a group of organisms (Nei and Kumar 2000). Phylogeny can be inferred from morphological characteristics, which are often not reliable as many different species have developed similar but non-homologous characteristics through convergent evolution. Phylogeny is more confidently inferred using molecular evidence, such as DNA, RNA, or protein, because these can be represented as short standardized sequences that can distinguish species and higher taxa based on genetic variation (Hajibabaei et al. 2007). Phylogenetic relationships of genes or organisms are usually presented in a phylogenetic tree, most commonly represented as a cladogram, which is a tree that depicts branching order (Gregory 2008). Any phylogenetic tree is merely a hypothesis, to be further tested with additional data; the true phylogeny is almost always unknown. The topology of the tree may be altered by a larger or different taxon sample or by changes to the parameters of the phylogenetic analysis

A phylogenetic tree can be analyzed using multivariate statistical analyses which are useful for analyzing large, complex data sets (Ramette 2007). The basic aim of multivariate, or exploratory analyses, is to represent the (dis)similarity between objects (eg. samples, sites) based on values of multiple variables associated with them (Ramette 2007). Multivariate analyses are useful to reveal patterns in large data sets with many interacting variables, but do not directly explain why those patterns exist (Ramette 2007). Traditionally, multivariate analyses are not able to incorporate phylogenetic data.

However, UniFrac, a program used in this study, is a suite of tools used that can run multivariate analyses on phylogenetic data.

1.7 Thesis rationale and objectives

Agaricomycetes provide free ecosystem services that maintain and alter soil quality by decomposing plant litter, forming mutualistic associations with plants, and by maintaining soil structure. Current farming practices dictate that much of agricultural land is subject to soil homogenization through tillage and ploughing and further alteration through chemical inputs such as pesticides and fertilizers. In order to utilize the ecosystem services that Agaricomycetes provide, a more comprehensive understanding on how tillage specifically affects agaricomycete community structure is the goal of this study. Previous studies by Lynch (2004), Bahnmann (2009), Thorn et al. (1996), Vranic and Thorn (unpublished (2005-2007)), and H. Deacon and Thorn (unpublished (2006-2009)) provide a larger rDNA data set from samples taken from KBS LTER in different years, and provided the basis for this study. Lynch's (2004) phylogenetic analysis revealed increased species and clade diversity with decreasing disturbance and certain species and clades were found only in certain treatments. Bahnmann (2009) had similar findings but repeated molecular sampling indicated that only some clades are stable and persistent throughout sampling years.

The objectives of this study were:

i) to detect patterns in the phylogeny that could not be detected in individual previous studies

ii) to use phylogeny-based and community-based analyses to compare composition of Agaricomycetes among four different agricultural treatments

With the use of a larger data set and new phylogenetic tools, and under the assumption that tillage, aboveground plant community composition, and chemical inputs affect community composition of soil Agaricomycetes, the goal of this thesis is to determine what differences in community composition can be seen among different land management practices, from conventional tillage to no tillage to successional plant communities. The Kellogg Biological Station Long Term Ecological Research (KBS LTER) site provided an ideal location to test the relationship between soil tillage and soil Agaricomycetes because experimental plots at KBS represent different degrees of agricultural disturbance by tillage as well as successional plant communities. This thesis will be more comprehensive than past research because it is based on a larger set of pooled data, whereas previous studies by Lynch (2004) and Bahnmann (2009) were conducted on individual sample sets. Futhermore, since 2004-2009, there has been an increase in the number of reference sequences available in GenBank, increasing the chances of related reference sequences for more of the unknown soil sequences. New trends may be resolved in this study due to advancements in phylogenetics and placement of previously unknown taxa of Agaricomycetes based on published (Hibbett et al. 2007, Matheny et al. 2007) and unpublished data (Thorn and Hibbett). Finally, phylogenetic analysis in combination with multivariate statistics that have not been previously applied to them may provide novel insights not recognized in past studies by Lynch (2004) and Bahnmann (2009). These tools will give more detailed insight into how tillage may affect the community composition of soil Agaricomycetes. Understanding this relationship must

underlie the analysis of consequences of anthropogenic land use change in agroecosystems.

CHAPTER 2: MATERIALS AND METHODS

2.1 Study Site

Data were obtained from soil samples collected at the Kellogg Biological Station Long Term Ecological Research site (KBS LTER). KBS is one of 26 sites that belong to the national LTER network. It was established in 1989 as a long-term research site for ecological and environmental studies on agroecosystems. KBS LTER is located in southwest Michigan, 50 km east of Lake Michigan (42° 24' N, 85° 24' W, elevation 288 m). The site covers 1600 ha of cropping systems, successional communities, and small lakes. The area surrounding the KBS site is mainly a rural landscape, where vegetation ranges from cultivated fields to early successional fields to old growth forests. A more detailed site description can be found on the KBS website (http://lter.kbs.msu.edu/about/site_description/index.php). Sampled sites at KBS include conventional till (CT) and no till (NT) plots of either corn, soybean, or wheat in alternating years, plots tilled until 1989 then allowed to enter succession (HTS), and a successional community that has never been tilled (NTS) (Appendix 1).

2.1.1 Soil

Soil in southwestern Michigan is characteristic of a mature glacial outwash plain and moraine complex as initially formed by the Wisconsin glaciation. Nowadays, soils in the region are mostly sandy-loam and silty clay loams of moderate fertility. Soils found at KBS fall into 15 soil series representing 4 general soil orders: alfisols, entisols, histosols, and mollisols. The dominant soil type at KBS is sandy-loam alfisol in the Main Cropping System Experiment (MCSE) (Broughton and Gross 2000).

2.1.2 Agricultural treatments

Experimental plots at KBS include different cropping systems, with a range of chemical-input intensities, and successional communities. Soil samples were collected by Thorn in June and July 1993, Lynch in June and October 2002, Bahnmann in November 2004 and November 2005, and Deacon in June 2006 from replicate plots of four treatments: conventional till (CT), no till (NT), historically tilled successional (HTS), and never tilled successional (NTS), located within the MCSE.

CT plots are tilled annually by chisel plow before seed planting in May. NT plots have not been tilled since 1989, and row crops were drill seeded. HTS plots have not been tilled since 1989 and have been allowed to turn into successional meadow, maintained by periodic burning. Finally, NTS plots are represented by a meadow that has not been tilled in recorded history and has been maintained by annual mowing since 1989. Each replicate plot is approximately 1 ha, except for NTS plots, which are approximately 0.1 ha in area and approximately 200 m off-site. Both CT and NT plots receive chemical inputs of fertilizer (N, P, K, lime) at agronomically relevant levels depending on the crop of the year. HTS and NTS plots have not received fertilizer since 1989; however, there is an N-subplot within NTS that was fertilized since 2001; no samples for this study came from that subplot. No pesticides have been applied to CT, NT, HTS or NTS plots.

2.1.3 Plant communities

Corn, soybean or wheat are planted each year in CT and NT plots and rotated among years (Table 2.1).

Table 2.1 Cover crop planted on conventional till (CT) and no till (NT) plots during sampling years

	1993	1994	2002	2004	2005	2006
Corn	X		X		X	
Soybean		X				X
Wheat				X		

HTS is an old field dominated by goldenrods (*Solidago canadensis*), clover (*Trifolium*), and non-native grasses (*Elymus*, *Poa* and *Phleum*). NTS is a meadow dominated by non-native grasses (*Bromus*, *Poa*, and *Arrhenatherum*), goldenrods (*Solidago canadensis*), brambles (*Rubus*), and clover (*Trifolium*). More detailed plant community information during years of sampling can be found by downloading the complete data table on the KBS website (http://lter.kbs.msu.edu/datatables/237).

2.2 Soil sample collection, washing and isolation of fungal cultures or DNA

All procedures described in this section were carried out by previous investigators (Thorn et al. 1996, Lynch 2004, Bahnmann 2009, Deacon, Vranic and Thorn, unpublished) and are reported as background information to my study. Soil cores (2.5 cm diameter by 15-30 cm depth) were collected from 5 or more locations within 4-6 replicate plots of each treatment, and were kept at 4°C until processed. Subsamples of 10 g (fresh weight) from pooled soil samples from each plot (Thorn et al. 1996, Bahnmann 2009, Deacon unpublished) or from individual sampling sites within plots (Lynch 2004) were suspended in 125-150 ml of 0.1 M sodium pyrophosphate, shaken for 5 minutes and washed through sieves of decreasing pore size: #16 (1.18 mm), #60 (0.25 mm), and #270 (0.053 mm), which were washed and sterilized with 70% ethanol between samples.

Washed organic material collected on the 0.053 mm mesh sieve (250-1000 µL) was used to isolate cultures of Basidiomycetes on selective agar media (Thorn et al. 1996, Vranic unpublished). Soil DNA was extracted using a bead beating proctocol with either the Power Soil DNA extraction Kit ® (Mo Bio Laboratories, Inc.) (Bahnmann 2009) or UltraClean™ Soil DNA Kits (Mo Bio Laboratories, Inc.) (Lynch 2004) and the FastPrep™ FP120 instrument (Bio101) with 4 cycles of 30 seconds at setting number 4 with breaks of 5 minutes on ice in between cycles (Lynch 2004, Bahnmann 2009, Deacon unpublished).

2.2.1 PCR amplification and TA cloning

Amplification of soil-extracted fungal DNA was carried out using primers with specificity for Basidiomycota (Lynch and Thorn 2006). The primers used were B001 (5' – GCTTTACCACATAAATCTGA – 3') and B2R+ (5' – TACCGTTGTAGTCTTAACAG – 3') (Lynch and Thorn 2006, Gardes and Bruns 1993). These primers yielded an amplification product approximately 2.4 kb in length spanning the nuclear ribosomal ITS region and approximately 1000 bp into the small (SSU or 18S) and large (LSU or 25S) rRNA genes. Amplification used a standard PCR protocol with 20-30 seconds at an annealing temperature of 55°C and 120 seconds extension at 72°C. Successful amplifications were gel-purified and used as a template for TOPO TA cloning (Invitrogen Corp., Mississauga, Canada). Twelve to 20 randomly selected clones from each cloning reaction were subjected to restriction fragment length polymorphism (RFLP) screening using either *Rsa*I or *Msp*I restriction enzymes (Fisher Scientific Ltd., Nepean, Canada). Clones with unique RFLP banding patterns within a cloning reaction

were sequenced using LR3 (5' – GGTCCGTGTTTCAAGAC – 3') sequencing primer described by Vilgalys and Hester (1990), yielding approximately 5' 600bp of the LSU gene.

2.3 Phylogenetic analysis

This section represents my own study methods. A set of 102 LSU sequences of Agaricomycetes representing the major phylogenetic groups of Agaricomycetes (Hibbett et al. 2007, Thorn and Hibbett, unpublished) were downloaded from GenBank to provide references and clustering points for KBS sequences. A list of reference sequences with GenBank accession numbers is included (Appendix 2). Five hundred ninety-one sequences of Agaricomycetes collected from KBS LTER and 123 of their closest nucleotide-nucleotide BLAST (Basic Local Alignment Search Tool) matches (http://www. ncbi.nlm.nih.gov) were compiled. Reference sequences, unknown sequences and their closest BLAST matches were the basis on which phylogenetic analysis was conducted.

All sequences were compiled into one FASTA file and were initially aligned using the ClustalW algorithm in MEGA5 (Tamura et al. 2011). However, the alignment was unsuccessful, probably due to the phylogenetically broad taxon sample and ragged sequence matrix (with some taxa having sequences that started or ended beyond others). To solve the latter issue, sequences were trimmed at the 5' end of the LSU gene using the identifier sequence CAAATCAG, and (where present) after the LR5 primer TTTCCCTCAGGA (Vilgalys and Hester 1990). The sequence region retained includes only LSU data, removing the highly variable ITS region; the ITS region was difficult to

align across the phylogenetically broad set of taxa included. The data matrix included up to approximately 900 bases of each sequence from most reference taxa, and 600 bases from unknown sequences from KBS LTER.

The file was split into two separate files to facilitate alignment, with the same three out-groups defined in each file: *Tremella aurantia*, *Trichosporon dulcitum*, and *Dacrymyces chrysospermus*. Species in the out-groups are from the classes Tremellomycetes and Dacrymycetes, of the sub-phylum Agaricomycotina. Both files were converted from FASTA to multi-FASTA format using DNA Baser (DNA Baser Sequence Assembler 2011) for alignment in SINA (v.1.2.9), which uses ribosomal structure to guide alignment of small and large subunit ribosomal RNA sequence data (Pruesse et al. 2007). The two alignment files were then merged using MUSCLE (Edgar 2004) and the alignment subsequently confirmed by visual inspection in SeaView (Gouy et al. 2010). The aligned file contained 816 sequences by 1274 bases, of which 259 were invariant and 794 were phylogenetically informative.

A constraint tree was defined for reference sequences such that they were constrained to a topology derived from multiple gene phylogenies of the Basidiomycota (Hibbett et al. 2007), while the unknown sequences were allowed to join the tree wherever their placement was optimal under Neighbor Joining (NJ) in PAUP 4.0b10 (Swofford 2002, Tamura et al. 2004). An NJ analysis with enforced backbone constraint was run in PAUP, using the BioNJ algorithm (Gascuel 1997). Bootstrap support was calculated for the NJ tree based on a heuristic search of 100 replicates, each with 10 random order sequence addition replicates (Swofford 2002). The resultant tree was edited in ClarisDraw to improve fonts and divide the tree into page-size portions. Operational

taxonomic units (OTUs) were defined as a single sequence or groups of sequences that had less than ~1% change in sequence identity.

2.4 Statistical methods and multivariate analysis

All community analyses were completed using the Unique Fraction (UniFrac) suite of software (Lozupone et al. 2006). UniFrac is a diversity measure that uses phylogenetic information to compare environmental samples by measuring the distance between communities based on the lineages they contain (Lozupone and Knight 2005; Lozupone et al. 2011). The web application required two input files; the phylogenetic tree generated in PAUP in NEXUS format and an environmental file describing the source of each sequence in the phylogenetic tree as a text file.

The UniFrac metric was used to determine whether communities in the sampled plots ('environments') were significantly different (Lozupone and Knight 2005). The UniFrac significance metric was calculated once using the "each environment individually" option and "all environments together" option. The "each environment individually" option measures each sample against the rest of the tree, treated as a single "other" environment and determines if that particular environment has a more unique branch length than expected by chance (Lozupone and Knight 2005). Its output is a table of P-Values for each separate environment indicating whether it is significantly different from the rest of the tree. This allows one to determine which particular environment is contributing to the unique branch length. The "all environments together" option determines whether or not sequences from all different environments, when measured against each other, are significantly different from each other (Lozupone and Knight

2005). In each case, the output is a corrected P-Value and a raw P-Value. A corrected P-Value is the raw P-Value that has been corrected for multiple comparisons when there are two or more. In each case, the number of tree permutations used to generate the random distribution of values to which the value of the true tree is compared when calculating a P-Value was set at 100. The number of permutations is a parameter that can be adjusted within this analysis that can re-analyze the data up to 100 times (Lozupone and Knight 2005).

The Parsimony test (P-test) in UniFrac was also performed (Martin 2002). The P-test determines whether the differences between environments are significant using phylogenetic information (Martin 2002). This was done in support of the UniFrac significance test. The P-test is more likely to give a significant result than the UniFrac significance metric when many sequences are very closely related to one another but are unique to one particular environment (Lozupone and Knight 2005). UniFrac determines two P-Values, raw and corrected. The Bonferroni correction is used to correct P-Values for multiple comparisons. Parsimony is employed by characterizing the tree by computing the minimum number of changes to explain the observed distribution of sequences between the different environments in the tree (Martin 2002). The output is a P-test value that is a fraction of random permutations of the labels that require the most parsimonious explanation. Therefore the P-test, using tree topology, tests the hypothesis that fewer changes from one environment to another are required to explain the observed distribution of sequences than would be required if the environments were randomly assigned to each sequence (Martin 2002). Again, the number of permutations was set at 100.

A jackknife environment cluster analysis was performed on the tree. Jackknifing is a statistical re-sampling technique that determines whether or not the same results could be found using only a random sub-sample of the data (Lozupone and Knight 2005). The number of permutations can be set to re-cluster the data 10 or 100 times (Lozupone and Knight 2005). The number of permutations was set at 100. Jackknifing is used to determine how robust the analysis is to both sample size and evenness by assessing how often the cluster nodes are recovered when smaller, even sets of sequences are sampled from each environment (Lozupone and Knight 2005). Jackknifing minimizes within-group variation and maximizes between-group variation in order to reveal well-defined groups or clusters of environments and therefore reduce the dimensionality of the data set to a manageable group of rows (Ramette 2007). Clustering environments are useful to determine patterns that could not have been determined from the pattern of significant differences alone (Lozupone and Knight 2005). The result is a tree-like diagram that shows how samples relate to one another by how strongly they are clustered, and this allows one to be more confident of clusters that are well-supported (Lozupone and Knight 2005).

While cluster diagrams are useful for showing which environments are most closely related to one another, principal coordinates analysis (PCoA), also known as metric multidimensional scaling, is useful to show how environments are distributed along multiple axes of variation (Lozupone and Knight 2005, Schmit and Lodge 2005). PCoA uses a linear mapping of distance or dissimilarities between objects, ‘sites’ or ‘replicate environments’ or ‘treatments’ for the purposes of this research, onto the ordination space (projection in a Cartesian space) so that similarities and differences

between replicate environments, which are represented as points in the ordination space, can be more easily visualized (Ramette 2007). Eigenvalues are used to measure how much variance is accounted for by the largest synthetic variables, or principal components (Ramette 2007). PCoA uses presence-absence data, which is useful as a variety of distance measures can be used (Schmit and Lodge 2005). PCoA was completed in UniFrac by calculating the distance matrix for each pair of environments using the UniFrac metric (Lozupone and Knight 2005). Distances are represented by points in a 2D space with a number of dimensions one less than the number of samples (Lozupone and Knight 2005). UniFrac provides an output of raw data with eigenvalues and eigenvectors (Lozupone and Knight 2005). PCoA summarizes the data with many variables to a smaller set of synthetic, derived variables, or principal components. The principal components, in descending order, describe how much of the variation each of the axes in this new space explains (Lozupone and Knight 2005). The first principal component separates out the data as much as possible, the second principal component provides the next most separation, and so on (Lozupone and Knight 2005). The reduction often leaves residual variation, information in the original data that is not retained by the new principal components in order, which allows for easier visualization.

CHAPTER 3: RESULTS

3.1 Phylogenetic Tree

Generation of a phylogenetic tree in PAUP 4.0b10 (Figure 3.1) using KBS sequences and GenBank reference sequences, provided the basis on which statistical tests and multivariate analysis were conducted. Bootstrap support for nodes was represented by numbers on branches to the left or right of sequence names. Bootstrap numbers ranged from 0 to 100, where 51 was the lowest bootstrap value reported, indicating weak support, and where 100 was the highest bootstrap value possible, indicating very strong support. Operational taxonomic units (OTUs) were indicated to the right of sequence names. Convential till plots are coloured in red, no till plots are coloured in orange, historically tilled successional plots are coloured in yellow, and never tilled successional plots are coloured in green. A total of 472 OTUs were defined based on the 591 sequences recovered from the four focal treatments at KBS LTER and the major clade Agaricales accounted for 330 (70%) of all OTUs. The remaining OTUs were assigned to 12 of the 19 major clades of Agaricomycetes: Tulasnellales – 19 OTUs; Cantharellales – 39 OTUs; Polyporales – 23 OTUs; Thelephorales – 3 OTUs; Hymenochaetales – 29 OTUs; Corticiales – 2 OTUs; Geastrales – 4 OTUs; Gomphoid/Phalloid clade – 3 OTUs; Sebacinales – 9 OTUs; Russulales – 2 OTUs; Trechisporales – 4 OTUs; Auriculariales – 5 OTUs.

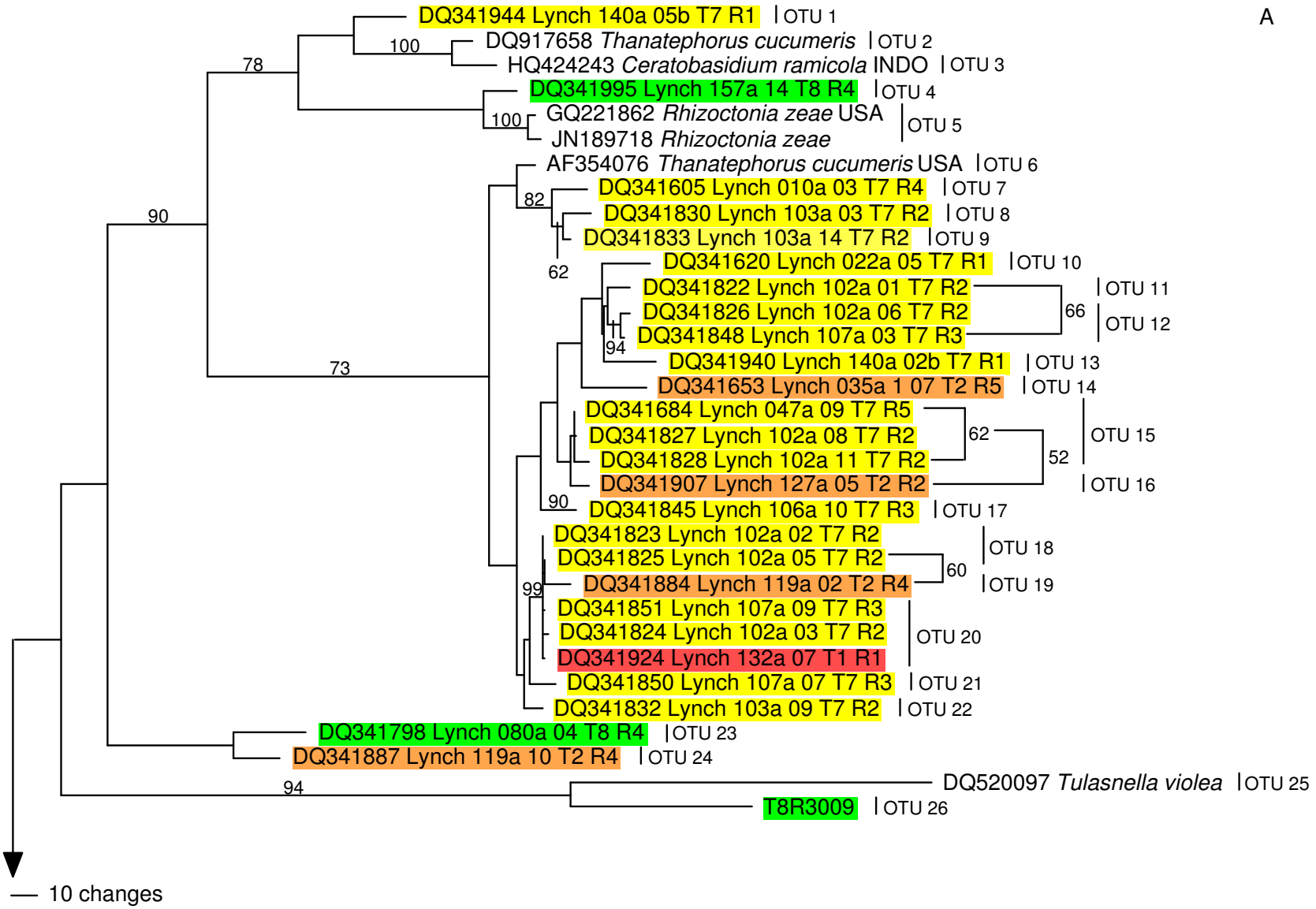
3.2 UniFrac significance test and P test

When sequences from all environments were tested individually using the UniFrac significance metric, the UniFrac P-Value given for each environment ranged

from ≤0.01 to 1.0 (Table 3.1). There was no significant difference for most environments when they were measured against the rest of the tree (treated as a single "other" environment). However, plots NT T2R2, HTS T7R4, HTS T7R5, and NTS T8R1-R4 had P-Values of ≤0.01, indicating that these seven plots were uniquely different in the phylogenetic makeup of their communities.

Figure 3.1. Neighbour-joining tree of reference sequences and sequences recovered from soils in KBS LTER sites: conventional till (CT); no till (NT); historically tilled successional (HTS); no till successional (NTS). Names coloured in red indicate CT; names coloured in orange indicate NT; names coloured in yellow indicate HTS; names coloured in green indicate NTS. Uncoloured names are reference sequences from GenBank, or other sequences not from the four study streatments at KBS LTER. The tree is scaled. The scale bar represents branch length corresponding to 10 changes for every 1000 bases. Major clades are (A) Tulasnellales; (B,C) Cantharellales; (D-F) Polyporales; (G-J) Agaricales: Clavarioid and Stephanosporaceae; (K-N) Agaricales: Agaricoid; (O,P) Agaricales: Tricholomoid; (Q) Agaricales: Schizophyllum-Lachnella, Maramioid; (R,S) Agaricales: Pluteoids: (T) Agaricales: Hygrophoroid, Tricholomoid; (U) Agaricales: Pterulaceae, Amylocorticales, Boletales, Atheliales, Jaapiales; (V) Thelephorales, Hymenochaetales, Corticiales, Gloeophyllales; (W) Geastrales, Gomphoid-Phalloid, Hymenochaetales; (X) Sebacinales, Russulales; (Y) Hymenochaetales; (Z) Dacrymycetes, Trechisporales, Auriculariales; (AA) Tremellomycetes

*"*Coprinus*" *cordisporus* name currently unresolved (Redhead et al. 2001)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JN189718 Rhizoctonia zeae | OTU 5
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DQ341823 Lynch 102a 02 T7 R2 | OTU 18
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94
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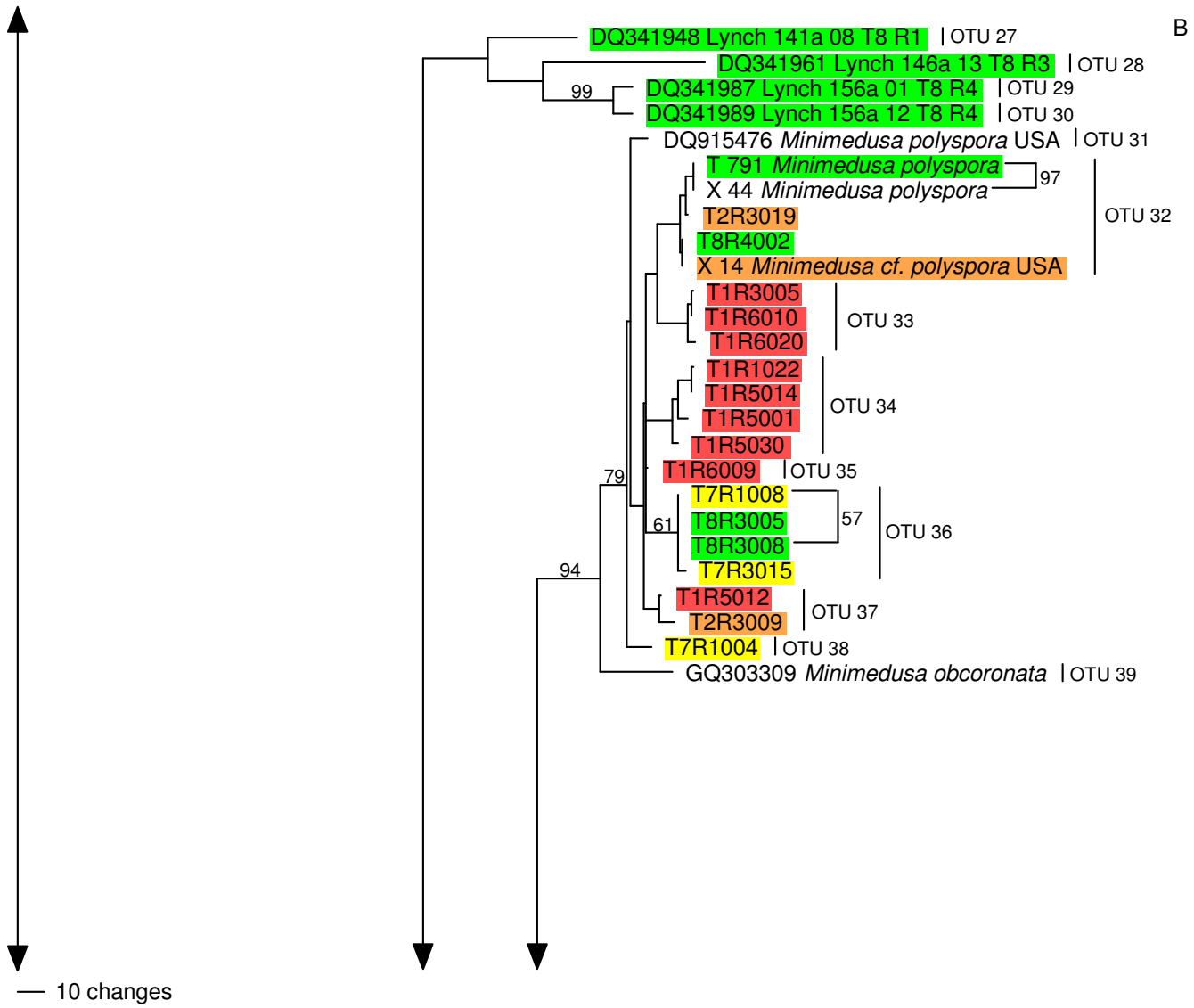
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OTU 28
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OTU 30
DQ915476 Minimedusa polyspora USA
OTU 31
T 791 Minimedusa polyspora
97
X 44 Minimedusa polyspora
T2R3019
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X 14 Minimedusa cf. polyspora USA
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T1R3005
T1R6010
T1R6020
OTU 33
T1R1022
T1R5014
T1R5001
T1R5030
OTU 34
79
T1R6009
OTU 35
T7R1008
61
T8R3005
T8R3008
57
T7R3015
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94
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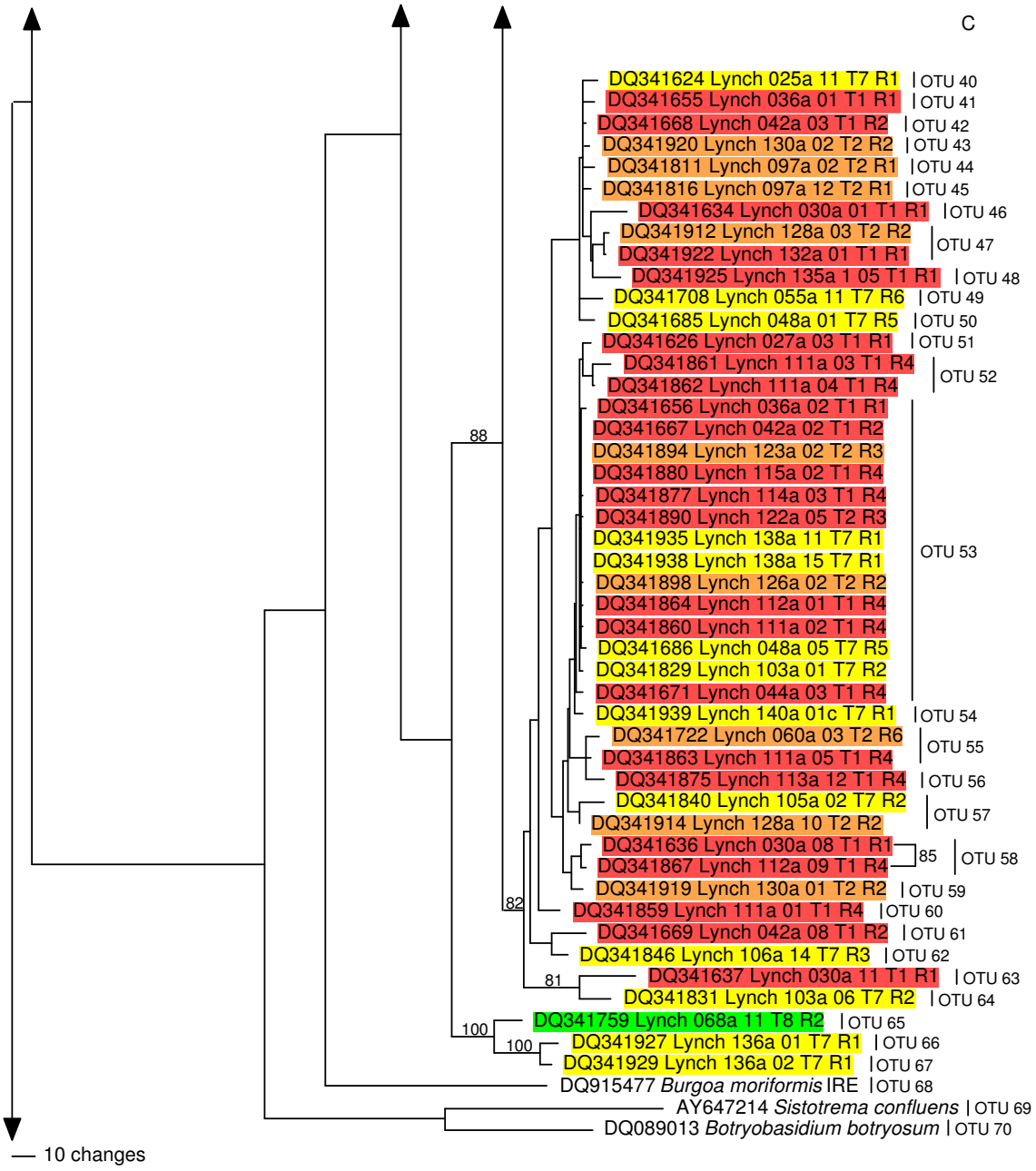
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DQ341626 Lynch 027a 03 T1 R1 | OTU 51
DQ341861 Lynch 111a 03 T1 R4
DQ341862 Lynch 111a 04 T1 R4 | OTU 52
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DQ341890 Lynch 122a 05 T2 R3
DQ341935 Lynch 138a 11 T7 R1
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OTU 53
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DQ341875 Lynch 113a 12 T1 R4 | OTU 56
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DQ341636 Lynch 030a 08 T1 R1
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85 | OTU 58
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DQ341927 Lynch 136a 01 T7 R1 | OTU 66
DQ341929 Lynch 136a 02 T7 R1 | OTU 67
DQ915477 Burgoa moriformis IRE | OTU 68
AY647214 Sistotrema confluens | OTU 69
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81
100
100
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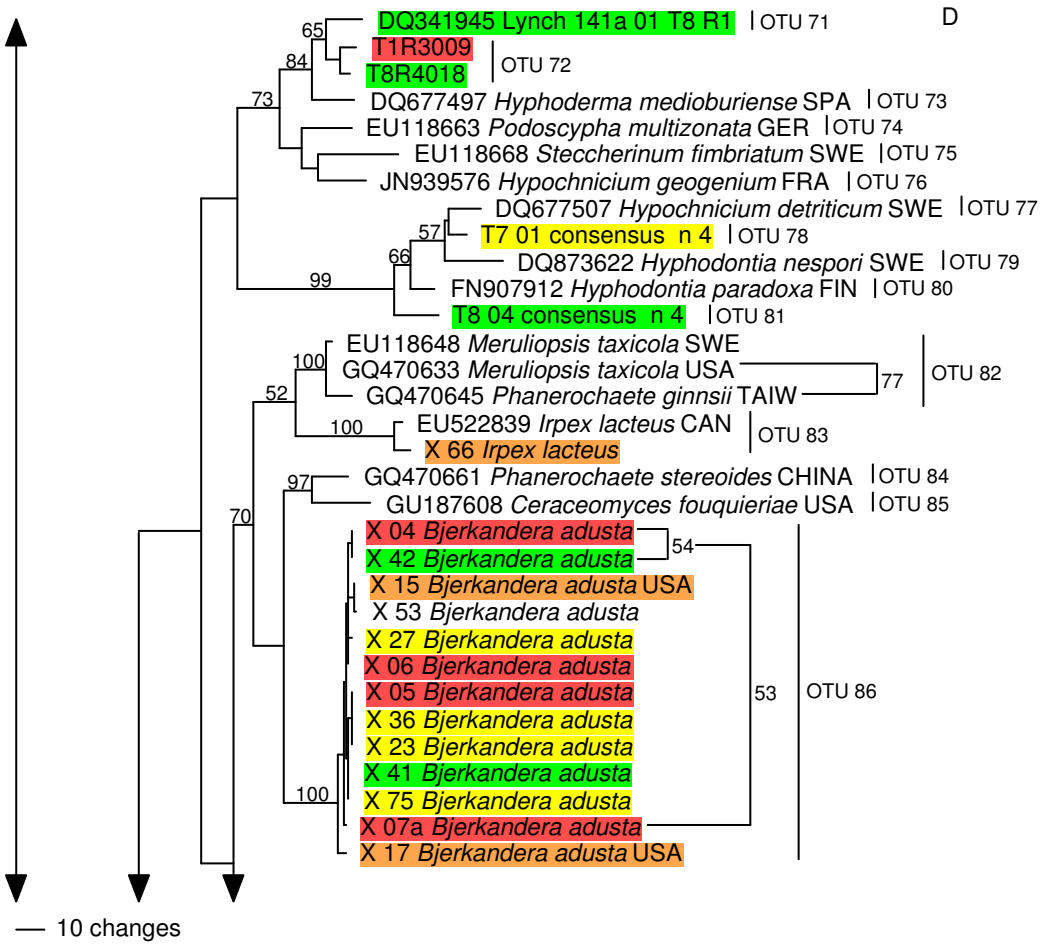
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OTU 74
EU118668 Steccherinum fimbriatum SWE
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JN939576 Hypochnicium geogenium FRA
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EU522839 Irpex lacteus CAN
X 66 Irpex lacteus
OTU 83
GQ470661 Phanerochaete stereoides CHINA
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GU187608 Ceraceomyces fouquieriae USA
OTU 85
X 04 Bjerkandera adusta
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X 27 Bjerkandera adusta
X 06 Bjerkandera adusta
X 05 Bjerkandera adusta
X 36 Bjerkandera adusta
X 23 Bjerkandera adusta
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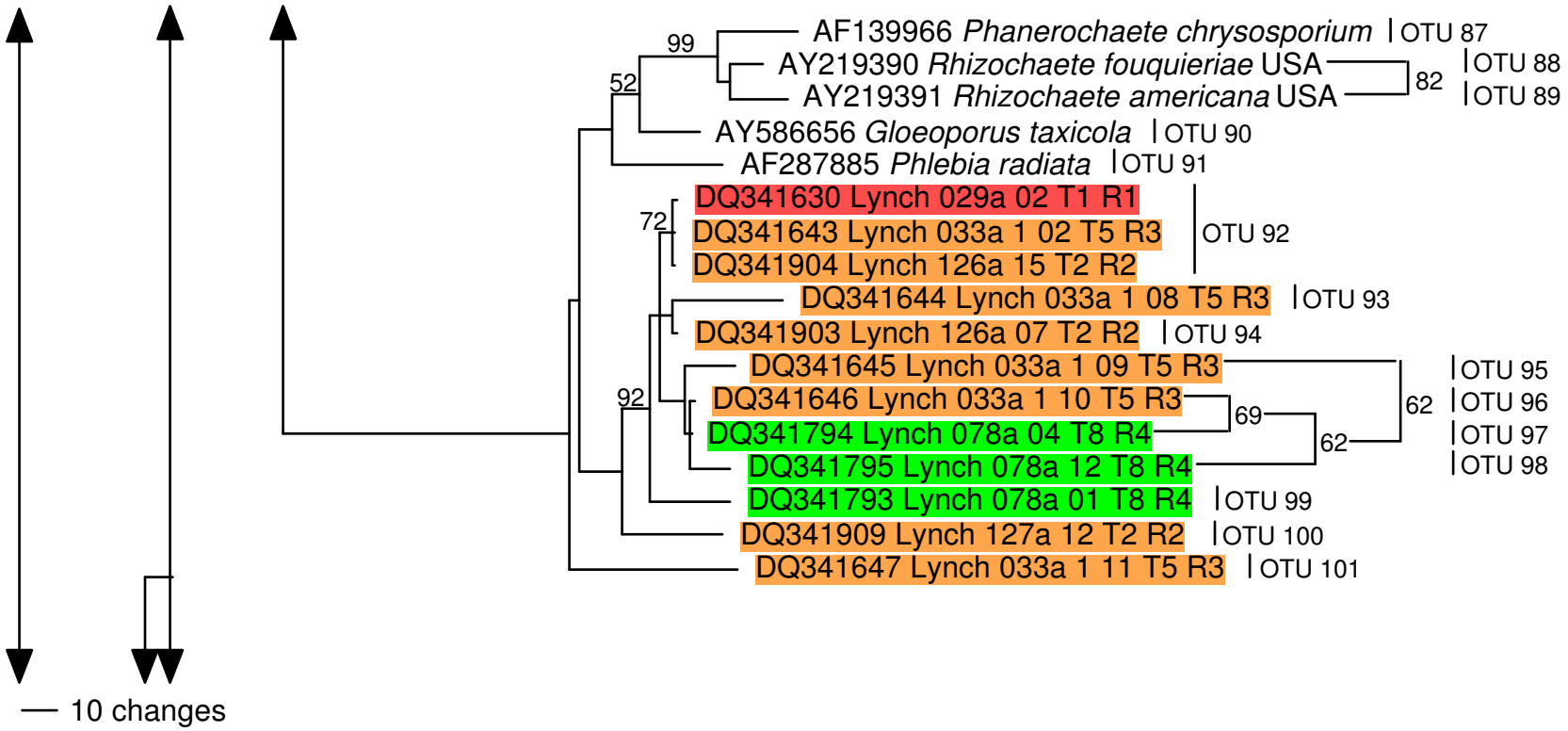
E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OTU 89
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OTU 90
AF287885 Phlebia radiata
OTU 91
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DQ341794 Lynch 078a 04 T8 R4
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OTU 95
OTU 96
OTU 97
OTU 98
DQ341793 Lynch 078a 01 T8 R4
OTU 99
DQ341909 Lynch 127a 12 T2 R2
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82
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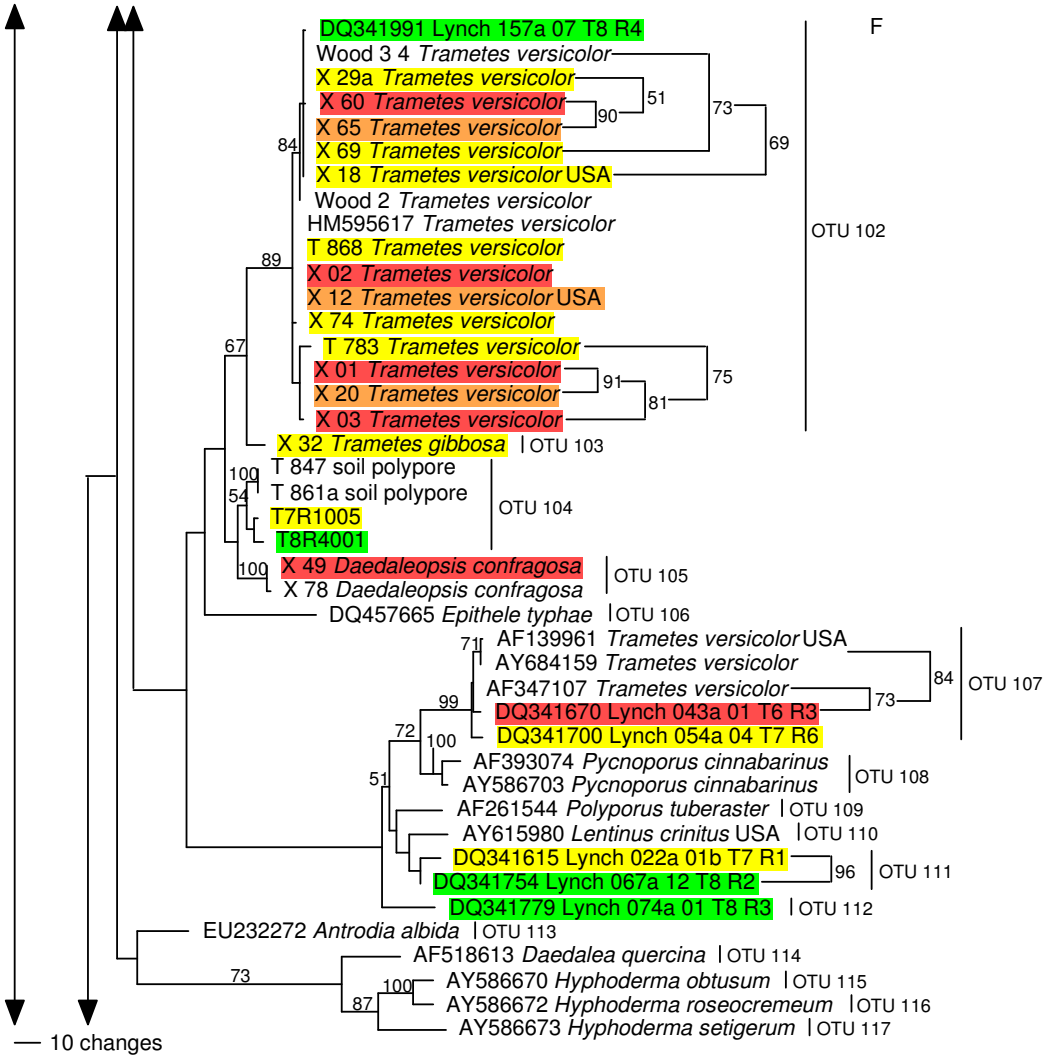
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X 29a Trametes versicolor
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X 65 Trametes versicolor
X 69 Trametes versicolor
X 18 Trametes versicolor USA
Wood 2 Trametes versicolor
HM595617 Trametes versicolor
T 868 Trametes versicolor
X 02 Trametes versicolor
X 12 Trametes versicolor USA
X 74 Trametes versicolor
T 783 Trametes versicolor
X 01 Trametes versicolor
X 20 Trametes versicolor
X 03 Trametes versicolor
OTU 102
X 32 Trametes gibbosa
OTU 103
T 847 soil polypore
T 861a soil polypore
T7R1005
T8R4001
OTU 104
X 49 Daedaleopsis confragosa
X 78 Daedaleopsis confragosa
OTU 105
DQ457665 Epithele typhae
OTU 106
AF139961 Trametes versicolor USA
AY684159 Trametes versicolor
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DQ341700 Lynch 054a 04 T7 R6
OTU 107
AF393074 Pycnoporus cinnabarinus
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OTU 108
AF261544 Polyporus tuberaster
OTU 109
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OTU 110
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OTU 112
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DQ341694 Lynch 053a 04 T6 R3
DQ341692 Lynch 053a 01 T6 R3
DQ341678 Lynch 046a 06 T7 R5
T8R2003
OTU 118
OTU 119
OTU 120
OTU 121
OTU 122
OTU 123
OTU 124
OTU 125
OTU 126
OTU 127
OTU 128
OTU 129
OTU 130
OTU 131
OTU 132
OTU 133
OTU 134
OTU 135
OTU 136
OTU 137
OTU 138
97
61
75
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72
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76
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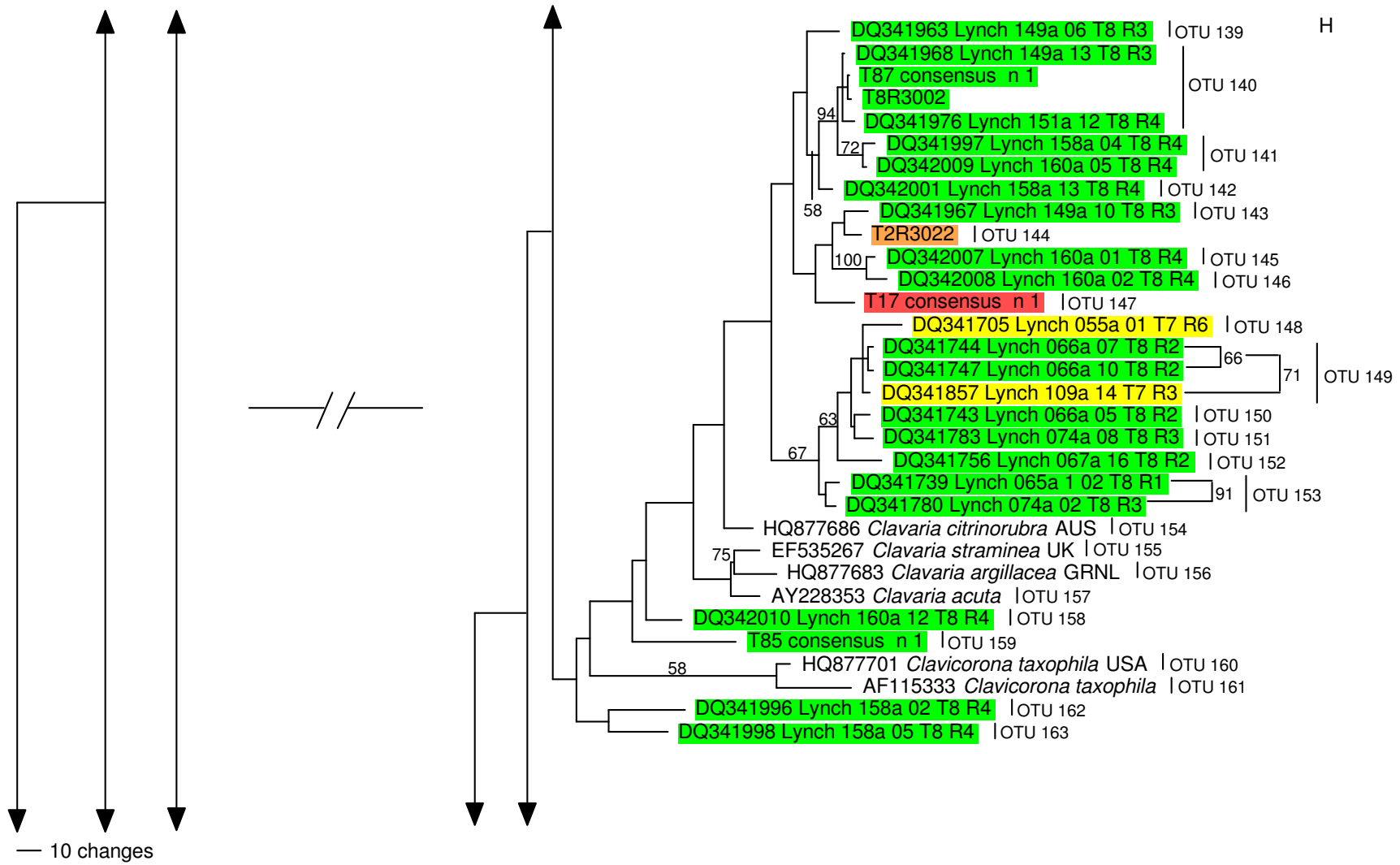
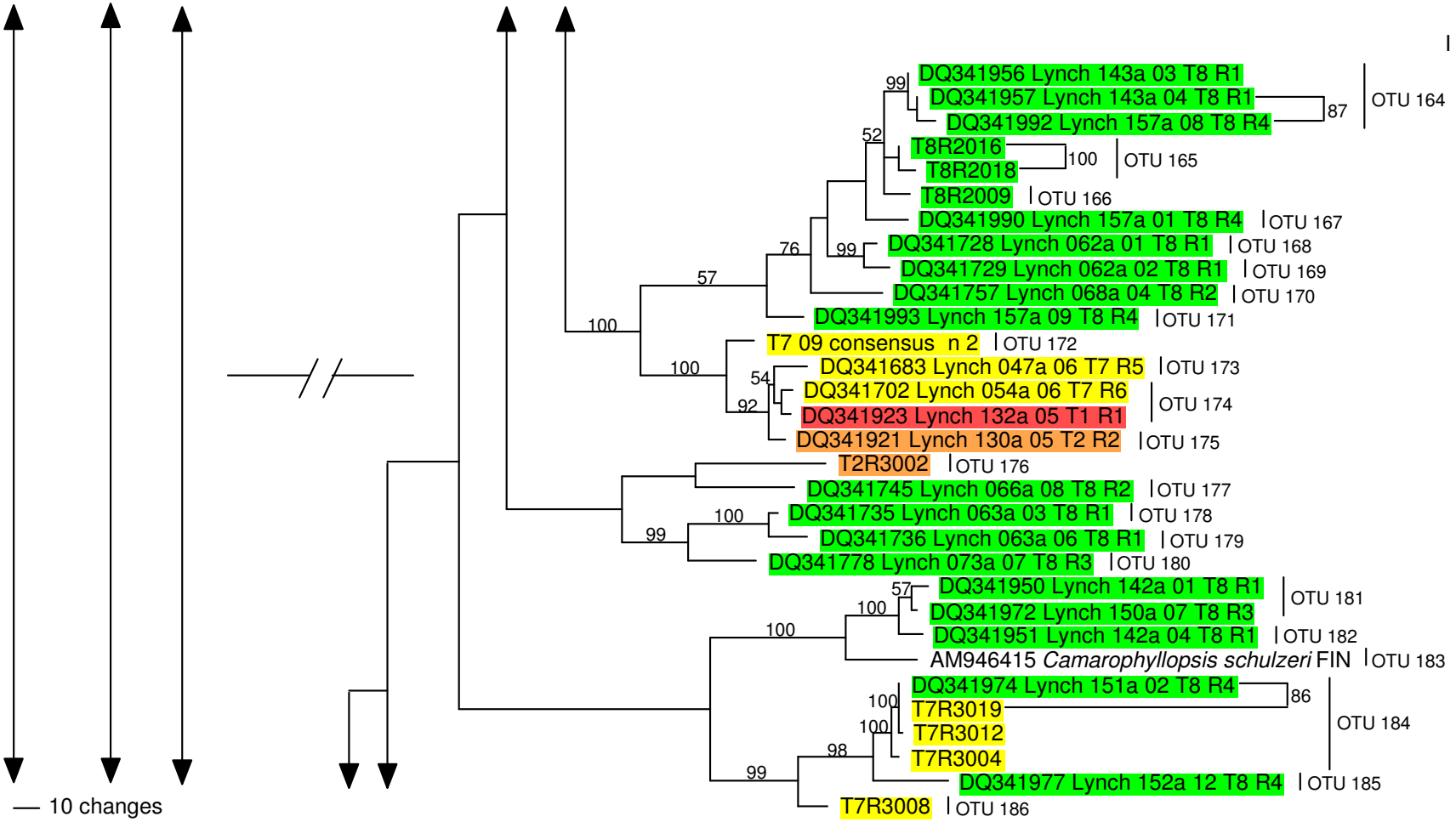
86
51
10 changes


H
DQ341963 Lynch 149a 06 T8 R3
OTU 139
DQ341968 Lynch 149a 13 T8 R3
T87 consensus n 1
T8R3002
OTU 140
94
DQ341976 Lynch 151a 12 T8 R4
72
DQ341997 Lynch 158a 04 T8 R4
DQ342009 Lynch 160a 05 T8 R4
OTU 141
DQ342001 Lynch 158a 13 T8 R4
OTU 142
58
DQ341967 Lynch 149a 10 T8 R3
OTU 143
T2R3022
OTU 144
100
DQ342007 Lynch 160a 01 T8 R4
OTU 145
DQ342008 Lynch 160a 02 T8 R4
OTU 146
T17 consensus n 1
OTU 147
DQ341705 Lynch 055a 01 T7 R6
OTU 148
DQ341744 Lynch 066a 07 T8 R2
66
DQ341747 Lynch 066a 10 T8 R2
71
DQ341857 Lynch 109a 14 T7 R3
OTU 149
63
DQ341743 Lynch 066a 05 T8 R2
OTU 150
DQ341783 Lynch 074a 08 T8 R3
OTU 151
67
DQ341756 Lynch 067a 16 T8 R2
OTU 152
DQ341739 Lynch 065a 1 02 T8 R1
91
DQ341780 Lynch 074a 02 T8 R3
OTU 153
HQ877686 Clavaria citrinorubra AUS
OTU 154
EF535267 Clavaria straminea UK
OTU 155
75
HQ877683 Clavaria argillacea GRNL
OTU 156
AY228353 Clavaria acuta
OTU 157
DQ342010 Lynch 160a 12 T8 R4
OTU 158
T85 consensus n 1
OTU 159
58
HQ877701 Clavicorona taxophila USA
OTU 160
AF115333 Clavicorona taxophila
OTU 161
DQ341996 Lynch 158a 02 T8 R4
OTU 162
DQ341998 Lynch 158a 05 T8 R4
OTU 163
10 changes


DQ341956 Lynch 143a 03 T8 R1
DQ341957 Lynch 143a 04 T8 R1
DQ341992 Lynch 157a 08 T8 R4
OTU 164
T8R2016
T8R2018
OTU 165
T8R2009
OTU 166
DQ341990 Lynch 157a 01 T8 R4
OTU 167
DQ341728 Lynch 062a 01 T8 R1
OTU 168
DQ341729 Lynch 062a 02 T8 R1
OTU 169
DQ341757 Lynch 068a 04 T8 R2
OTU 170
DQ341993 Lynch 157a 09 T8 R4
OTU 171
T7 09 consensus n 2
OTU 172
DQ341683 Lynch 047a 06 T7 R5
OTU 173
DQ341702 Lynch 054a 06 T7 R6
DQ341923 Lynch 132a 05 T1 R1
OTU 174
DQ341921 Lynch 130a 05 T2 R2
OTU 175
T2R3002
OTU 176
DQ341745 Lynch 066a 08 T8 R2
OTU 177
DQ341735 Lynch 063a 03 T8 R1
OTU 178
DQ341736 Lynch 063a 06 T8 R1
OTU 179
DQ341778 Lynch 073a 07 T8 R3
OTU 180
DQ341950 Lynch 142a 01 T8 R1
OTU 181
DQ341972 Lynch 150a 07 T8 R3
DQ341951 Lynch 142a 04 T8 R1
OTU 182
AM946415 Camarophyllopsis schulzeri FIN
OTU 183
DQ341974 Lynch 151a 02 T8 R4
T7R3019
T7R3012
T7R3004
OTU 184
DQ341977 Lynch 152a 12 T8 R4
OTU 185
T7R3008
OTU 186
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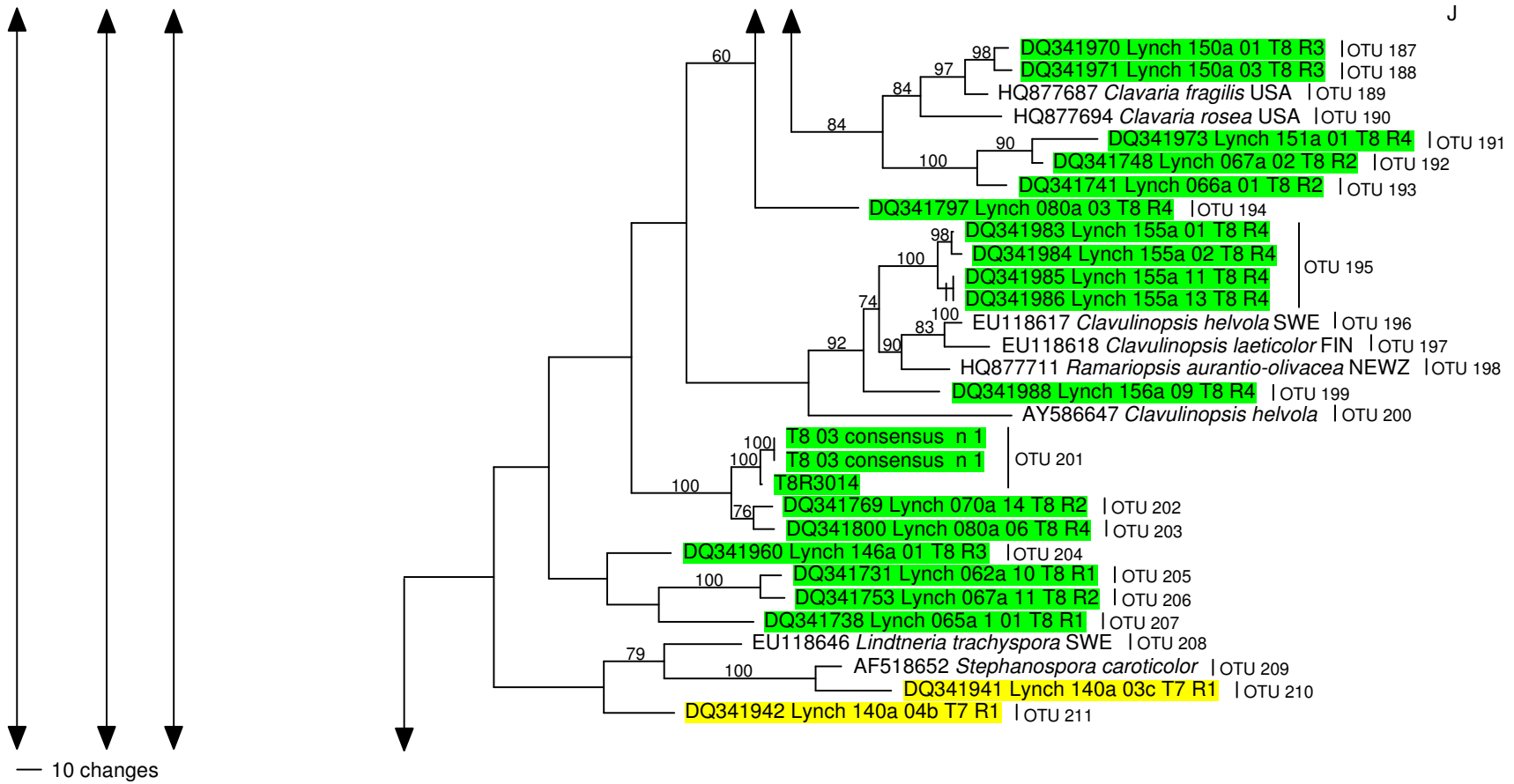
J
DQ341970 Lynch 150a 01 T8 R3 | OTU 187
DQ341971 Lynch 150a 03 T8 R3 | OTU 188
HQ877687 Clavaria fragilis USA | OTU 189
HQ877694 Clavaria rosea USA | OTU 190
DQ341973 Lynch 151a 01 T8 R4 | OTU 191
DQ341748 Lynch 067a 02 T8 R2 | OTU 192
DQ341741 Lynch 066a 01 T8 R2 | OTU 193
DQ341797 Lynch 080a 03 T8 R4 | OTU 194
DQ341983 Lynch 155a 01 T8 R4
DQ341984 Lynch 155a 02 T8 R4
DQ341985 Lynch 155a 11 T8 R4
DQ341986 Lynch 155a 13 T8 R4
OTU 195
EU118617 Clavulinopsis helvola SWE | OTU 196
EU118618 Clavulinopsis laeticolor FIN | OTU 197
HQ877711 Ramariopsis aurantio-olivacea NEWZ | OTU 198
DQ341988 Lynch 156a 09 T8 R4 | OTU 199
AY586647 Clavulinopsis helvola | OTU 200
T8 03 consensus n 1
T8 03 consensus n 1
T8R3014
OTU 201
DQ341769 Lynch 070a 14 T8 R2 | OTU 202
DQ341800 Lynch 080a 06 T8 R4 | OTU 203
DQ341960 Lynch 146a 01 T8 R3 | OTU 204
DQ341731 Lynch 062a 10 T8 R1 | OTU 205
DQ341753 Lynch 067a 11 T8 R2 | OTU 206
DQ341738 Lynch 065a 1 01 T8 R1 | OTU 207
EU118646 Lindtneria trachyspora SWE | OTU 208
AF518652 Stephanospora caroticolor | OTU 209
DQ341941 Lynch 140a 03c T7 R1 | OTU 210
DQ341942 Lynch 140a 04b T7 R1 | OTU 211
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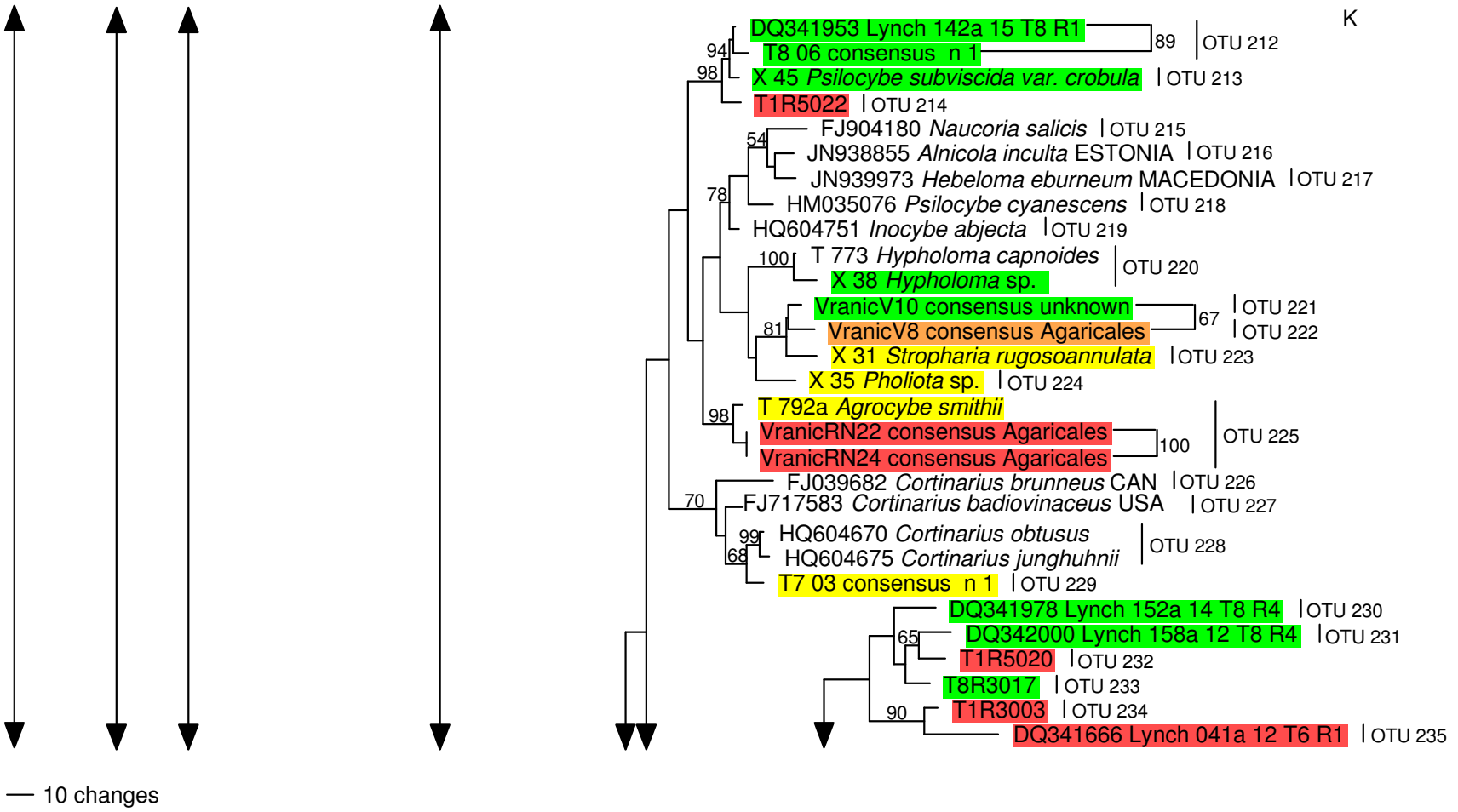
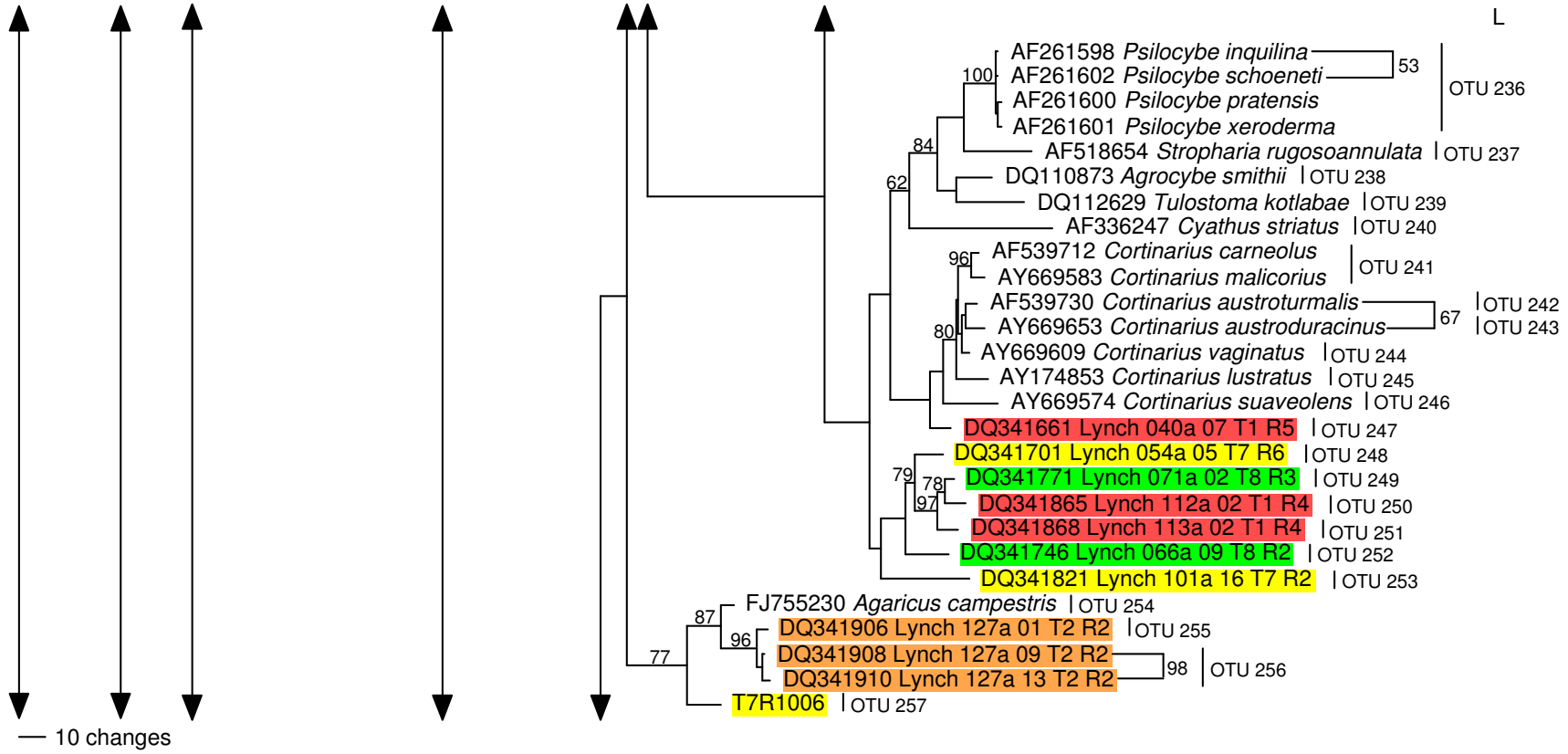

K
DQ341953 Lynch 142a 15 T8 R1
89
OTU 212
94
T8 06 consensus n 1
98
X 45 Psilocybe subviscida var. crobula
OTU 213
T1R5022
OTU 214
FJ904180 Naucoria salicis
OTU 215
54
JN938855 Alnicola inculta ESTONIA
OTU 216
JN939973 Hebeloma eburneum MACEDONIA
OTU 217
78
HM035076 Psilocybe cyanescens
OTU 218
HQ604751 Inocybe abjecta
OTU 219
100
T 773 Hypholoma capnoides
OTU 220
X 38 Hypholoma sp.
VranicV10 consensus unknown
67
OTU 221
81
VranicV8 consensus Agaricales
OTU 222
X 31 Stropharia rugosoannulata
OTU 223
X 35 Pholiota sp.
OTU 224
98
T 792a Agrocybe smithii
VranicRN22 consensus Agaricales
100
OTU 225
VranicRN24 consensus Agaricales
FJ039682 Cortinarius brunneus CAN
OTU 226
70
FJ717583 Cortinarius badiovinaceus USA
OTU 227
99
HQ604670 Cortinarius obtusus
68
HQ604675 Cortinarius junghuhnii
OTU 228
T7 03 consensus n 1
OTU 229
DQ341978 Lynch 152a 14 T8 R4
OTU 230
65
DQ342000 Lynch 158a 12 T8 R4
OTU 231
T1R5020
OTU 232
T8R3017
OTU 233
90
T1R3003
OTU 234
DQ341666 Lynch 041a 12 T6 R1
OTU 235
10 changes


L
AF261598 Psilocybe inquilina
AF261602 Psilocybe schoeneti
AF261600 Psilocybe pratensis
AF261601 Psilocybe xeroderma
53
OTU 236
100
AF518654 Stropharia rugosoannulata
OTU 237
84
DQ110873 Agrocybe smithii
OTU 238
62
DQ112629 Tulostoma kotlabae
OTU 239
AF336247 Cyathus striatus
OTU 240
AF539712 Cortinarius carneolus
AY669583 Cortinarius malicorius
OTU 241
96
AF539730 Cortinarius austroturmalis
AY669653 Cortinarius austroduracinus
67
OTU 242
OTU 243
80
AY669609 Cortinarius vaginatus
OTU 244
AY174853 Cortinarius lustratus
OTU 245
AY669574 Cortinarius suaveolens
OTU 246
DQ341661 Lynch 040a 07 T1 R5
OTU 247
DQ341701 Lynch 054a 05 T7 R6
OTU 248
79
78
DQ341771 Lynch 071a 02 T8 R3
OTU 249
97
DQ341865 Lynch 112a 02 T1 R4
OTU 250
DQ341868 Lynch 113a 02 T1 R4
OTU 251
DQ341746 Lynch 066a 09 T8 R2
OTU 252
DQ341821 Lynch 101a 16 T7 R2
OTU 253
FJ755230 Agaricus campestris
OTU 254
87
DQ341906 Lynch 127a 01 T2 R2
OTU 255
96
DQ341908 Lynch 127a 09 T2 R2
DQ341910 Lynch 127a 13 T2 R2
98
OTU 256
77
T7R1006
OTU 257
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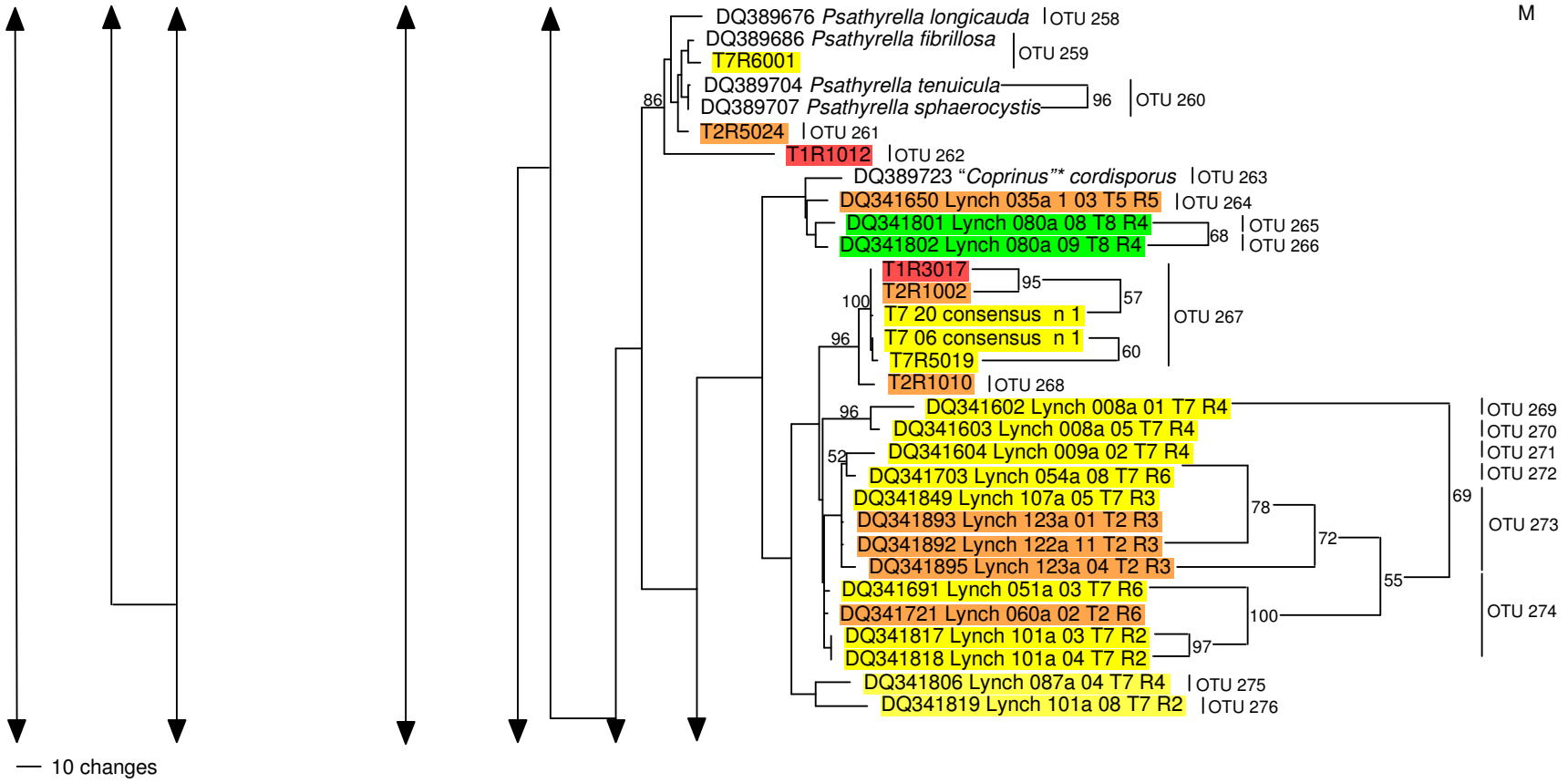
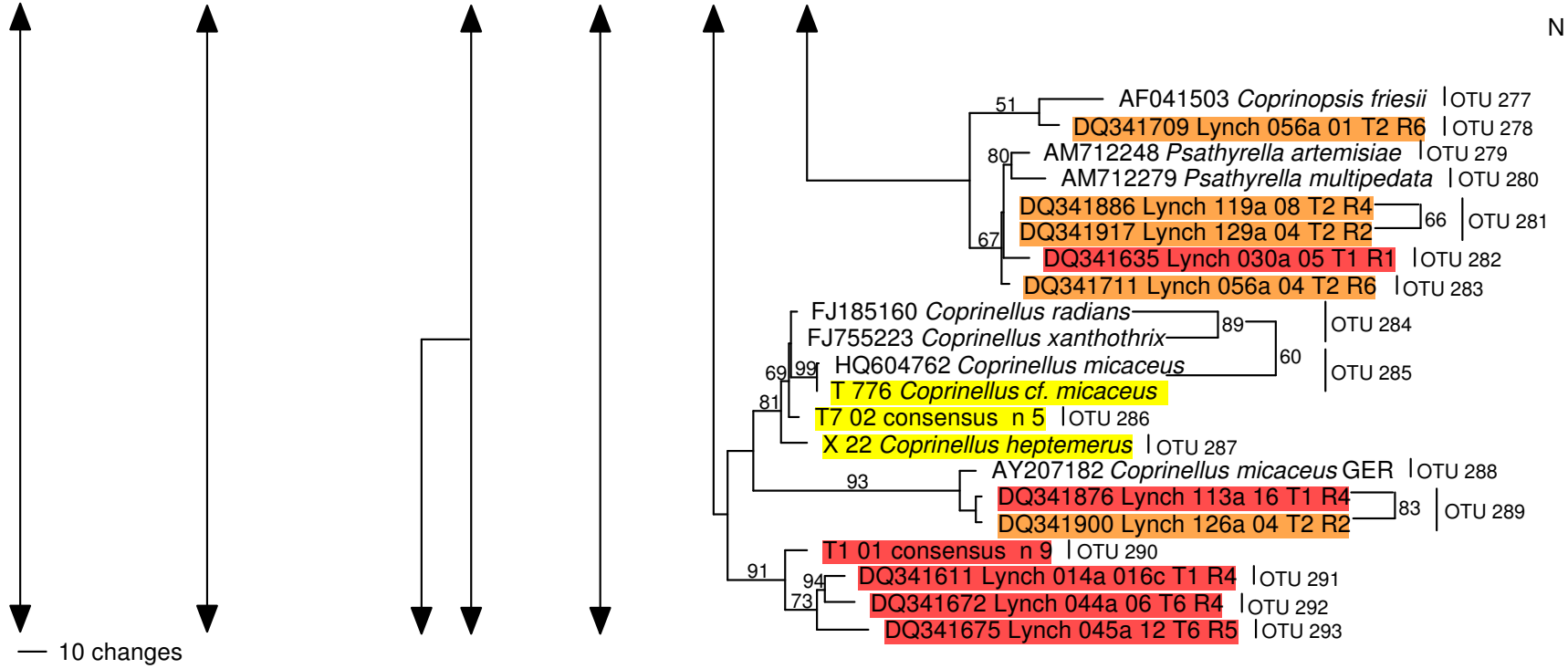
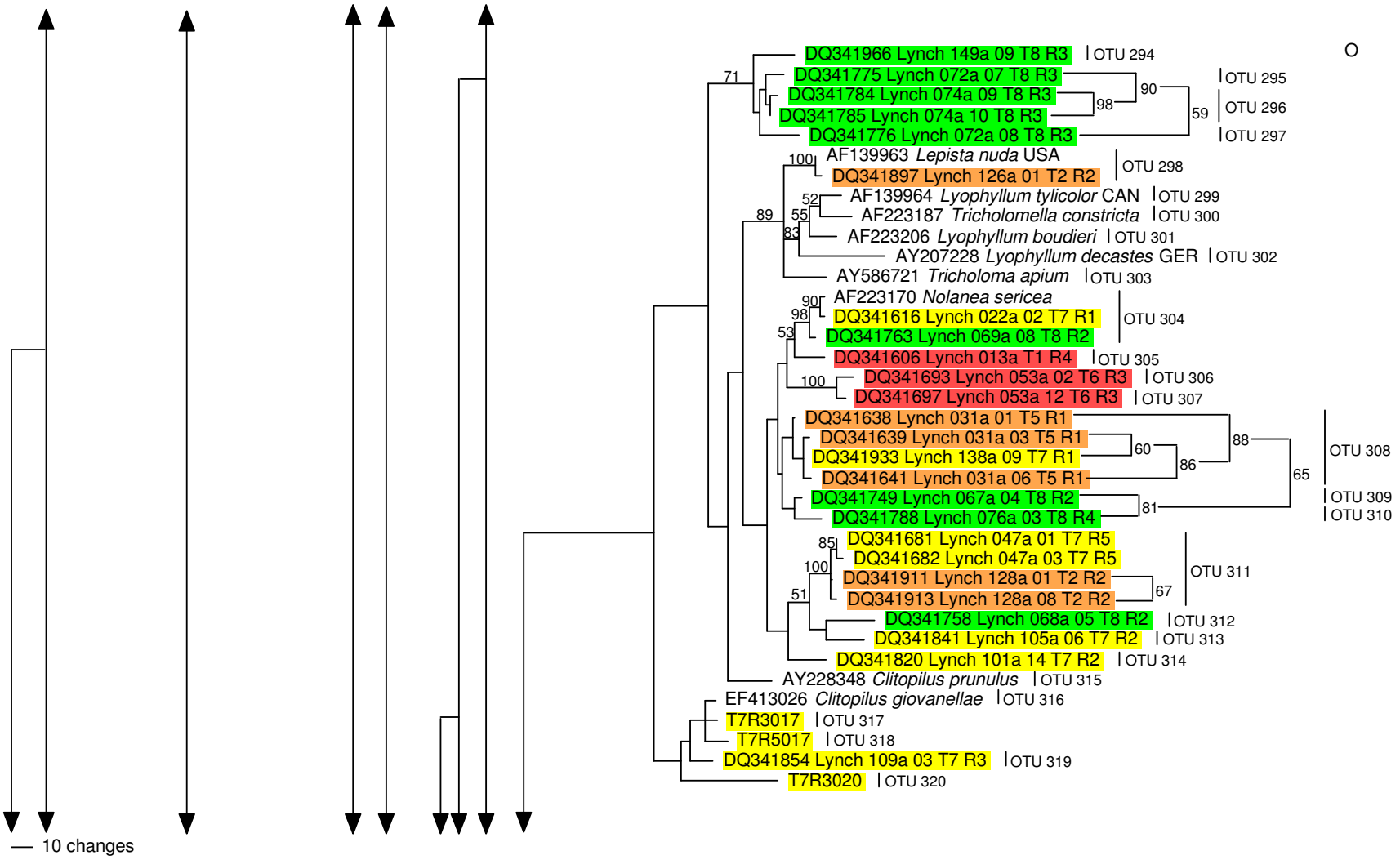

M
DQ389676 Psathyrella longicauda
OTU 258
DQ389686 Psathyrella fibrillosa
T7R6001
OTU 259
DQ389704 Psathyrella tenuicula
DQ389707 Psathyrella sphaerocystis
96
OTU 260
86
T2R5024
OTU 261
T1R1012
OTU 262
DQ389723 "Coprinus"* cordisporus
OTU 263
DQ341650 Lynch 035a 1 03 T5 R5
OTU 264
DQ341801 Lynch 080a 08 T8 R4
DQ341802 Lynch 080a 09 T8 R4
68
OTU 265
OTU 266
T1R3017
T2R1002
95
57
100
T7 20 consensus n 1
T7 06 consensus n 1
96
60
T7R5019
OTU 267
T2R1010
OTU 268
96
DQ341602 Lynch 008a 01 T7 R4
DQ341603 Lynch 008a 05 T7 R4
52
DQ341604 Lynch 009a 02 T7 R4
DQ341703 Lynch 054a 08 T7 R6
DQ341849 Lynch 107a 05 T7 R3
78
DQ341893 Lynch 123a 01 T2 R3
72
DQ341892 Lynch 122a 11 T2 R3
DQ341895 Lynch 123a 04 T2 R3
55
69
OTU 269
OTU 270
OTU 271
OTU 272
OTU 273
DQ341691 Lynch 051a 03 T7 R6
DQ341721 Lynch 060a 02 T2 R6
100
OTU 274
DQ341817 Lynch 101a 03 T7 R2
DQ341818 Lynch 101a 04 T7 R2
97
DQ341806 Lynch 087a 04 T7 R4
OTU 275
DQ341819 Lynch 101a 08 T7 R2
OTU 276
10 changes


N
AF041503 Coprinopsis friesii
OTU 277
DQ341709 Lynch 056a 01 T2 R6
OTU 278
AM712248 Psathyrella artemisiae
OTU 279
AM712279 Psathyrella multipedata
OTU 280
DQ341886 Lynch 119a 08 T2 R4
DQ341917 Lynch 129a 04 T2 R2
OTU 281
DQ341635 Lynch 030a 05 T1 R1
OTU 282
DQ341711 Lynch 056a 04 T2 R6
OTU 283
FJ185160 Coprinellus radians
FJ755223 Coprinellus xanthothrix
OTU 284
HQ604762 Coprinellus micaceus
T 776 Coprinellus cf. micaceus
OTU 285
T7 02 consensus n 5
OTU 286
X 22 Coprinellus heptemerus
OTU 287
AY207182 Coprinellus micaceus GER
OTU 288
DQ341876 Lynch 113a 16 T1 R4
DQ341900 Lynch 126a 04 T2 R2
OTU 289
T1 01 consensus n 9
OTU 290
DQ341611 Lynch 014a 016c T1 R4
OTU 291
DQ341672 Lynch 044a 06 T6 R4
OTU 292
DQ341675 Lynch 045a 12 T6 R5
OTU 293
10 changes


O
DQ341966 Lynch 149a 09 T8 R3
OTU 294
DQ341775 Lynch 072a 07 T8 R3
DQ341784 Lynch 074a 09 T8 R3
DQ341785 Lynch 074a 10 T8 R3
DQ341776 Lynch 072a 08 T8 R3
OTU 295
OTU 296
OTU 297
AF139963 Lepista nuda USA
DQ341897 Lynch 126a 01 T2 R2
OTU 298
AF139964 Lyophyllum tylicolor CAN
OTU 299
AF223187 Tricholomella constricta
OTU 300
AF223206 Lyophyllum boudieri
OTU 301
AY207228 Lyophyllum decastes GER
OTU 302
AY586721 Tricholoma apium
OTU 303
AF223170 Nolanea sericea
DQ341616 Lynch 022a 02 T7 R1
DQ341763 Lynch 069a 08 T8 R2
OTU 304
DQ341606 Lynch 013a T1 R4
OTU 305
DQ341693 Lynch 053a 02 T6 R3
OTU 306
DQ341697 Lynch 053a 12 T6 R3
OTU 307
DQ341638 Lynch 031a 01 T5 R1
DQ341639 Lynch 031a 03 T5 R1
DQ341933 Lynch 138a 09 T7 R1
DQ341641 Lynch 031a 06 T5 R1
DQ341749 Lynch 067a 04 T8 R2
DQ341788 Lynch 076a 03 T8 R4
OTU 308
OTU 309
OTU 310
DQ341681 Lynch 047a 01 T7 R5
DQ341682 Lynch 047a 03 T7 R5
DQ341911 Lynch 128a 01 T2 R2
DQ341913 Lynch 128a 08 T2 R2
OTU 311
DQ341758 Lynch 068a 05 T8 R2
OTU 312
DQ341841 Lynch 105a 06 T7 R2
OTU 313
DQ341820 Lynch 101a 14 T7 R2
OTU 314
AY228348 Clitopilus prunulus
OTU 315
EF413026 Clitopilus giovanellae
OTU 316
T7R3017
OTU 317
T7R5017
OTU 318
DQ341854 Lynch 109a 03 T7 R3
OTU 319
T7R3020
OTU 320
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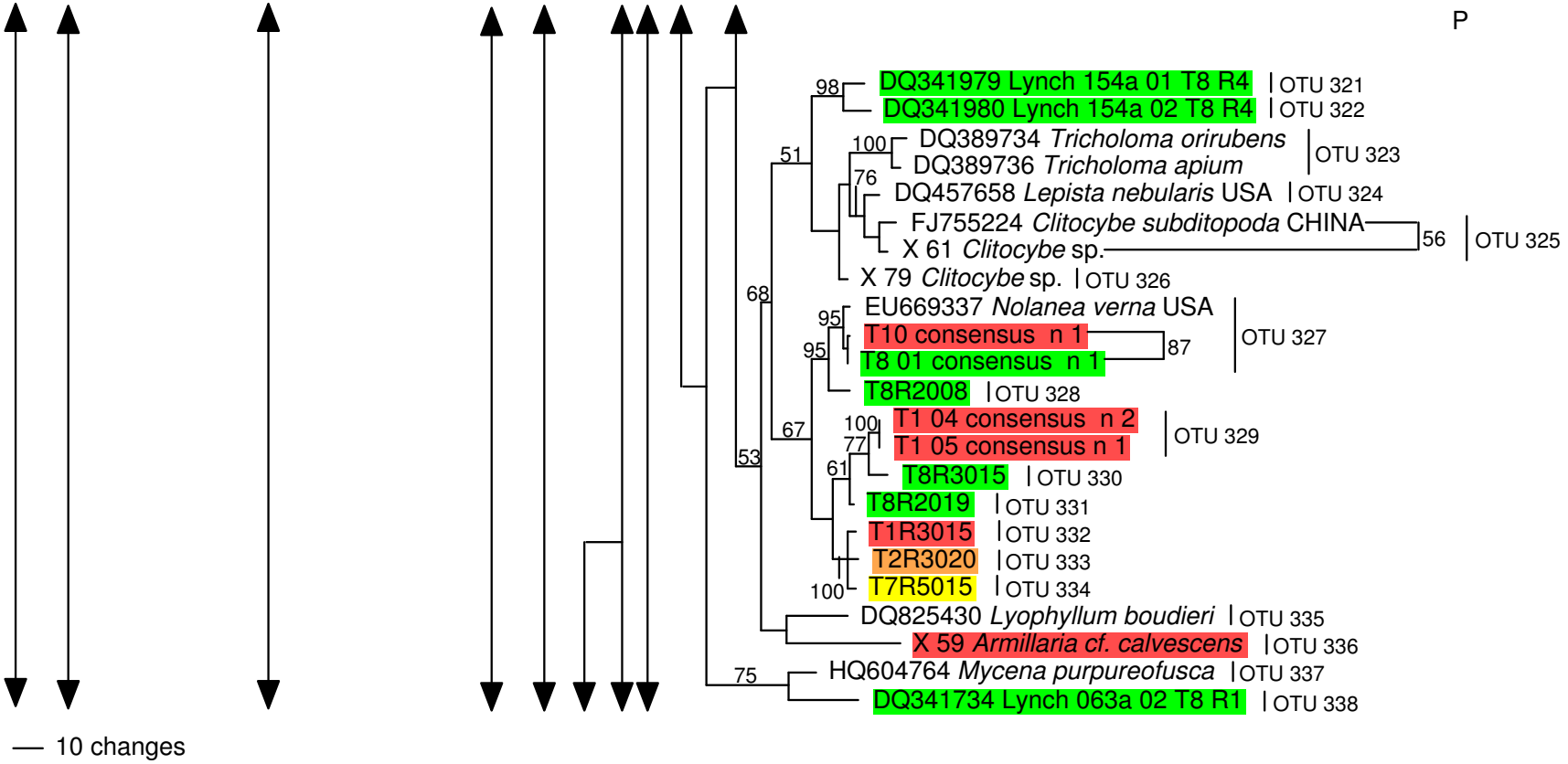
P
DQ341979 Lynch 154a 01 T8 R4 | OTU 321
DQ341980 Lynch 154a 02 T8 R4 | OTU 322
DQ389734 Tricholoma orirubens
DQ389736 Tricholoma apium | OTU 323
DQ457658 Lepista nebularis USA | OTU 324
FJ755224 Clitocybe subditopoda CHINA
X 61 Clitocybe sp. | OTU 325
X 79 Clitocybe sp. | OTU 326
EU669337 Nolanea verna USA
T10 consensus n 1
T8 01 consensus n 1 | OTU 327
T8R2008 | OTU 328
T1 04 consensus n 2
T1 05 consensus n 1 | OTU 329
T8R3015 | OTU 330
T8R2019 | OTU 331
T1R3015 | OTU 332
T2R3020 | OTU 333
T7R5015 | OTU 334
DQ825430 Lyophyllum boudieri | OTU 335
X 59 Armillaria cf. calvescens | OTU 336
HQ604764 Mycena purpureofusca | OTU 337
DQ341734 Lynch 063a 02 T8 R1 | OTU 338
98
100
51
76
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100
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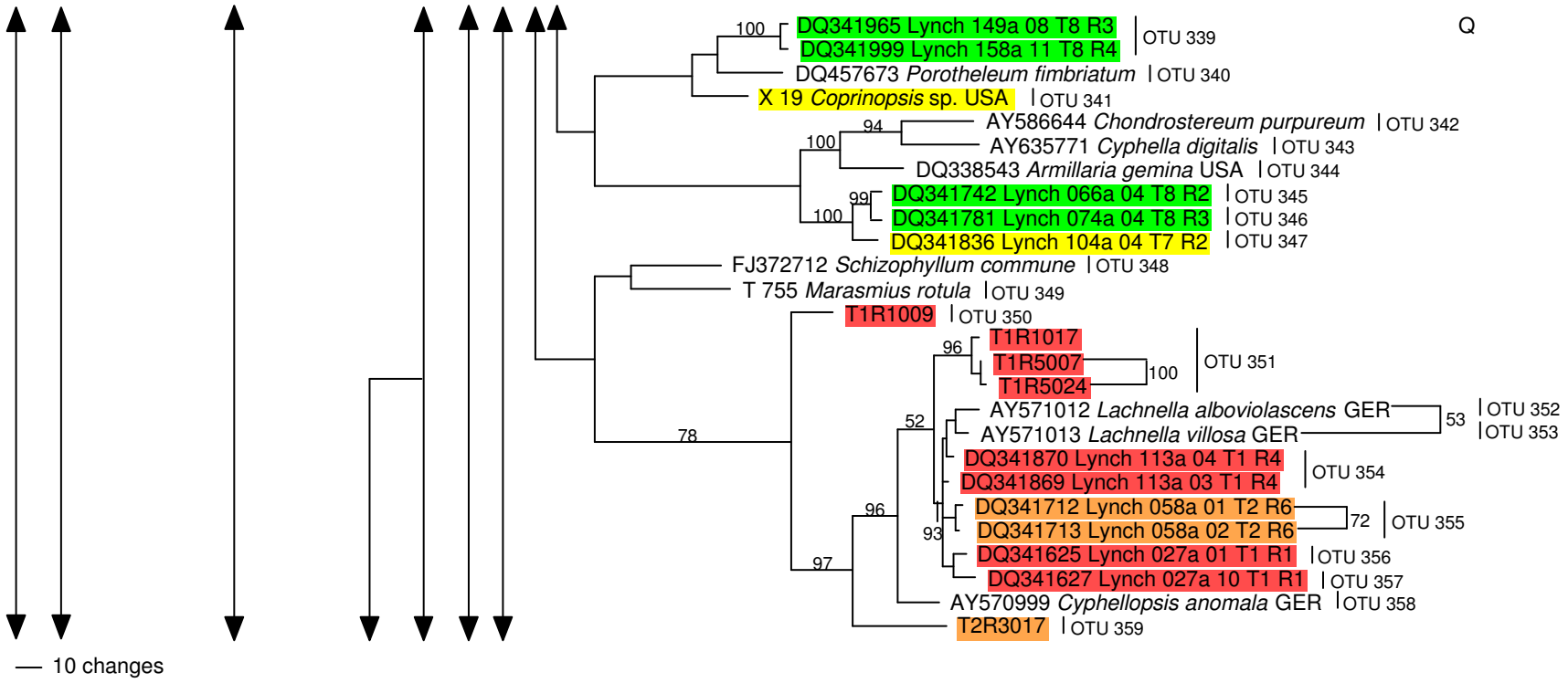
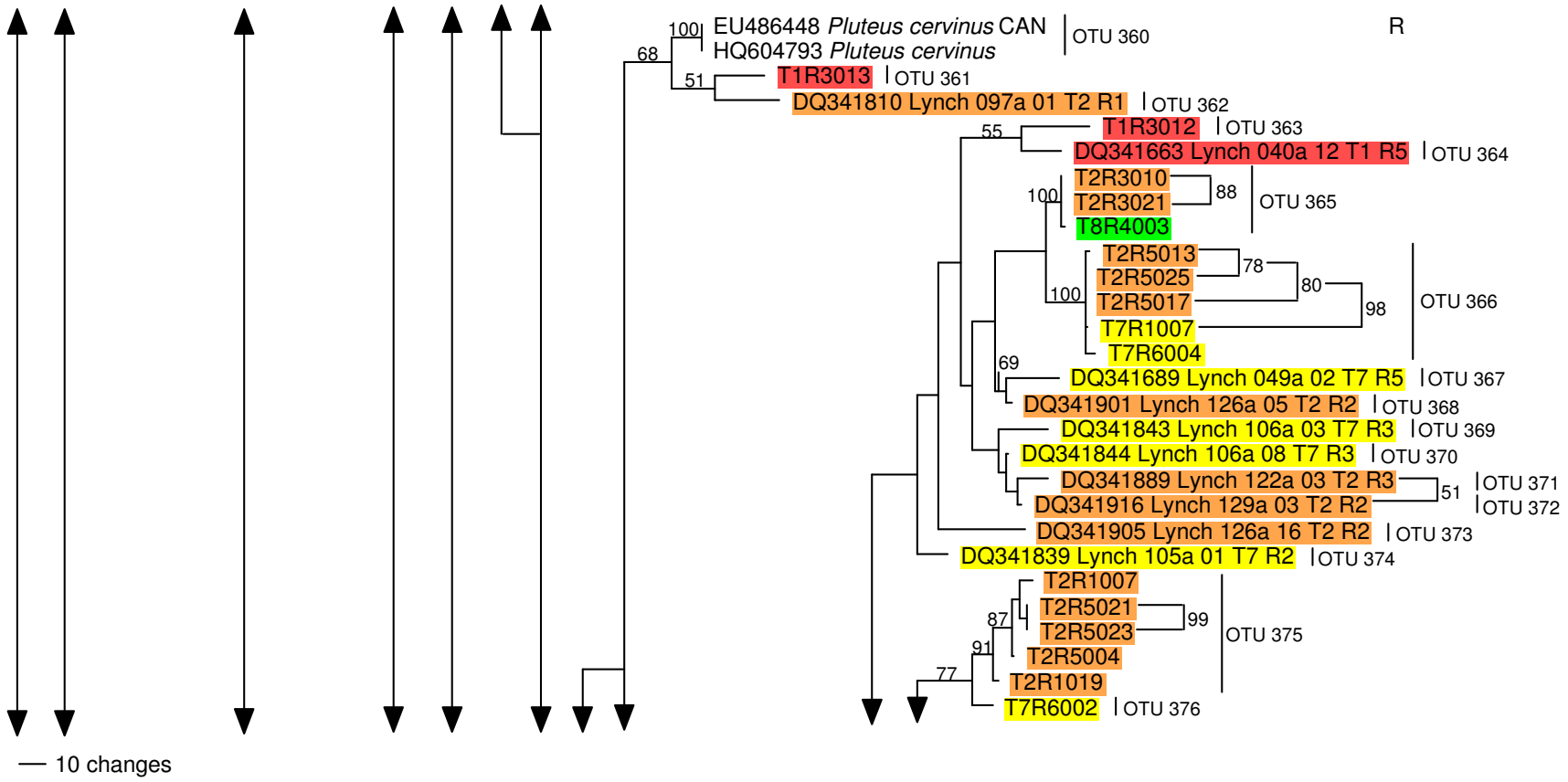
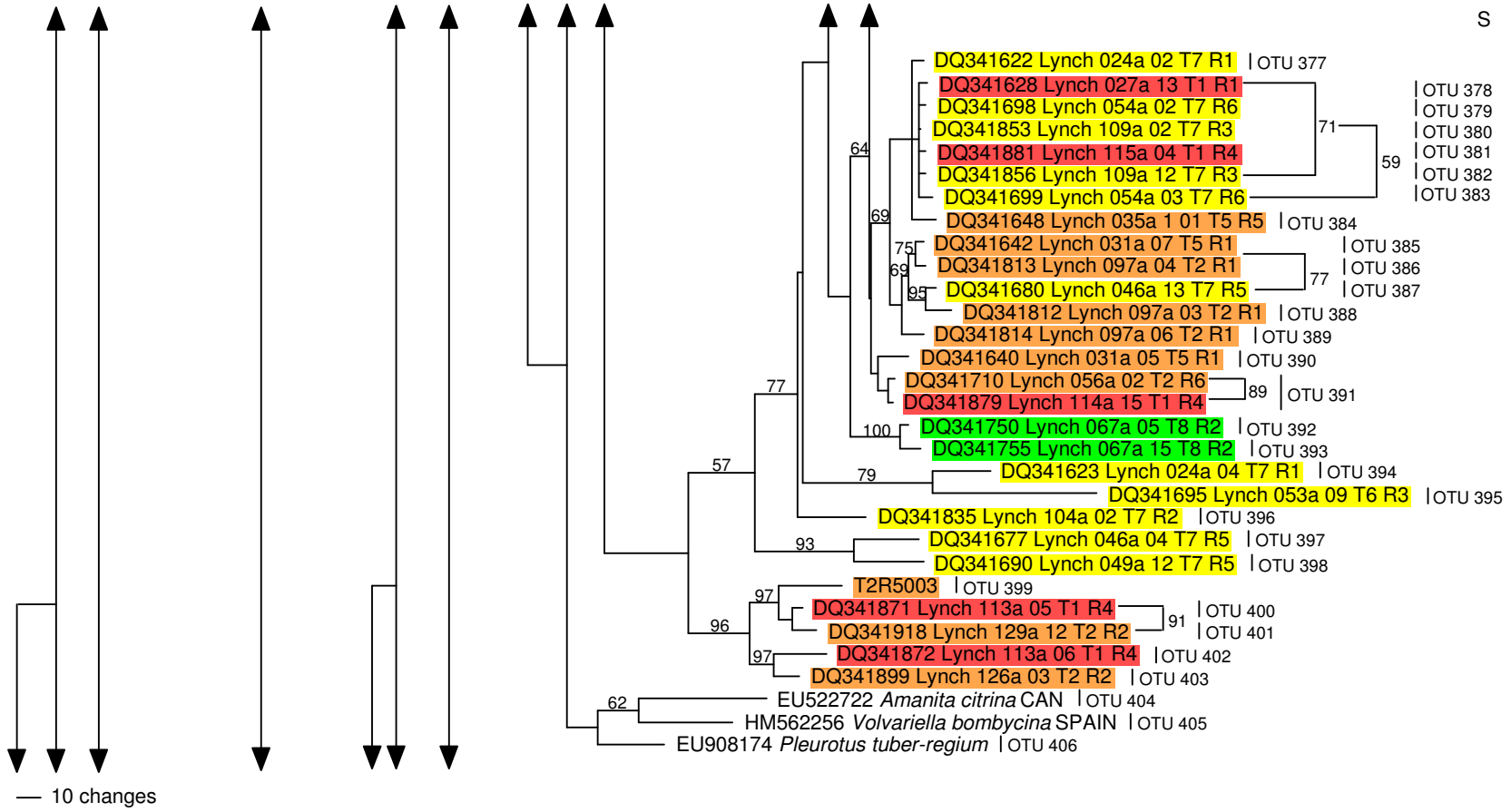
100
75
10 changes


Q
100
DQ341965 Lynch 149a 08 T8 R3
DQ341999 Lynch 158a 11 T8 R4
OTU 339
DQ457673 Porotheleum fimbriatum
OTU 340
X 19 Coprinopsis sp. USA
OTU 341
94
AY586644 Chondrostereum purpureum
OTU 342
100
AY635771 Cyphella digitalis
OTU 343
DQ338543 Armillaria gemina USA
OTU 344
99
DQ341742 Lynch 066a 04 T8 R2
OTU 345
100
DQ341781 Lynch 074a 04 T8 R3
OTU 346
DQ341836 Lynch 104a 04 T7 R2
OTU 347
FJ372712 Schizophyllum commune
OTU 348
T 755 Marasmius rotula
OTU 349
T1R1009
OTU 350
96
T1R1017
T1R5007
100
T1R5024
OTU 351
AY571012 Lachnella alboviolascens GER
53
OTU 352
52
AY571013 Lachnella villosa GER
OTU 353
78
DQ341870 Lynch 113a 04 T1 R4
DQ341869 Lynch 113a 03 T1 R4
OTU 354
96
DQ341712 Lynch 058a 01 T2 R6
72
OTU 355
93
DQ341713 Lynch 058a 02 T2 R6
DQ341625 Lynch 027a 01 T1 R1
OTU 356
97
DQ341627 Lynch 027a 10 T1 R1
OTU 357
AY570999 Cyphellopsis anomala GER
OTU 358
T2R3017
OTU 359
10 changes


EU486448 Pluteus cervinus CAN
HQ604793 Pluteus cervinus
OTU 360
R
T1R3013
OTU 361
DQ341810 Lynch 097a 01 T2 R1
OTU 362
T1R3012
OTU 363
DQ341663 Lynch 040a 12 T1 R5
OTU 364
T2R3010
T2R3021
T8R4003
OTU 365
T2R5013
T2R5025
T2R5017
T7R1007
T7R6004
OTU 366
DQ341689 Lynch 049a 02 T7 R5
OTU 367
DQ341901 Lynch 126a 05 T2 R2
OTU 368
DQ341843 Lynch 106a 03 T7 R3
OTU 369
DQ341844 Lynch 106a 08 T7 R3
OTU 370
DQ341889 Lynch 122a 03 T2 R3
OTU 371
DQ341916 Lynch 129a 03 T2 R2
OTU 372
DQ341905 Lynch 126a 16 T2 R2
OTU 373
DQ341839 Lynch 105a 01 T7 R2
OTU 374
T2R1007
T2R5021
T2R5023
T2R5004
T2R1019
OTU 375
T7R6002
OTU 376
10 changes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DQ341628 Lynch 027a 13 T1 R1 OTU 378
DQ341698 Lynch 054a 02 T7 R6 OTU 379
DQ341853 Lynch 109a 02 T7 R3 OTU 380
DQ341881 Lynch 115a 04 T1 R4 OTU 381
DQ341856 Lynch 109a 12 T7 R3 OTU 382
DQ341699 Lynch 054a 03 T7 R6 OTU 383
DQ341648 Lynch 035a 1 01 T5 R5 OTU 384
DQ341642 Lynch 031a 07 T5 R1 OTU 385
DQ341813 Lynch 097a 04 T2 R1 OTU 386
DQ341680 Lynch 046a 13 T7 R5 OTU 387
DQ341812 Lynch 097a 03 T2 R1 OTU 388
DQ341814 Lynch 097a 06 T2 R1 OTU 389
DQ341640 Lynch 031a 05 T5 R1 OTU 390
DQ341710 Lynch 056a 02 T2 R6 OTU 391
DQ341879 Lynch 114a 15 T1 R4
DQ341750 Lynch 067a 05 T8 R2 OTU 392
DQ341755 Lynch 067a 15 T8 R2 OTU 393
DQ341623 Lynch 024a 04 T7 R1 OTU 394
DQ341695 Lynch 053a 09 T6 R3 OTU 395
DQ341835 Lynch 104a 02 T7 R2 OTU 396
DQ341677 Lynch 046a 04 T7 R5 OTU 397
DQ341690 Lynch 049a 12 T7 R5 OTU 398
T2R5003 OTU 399
DQ341871 Lynch 113a 05 T1 R4 OTU 400
DQ341918 Lynch 129a 12 T2 R2 OTU 401
DQ341872 Lynch 113a 06 T1 R4 OTU 402
DQ341899 Lynch 126a 03 T2 R2 OTU 403
EU522722 Amanita citrina CAN OTU 404
HM562256 Volvariella bombycina SPAIN OTU 405
EU908174 Pleurotus tuber-regium OTU 406
64
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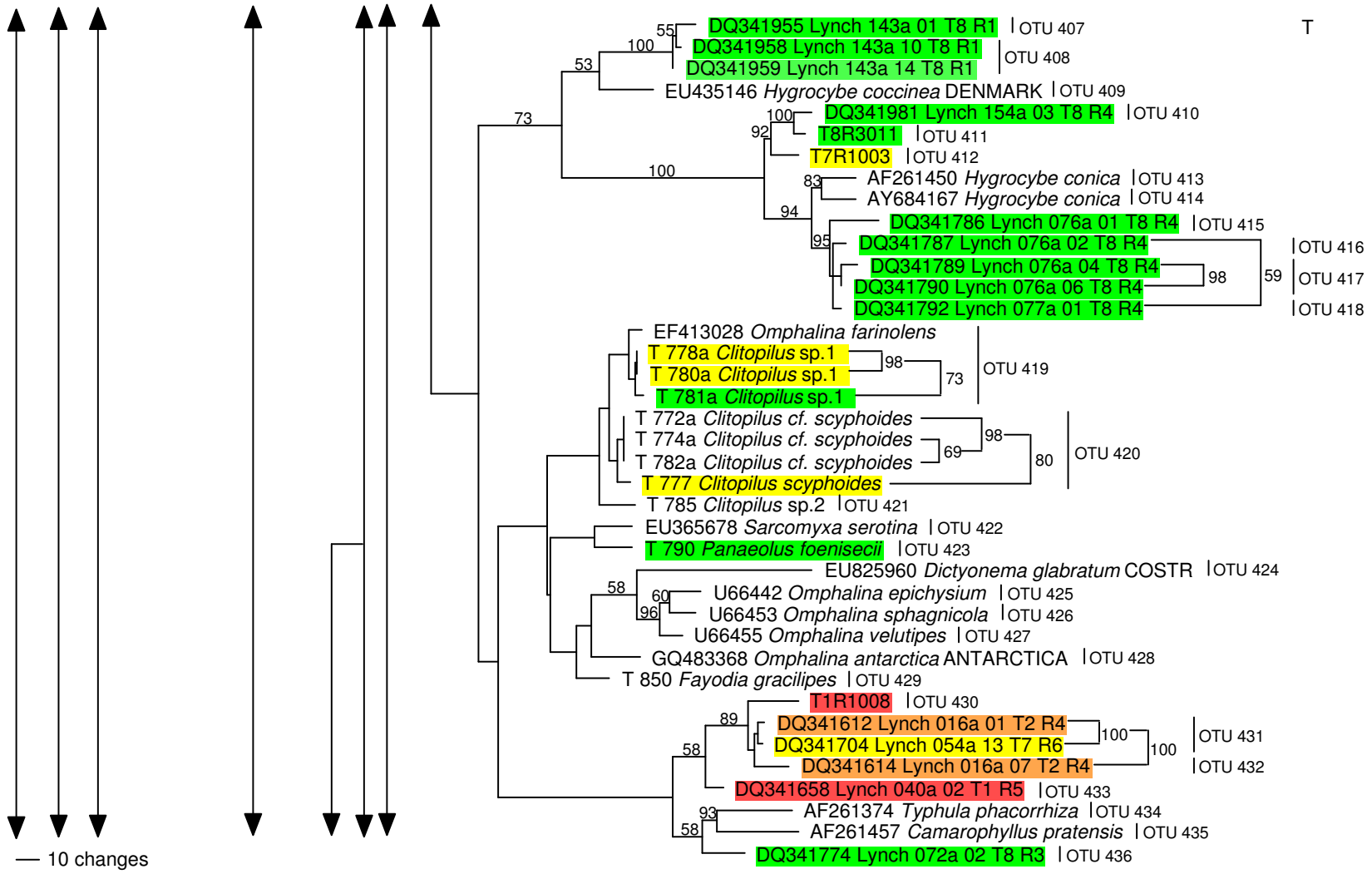
DQ341955 Lynch 143a 01 T8 R1 OTU 407
DQ341958 Lynch 143a 10 T8 R1 OTU 408
DQ341959 Lynch 143a 14 T8 R1
EU435146 Hygrocybe coccinea DENMARK OTU 409
DQ341981 Lynch 154a 03 T8 R4 OTU 410
T8R3011 OTU 411
T7R1003 OTU 412
AF261450 Hygrocybe conica OTU 413
AY684167 Hygrocybe conica OTU 414
DQ341786 Lynch 076a 01 T8 R4 OTU 415
DQ341787 Lynch 076a 02 T8 R4 OTU 416
DQ341789 Lynch 076a 04 T8 R4 OTU 417
DQ341790 Lynch 076a 06 T8 R4
DQ341792 Lynch 077a 01 T8 R4 OTU 418
EF413028 Omphalina farinolens
T 778a Clitopilus sp.1
T 780a Clitopilus sp.1
T 781a Clitopilus sp.1 OTU 419
T 772a Clitopilus cf. scyphoides
T 774a Clitopilus cf. scyphoides
T 782a Clitopilus cf. scyphoides
T 777 Clitopilus scyphoides OTU 420
T 785 Clitopilus sp.2 OTU 421
EU365678 Sarcomyxa serotina OTU 422
T 790 Panaeolus foenisecii OTU 423
EU825960 Dictyonema glabratum COSTR OTU 424
U66442 Omphalina epichysium OTU 425
U66453 Omphalina sphagnicola OTU 426
U66455 Omphalina velutipes OTU 427
GQ483368 Omphalina antarctica ANTARCTICA OTU 428
T 850 Fayodia gracilipes OTU 429
T1R1008 OTU 430
DQ341612 Lynch 016a 01 T2 R4
DQ341704 Lynch 054a 13 T7 R6 OTU 431
DQ341614 Lynch 016a 07 T2 R4 OTU 432
DQ341658 Lynch 040a 02 T1 R5 OTU 433
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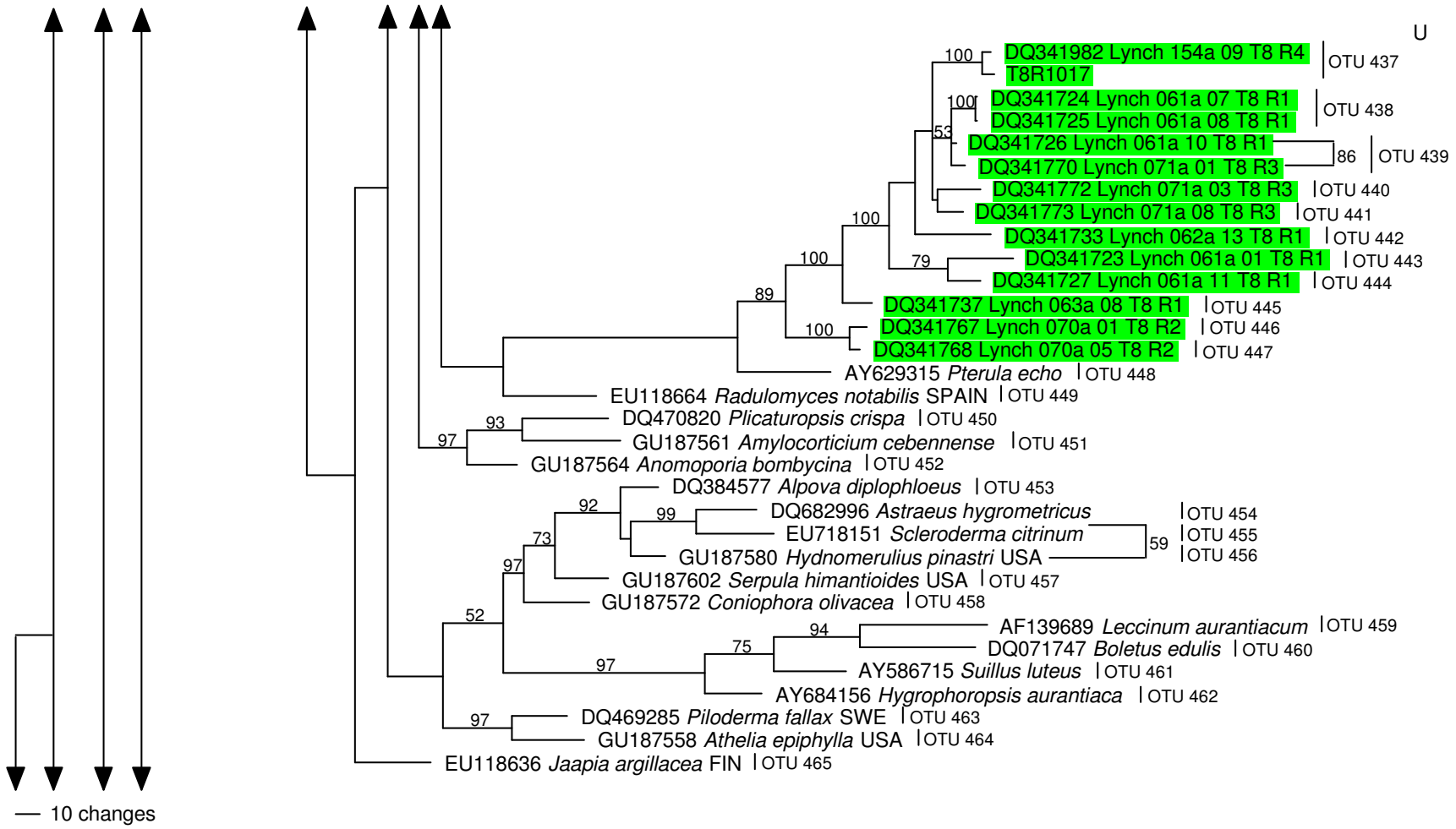
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OTU 441
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OTU 442
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OTU 443
DQ341727 Lynch 061a 11 T8 R1
OTU 444
DQ341737 Lynch 063a 08 T8 R1
OTU 445
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OTU 446
DQ341768 Lynch 070a 05 T8 R2
OTU 447
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OTU 448
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DQ470820 Plicaturopsis crispa
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GU187561 Amylocorticium cebennense
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GU187564 Anomoporia bombycina
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DQ682996 Astraeus hygrometricus
OTU 454
EU718151 Scleroderma citrinum
OTU 455
GU187580 Hydnomerulius pinastri USA
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GU187602 Serpula himantioides USA
OTU 457
GU187572 Coniophora olivacea
OTU 458
AF139689 Leccinum aurantiacum
OTU 459
DQ071747 Boletus edulis
OTU 460
AY586715 Suillus luteus
OTU 461
AY684156 Hygrophoropsis aurantiaca
OTU 462
DQ469285 Piloderma fallax SWE
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GU187558 Athelia epiphylla USA
OTU 464
EU118636 Jaapia argillacea FIN
OTU 465
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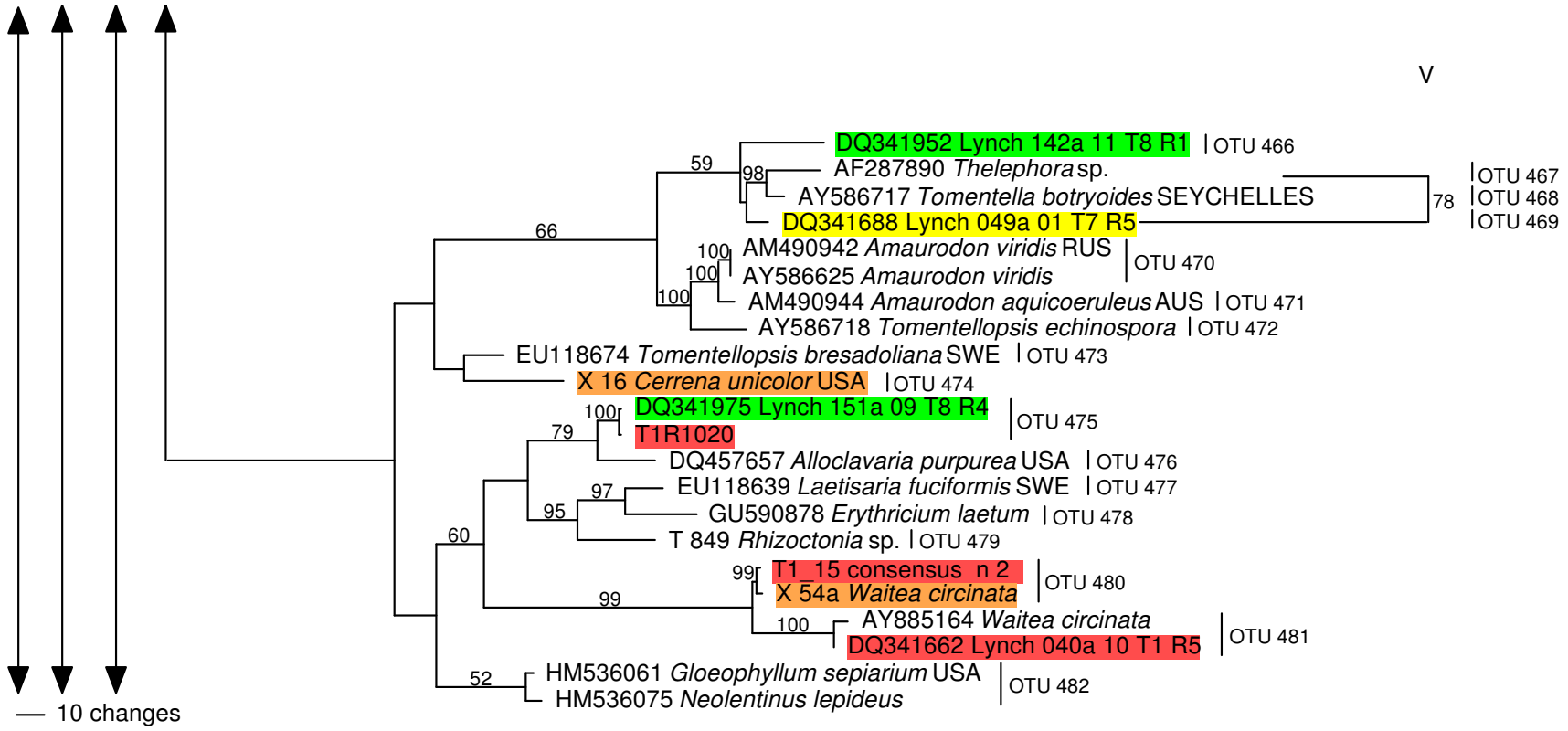
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AY586717 Tomentella botryoides SEYCHELLES
DQ341688 Lynch 049a 01 T7 R5
OTU 467
OTU 468
OTU 469
AM490942 Amaurodon viridis RUS
AY586625 Amaurodon viridis
OTU 470
AM490944 Amaurodon aquicoeruleus AUS
OTU 471
AY586718 Tomentellopsis echinospora
OTU 472
EU118674 Tomentellopsis bresadoliana SWE
OTU 473
X 16 Cerrena unicolor USA
OTU 474
DQ341975 Lynch 151a 09 T8 R4
T1R1020
OTU 475
DQ457657 Alloclavaria purpurea USA
OTU 476
EU118639 Laetisaria fuciformis SWE
OTU 477
GU590878 Erythricium laetum
OTU 478
T 849 Rhizoctonia sp.
OTU 479
T1_15 consensus n 2
X 54a Waitea circinata
OTU 480
AY885164 Waitea circinata
DQ341662 Lynch 040a 10 T1 R5
OTU 481
HM536061 Gloeophyllum sepiarium USA
HM536075 Neolentinus lepideus
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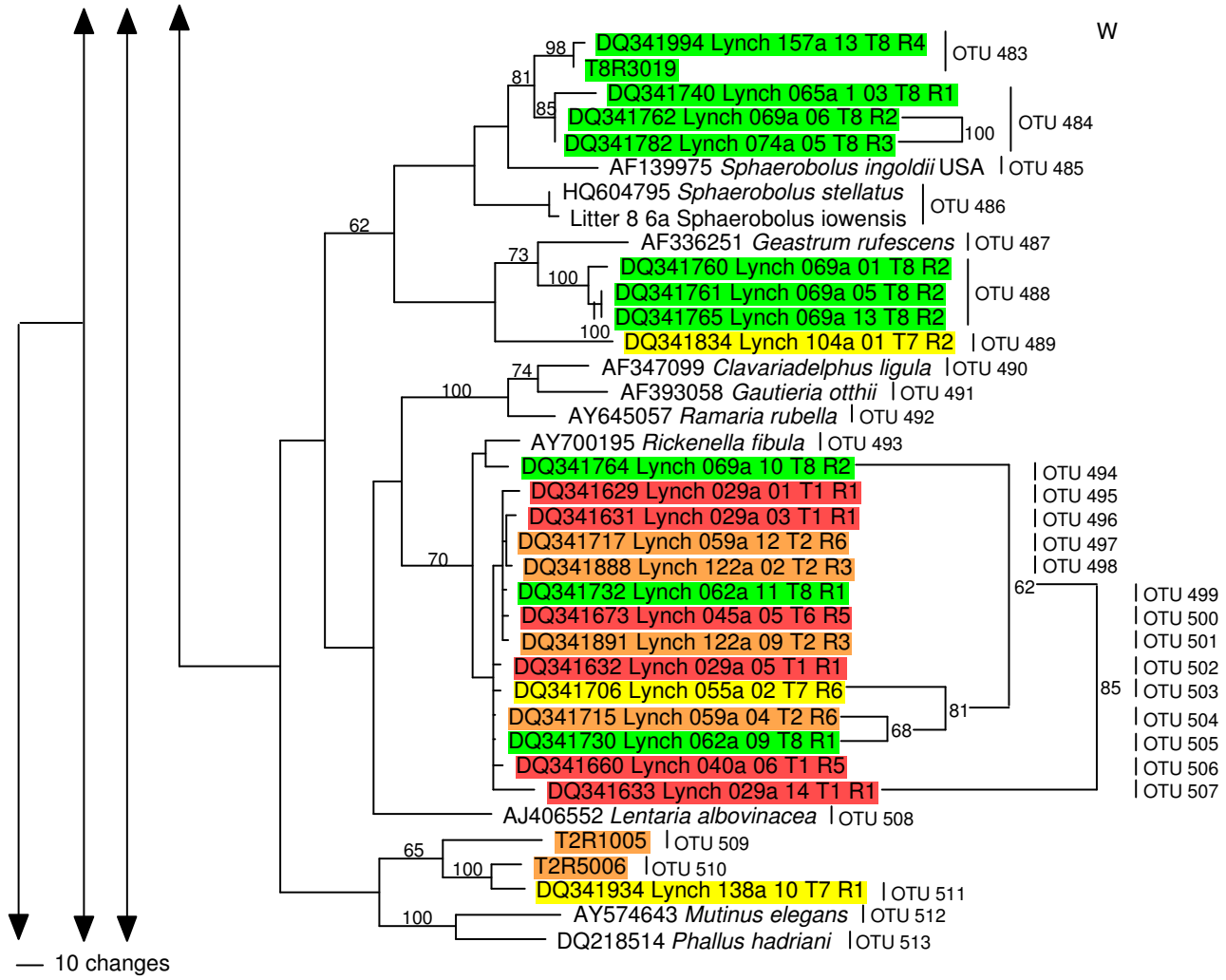
10 changes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DQ341762 Lynch 069a 06 T8 R2
DQ341782 Lynch 074a 05 T8 R3
OTU 484
AF139975 Sphaerobolus ingoldii USA
OTU 485
HQ604795 Sphaerobolus stellatus
Litter 8 6a Sphaerobolus iowensis
OTU 486
AF336251 Geastrum rufescens
OTU 487
DQ341760 Lynch 069a 01 T8 R2
DQ341761 Lynch 069a 05 T8 R2
DQ341765 Lynch 069a 13 T8 R2
OTU 488
DQ341834 Lynch 104a 01 T7 R2
OTU 489
AF347099 Clavariadelphus ligula
OTU 490
AF393058 Gautieria otthii
OTU 491
AY645057 Ramaria rubella
OTU 492
AY700195 Rickenella fibula
OTU 493
DQ341764 Lynch 069a 10 T8 R2
DQ341629 Lynch 029a 01 T1 R1
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DQ341673 Lynch 045a 05 T6 R5
DQ341891 Lynch 122a 09 T2 R3
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DQ341715 Lynch 059a 04 T2 R6
DQ341730 Lynch 062a 09 T8 R1
DQ341660 Lynch 040a 06 T1 R5
DQ341633 Lynch 029a 14 T1 R1
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OTU 498
OTU 499
OTU 500
OTU 501
OTU 502
OTU 503
OTU 504
OTU 505
OTU 506
OTU 507
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OTU 508
T2R1005
OTU 509
T2R5006
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AY574643 Mutinus elegans
OTU 512
DQ218514 Phallus hadriani
OTU 513
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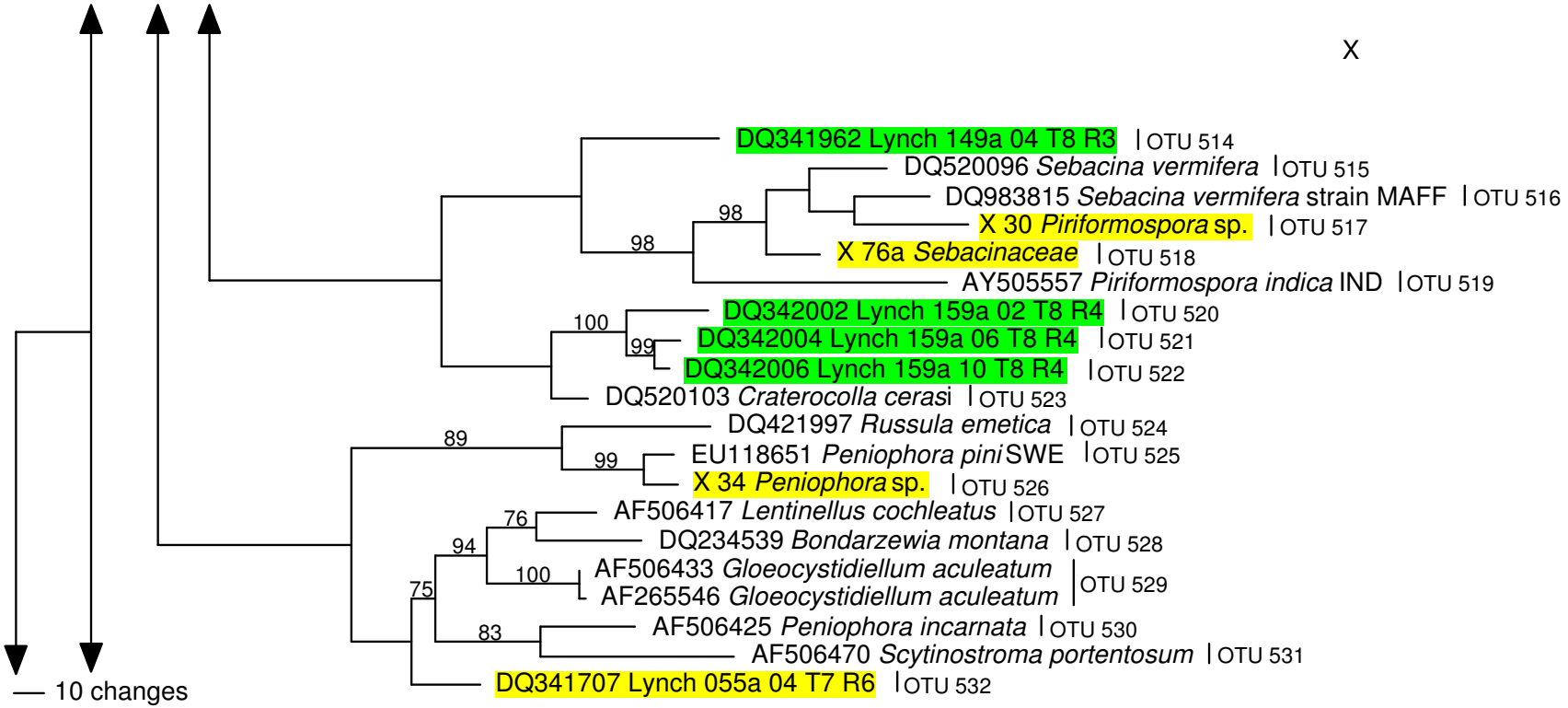
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DQ983815 Sebacina vermifera strain MAFF | OTU 516
X 30 Piriformospora sp. | OTU 517
X 76a Sebacinaceae | OTU 518
AY505557 Piriformospora indica IND | OTU 519
DQ342002 Lynch 159a 02 T8 R4 | OTU 520
DQ342004 Lynch 159a 06 T8 R4 | OTU 521
DQ342006 Lynch 159a 10 T8 R4 | OTU 522
DQ520103 Craterocolla cerasi | OTU 523
DQ421997 Russula emetica | OTU 524
EU118651 Peniophora pini SWE | OTU 525
X 34 Peniophora sp. | OTU 526
AF506417 Lentinellus cochleatus | OTU 527
DQ234539 Bondarzewia montana | OTU 528
AF506433 Gloeocystidiellum aculeatum
AF265546 Gloeocystidiellum aculeatum | OTU 529
AF506425 Peniophora incarnata | OTU 530
AF506470 Scytinostroma portentosum | OTU 531
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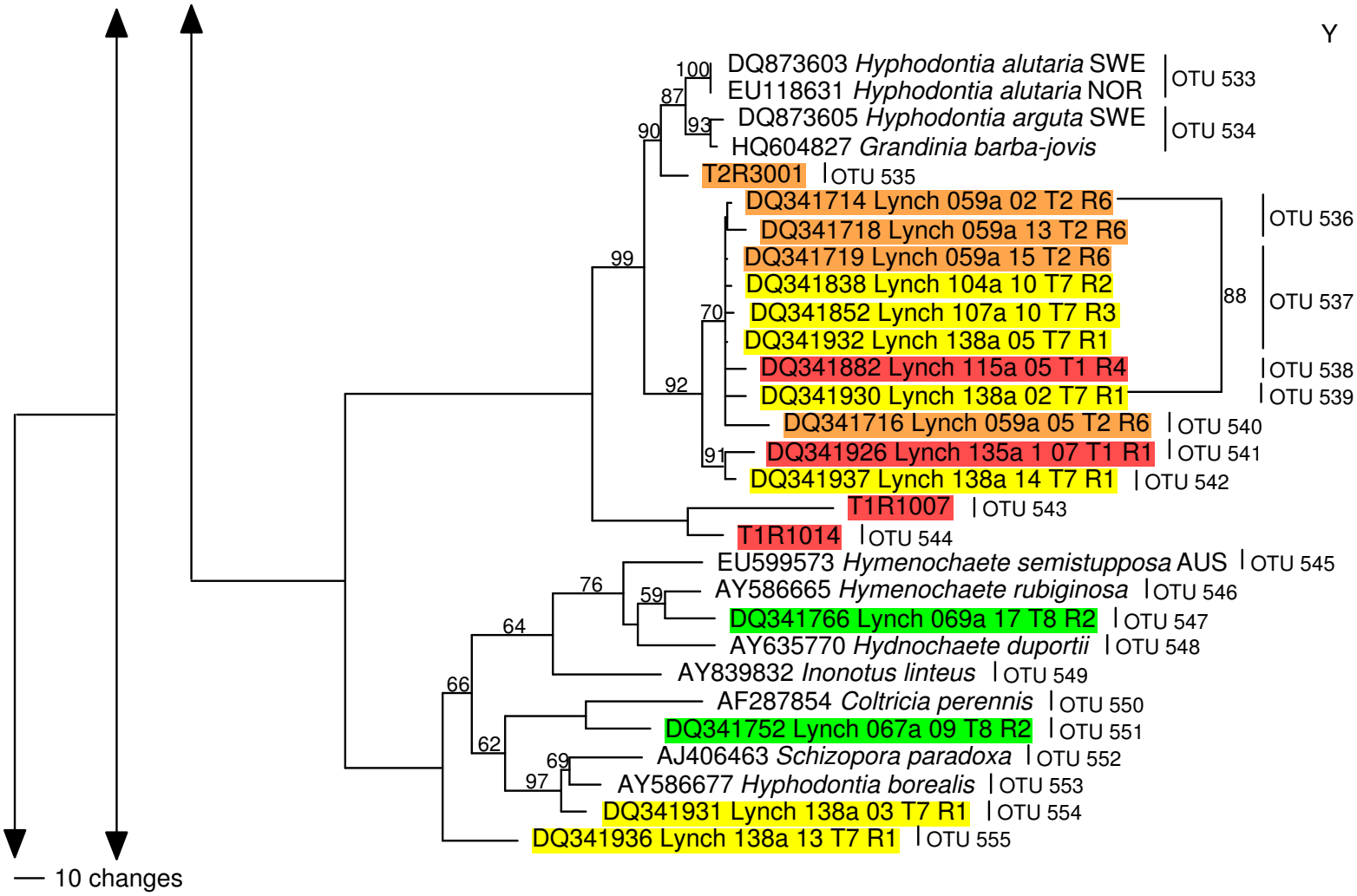
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EU118631 Hyphodontia alutaria NOR
DQ873605 Hyphodontia arguta SWE
HQ604827 Grandinia barba-jovis
T2R3001
DQ341714 Lynch 059a 02 T2 R6
DQ341718 Lynch 059a 13 T2 R6
DQ341719 Lynch 059a 15 T2 R6
DQ341838 Lynch 104a 10 T7 R2
DQ341852 Lynch 107a 10 T7 R3
DQ341932 Lynch 138a 05 T7 R1
DQ341882 Lynch 115a 05 T1 R4
DQ341930 Lynch 138a 02 T7 R1
DQ341716 Lynch 059a 05 T2 R6
DQ341926 Lynch 135a 1 07 T1 R1
DQ341937 Lynch 138a 14 T7 R1
T1R1007
T1R1014
EU599573 Hymenochaete semistupposa AUS
AY586665 Hymenochaete rubiginosa
DQ341766 Lynch 069a 17 T8 R2
AY635770 Hydnochaete duportii
AY839832 Inonotus linteus
AF287854 Coltricia perennis
DQ341752 Lynch 067a 09 T8 R2
AJ406463 Schizopora paradoxa
AY586677 Hyphodontia borealis
DQ341931 Lynch 138a 03 T7 R1
DQ341936 Lynch 138a 13 T7 R1
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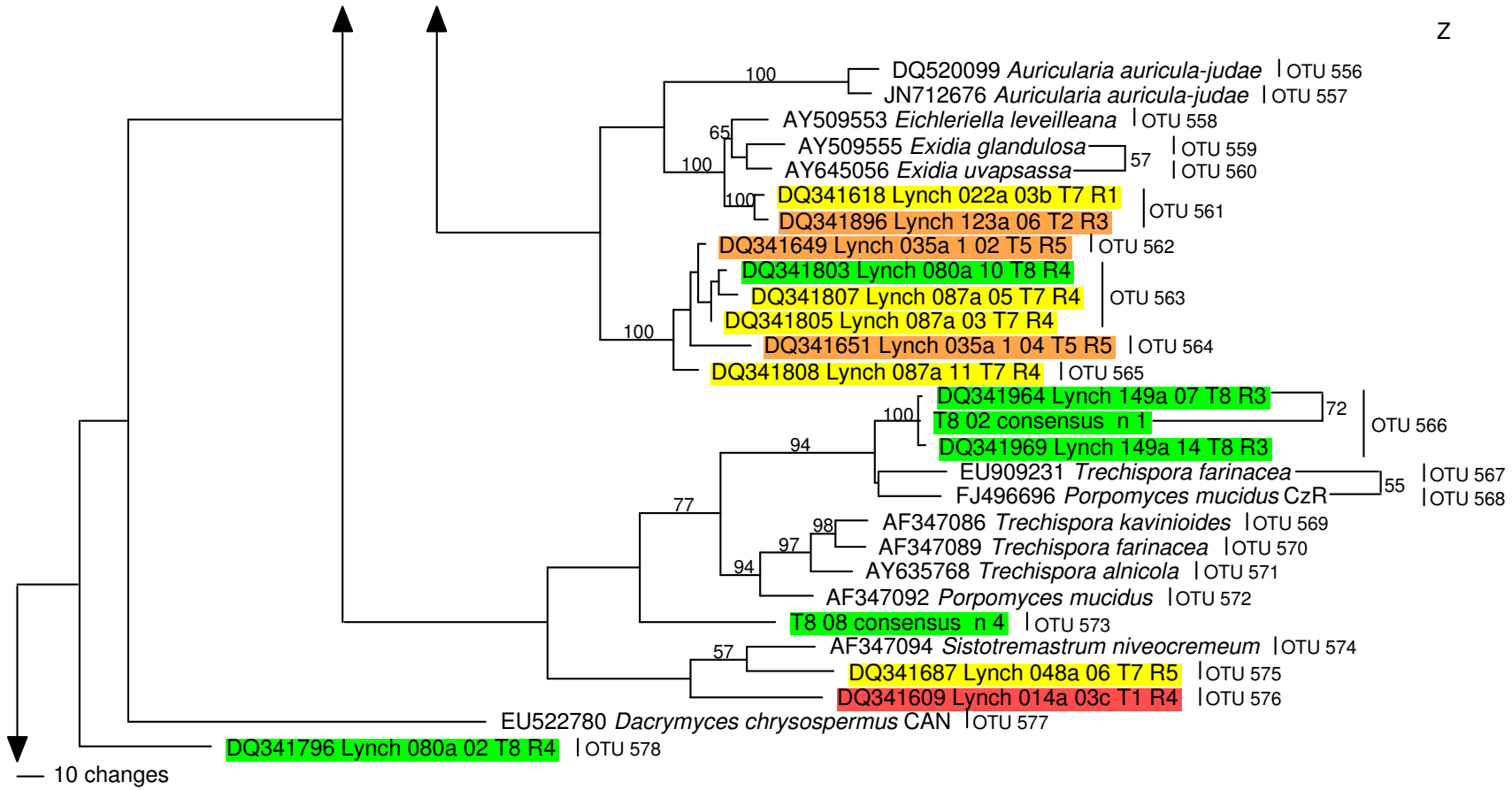
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OTU 557
AY509553 Eichleriella leveilleana
OTU 558
AY509555 Exidia glandulosa
AY645056 Exidia uvapsassa
57
OTU 559
OTU 560
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DQ341896 Lynch 123a 06 T2 R3
OTU 561
DQ341649 Lynch 035a 1 02 T5 R5
OTU 562
DQ341803 Lynch 080a 10 T8 R4
DQ341807 Lynch 087a 05 T7 R4
DQ341805 Lynch 087a 03 T7 R4
OTU 563
DQ341651 Lynch 035a 1 04 T5 R5
OTU 564
DQ341808 Lynch 087a 11 T7 R4
OTU 565
DQ341964 Lynch 149a 07 T8 R3
T8 02 consensus n 1
DQ341969 Lynch 149a 14 T8 R3
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OTU 566
EU909231 Trechispora farinacea
FJ496696 Porpomyces mucidus CzR
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OTU 567
OTU 568
AF347086 Trechispora kavinioides
OTU 569
AF347089 Trechispora farinacea
OTU 570
AY635768 Trechispora alnicola
OTU 571
AF347092 Porpomyces mucidus
OTU 572
T8 08 consensus n 4
OTU 573
AF347094 Sistotremastrum niveocremeum
OTU 574
DQ341687 Lynch 048a 06 T7 R5
OTU 575
DQ341609 Lynch 014a 03c T1 R4
OTU 576
EU522780 Dacrymyces chrysospermus CAN
OTU 577
DQ341796 Lynch 080a 02 T8 R4
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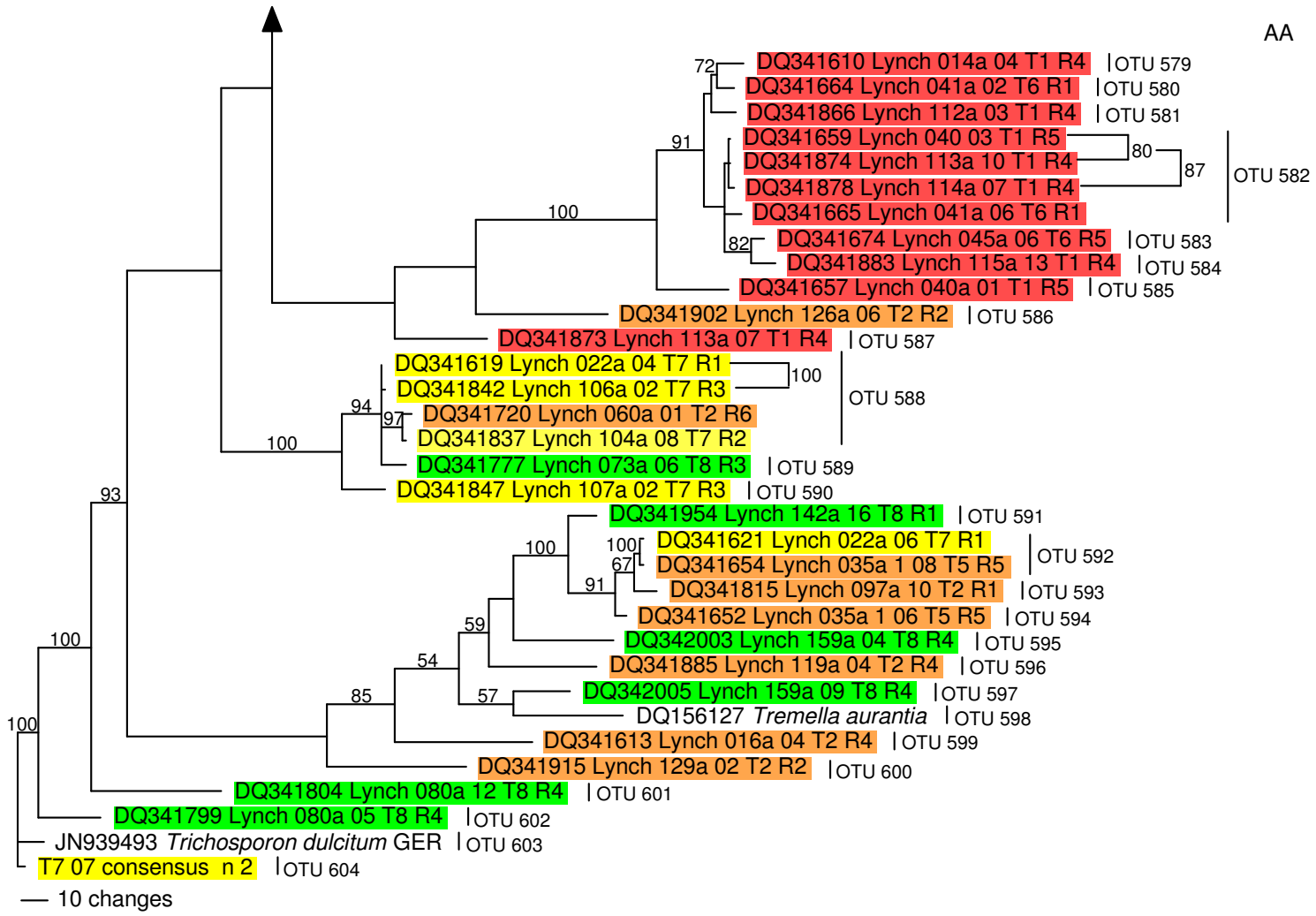
10 changes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DQ341659 Lynch 040 03 T1 R5
DQ341874 Lynch 113a 10 T1 R4
DQ341878 Lynch 114a 07 T1 R4
DQ341665 Lynch 041a 06 T6 R1
OTU 582
DQ341674 Lynch 045a 06 T6 R5
OTU 583
DQ341883 Lynch 115a 13 T1 R4
OTU 584
DQ341657 Lynch 040a 01 T1 R5
OTU 585
DQ341902 Lynch 126a 06 T2 R2
OTU 586
DQ341873 Lynch 113a 07 T1 R4
OTU 587
DQ341619 Lynch 022a 04 T7 R1
DQ341842 Lynch 106a 02 T7 R3
OTU 588
DQ341720 Lynch 060a 01 T2 R6
DQ341837 Lynch 104a 08 T7 R2
DQ341777 Lynch 073a 06 T8 R3
OTU 589
DQ341847 Lynch 107a 02 T7 R3
OTU 590
DQ341954 Lynch 142a 16 T8 R1
OTU 591
DQ341621 Lynch 022a 06 T7 R1
DQ341654 Lynch 035a 1 08 T5 R5
OTU 592
DQ341815 Lynch 097a 10 T2 R1
OTU 593
DQ341652 Lynch 035a 1 06 T5 R5
OTU 594
DQ342003 Lynch 159a 04 T8 R4
OTU 595
DQ341885 Lynch 119a 04 T2 R4
OTU 596
DQ342005 Lynch 159a 09 T8 R4
OTU 597
DQ156127 Tremella aurantia
OTU 598
DQ341613 Lynch 016a 04 T2 R4
OTU 599
DQ341915 Lynch 129a 02 T2 R2
OTU 600
DQ341804 Lynch 080a 12 T8 R4
OTU 601
DQ341799 Lynch 080a 05 T8 R4
OTU 602
JN939493 Trichosporon dulcitum GER
OTU 603
T7 07 consensus n 2
OTU 604
10 changes

Table 3.1 Environmental distance P-Values for each environment tested individually

Environment	P-Value
CT T1R1	0.73
CT T1R2	1.0
CT T1R3	1.0
CT T1R4	1.0
CT T1R5	0.23
CT T1R6	0.27
NT T2R1	0.34
NT T2R2	0.02
NT T2R3	0.06
NT T2R4	1.0
NT T2R5	$\leq$0.01
NT T2R6	0.24
HTS T7R1	0.13
HTS T7R2	0.49
HTS T7R3	0.42
HTS T7R4	$\leq$0.01
HTS T7R5	$\leq$0.01
HTS T7R6	0.29
NTS T8R1	$\leq$0.01
NTS T8R2	$\leq$0.01
NTS T8R3	$\leq$0.01
NTS T8R4	$\leq$0.01

When sequences from all environments were tested together to determine if the different environments in the tree were significantly different from each other, the UniFrac P-Value was reported as <<0.001, indicating highly significant differences among the different sites.

The P test (Martin 2002) significance value for all environments tested together was <<0.001, again indicating highly significant differences among the different sites.

3.3 Jackknife environment cluster

When a statistical resampling technique called jackknife environment cluster was performed, all sites were strongly clustered together, >99.9% at the furthest node (Figure 3.2). However at the second furthest node, all environments were clustered together with 50-70% support, excluding NT T2R4 and NT T2R5. At the remaining nodes, environments were clustered together with <50% support.

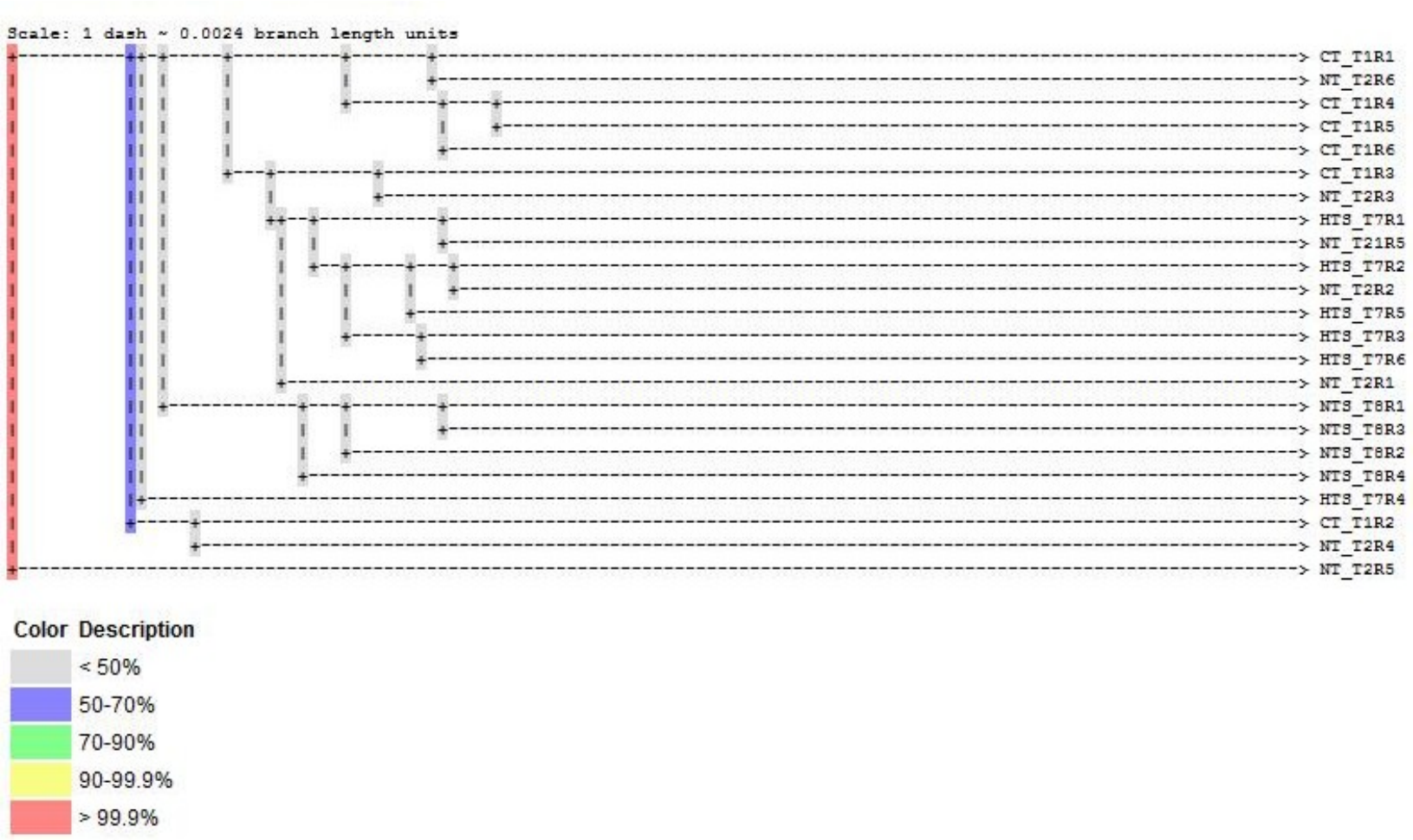
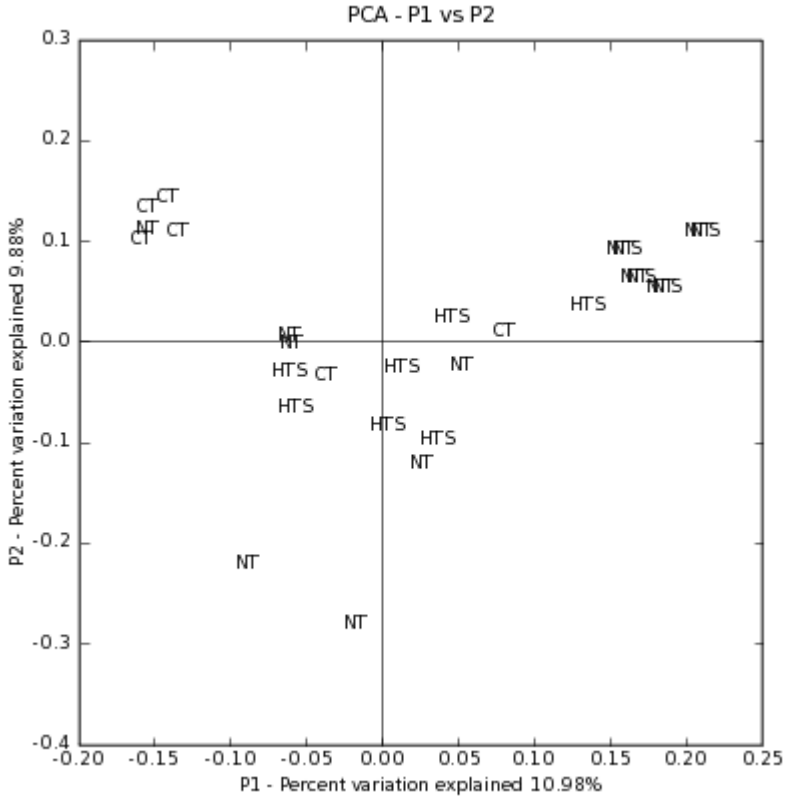


Figure 3.2 Jackknife environment cluster for all environments

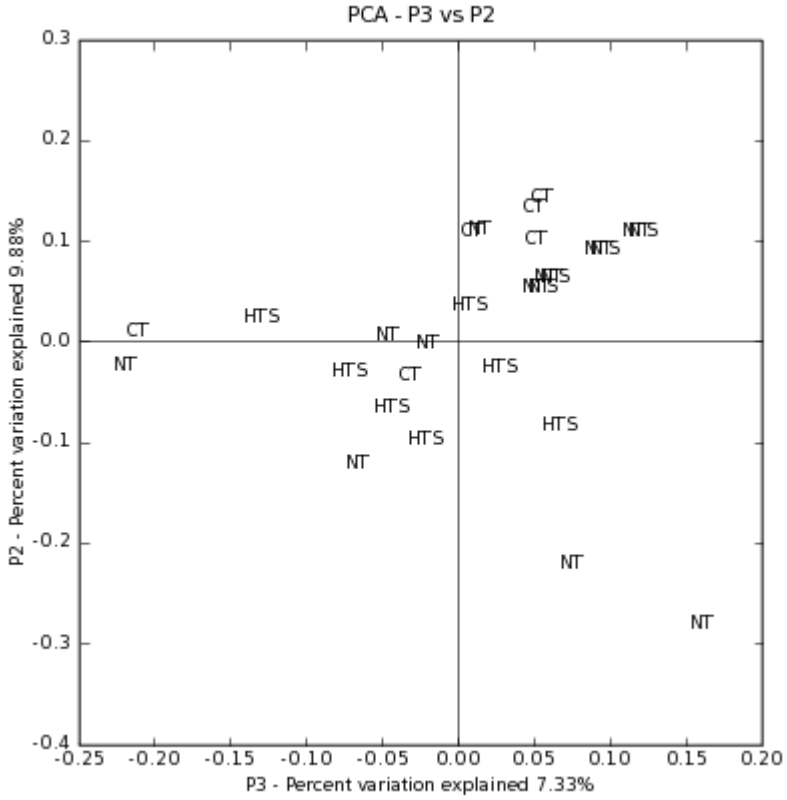
3.4 Principal coordinates analysis (PCoA)

Principal coordinates analysis of the data distinguished NTS from the other sites, CT, NT, and HTS with NTS having the greatest distance from agricultural treatments, CT and NT (Figure 3.3A-C). NTS was furthermore distinguished as NTS sites were grouped closely together. CT was generally distinct from the rest of the sites as 4 of the 6 CT sample sites were grouped closely together and relatively away from the other sites, in the top left corner (Figure 3.3 A). Two NT sites near the bottom right corner were relatively distinct from the other sites as the distance between them and the other sites was comparatively greater (Figure 3.3 A). Finally, HTS sites are not in close proximity to one another, not being tightly clustered, and the juxtaposition of HTS sites with different treatments suggested that the lineages of Agaricomycetes found in HTS are not distinct from those of sites within CT, NT, and NTS. Principal components 1 (P1), 2 (P2), and 3 (P3) explain 10.98%, 9.88%, and 7.33% of the variation, respectively.

A)


PCA - P1 vs P2
P2 - Percent variation explained 9.88%
P1 - Percent variation explained 10.98%
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.20
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
0.20
0.25
CT
NT
HTS

B)


PCA - P3 vs P2
P2 - Percent variation explained 9.88%
P3 - Percent variation explained 7.33%
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.25
-0.20
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
0.20
CT
NT
HTS

C)

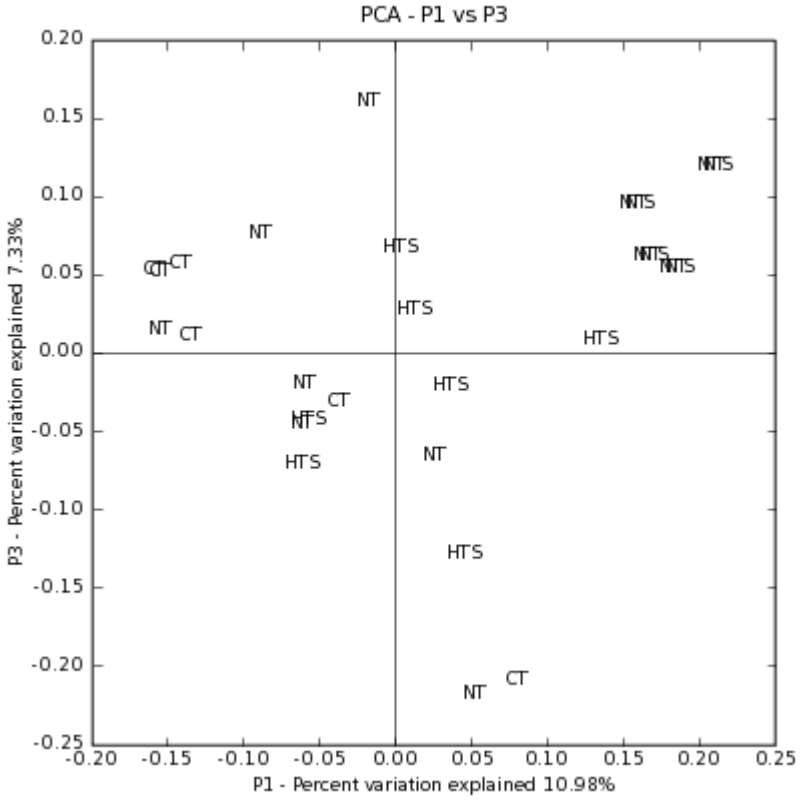


Figure 3.3 Principal coordinates analysis of KBS LTER sites based on UniFrac values from KBS and reference sequences. (A) PCoA for principal component 1 (P1) measured against principal component 2 (P2). (B) PCoA for principal component 3 (P3) measured against P2. (C) PCoA for P1 measured against P3.

3.5 Taxonomic representation in treatments studied

The recovered sequences from KBS LTER represented taxa from 13 of the 19 major clades of Agaricomycetes identified by Hibbett (2006). Table 3.2 provides a summary of the major clades and which treatments they were found in; more detailed description of the major clades can be found in Discussion. The majority of the sequences

were within the Agaricales clade, similar to findings of Bahnmann (2009). The Cantharellales, Hymenochaetales, Polyporales, and Agaricales were found across all treatments. The Auriculariales and Tulasnellales were found in NT, HTS, and NTS treatments but were absent from the CT treatment. The Sebacinales, Geastrales, and Thelephorales were found in only HTS and NTS treatments. Trechisporales was found in CT, HTS, and NTS treatments and was absent in the NT treatment. Gomphoid/Phalloid was present in NT and HTS treatments. Corticiales was present in CT and NT treatments and Russulales was found only in the HTS treatment.

Table 3.2 Major clade distribution in CT, NT, HTS, and NTS treatments at KBS LTER

CLADE	Treatment found	Role in samples from KBS
Tulasnellales	NT, HTS, NTS	ECM, saprotroph, root pathogen of crops
Cantharellales	All	Saprotroph
Polyporales	All	Saprotroph
Agaricales	All	ECM, saprotroph
Thelephorales	HTS, NTS	ECM
Hymenochaetales	All	Saprotroph
Corticiales	CT, NT	Root pathogen of corn
Geastrales	HTS, NTS	Saprotroph
Gomphoid/Phalloid	NT, HTS	Saprotroph
Sebacinales	HTS, NTS	ECM, saprotroph
Russulales	HTS	Saprotroph
Auriculariales	NT, HTS, NTS	Saprotroph
Trechisporales	CT, HTS, NTS	Saprotroph

As can be seen in Figure 3.1, similar OTUs are not strictly clustered together and separated by treatment alone. Though clusters in the tree where related OTUs may only be found in plots that are similar, like HTS and NTS, there are groups of OTUs that are shown to be related to taxa in completely different plots. This is not surprising as all the

major clades, except for Russulales found in only HTS, are detected in at least 2 different treatments, due to the relatively diverse nutritional roles of many of the major clades.

CHAPTER 4: DISCUSSION

4.1 Broad phylogenetic placement

Broad phylogenetic placement by the Neighbour-Joining method produced a large tree that is not easily comparable to the results of previous studies from the same sites, simply due to the larger data set. Constraint of the backbone of the tree forced reference sequences to group in phylogenetically appropriate clades, whilst allowing unknown sequences to fall where appropriate. Bootstrap support for pairs or groups of sequences was very high (>95%) for over 150 pairs or groups. The topology of the tree for these groups is stable and nearly all the characters informative for those groupings are adequate to validate the topology (Berry and Gascuel 1996, Bremer 1988). High bootstrap values can be considered to be statistically significant and indicate uniform support for a clade (Felsenstein 1985, Berry and Gascuel 1996, Soltis and Soltis 2003).

Analysis of 816 sequences (including reference sequences) representing 604 OTUs (including reference sequences), found that the largest proportion of sequences (13.8% of all OTUs) fell into the minor clade Clavarioid of Agaricales. The Agaricoid clade of Agaricales accounted for the second greatest proportion of sequences (11.4% of all OTUs).

4.2 Tulasnellales (Figure 3.1. A)

The Tulasnellales often form mycorrhizal associations with terrestrial plants, including orchids and liverworts (Langer 1994, Kottke et al. 2003). Other members of this clade are root pathogens of crops or are saprotrophic and found in soil (Langer 1994, Kottke et al. 2003). This clade contains 21 (4.3%) of the OTUs found at the KBS LTER

sites. Bootstrap support for most inner nodes is >82% with the exception of one node with 62% support. The outer-most nodes of the Tulasnellales clade have 90% and 94% bootstrap support. Fourteen of 21 OTUs were from HTS plots, and the rest were from NTS and NT plots. The absence of OTUs from CT plots suggests that members of this clade may be particularly sensitive to soil disruption by tillage yet persist well in plots that are not tilled.

4.3 Cantharellales (Figure 3.1. B, C)

The Cantharellales contain fungi with a variety of morphologies as well as ecological roles: ectomycorrhizal, saprotrophic, and even pathogenic (Hibbett and Thorn 2001, Moncalvo et al. 2006). However, some fungi within this order form mutually beneficial associations with trees, shrubs, and other plants (Moncalvo et al. 2006). The Cantharellales are represented by 39 OTUs that are allied to *Minimedusa* and *Burgoa*. The species of *Minimedusa* that are allied with these OTUs are usually found on fresh leaves (Kuthubutheen and Muid 1984, Matsushima 1995, Peláez et al. 2001). However, most other *Minimedusa* sp. have been found growing over corticolous lichens and *Burgoa* is generally lichenicolous (Diederich and Lawrey 2007, Humphrey et al. 2002). These OTUs are widely distributed across the different treatments but most prominent (~40% of OTUs) in the CT plots and the least in NTS plots. It is likely that members of this clade detected at KBS are saprotrophic. The variety of substrates that members of this clade can utilize may contribute to their presence in all treatments sampled.

4.4 Polyporales (Figure 3.1. D-F)

The Polyporales play ecologically important roles as wood-decayers, timber pathogens, and fungi that produce white-rot (Binder et al. 2005). However, there are no documented mycorrhizal species (Binder et al. 2005). This clade contains 23 OTUs, representing 3.8% of all OTUs from KBS LTER. Bootstrap support for inner nodes is varied in this clade, with support as low as 54% yet as high as 100%. The clade with the third most OTUs, the Polyporales, seem to be well sampled in all treatments, with roughly 12-15 recovered sequences in each treatment. High sampling frequency of the Polyporales in all four sites suggests that this clade is fairly persistent where there is appropriate substrate and furthermore it does not seem to be negatively affected by tillage. However, their presence in the HTS and NTS plots can be explained by their affinity to lignin-rich substrate, such as coarse-textured herbaceous plants like *Solidago*, dominant in non-agricultural sites at KBS.

4.5 Agaricales (Figure 3.1. G-U)

The Agaricales is the largest clade of mushroom-forming fungi and includes more than half of all known species of the Agaricomycetes (Hibbett et al. 1997, Hibbett and Thorn 2001). Many of the fungi in Agaricales have fruiting bodies with stem and umbrella-like caps, but others are resupinate and jelly fungi (Hibbett 2006). Species in this major clade are primarily ectomycorrhizal or saprotrophic, causing white or brown-rot (Hibbett 2006). In addition, *Clavaria*, *Hygrocybe*, and *Camarophyllopsis* are indicators of grassland health (Newton et al. 2003). As previously mentioned, this major clade accounted for 330 (~70%) of all OTUs from KBS LTER. Bahnmann (2009) found

69% of all of her OTUs were Agaricales. By far, this is the most prominent clade in this study as well; it can be further subdivided into 9 minor clades: Clavariaceae, Stephanosporaceae, Agaricoid, Tricholomoid, *Schizophyllum-Lachnella*, Marasmioid, Pluteoid, Hygrophoroid, and Pterulaceae.

4.5.1 Clavarioid (Figure 3.1. G-J)

The Clavariaceae or Clavarioid clade accounted for 65 OTUs or 19.7% of Agaricales. Most (>94%) of the OTUs in this minor clade were found in HTS and NTS sites, with the exception of 3 OTUs from two NT plots. Bootstrap support for the OTUs in this clade is varied, with some inner nodes weakly supported at 51% whereas some inner nodes are strongly supported at 100%. Members of this clade are saprotrophic and terrestrial in soil or among leaf litter in grasslands or in hardwood forests (Breitenbach and Kränzlin 1986, Matheny et al. 2006) and some are mycorrhizal associates of Ericaceae (Petersen and Litten 1989). Some species are also grassland fungi which may explain an ecological role that they may play in HTS; as with species of *Hygrocybe*, they have been suggested as indicators of grassland health (Keizer 1993, McHugh et al. 2001, Newton et al. 2003). Also, Clavarioids may simply be more sensitive to soil disturbance and may establish and persist best in plots with suitable substrate at the soil surface. This clade may act as a particularly good indicator of non-agricultural sites.

4.5.2 Stephanosporaceae (Figure 3.1. J)

This minor clade contains only 4 OTUs, of which 2 are reference sequences and 2 were from a single HTS plot. The absence of this clade from NTS, NT, and CT plots and

low numbers of OTUs recovered may be due to inefficient sampling due to the rarity of this clade in KBS LTER sites.

4.5.3 Agaricoid clade (Figure 3.1. K-N)

Members of this clade are diverse - some are ectomycorrhizal (*Cortinarius* and *Hebeloma*) and others are saprotrophic; some taxa produce the hallucinogenic compound psilocybin (*Inocybe* and *Psilocybe*) and some members of this clade, namely the Agaricaceae, have a symbiotic relationship with attine or fungus gardening ants (Matheny et al. 2006, Vellinga 2004, Watling and Gregory 1987, Chapela et al. 1994, Mueller et al. 1998). The Agaricoid clade accounts for 54 recovered OTUs, which are represented almost equally in CT, NT, HTS, and NTS. Bootstrap support for this clade is varied with inner nodes ranging from 51% to 100% support. Like some of the previously mentioned clades, the Agaricoid presence in all 4 treatments indicates that members of this clade have diverse ecologies and can perhaps colonize a wide variety of substrates and may also be quite resistant to soil disturbance.

4.5.4 Tricholomoid clade (Figure 3.1. O, P, T)

This clade is represented by 32 OTUs, including species found in the minor clade, Entolomatoid. Bootstrap support for this clade is varied, showing as weak support as 52% to as strong support as 100%. OTUs of sequences recovered from KBS soils were found in all treatments. This could indicate that this clade is fairly persistent in soils subject to different degrees of disturbance by tillage, from no disturbance to high disturbance. Furthermore, the members of this clade may not require substrate at the soil

surface to persist, as the Tricholomoids can be found in CT plots where crop residue would be tilled into the soil. Members of this clade are typically saprotrophic on litter and in soil in grassland ecosystems and have a broad distribution, which can help explain their distribution across all four treatments at KBS (Noordeloos 1988). Five OTUs were closely allied to *Nolanea*, a member of the Entolomatoid subclade which is often used as an indicator of undisturbed grassland habitats (Newton et al. 2003).

4.5.5 *Schizophyllum-Lachnella* clade (Figure 3.1. Q)

This minor clade is represented by 8 OTUs from KBS LTER. Bootstrap support is moderate for one group (72%), while the rest of the groups are strongly supported at >93%. OTUs from this clade were from NTS, NT, and CT plots. OTUs grouping around reference sequences of *Lachnella* are mainly from NT and CT plots, which is surprising as *Lachnella* are generally saprotrophic on wood (Unterseher et al. 2005). However, *Lachnella* may also be saprotrophic on herbaceous stems and Lynch (2004) considered it is likely that species here were on stems of corn, soybean or wheat.

4.5.6 Marasmioid clade (Figure 3.1. Q)

The Marasmioid clade has 4 OTUs recovered from KBS soils and 4 OTUs that are reference sequences. One OTU grouping in this clade has moderate bootstrap support (75%) whereas the other OTU groupings have >99% support. Recovered sequences that are represented as OTUs were found mainly in the NTS plots with one OTU being found in the HTS plot, indicating that this clade may be associated with aboveground successional communities and is not persistent in soils that are regularly disturbed by

tillage. OTUs were allied closely with *Mycena purpureofusca*, a white-rot fungus and *Armillaria gemina*, a root-inhabiting fungus (Sun et al. 2012, Bérubé and Dessureault 1989).

4.5.7 Pluteoid clade (Figure 3.1. R, S)

The Pluteoid clade consists of 43 OTUs, allied with *Pluteus* sp., *Volvariella*, *Amanita*, and *Pleurotus*, with varied bootstrap support for inner nodes, from 51%-100%. Most of the taxa in this clade are decomposers except for *Amanita* which is ectomycorrhizal (Matheny et al. 2006). Members of *Pleurotus* can even attack nematodes (Thorn et al. 2000). Most of the OTUs were recovered from HTS and NT plots. Only 5.4% of the OTUs in this group were from NTS while 20.9% of the OTUs from this clade were from CT plots. While most of the OTUs were from two very different treatments, HTS and NT, this clade's presence in all plots indicates that it is fairly persistent and resistant to different types of agricultural disturbance, whether that disturbance may be soil disturbance by tillage or aboveground plant community disturbance by mowing. This is the clade referred to as "Sister clade to *Volvariella*" by Lynch (2004) and Bahnmann (2009). The members are now resolved as part of the Pluteoid clade but still do not have named reference sequences in GenBank.

4.5.8 Hygrophoroid clade (Figure 3.1. T)

This minor clade has 14 OTUs from KBS LTER. All but one of the 9 OTUs that clustered with *Hygrocybe* were from NTS, and the other was from HTS. The five other OTUs in this clade from KBS LTER clustered around *Typhula* and *Camaprophyllus* and

were found in HTS, NT, and CT plots. The proportion of Hygrophoroids found in NTS plots suggests that this clade typically does best in plots where there is no tillage and a diverse assemblage of plants. Indeed, species in the Hygrophoroid clade have been thought to be mainly terrestrial litter decomposers and *Hygrocybe* sp. are classically associated with high diversity grasslands (Tanesaka et al. 1993, McHugh et al. 2001). However, recent evidence from stable isotope analyses suggests they may be associated with bryophytes or algae and it is unlikely that they are decomposers (Seitzman et al. 2011).

4.5.9 Pterulaceae clade (Figure 3.1. U)

This minor clade contains 11 OTUs, only one of which is a reference sequence, *Pterula echo*, a wood-inhabiting fungus (Munkacsi et al. 2004). Unfortunately, there is no bootstrap support for the monophyly of the OTUs from KBS LTER with the reference sequence *Pterula echo*. The 10 OTUs in this clade were found in each of NTS plots but in no other treatments, indicating that this clade may be well-associated with established aboveground plant communities not subject to disturbance by tillage.

4.6 Thelephorales (Figure 3.1. V)

The Thelephorales is the sister group to Polyporales, which is surprising as all species in the Thelephorales are mycorrhizal whereas the species found within the Polyporales are all saprotrophic (Hibbett 2006). The Thelephorales clade is represented by only 3 OTUs recovered from KBS soils. These OTUs are linked most closely with *Thelephora* sp. and *Tomentella botryoides* with low bootstrap support and were recovered

from HTS and NTS soils. Detection by Lynch (2004) and not by Bahnmann (2009) may suggest that these rare OTUs from Thelephorales may be detected only with a larger sampling effort.

4.7 Hymenochaetales (Figure 3.1. V, W, Y)

Species in this clade are mainly saprotrophic (Larsson et al. 2006). The majority of these species are primary decomposers and cause white-rot (Larsson et al. 2006). The Hymenochaetales also contains species that exhibit many different life strategies; some colonize living trees, blurring the distinction between saprotrophic and parasitic strategies (Larsson et al. 2006). Some, including *Rickenella* (OTUs 493-507) are capable of fruiting on or in association with Bryophytes (Larsson et al. 2006). *Coltricia perennis* forms an ectomycorrhizal association with *Pinus banksiana* (jack pine) (Larsson et al. 2006). One of the most interesting groups in the Hymenochaetales is *Hyphoderma*, which has nematode-capturing abilities (Tzean and Liou 1993). Specialized cells on the hyphae called stephanocysts and echinocysts are covered by an adhesive mucilage and attach easily to the nematode cuticle; captured nematodes are killed and the bodies penetrated by hyphae (Tzean and Liou 1993).

The Hymenochaetales is represented by 29 OTUs, accounting for 5% of OTUs. Many of the sequences have grouped with different reference sequences representing different genera of Hymenochaetales, indicating the variety of Hymenochaetales found in KBS soils. Hymenochaetales is found equally in CT, NT, and HTS, but less in NTS. This could mean that the clade Hymenchaetales is resistant to disturbance or may even persist because of disturbance. Furthermore, the variety of substrates and life strategies

exhibited by species in this clade may have allowed for a higher sampling frequency than other clades. Indeed, suitable saprobic substrates as well as bryophyetes were available in all sites (Lynch 2004).

4.8 Corticiales (Figure 3.1. V)

Species in this order have diverse nutritional roles but most have resupinate fruiting bodies (Lawrey et al. 2008). Some nutritional ecologies include mutualistic and pathogenic forms (*Waitea* and *Laetisaria*) as well as lignicolous saprobes (Diederich et al. 2003, Binder et al. 2005, DePriest et al. 2005, Lawrey et al. 2007, Stalpers and Loerakker 1982). Internal nodes show >95% bootstrap support. Corticiales is represented by only 2 OTUs recovered from CT and NT that are allied with *Waitea*, root pathogens of many different plants including corn, rice, and turfgrass (Leiner and Carling 1994).

4.9 Geastrales (Figure 3.1. W)

Commonly known as “earthstars”, this order is named after the star-like fruiting bodies (Hosaka et al. 2006). Members of this clade are typically saprotrophic, on rotting wood or soil, and are common in horticultural gardens with wood-chip mulch (Flegler 1984, Sunhede 1989, Pegler et al. 1995, Geml et al. 2005). This clade contains only 4 OTUs from KBS LTER, 3 from NTS plots, and 1 from HTS. This could indicate that this particular clade is sensitive to soil disturbance by tillage or that its presence is facilitated by a diverse assemblage of aboveground plant communities, as are present in the NTS and HTS plots. Mainly, Geastrales may be present in NTS and HTS plots as there may be more suitable substrate available for decomposition, compared to CT or NT

plots.

4.10 Gomphoid/Phalloid (Figure 3.1. W)

The Gomphoid/Phalloid clade contains of a group of fungi that is both morphologically and ecologically diverse (Hosaka et al. 2006). Fruiting body morphologies include, stink-horns, coral fungi, club fungi, gilled mushrooms, resupinate fungi, and false truffles (Hosaka et al. 2006). Both ectomycorrhizal and saprobic taxa are represented by this clade (Hosaka et al. 2006). One of the less represented clades in the phylogeny, the Gomphoid/Phalloid clade accounts for only 3 OTUs from NT and HTS plots. Poor representation of this clade may also be due to undersampling or the preference of these fungi for richer or less disturbed sites (Pegler et al. 1995). Phalloids, represented by OTUs 509-511 are allied with *Mutinus elegans*, usually found on rotting wood and *Phallus hadriani*, often found in sandy soils (Pegler et al. 1995).

4.11 Sebacinales (Figure 3.1. X)

This clade is made up of fungi that are mainly terrestrial and form mycorrhizal associations with plants (Weiss et al. 2004). Mycorrhizal taxa of *Sebacinaceae* include mycobionts of ectomycorrhizas, orchid mycorrhizas, ericoid mycorrhizas, and jungermannioid mycorrhizas (Weiss et al. 2004, Duckett et al. 2006). Sebacinales is divided into two distinct clades, A and B, which differ in their ecology (Weiss et al. 2004). Clade A represents Sebacinales that form mycorrhizal associations with the achlorophyllous orchids, *Neottia nidus-avis* and *Hexalectris spicata*, and other related photosynthetic orchids (Julou et al. 2005, McKendrick et al. 2002, Selosse et al. 2002,

Selosse et al. 2004, Taylor et al. 2003). At the same time, many of the Sebacinoids in clade A form ectomycorrhizal associations with tree and plant roots (Selosse et al. 2002, Urban et al. 2003, Walker and Parrent 2004, Moyersoen 2006). Sebacinoids in clade B have a wider array of associations, some even associating with liverwort thalli (Kottke et al. 2003). Clade B contains the important, nonspecific root endophyte, *Piriformospora indica* (Verma et al. 1998). Sebacinales have been widely shown to form mycorrhizal associations with the Ericaceae (Weiss et al. 2004). The Sebacinales clade was represented by 9 separate OTUs with >98% bootstrap support. OTU 518 has 98% bootstrap support with *Piriformospora indicia*, a non-specific root endophyte, and related species (Verma et al. 1998). The 3 remaining unknown KBS sequences share 100% bootstrap support with *Craterocolla cerasi*, which is saprotrophic on dead wood (Breitenbach and Kränzlin 1986). OTUs from this clade are found only in HTS and NTS plots, so although *Piriformospora* can form associations with crop plants such as wheat and maize (Varma et al. 1999), it was not found in agricultural plots in this study.

4.12 Russulales (Figure 3.1. X)

The Russulales are morphologically diverse, containing a variety of fruiting body forms including resupinate, discoid, effused-reflexed, clavarioid, pileate, and gasteroid (Miller et al. 2006). Some species in this clade are saprotrophic, causing white-rot and some act as timber pathogens (Hibbett 2006, Miller et al. 2006). However there are some species in this clade that are ectomycorrhizal, root parasites, and even insect symbionts (Miller et al. 2006). This clade contains only 2 OTUs from KBS, unknown sequences that are linked closely with *Peniophora* sp (a white-rotting saprotroph). Both unknown OTUs

were found in HTS plots, presumably where there would be appropriate woody or coarse herbaceous substrate that would be absent from NT and CT plots. Again, poor representation of this clade may be due to undersampling.

4.13 Auriculariales (Figure 3.1. Z)

The Auriculariales clade consists of fungi that are saprotrophic, growing mainly on dead wood (Weiss and Oberwinkler 2001). The phylogenetic placement of Auriculariales is close to Sebacinales and Trechisporales (Hibbett et al. 2007). Five OTUs, detected by Lynch (2004), were found in this clade with bootstrap support of 100%. The OTUs derived here all came from NT, HTS, and NTS plots. In Lynch's (2004) study, clusters of OTUs around *Exidia* and *Exidiopsis*, both reference sequences for Auriculariales, occurred in all other treatments except CT. These plots differ in their plant community composition but none of these treatments is tilled suggesting that members of this clade may persist in soils that are not disturbed by tillage regardless of the aboveground community composition. Since members of this clade are found on decaying woody materials, their distribution in predominantly non-agricultural treatments is consistent with the ecological description of Auriculariales as there is more litter available on the surface of these plots than is available on CT plots.

4.14 Trechisporales (Figure 3.1. Z)

The Trechisporales are composed of mainly resupinate species that give the impression of being soil-dwelling saprotrophs, but there is no indication of a mycorrhizal habit (Larsson et al. 2004, Liberta 1973). Trechisporales represent 4 OTUs with strong

(>98%) bootstrap support for groupings with the exception of one pair with weak 57% support. Of the OTUs recovered from KBS soils, two are from NTS plots and one each from HTS and CT plots. No sequences were recovered from NT plots. Although the OTU detected in the CT plot has 57% bootstrap support to *Sistotremastrum niveocremeum*, a wood-decaying fungus (Larsson et al. 2004), the occurrence of this clade in plots that did not contain woody substrate suggests that these saprotrophs are not limited to wood.

4.15 Non-represented clades

Although Agaricomycetes are generally important saprotrophs and ectomycorrhizal fungi, not all the clades in the Agaricomycetes are represented in this study. While reference sequences were given for each clade, no sequences from Hysterangiales, Gloeophyllales, Jaapiales, Atheliales, Boletales, or Amylocorticiales were recovered from KBS soils. Ectomycorrhizal fungi (e.g. Hysterangiales and most Boletales) were not expected in KBS plots since no suitable hosts are present in CT or NT and few or none are in plots of HTS or NTS. Gloeophyllales is an order of brown-rot fungi, and these along with the saprotrophic, brown-rot members of Boletales are associated with conifers, not present in the KBS plots sampled. As far as they are known at present, the Jaapiales, Atheliales, and Amylocorticiales are small groups with narrow ecological niches (Binder et al. 2005, Larsson et al. 2004), thus their apparent absence from KBS soils is not surprising.

4.16 Representation in CT plots

Of the 13 major clades detected, representatives from 6 clades, Cantharellales, Trechisporales, Hymenochaetales, Corticiales, Polyporales, and Agaricales, were found in CT plots. The clades with the greatest presence were the Cantharellales clade with 17 OTUs and the Agaricales with 37 OTUs. The Cantharellales found were allied to taxa that are saprobic on leaves. The Agaricales clade contains a wide variety of minor clades that have diverse ecological roles. The diversity of niches to be exploited by members of this major clade allow for the dominating presence of Agaricales, especially as decomposers (Matheny et al. 2006). The remaining four clades all contained less than 4 OTUs in CT plots. Of all the treatments, CT contains the fewest major clades. This is likely due to the effect of tillage on fungal hyphae as well as appropriate substrates for decomposers being turned into the soil rather than being left on the surface. Furthermore, CT plots are planted in corn, soybean, or wheat during the growing year. The lack of plant diversity and the transience of corn, soybean, or wheat crops are likely causes of the lack of fungal diversity when compared to NTS and HTS plots. The reduction in plant diversity may affect the availability of growth-limiting resources for fungi (Tilman 1982, 1987). UniFrac p-test significance for all environments tested together and P-test significance both gave values of $<<0.001$, isolating each site from the others as significantly different. PCoA of the data show that 4 of the 6 CT replicate plots are clustered tightly together and away from the rest of the treatments, with the exception of one NT site found in the middle of the CT cluster (Figure 3.3A). The remaining two CT plots are near the middle of the ordination space. It appears that 4 of 6 CT plots are phylogenetically distinct from the other sites, while 2 of the CT plots appear to contain

lineages of Agaricomycetes that are similar to those in HTS plots. This is not surprising as the 6 major clades that were detected from CT plots are also detected in HTS plots, with the exception of Corticiales.

4.17 Representation in NT plots

Eight major clades, the Auriculariales, Tulasnellales, Cantharellales, Gomphoid/Phalloid, Hymenochaetales, Corticiales, Polyporales, and Agaricales were present to some degree in NT plots. Similar to the findings in the CT plots, most of the OTUs in NT plots belong to the Agaricales. Naturally, as the largest clade with the most OTUs overall, this is not unexpected, with Agaricales contributing to ~55% of the OTUs in NT plots. Again, Agaricales may be present due to many minor clades whose main nutritional roles are that of a mycorrhizal symbiont to higher plants and decomposer of litter (Matheny et al. 2006). The increase in number and diversity of clades found in NT plots compared to CT plots may be attributed to the absence of tillage. All clades found in CT plots were also found in NT plots, with the exception of Trechisporales (which was recovered once each in CT and HTS plots and twice in NTS). PCoA analysis (Figure 3.3) shows no particular trend in the ordination of NT sites. Two particular NT plots are clustered very closely together, suggesting that these two plots contain a very similar assemblage of closely related species. The remaining NT sites are scattered throughout the ordination space indicating that species found in NT sites are not unique to NT sites.

4.18 Representation in HTS plots

All of the 13 major clades detected in this study were detected in HTS plots with the exception of one, the Corticiales. Two OTUs in Corticiales detected in KBS LTER were linked only with *Waitea*, a pathogen of grasses including the crops wheat and maize, present only in CT and NT plots.

As in the CT and NT plots, the highest number of OTUs in HTS came from the Agaricales clade, accounting for ~55% of all OTUs detected in these plots. The second highest number of OTUs in HTS came from Cantharellales and Tulasnellales clades, while the remaining clades accounted for less than 6% of the OTUs. OTUs 517-518 from HTS are strongly linked with the root associates *Sebacina* and *Piriformospora* but OTUs 514 and 520-522 from NTS are not strongly linked with any reference taxon in the Sebacinales; the latter three are weakly linked with the saprotrophic *Craterocolla.* All Cantharellales recovered from KBS LTER are phylogenetically close to saprotrophic species of *Minimedusa*; isolates T-791 (NTS), X-14 (NT), and X-44 (from soil of a nearby deciduous forest) grew vigorously in culture and are clearly saprotrophic (Thorn, unpublished). PCoA of the data show HTS plots to be not as closely clustered together as NTS plots and the distance between HTS sites in the ordination suggest that HTS contains a wide array of different clades of Agaricomycetes and indeed HTS plots contain the greatest number of clades of Agaricomycetes. The plant communities in HTS plots are diverse and not uniform between replicate plots (e.g. one plot had developed a considerable stand of saplings of black locust (*Robinia pseudoacacia*) by 2005 (http://lter.kbs.msu.edu/datatables/237), so it is not surprising to find a diverse and heterogeneous community of Agaricomycetes in these plots as well. Also, the diversity of

fungi found here may be attributed to the lack of tillage, as these plots are not disrupted by soil homogenization.

4.19 Representation in NTS plots

Somewhat fewer clades were found in the NTS plots compared to the HTS plots; 10 of 19 major clades found were Auriculariales, Sebacinales, Tulasnellales, Cantharellales, Trechisporales, Geastrales, Hymenochaetales, Polyporales, Thelephorales, and Agaricales. Gomphoid/Phalloid, Corticiales, and Russulales were not found in NTS plots yet were found in HTS plots; this could indicate that these clades may be associated with non-agricultural sites with mild disturbance in the past. As in the previous treatments, Agaricales dominated with ~75% of the OTUs in NTS belonging to Agaricales. The remaining clades contained OTUs that each contributed to less than 5% of OTUs found in NTS plots. Finding large numbers of OTUs of Agaricales can be explained by their overall large presence within the Agaricomycetes and the many functional roles they play, as previously discussed. NTS is characterized by a distinct group of Agaricomycetes: Auriculariales, Sebacinales, Tulasnellales, Geastrales, Thelephorales, and all minor clades of Agaricales. Almost half of all detected clades in these plots are not detected in CT plots. This suggests that these particular clades may be more sensitive to tillage than clades that are present in both NTS and CT plots. Furthermore, the detection of these clades in NTS plots and not in CT plots may be due to the greater diversity of plants. PCoA of the data suggest that Agaricomycetes found in the NTS plots are significantly different from fungi found in other treatments; also, the extremely close clustering of NTS sites in Figure 3.3 suggests that fungi in the NTS plots

are phylogenetically closely related to one another. UniFrac P-Values of $\leq$0.01 for all NTS plots indicate that these plots differ significantly from the rest of the tree in the lineages they contain.

4.20 Conservation of fungi through sustainable practices

Major clades of Agaricomycetes provide ecosystem services that are beneficial to soil fertility and play an essential role in nutrient cycling (Ananyeva et al. 1999). The goal of sustainable agriculture is to enhance natural ecosystem services while maintaining viable agricultural production. The overarching goal is to ensure that future generations are well-supported and that our actions today are facilitating this goal. The conservation of fungi in general is overlooked as a goal to sustainable agriculture but it is evident that by the ecological descriptions and results of this and previous studies that some agricultural practices are detrimental to (at least to some members of) Agaricomycete communities.

Though successional communities, HTS and NTS, had the greatest diversity of Agaricomycetes, these communities are not present in most large-scale agricultural regimes. NT plots had 25% more clades of Agaricomycetes than did CT plots. The species of fungi found in NT plots have diverse ecological roles as decomposers and mycorrhizal fungi that contribute to the free ecological services mentioned above. Therefore, it is suggested that, in order to enhance or maintain diversity of fungal communities in agricultural regimes, reduction in soil disturbance and cropping rotations are adopted (Stromberger 2005). Adopting conservative agricultural practices is not only beneficial to maintaining diverse fungal communities but also contributes to ecosystem

health – objectives of sustainable agriculture (Doran 2002). These practices are also of immediate value to farmers as their crops are dependent on soil health.

Conversion from conventional tillage to no tillage management or reduced-tilllage will reduce soil physical disturbance. Fungal hyphae will escape being broken up and this should increase the length of fungal hyphae and increase the proportion of fungal biomass, since organic matter would remain available at the surface for degradation (Stromberger 2005). Beare et al. (1997) reported lengths of fungal hyphae in surface soil under no till agricultural treatments were 1.3-1.5 times longer than fungal hyphal lengths under conventional tillage. Cropping rotations that incorporate different crops will increase in the heterogeneity of niches available for Agaricomycetes via more diversified substrate resources (Stomberger 2005, Bossio et al. 1998, Schutter et al. 2001) and the increase in plant diversity will likely promote ecosystem functioning (Reich et al. 2012). Maintaining a diversity of Agaricomycetes could be further incorporated as a solution for sustainable agriculture by planting varied habitats surrounding large agricultural fields or even the preserving similar surrounding habitats, like non-tilled land or natural vegetation. In this way, a greater diversity of taxa of Agaricomycetes and likely other fungi, such as vesicular abuscular mycorrhizal fungi, may be made available to planted crops.

CHAPTER 5: CONCLUSION

Sustainable agricultural practices are beginning to be adopted to help conserve soil as a resource and protect it from further degradation. However, the degree to which certain agricultural practices, like tillage, affect soil Agaricomycetes is only beginning to be understood. In general, soil fungi are affected by conventional tillage, likely through soil homogenization and damage to fungal hyphae. CT plots had the lowest Agaricomycete species diversity of all the plots. NT plots had more clades of Agaricomycetes when compared to CT plots. The non-agricultural HTS and NTS plots had the greatest diversity of Agaricomycetes. These results support previous studies on tillage affecting soil Agaricomycetes, from the same area.

5.1 Direction of future research

Lynch (2004) and Bahnmann (2009) found similar overall patterns of minor clade distribution among treatments; however, numerous unknown species were detected in multiple plots within each study year. Lynch (2004) found many situations in which entire clades had no relevant ingroups to aid in phylogenetic resolution; this was a function of poor database coverage in GenBank. However, with improved GenBank data base coverage, an increase in the number of GenBank reference sequences, and the placement of previously unknown taxa of Agaricomycetes based on published (Hibbett et al. 2007, Matheny et al. 2007) and unpublished data (Thorn and Hibbett), these clades are now more resolved and better supported, and previously unknown species have been identified accordingly. Even with improvements in GenBank reference sequences, the clade referred to as “Sister clade to *Volvariella*” by Lynch (2004) and Bahnmann (2009)

is now resolved as part of the Pluteoid clade but still does not have named reference sequences in GenBank. In addition, PCoA showed how different plots were separated by the phylogenetic lineages of Agaricomycetes they contained, which was not shown in previous work.

Lynch's (2004) and Bahnmann's (2009) findings on the effects of niche heterogeneity on Agaricomycete diversity are mirrored in this study. Even with a larger data set, the general trend remains the same: as niche heterogeneity (e.g. plant diversity) increased there was an increase in diversity of Agaricomycetes. However, these results are limited to KBS LTER in Michigan. It may be necessary to test the research questions put forth by this study to other comparable locations. It would be interesting to see if the trends exhibited here are supported in other locations as well. This will be central to advancing our knowledge on Agaricomycete presence in agricultural and successional soils.

Next generation sequencing (NGS), a sensitive sequencing technique that can produce millions of sequences from the same volume of samples and can provide a more comprehensive data set to work with. Using single DNA molecules and skipping traditional DNA amplification, short reads of sequences (now approximately 250 to 400 base pairs) can be produced (ten Bosch and Grody 2008, Voelkerding et al. 2009, Tucker et al. 2009, Fullwood et al. 2009, Morozova and Marra 2008, Petterson et al. 2009). NGS would help to provide a more quantitative data set that may reveal trends not detectable at this level of study. Rare taxa may be better represented due to an increase in sequences and may be more easily resolved. Furthermore, NGS may obtain a more quantitative measure of the relative abundance of predominant taxa. Obtaining combined sequence

data for both plants and fungi could allow for identification of plant community members associated with particular clades or OTUs of fungi, greatly improving the speculations made herein about the possible ecological relations of the fungi detected. Sustainable agriculture will require changes in many agricultural practices, yet further investigation is necessary to determine which of the interacting agricultural practices has the greatest impact on Agaricomycetes in the Michigan region and other agriculturally relevant areas of North America.

This study demonstrates that tillage affects the community assemblage of Agaricomycetes and that, in general, the diversity of Agaricomycetes in soils that are conventionally tilled is less than in soils under no till management, but each of these has less fungal diversity than areas with a diverse plant community, such as in the successional treatments at KBS LTER. However the effects of conventional tillage on community structure of Agaricomycetes may indeed be synergistic with the effects of other traditionally unsustainable agricultural practices such as mono-cropping, chemical fertilizer inputs, and herbicide and pesticide usage. Niche heterogeneity, aboveground plant diversity, and chemical inputs are factors potentially affecting community assembly of Agaricomycetes that should be examined in future studies.

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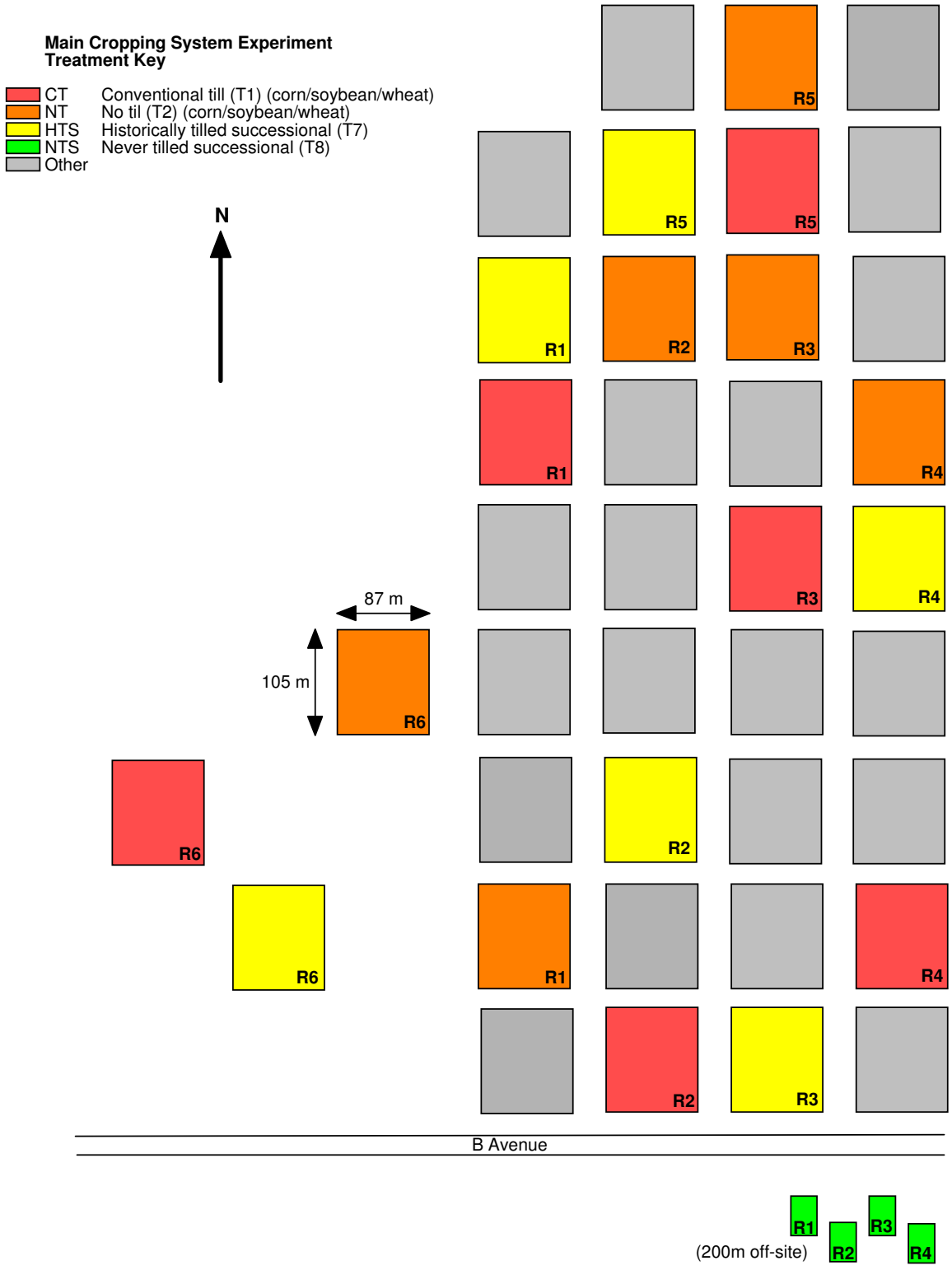
Appendix 1. Main Cropping System Experiment (MCSE) at Kellogg Biological Station Long Term Ecological Research (KBS LTER) site. Numbers preceded by "R" are replicate plots within treatments. Distances between plots are not to scale.



Appendix 2. GenBank accession numbers for reference sequences used in phylogenetic analysis. Exemplar sequences shown in **bold** and closest BLAST matches shown in regular font.

Species	Accession Number	Clade
Agaricus campestris	FJ755230	Agaricoid
Agrocybe smithii	DQ110873	Agaricoid
Alloclavaria purpurea	DQ457657	Hymenochaetales
Alnicola inculta	JN938855	Agaricoid
Alpova diplophloeus	DQ384577	Boletales
Amanita citrina	EU522722	Pluteoid
Amaurodon aquicoeruleus	AM490944	Thelephorales
Amaurodon viridis	AM490942	Thelephorales
Amaurodon viridis	AY586625	Thelephorales
Amylocorticium cebennense	GU187561	Amylocorticiales
Anomoporia bombycina	GU187564	Amylocorticiales
Antrodia albida	EU232272	Antrodia clade
Armillaria gemina	DQ338543	Marasmioid
Astraeus hygrometricus	DQ682996	Boletales
Athelia epiphylla	GU187558	Atheliales
Auricularia auricula-judae	DQ520099	Auriculariales
Auricularia auricula-judae	JN712676	Auriculariales
Boletus edulis	DQ071747	Boletales
Bondarzewia montana	DQ234539	Russulales
Botryobasidium botryosum	DQ089013	Cantharellales
Burgoa moriformis	DQ915477	Cantharellales
Camarophyllopsis hymenocephala	DQ457679	Clavarioid
Camarophyllopsis hymenocephala	EF561628	Clavarioid
Camarophyllopsis schulzeri	AM946415	Clavarioid
Camarophyllus pratensis	AF261457	Hygrophoroid
Ceraceomyces fouquieriae	GU187608	Phlebia clade
Ceratobasidium ramicola	HQ424243	Tulasnellales
Chondrostereum purpureum	AY586644	Marasmioid
Clavaria alboglobospora	HQ877682	Clavarioid
Clavaria argillacea	HQ877683	Clavarioid
Clavaria acuta	AY228353	Clavarioid
Clavaria acuta	EF535278	Clavarioid
Clavaria acuta	HQ877679	Clavarioid
Clavaria citrinorubra	HQ877686	Clavarioid
Clavaria falcata	Thorn, unpublished	Clavarioid

Species	Accession Number	Clade
Clavaria fragilis	HQ877687	Clavarioid
Clavaria fuscata	HQ877691	Clavarioid
Clavaria rosea	HQ877694	Clavarioid
Clavaria straminea	EF535267	Clavarioid
Clavaria subacuta	HQ877699	Clavarioid
Clavariadelphus ligula	AF347099	Gomphales
Clavicorona taxophila	AF115333	Clavarioid
Clavicorona taxophila	HQ877701	Clavarioid
Clavulinopsis helvola	AY586647	Clavarioid
Clavulinopsis helvola	EU118617	Clavarioid
Clavulinopsis laeticolor	EU118618	Clavarioid
Clitocybe subditopoda	FJ755224	Tricholomoid
Clitopilus giovanellae	EF413026	Tricholomoid
Clitopilus prunulus	AY228348	Tricholomoid
Coltricia perennis	AF287854	Hymenochaetales
Coniophora olivacea	GU187572	Boletales
Coprinellus micaceus	AY207182	Agaricoid
Coprinellus micaceus	HQ604762	Agaricoid
Coprinellus radians	FJ185160	Agaricoid
Coprinellus xanthothrix	FJ755223	Agaricoid
Coprinus cordisporus	DQ389723	Agaricoid
Coprinus friesii	AF041503	Agaricoid
Cortinarius austroduracinus	AY669653	Agaricoid
Cortinarius austroturmalis	AF539730	Agaricoid
Cortinarius badiovinaceus	FJ717583	Agaricoid
Cortinarius brunneus	FJ039682	Agaricoid
Cortinarius carneolus	AF539712	Agaricoid
Cortinarius junghuhnii	HQ604675	Agaricoid
Cortinarius lustrates	AY174853	Agaricoid
Cortinarius malicorius	AY669583	Agaricoid
Cortinarius obtusus	HQ604670	Agaricoid
Cortinarius suaveolens	AY669574	Agaricoid
Cortinarius vaginatus	AY669609	Agaricoid
Craterocolla cerasi	DQ520103	Sebacinales
Cyathus striatus	AF336247	Agaricoid
Cyphella digitalis	AY635771	Cyphellaceae
Cyphellopsis anomala	AY570999	Schizophyllaceae
Dacrymyces chrysospermus	EU522780	Dacrymycetales

Species	Accession Number	Clade
Daedalea quercina	AF518613	Antrodia clade
Dictyonema glabratum	EU825960	Hygrophoroid
Eichleriella leveilleana	AY509553	Auriculariales
Epithele typhae	DQ457665	CorePolypores
Erythricium laetum	GU590878	Corticiales
Exidia glandulosa	AY509555	Auriculariales
Exidia uvapsassa	AY645056	Auriculariales
Gautieria otthii	AF393058	Gomphales
Geastrum rufescens	AF336251	Geastrales
Gloeocystidiellum aculeatum	AF265546	Russulales
Gloeocystidiellum aculeatum	AF506433	Russulales
Gloeophyllum sepiarium	HM536061	Gloeophyllales
Gloeoporus taxicola	AY586656	Phlebia clade
Grandinia barba-jovis	HQ604827	Cantharellales
Hebeloma eburneum	JN939973	Agaricoid
Hydnochaete duportii	AY635770	Hymenochaetales
Hydnomerulius pinastri	GU187580	Boletales
Hygrocybe coccinea	EU435146	Hygrophoroid
Hygrocybe conica	AF261450	Hygrophoroid
Hygrocybe conica	AY684167	Hygrophoroid
Hygrophoropsis aurantiaca	AY684156	Boletales
Hymenochaete rubiginosa	AY586665	Hymenochaetales
Hymenochaete semistupposa	EU599573	Hymenochaetales
Hygrophoropsis aurantiaca	AY684156	Boletales
Hyphoderma medioburiense	DQ677497	Hymenochaetales
Hyphoderma obtusum	AY586670	Hymenochaetales
Hyphoderma roseocremeum	AY586672	Hymenochaetales
Hyphoderma setigerum	AY586673	Hymenochaetales
Hyphodontia alutaria	DQ873603	Hymenochaetales
Hyphodontia alutaria	EU118631	Hymenochaetales
Hyphodontia arguta	DQ873605	Hymenochaetales
Hyphodontia borealis	AY586677	Hymenochaetales
Hyphodontia nespori	DQ873622	Hymenochaetales
Hyphodontia paradoxa	FN907912	Hymenochaetales
Hypochnicium detriticum	DQ677507	Residual Polypores
Hypochnicium geogenium	JN939576	Residual Polypores
Inocybe abjecta	HQ604751	Agaricoid
Inonotus linteus	AY839832	Hymenochaetales

Species	Accession Number	Clade
Irpex lacteus	EU522839	Phlebia clade
Jaapia argillacea	EU118636	Jaapiales
Lachnella alboviolascens	AY571012	Schizophyllaceae
Lachnella villosa	AY571013	Schizophyllaceae
Laetisaria fuciformis	EU118639	Corticiales
Leccinum aurantiacum	AF139689	Boletales
Lentaria albovinacea	AJ406552	Gomphales
Lentinellus cochleatus	AF506417	Russulales
Lentinus crinitus	AY615980	Core Polypores
Lepista nebularis	DQ457658	Tricholomoid
Lepista nuda	AF139963	Tricholomoid
Lindtneria trachyspora	EU118646	Stephanosporaceae
Lyophyllum boudieri	AF223206	Tricholomoid
Lyophyllum boudieri	DQ825430	Tricholomoid
Lyophyllum decastes	AY207228	Tricholomoid
Lyophyllum tylicolor	AF139964	Tricholomoid
Meruliopsis taxicola	EU118648	Phlebia clade
Meruliopsis taxicola	GQ470633	Phlebia clade
Minimedusa obcoronata	GQ303309	Cantharellales
Minimedusa polyspora	DQ915476	Cantharellales
Mutinus elegans	AY574643	Phallales
Mycena purpureofusca	HQ604764	Marasmioid
Naucoria salicis	FJ904180	Agaricoid
Neolentinus lepideus	HM536075	Gloeophyllales
Nolanea verna	EU669337	Tricholomoid
Nolanea sericea	AF223170	Tricholomoid
Omphalina antarctica	GQ483368	Hygrophoroid
Omphalina farinolens	EF413028	Hygrophoroid
Peniophora incarnata	AF506425	Russulales
Peniophora pini	EU118651	Russulales
Phallus hadriani	DQ218514	Phallales
Phanaerochaete chrysosporium	AF139966	Phlebia clade
Phanaerocheate ginnsii	GQ470645	Phlebia clade
Phanaerocheate stereoides	GQ470661	Phlebia clade
Phlebia radiata	AF287885	Phlebia clade
Piloderma fallax	DQ469285	Atheliales
Piriformospora indica	AY505557	Sebacinales
Pleurotus tuber-regium	EU908174	Pluteoid

Species	Accession Number	Clade
Plicaturopsis crispa	DQ470820	Amylocorticiales
Pluteus cervinus	EU486448	Pluteoid
Pluteus cervinus	HQ604793	Pluteoid
Polyporus tuberaster	AF261544	Core Polypores
Podoscypha multizonata	EU118663	Residual Polypores
Porotheleum fimbriatum	DQ457673	Schizophyllaceae
Porpomyces mucidus	AF347092	Trechisporales
Porpomyces mucidus	FJ496696	Trechisporales
Psathyrella artemisiae	AM712248	Agaricoid
Psathyrella fibrillosa	DQ389686	Agaricoid
Psathyrella longicauda	DQ389676	Agaricoid
Psathyrella multipedata	AM712279	Agaricoid
Psathryrella sphaerocystis	DQ389707	Agaricoid
Psathyrella tenuicula	DQ389704	Agaricoid
Psilocybe cyanescens	HM035076	Agaricoid
Psilocybe inquilina	AF261598	Agaricoid
Psilocybe pratensis	AF261600	Agaricoid
Psilocybe schoeneti	AF261602	Agaricoid
Psilocybe xeroderma	AF261601	Agaricoid
Pterula echo	AY629315	Pterulaceae
Pycnoporus cinnibarinus	AF393074	Core Polypores
Pycnoporus cinnibarinus	AY586703	Core Polypores
Radulomyces notabilis	EU118664	Pterulaceae
Ramaria rubella	AY645057	Gomphales
Ramariopsis aurantio-olivaea	HQ877711	Clavarioid
Rhizochaete americana	AY219391	Phlebia clade
Rhizochaete fouquieriae	AY219390	Phlebia clade
Rhizoctonia zeae	GQ221862	Tulasnellales
Rhizoctonia zeae	JN189718	Tulasnellales
Rickenella fibula	AY700195	Hymenochaetales
Russula emetica	DQ421997	Russulales
Sarcomyxa serotina	EU365678	Hygrophoroid
Schizophyllum commune	FJ372712	Schizophyllaceae
Schizopora paradoxa	AJ406463	Hymenochaetales
Scleroderma citrinum	EU718151	Boletales
Scytinostroma portentosum	AF506470	Russulales
Sebacina vermifera	DQ520096	Sebacinales
Sebacina vermifera	DQ520103	Sebacinales

Species	Accession Number	Clade
Sebacina vermifera	DQ983815	Sebacinales
Serpula himantioides	GU187602	Boletales
Sistotrema confluens	AY647214	Cantharellales
Sistrotremastrum niveocremeum	AF347094	Trechisporales
Sphaerobolus ingoldii	AF139975	Geastrales
Sphaerobolus stellatus	HQ604795	Geastrales
Steccherinum fimbriatrum	EU118668	Phlebia clade
Stephanospora caroticolor	AF518652	Stephanosporaceae
Stropharia rugosoannulata	AF518654	Agaricoid
Suillus luteus	AY586715	Boletales
Thanatephorus cucumeris	AF354076	Tulasnellales
Thanatephorus cucumeris	DQ917658	Tulasnellales
Thelephora sp	AF287890	Thelephorales
Tomentella botryoides	AY586717	Thelephorales
Tomentellopsis bresadoliana	EU118674	Thelephorales
Tomentellopsis echinospora	AY586718	Thelephorales
Trametes versicolor	AF139961	Core Polypores
Trametes versicolor	AF347107	Core Polypores
Trametes versicolor	AY684159	Core Polypores
Trametes versicolor	HM595617	Core Polypores
Trechispora farinacea	AF347089	Trechisporales
Trechispora farinacea	EU909231	Trechisporales
Trechispora alnicola	AY635768	Trechisporales
Trechispora kavinioides	AF347086	Trechisporales
Tremella aurantia	DQ156127	Tremellales
Tricholoma apium	AY586721	Tricholomoid
Tricholoma apium	DQ389736	Tricholomoid
Tricholoma orirubens	DQ389734	Tricholomoid
Tricholomella constricta	AF223187	Tricholomoid
Trichosporon dulcitum	JN939493	Tremellales
Tulasnella violea	DQ520097	Tulasnellales
Tulostoma kotlabae	DQ112629	Agaricoid
Typhula phacorrhiza	AF261374	Hygrophoroid
Volvariella bombycina	HM562256	Pluteoid
Waitea circinata	AY885164	Corticiales

CURRICULUM VITAE

Name:	Jessie Rachel Wong
Education:	University of Western Ontario London, Ontario, Canada 2006-2010, B.Sc. in Biology and Environmental Science
	University of Western Ontario London, Ontario, Canada 2010-2012, M.Sc. in Biology with Environment and Sustainability
Awards:	Western Graduate Research Scholarship 2010-2012
Relevant Work Experience:	Research Associate Dr. R. G. Thorn The University of Western Ontario Sept 2010-April 2010
	Teaching Assistant The University of Western Ontario 2010-2012